Theor Appl Genet (1992) 83:1027–1034

THEORETICAL AND APPLIED GENETICS

Lycopersicon esculentum lines containing small overlapping introgressions from *L. pennellii*

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Received June 27, 1991; Accepted October 1, 1991
Communicated by F. Salamini

Summary. The objective of this project was to introgress small overlapping chromosome segments which cover the genome of *L. pennellii* into *Lycopersicon esculentum* lines. The interspecific hybrid was backcrossed to *L. esculentum*, and a map of 981 cM, based on 146 molecular markers covering the entire genome, was produced. A similar backcross 1 population was selfed for six generations, under strong selection for cultivated tomato phenotypes, to produce 120 introgression lines. The introgression lines were assayed for the above-mentioned molecular markers, and 21 lines covering 936 cM of *L. pennellii*, with an average introgression of 86 cM, were selected to provide a resource for the mapping of new DNA clones. The rest of the lines have shorter introgressions consisting of specific regions with an average size of 38 cM. The proportion of the *L. pennellii* genome in the introgression lines was lower than expected (252 cM) because of strong selection against the wild-parent phenotype. The mean introgression rate for ends of linkage groups in the 120 lines was 3 times higher than for other regions of the genome. The introgression lines can assist in RFLP-based gene cloning by allowing the rapid selection of DNA markers that map to specific chromosome segments. The introgression lines also provide a base population for the mapping and breeding for quantitative traits such as salt and drought tolerance that characterize the wild species *L. pennellii*.

Key words: *Lycopersicon esculentum* – *L. pennellii* – RFLP – Introgression lines – Breeding

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Introduction

Restriction fragment length polymorphisms (RFLPs) provide a system for high resolution mapping of genomes. In plants, various populations have been subjected to RFLP mapping analysis. (1) F_2 and backcross (BC) generations; these traditional mapping populations were produced either within or between species, depending on the level of RFLPs in the analyzed genomes (Tanksley et al. 1989). (2) Recombinant inbreds (RI); such lines have been developed in maize, which is a highly polymorphic species, as a result of the inbreeding of F_2 populations (Burr et al. 1988). The main advantage of RI over the F_2 and BC populations is that it constitutes a permanent mapping resource that can be propagated from seed and analyzed by a number of investigators whose results are cumulative. RI lines can be used for the analysis of quantitative traits since each inbred genotype can be planted in replicated trials (Burr and Burr 1991). (3) Cytogenetic variants; uneuploid stocks allow a rapid assignment of DNA clones to chromosomes through a comparison of the relative intensities of the bands produced in Southern blots (Young et al. 1987; Sharp et al. 1989). In maize, RFLP loci can be mapped to chromosome regions using B–A translocations (Weber and Helentjaris 1989). (4) Nearly isogenic lines (NILs); lines differing in only a small chromosome segment were used to map DNA clones that are tightly linked to disease resistance genes in tomato (Young et al. 1988; Sarfatti et al. 1989; Levesque et al. 1990; Messeguer et al. 1991; Klein-Lankhorst et al. 1991 a; Martin et al. 1991; Sarfatti et al. 1991; Behare et al. 1991). Such clones can be identified because resistance genes have been introgressed into cultivated varieties from wild relatives and the process of introgression has left a residual chromosome segment of polymorphic DNA flanking the gene of interest.

The introgression lines described in this study provide a set of NILs for a large portion of the tomato genome.

Lycopersicon pennellii is a green-fruited species commonly used as the material of choice for genetic studies in tomato. The accession LA716, which was collected in the Peruvian desert, is self-compatible, highly inbred and can be easily hybridized to *L. esculentum* (Rick and Tanksley 1981). The tomato RFLP map, which includes more than 600 markers covering the 12 chromosomes, was developed through analysis of an F_2 population resulting from a cross between the cultivated tomato *L. esculentum* and *L. pennellii* (Tanksley et al. 1989). The interspecific population was used for mapping, since the degree of polymorphism detected by RFLP analysis between *L. esculentum* and *L. pennellii* is more than 15 times higher than between *L. esculentum* accessions (Miller and Tanksley 1990). In this study we used the tomato RFLP system to construct a population of plants containing short overlapping introgressions of the *L. pennellii* genome in a processing tomato genetic background.

Materials and methods

BC1 mapping population

For mapping analysis *L. esculentum* cv 'Vendor' was crossed as the female parent to *L. pennellii* (LA716). The interspecific hybrid was backcrossed as the male parent to 'Vendor', and 119 BC1 progenies were grown and subjected to RFLP analysis.

Introgression lines

The *L. esculentum* processing tomato cv. 'M82-1-8' was crossed to *L. pennellii* (LA716), and the F_1 hybrid backcrossed to 'M82-1-8'. Of the 600 BC1 plants grown in the field in 1984, 99 were selected on the basis of seed availability. During the following year 12 individuals from each of the 99 selfed BC1 plants (BC1S1) were grown and selected for *L. esculentum* characteristics such as growth habit, fruit color, fruit size and yield. This basic scheme of selfing and single plant selections in the field (an average of 1500 plants grown annually, of which approximately 100 were selected for the next generation) was continued until BC1S6, at which stage 120 plants representing 20 of the original BC1 individuals were chosen for RFLP analysis. Field selection was conducted in the Negev Desert, Israel, where plants were drip-irrigated with saline water of 10–20 dS/m according to Saranga et al. (1991).

RFLP analysis

DNA was extracted from 'Vendor', 'M82-1-8', *L. pennellii*, the interspecific hybrids, the BC1 mapping population and the BC1S6 plants. DNA isolation, restriction digests, electrophoresis on agarose gels, Southern blots, hybridizations and autoradiography were as described by Bernatzky and Tanksley (1986), except that filters were probed with random hexamer-labeled plasmids (Feinberg and Vogelstein 1983).

In order to determine the RFLPs for analysis of the BC1 and BC1S6 populations, 134 random genomic and cDNA clones (of which 5 identified unlinked duplicated loci) covering the entire genome were hybridized to survey filters carrying DNA of *L. esculentum* ('Vendor' and 'M82-1-8'), *L. pennellii* (LA716) and the interspecific hybrids digested with the following restriction enzymes: *Eco*RI, *Eco*RV, *Dra*I, *Hae*III, *Xba*I and *Bst*NI. Clones were hybridized individually to the populations on the basis of the appropriate polymorphism between the parents. Markers that cosegregated in the BC1 population were not assayed in the BC1S6 (Table 1).

Isozyme analysis for the loci *Prx-1*, *Pgm-2*, *Aps-1*, *Aps-2*, *Got-2*, *6Pgdh-2* and *Pgi-1* was performed according to Zamir and Tal (1987).

Mapping analysis

Linkage maps were constructed using MapMaker (Lander et al. 1987) and employing the Kosambi mapping function. Markers were ordered along the linkage groups only if they exhibited preferred orientation with a Lod score greater than two. The order was verified by manual examination of the data in three-point linkage tests for minimum number of double recombinants.

Genome composition

Genome composition in the BC1 and introgression lines was estimated according to Paterson et al. (1991). When consecutive markers along the chromosomes of an individual showed the same genotype, it was assumed that the region intervening between the markers was composed entirely of the marker genotype. When consecutive markers along the chromosomes of an individual showed different genotypes, it was assumed that half of each genotype made up the whole interval. Such estimates disregard the possibility of double recombinants within an interval. To compare the BC1 population and the introgression lines, we calculated genome composition on the basis of a haploid genome. This approach yields the same composition estimate for an introgression line homozygous to an *L. pennellii* segment as it does for an introgression lines heterozygous to the same segment.

The expected total length (ETL) of chromosome segments carrying the *L. pennellii* alleles, either in the homozygous or heterozygous state, out of the total map length (TML) after n generations of selfing of a BC1 population is: $ETL = \{[0.5^n + (0.5 - 0.5^n)/2] \times TML\}$. The expected ratio (ER) of heterozygous introgressed segments out of the total introgressed segments after n generations of selfing of a BC1 population is: $ER = \{(0.5^n)/[0.5^n + (0.5 - 0.5^n)/2]\}$.

Results

The BC1 mapping analysis

The RFLP map, based on 119 BC1 individuals, includes 146 molecular markers (Fig. 1). The restriction enzymes used for mapping and the number of BC1 plants scored are shown in Table 1. Total map length was 981 cM and the average distance between the markers was 8.4 cM. The mean number of recombination events for a BC1 individual over its entire genome was 8.8, with a minimum of 3 and a maximum of 17.

An average diploid BC1 individual would be expected to contain 50% of the *L. pennellii* genome (490 cM) in a heterozygous state. In our population an average BC1 plant contained 519 cM from *L. pennellii* with a minimum of 237 cM and a maximum of 794 cM (Fig. 2). The

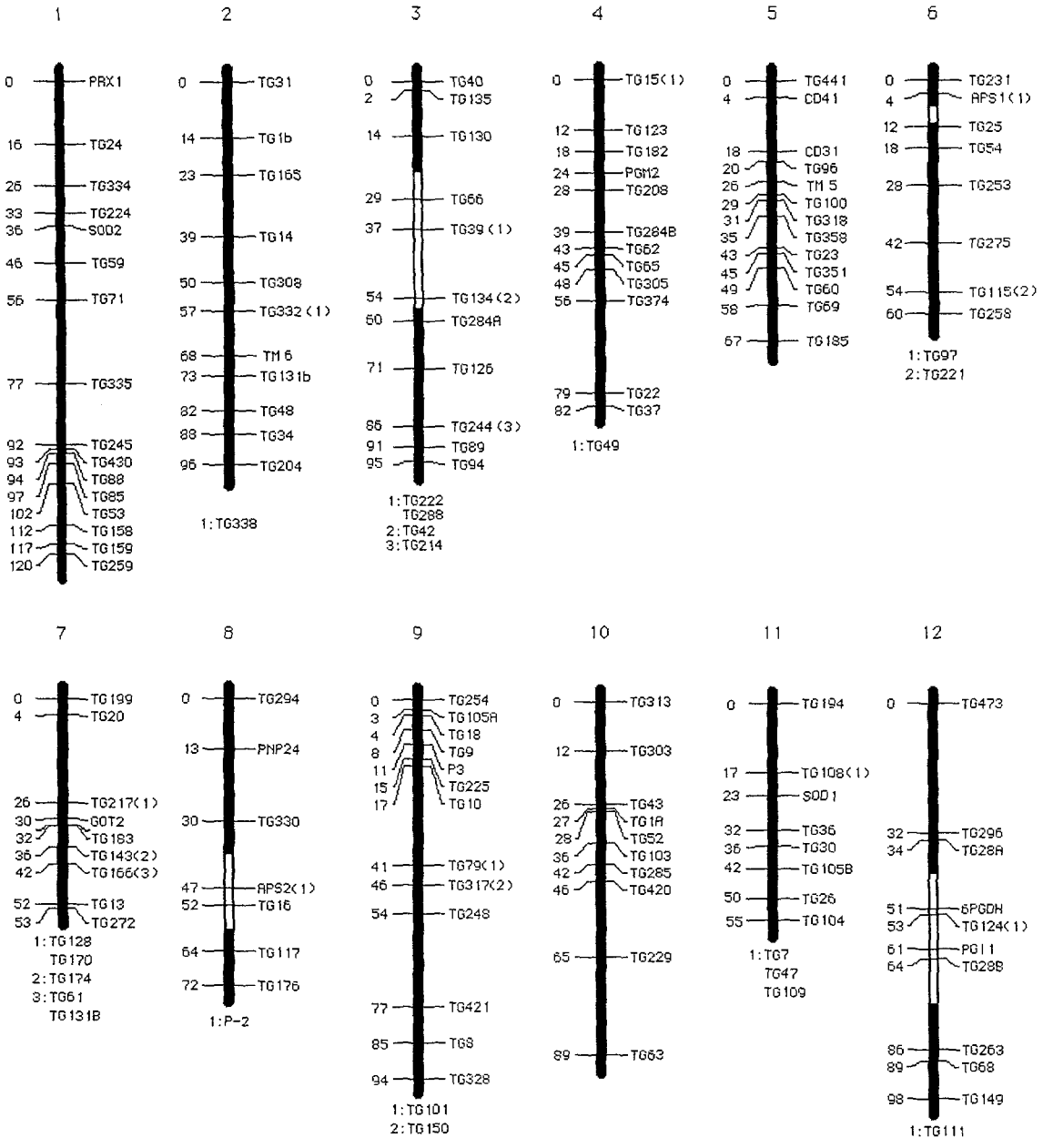

Fig. 1. RFLP linkage map of the tomato developed by segregation analysis in a BC1 generation derived from an *L. esculentum* × *L. pennellii* cross. Markers at the bottom of the chromosomes cosegregated with the markers having the same numbers in *brackets*. The *black regions* represent the portion of the genome included in the introgression lines

higher than expected inclusion of *L. pennellii* chromosome segments in the BC1 resulted from deviations from the expected Mendelian ratios. Regions on chromosomes 1, 2, 6, 9, 10, 11 and 12 showed a significant excess of *L. pennellii* alleles, and the *L. esculentum* alleles were favored only for chromosome 8 (Table 1).

Introgression lines

From the selected BC1S6 lines, 120 plants were scored for 129 molecular markers. With no selection, an average BC1S6 plant would be expected to contain 252.5 cM of the wild parent, of which 88.5% would be in a homozygous state. The genome composition of the BC1S6 population indicates that the mean inclusion of the wild-parent genome was 47 cM (Fig. 2), of which 68% were in a homozygous condition. The mean number of independent introgressions for an average plant in the population was 2.61.

Four regions on chromosomes 3, 6, 8 and 12 were selected out during the breeding of the introgression lines (Fig. 1). The data indicate that, in general, the chromo-

Table 1. Segregation of 129 markers in a BC1 generation derived from an *L. esculentum* × *L. pennellii* hybrid and the number of BC1S6 introgression lines (ILs) containing the *L. pennellii* allele at each locus

Locus	Mapping enzyme[a]	EE:EP[b]	Number of ILs with *L. pennellii* allele
Chromosome 1			
Prx-1		54:53	2/120 (1)[c]
TG24	2	54:52	1/119
TG334	5	43:45	1/120
TG224	2	43:45	2/120
Sod-2	3	52:61	3/119
TG59	4	59:54	3/115
TG71	4	56:56	2/120
TG335	5	33:55*	2/120
TG245	5	31:58**	7/120
TG430	3	27:41	7/120
TG88	3	36:46	8/120
TG85	4	41:56	8/120
TG53	4	47:58	7/116
TG158	5	37:51	10/120
TG159	5	40:49	8/120
TG259	6	51:56	8/120
Chromosome 2			
TG31	3	44:65	7/120 (5)
TG1B	1	45:67*	8/120
TG165	3	20:31	8/120
TG14	1	48:66	8/116
TG308	2	33:43	12/120
TG332	5	36:49	13/120
TM6	5	43:49	12/120
TG131B	3	48:68	12/120
TG48	2	50:61	4/120
TG34	6	52:65	4/119
TG204	4	47:56	8/108 (4)
Chromosome 3			
TG40	2	46:48	18/120 (7)
TG135	6	61:55	11/120
TG130	4	63:56	2/120
TG66	1	59:60	0/120
TG39	2	56:58	0/120
TG134	1	54:54	0/120
TG284A	3	38:47	1/120
TG126	4	40:45	1/120
TG244	5	54:44	3/120 (2)
TG89	4	63:54	1/117
TG94	4	23:29	2/120 (1)
Chromosome 4			
TG15	2	49:50	4/116
TG123	3	59:58	9/120
TG182	2	23:36	9/118
Pgm-2		54:59	2/120
TG208	2	41:38	2/120
TG284B	3	46:31	3/116
TG62	2	49:44	2/119
TG65	4	46:55	2/120
TG305	1	39:28	6/118 (1)
TG374	2	32:25	5/120
TG22	3	47:42	6/120
TG37	5	56:37	6/120

Table 1. cont.

Locus	Mapping enzyme[a]	EE:EP[b]	Number of ILs with *L. pennellii* allele
Chromosome 5			
TG441	4	33:33	3/120 (1)
CD41	4	45:52	4/120 (2)
CD31	6	54:49	2/120
TG96	4	56:56	2/120
TM5	1	53:54	2/120
TG100	2	58:58	4/120
TG318	2	57:59	2/120
TG358	3	41:26	3/120 (1)
TG23	1	56:63	9/119 (1)
TG351	3	36:36	8/120
TG60	3	53:61	11/120
TG69	3	44:53	13/120
TG185	4	42:54	16/119 (7)
Chromosome 6			
TG231	4	26:48*	4/119
Aps-1		53:65	4/120
TG25	1	44:51	1/118
TG54	3	44:59	2/120
TG253	3	47:53	2/120
TG275	1	54:61	1/120
TG115	3	58:60	5/120
TG258	3	47:52	7/120 (2)
Chromosome 7			
TG199	1	50:68	5/111 (2)
TG20	2	49:61	3/120
TG217	6	51:68	4/120
Got-2		48:53	7/120
TG183	4	54:64	16/117
TG143	2	53:66	16/117
TG166	3	52:67	3/120
TG13	3	52:67	3/120
TG272	2	37:49	1/120
Chromosome 8			
TG294	2	54:40	11/120 (5)
PNP24	3	64:43	6/116
TG330	2	54:37	5/120
Aps-2		72:46*	0/120
TG16	4	72:45**	0/120
TG117	6	63:47*	2/120 (1)
TG176	3	51:41	1/119
Chromosome 9			
TG254	1	32:53*	7/119 (1)
TG105A	3	42:63*	6/120
TG18	1	41:71**	7/105
TG9	1	38:66**	7/108
P3	2	49:59	11/120
TG225	2	36:53	15/120
TG10	1	52:63	13/119
TG79	4	59:60	1/120
TG317	1	60:58	3/120
TG248	2	39:43	1/119
TG421	3	33:40	1/120
TG8	3	57:53	1/120
TG328	5	44:44	7/120 (6)

Table 1. cont.

Locus	Mapping enzyme[a]	EE:EP[b]	Number of ILs with *L. pennellii* allele
Chromosome 10			
TG313	1	33:34	5/119
TG303	4	32:33	6/120
TG43	2	28:43	3/117
TG1A	1	54:65	5/119
TG52	1	53:63	5/119
TG103	4	56:55	6/118
TG285	3	27:47*	6/120
TG420	1	24:38	3/119
TG229	1	22:70***	15/117 (3)
TG63	3	31:67***	22/120 (10)
Chromosome 11			
TG194	3	33:46	29/119 (11)
TG108	2	37:81***	18/119
Sod-1	2	38:75***	9/110
TG36	1	31:73***	12/120
TG30	4	24:63***	11/119
TG105B	3	40:77***	11/120
TG26	4	48:70*	11/119
TG104	2	22:33	21/117 (10)
Chromosome 12			
TG473	2	37:30	10/119 (10)
TG296	2	32:44	1/120
TG28A	1	44:61	1/120
6Pgdh-2		37:81***	0/120
TG124	4	30:75***	0/120
Pgi-1		41:78***	0/120
TG28B	1	43:74***	0/120
TG263	3	23:43**	4/120
TG68	2	55:63	4/120
TG149	3	48:50	23/103 (19)

[a] 1, *Eco*RI; 2, *Eco*RV; 3, *Dra*I; 4, *Hae*III; 5, *Bst*NI; 6 *Xba*I

[b] EE, Homozygous to the *L. esculentum* allele; EP, heterozygous to the *L. pennellii* allele. Chi-square values greater than this would be expected by chance at a probability (P): * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$

[c] The number of point introgressions are enclosed in brackets

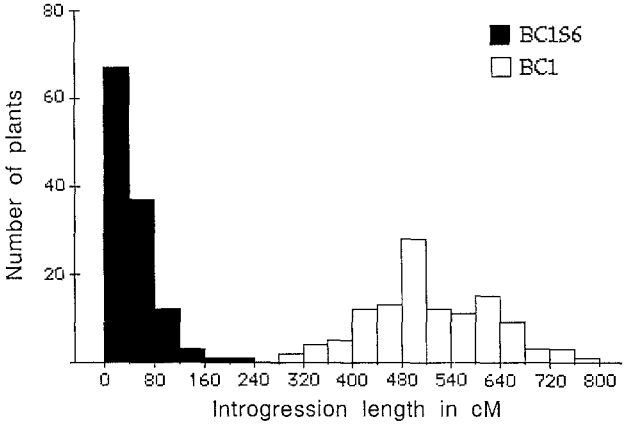

Fig. 2. Frequency distribution for the length of the *L. pennellii* introgressions (cM) in the BC1 generation derived from an *L. esculentum* × *L. pennellii* cross and the BC1S6 introgression lines

Table 2. Genetic composition of the mapping introgression lines covering 936 cM of the tomato genome. *L. pennellii* introgressions are indicated by the chromosome number followed by position and size of the introgression according to Fig. 1

Line	*L. pennellii* introgressions
1	1(0–33), 2(39–57), 3(0–14), 6(54–60), 11(O)
2	1(36–94)[a], 2(39–57), 3(0–14), 4(79–82), 6(54–60), 11(O)[a]
3	1(92–120), 6(18–54)[a], 7(26–36)[a]
4	2(0–23)[a], 9(11–17), 10(0–12)[a], 11(0)[a], 12(0)
5	2(14–73)[a], 5(0–29)[a], 10(65–89)[a], 11(17–42)[a]
6	2(39–96)
7	1(33–77)[a], 3(71–95)[a], 4(39–82)[a], 10(27–89)
8	4(0–18), 11(17–55)
9	4(18–39), 8(0–13)[a], 11(0)
10	5(43–67)
11	3(0), 5(29–67), 6(54–60)[a], 11(17–55)
12	4(56–82)[a], 6(0–4), 7(32–36), 12(0–34)[a]
13	6(12–28)[a], 10(65–89)[a]
14	7(0–36), 10(65–89)[a]
15	2(0)[a], 4(12–18)[a], 7(30–53)[a]
16	8(0–30), 10(65–89), 11(0)
17	4(48–82)[a], 8(64–72), 9(54–94)[a], 11(0–23)
18	9(0–17)
19	9(15–54)[a]
20	2(14–23)[a], 5(58–67), 6(54–60)[a], 10(0–42)[a], 11(50–55)
21	9(11–17), 12(86–98)

[a] The introgression is in a heterozygous state

some segments with highest introgression rates were mostly at the ends of the linkage groups (Table 1). An average marker in the population was introgressed into 3 of the 118 introgression lines, while the mean introgression rate for the 24 markers at the ends of the linkage groups was 9 (Table 1). The highest rate of introgression was detected for TG194 on chromosome 11 (Table 1). At the ends of linkage groups we also found the highest proportion of introgressions detected only by a single marker (point introgression).

Since the introgression lines were probed with RFLP markers spaced on average every 8.4 cM, small *L. pennellii* introgressions could have escaped detection. To determine the presence of small unidentified introgressions we probed the lines with a higher density of markers in three regions: chromosome 1(92–120) was probed with 8 markers, chromosome 5(18-49) with 9 markers and chromosome 9(0-17) with 7 markers. No introgression of a single RFLP marker was detected for chromosomes 1 and 9, and 2 independent single marker introgressions were found for chromosome 5 (Table 1). In all three chromosome regions only 2 of the 34 introgressions would have escaped detection if the spacing between the markers was 8.4 cM.

The 120 introgression lines were divided into two groups. In order to facilitate the mapping of new DNA markers we selected 21 lines (Table 2), with an average

Table 3. Genome composition of additional *L. pennellii* introgression lines. *L. pennellii* introgressions are indicated by the chromosome number followed by position and size of the introgression according to Fig. 1

Line	*L. pennellii* introgressions	Line	*L. pennellii* introgressions
22	0	72	7(32–36)[a], 11(55)
23	0	73	11(0), 12(98)
24	0	74	3(0–2), 10(89)
25	12(86–98)	75	4(48), 5(0)
26	2(39–73)	76	2(50–74), 12(98)[a]
27	12(98)[a]	77	7(32–36), 10(65–89)
28	10(65)	78	7(32–36), 8(64)[a]
29	11(55)	79	7(32–36), 10(89)
30	11(55)	80	9(0–17), 11(42–55)
31	1(92–112)	81	5(4)[a], 11(55)
32	1(92–120)	82	1(92–120), 5(35), 5(45–58)
33	10(65–89)	83	8(30), 11(0), 11(55)
34	9(0–15)	84	1(94–120)[a], 8(0–13), 11(0–17)
35	2(14–23)	85	2(96), 3(95)[a], 9(11–46)[a]
36	9(0–17)[a]	86	1(36–40)[a], 4(0–12), 6(0–4)
37	11(55)	87	10(89)[a], 11(0–17)[a], 12(98)[a]
38	5(45–58)[a]	88	5(67), 11(0), 11(36–55)
39	8(30)[a]	89	8(0–13), 10(65–89), 11(0)
40	5(58–67)	90	10(65–89), 11(55)[a], 12(0)
41	3(0–2)	91	4(18–39), 8(0–13), 11(0)
42	7(32–36)	92	2(96), 9(11–46)[a], 12(86–98)[a]
43	5(43–67)[a]	93	3(0), 5(29–67), 6(54–60)[a]
44	5(43–67)	94	2(0)[a], 5(67)[a], 12(98)
45	4(0–18)[a], 12(98)	95	2(39–96), 3(0)[a], 12(98)
46	11(0), 11(23–55)[a]	96	4(48–82)[a], 9(0–3), 11(0–23)[a]
47	2(14–23), 11(0)	97	2(0)[a], 4(48–82)[a], 11(0)
48	8(0–13)[a], 11(0–17)	98	7(42–52), 8(0), 11(0–17)[a]
49	2(39–73), 5(43–67)	99	2(68–73), 5(0–22)[a], 10(27–89)
50	4(0–18), 5(43–67)[a]	100	3(0–2), 4(39–48)[a], 10(89)
51	5(43–67), 12(98)	101	5(4)[a], 9(15–17)[a], 11(55)
52	2(39–73)[a], 5(43–67)[a]	102	2(0), 6(54–60)[a], 7(0)[a], 12(98)
53	11(0–17), 12(98)	103	2(39–50)[a], 8(30), 11(0–23), 11(55)
54	4(0–12), 11(0–55)[a]	104	4(12–18), 10(27–46)[a], 11(0–17)[a], 12(98)
55	7(36), 11(55)	105	5(67), 11(0), 11(49–55)[a], 12(98)
56	1(92–112), 7(30–36)	106	2(96)[a], 3(0), 4(12–18)[a], 12(98)
57	1(92–120), 7(30–36)	107	7(0–26)[a], 7(26–36), 8(12–30), 10(65–89)
58	10(42–89), 12(86–98)	108	3(0–2), 8(0), 9(94), 10(89)
59	3(0), 12(98)	109	9(0–17), 11(17–23), 11(23–42)[a], 11(42–55)
60	3(0)[a], 12(98)	110	2(14–23), 9(4–17), 10(0–12)[a], 11(0), 12(0)
61	2(0–23)[a], 11(0–36)	111	2(57–96), 9(15–17), 11(0–17), 11(55), 12(98)
62	2(0)[a], 5(43)[a]	112	2(57–96), 4(12–18)[a], 10(27–89)[a], 11(0–17), 12(98)
63	2(14–23), 11(0)	113	2(0)[a], 4(12–18)[a], 6(54–60)[a], 7(0–36), 12(98)
64	2(50–73), 12(98)	114	1(0–16), 3(0–2), 8(0), 9(94), 10(89)
65	2(39–73), 12(98)	115	3(0–2)[a], 7(42–52), 8(0)[a], 9(94)[a], 11(0–17)[a]
66	7(0), 11(55)	116	3(0–2), 8(0), 9(94), 10(89), 12(0)
67	7(0)[a], 11(23–55)	117	1(112–120), 5(66), 10(0–12), 10(65), 12(0)
68	7(32–36), 11(23–55)	118	3(0–2), 6(0–4), 8(0), 9(94)[a], 10(89), 12(0)
69	7(32–36), 11(55)	119	1(112–120), 3(86), 5(66), 10(0–12), 10(65), 12(0)
70	7(32–36), 11(55)	120	1(112–120), 3(86), 5(66), 6(0–4), 10(0–12), 10(65), 12(0)
71	4(18–28)[a], 9(0–17)		

[a] The introgression is in a heterozygous state

introgression of 86 cM per line that give the maximum overlapping coverage of 936 cM of the *L. pennellii* genome. These lines can be used for the mapping of DNA clones in the following manner: The clones are first screened for RFLPs between *L. esculentum* and *L. pennellii* and then hybridized to DNA isolated from the 21 introgression lines digested with the appropriate enzyme. The line or lines showing the *L. pennellii* polymorphism are identified. On the basis of the mapping introgression data in Table 2 it is possible to identify the regions shared by the positive lines only, and to associate the clone with one or more possible locations on the map. If none of the

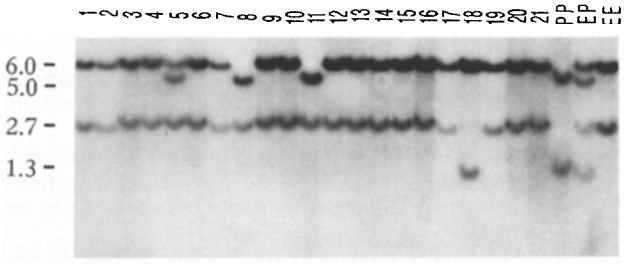

Fig. 3. Hybridization of the duplicate genomic clone TG105 to DNA from the introgression lines 1–21, *L. pennellii* (*PP*) the interspecific hybrid (*EP*) and the *L. esculentum* parent (*EE*) digested with *Dra*I. The *L. pennellii* introgressions of lines 1–21 are shown in Table 2, and the mapping strategy is described in the text. *Left-hand margin* indicates molecular weights in kb

lines show the *L. pennellii* polymorphism the clone can then be associated to regions not covered by the introgressions. This mapping strategy is demonstrated in Fig. 3 where the duplicate clone *TG105* was mapped to chromosome 9 between 0 and 15 cM and chromosome 11 between 23 and 50 cM. Since some of the introgressions are in a heterozygous state it is necessary to isolate the DNA from a bulk of progenies from each of the lines. In some cases higher resolution mapping and verification of the results can be obtained upon, the analysis of selected plants from the 99 lines that provide smaller introgressions of specific *L. pennellii* chromosome segments (Table 3). The average introgression size in these lines is 38 cM, with a minimum of 0 (3 lines) and a maximum of 144 cM.

Discussion

The BC1 map described in this study was compared with the map obtained for an F_2 generation of the same interspecific cross (Miller and Tanksley 1990). The map length of 12 linkage groups delimited by identical markers indicates that the BC1 map length was 786 cM compared to 1005 cM for the F_2 map. In the BC1 only paternal recombination is detectable, while in the F_2 both male and female gametes recombine. The difference between the maps is apparently due to the higher recombination frequencies in maternal than in paternal gametes (Gadish and Zamir 1987; Paterson et al. 1991).

The selection for horticultural characteristics carried out during the breeding of the introgression lines had a marked effect on the genome composition of the BC1S6 generation; with no selection an average line is expected to contain 252 cM of *L. pennellii*, whereas the mean introgression in the 120 selected lines was 47 cM. It is reasonable to assume that major genes contributing to unacceptable phenotypes are located in the four chromosome regions that are not represented in the introgression lines. An interesting trend of high introgression rates of linkage group ends and of single markers (point introgressions) was detected in the lines (Table 1); this phenomenon is associated with the response of the population to selection pressures applied in the field (Eshed and Zamir, unpublished results). The proportion of heterozygous loci detected after six generations of selfing was 3 times higher than the expected 11.5% because of the reduced fertility of *L. pennellii* homozygotes in the recurrent parent background.

The introgression lines can be used as a highly polymorphic perpetual mapping resource for the placing of new clones to the tomato genome in a manner described in the results section. To simplify such mapping we are presently selecting for homozygous lines having an introgression from a single linkage group.

Controlled introgression of five chromosome segments of *L. pennellii* (LA716) into *L. esculentum* was carried out by Rick (1969) in order to investigate their effects of the segregation and recombination of marker genes. The wild species was crossed to separate *L. esculentum* stocks each carrying a group of linked recessive morphological markers; successive backcrosses and selection for wild-type phenotypes provided the introgression lines. Recently, Klein-Lankhorst et al. (1991 a) have used a chromosome 6-*L. pennellii* introgression line developed by Rick (1969) as an aid for selecting DNA markers linked to the nematode resistance gene, *Mi*, which maps to the introgressed region. DNA markers tightly linked to *Mi* provide starting points for a chromosome walk aimed at cloning of the gene. The introgression line and the *L. esculentum* stock used for the backcrosses are NILs for the introgressed segment. Southern blots carrying DNA of the NILs were probed with genomic clones, and those that showed polymorphism between the lines were mapped to the region of interest. Screening of the NILs was also performed using random amplified polymorphic DNA (RAPD; Klein-Lankhorst et al. 1991 b, Martin et al. 1991). The population described in this study provides a tool for the identification of markers that map to a particular chromosome segment since 95% of the genome is covered and some of the introgressed regions are small.

The tomato is a useful model system for the analysis of Mendelian factors underlying quantitative traits. Using RFLP linkage maps in interspecific crosses, it has been possible to identify quantitative loci affecting fruit size, soluble solids concentrations (Paterson et al. 1988, 1990, 1991), water use efficiency (Martin et al. 1989) and insect resistance (Nienhuis et al. 1987).

L. pennellii (LA716) has been characterized as having a high tolerance to drought (Rick 1973) and to salt (Saranga et al. 1991). The breeding of tomato cultivars that can produce economic yield under dry and saline conditions can be enhanced by RFLP mapping of quantitative loci affecting the response of the plants to these

environmental stresses. However, analysis of economic yield is more difficult in early segregating generations of the interspecific cross since the plants are partially sterile and segregate for many undesirable traits. Using the introgression lines it is possible to estimate the contribution of each chromosome region to economic yield under the stress conditions in replicated field trials.

Acknowledgements. We thank S.D. Tanksley for providing us with the DNA probes; we also thank T. Pleban, H. Van-Oss and T. Bloch for technical assistance and S. Smith for editing. This research was supported by Grant No. 1822-90 from BARD, The United States – Israel Binational Research and Development Fund.

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