Integration of the Classical and Molecular Linkage Maps of Tomato Chromosome *6*

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ABSTRACT

In the past, a classical map of the tomato genome has been established that is based on linkage data from intraspecific *Lycopersicon esculentum* crosses. In addition, a high density molecular linkage map has recently been constructed using a *L. esculentum* × *L. pennellii* cross. As the respective maps only partially match, they provide limited information about the relative positions of classical and molecular markers. In this paper we describe the construction of an integrated linkage map of tomato chromosome *6* that shows the position of cDNA-, genomic DNA- and RAPD markers relative to 10 classical markers. Integration was achieved by using a *L. esculentum* line containing an introgressed chromosome *6* from *L. pennellii* in crosses to a variety of *L. esculentum* marker lines. In addition, an improved version of the classical linkage map is presented that is based on a combined analysis of new linkage data for 16 morphological markers and literature data. Unlike the classical map currently in use, the revised map reveals clustering of markers into three major groups around the *yv*, *m-2* and *c* loci, respectively. Although crossing-over rates are clearly different when comparing intraspecific *L. esculentum* crosses with *L. esculentum* × *L. pennellii* crosses, the clusters of morphological markers on the classical map coincide with clusters of genomic- and cDNA-markers on the molecular map constructed by Tanksley and coworkers.

THE past decade has witnessed a major advance in the development of gene mapping and gene isolation strategies. Nowadays, essentially every segment of a eukaryote genome is accessible to detailed characterization and manipulation. Techniques for analyzing complex eukaryote genomes have become so powerful that, in principle, entire chromosomes or even genomes are amenable to physical mapping and sequence analysis. Accordingly, for a variety of organisms including yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, mouse, man, rice and *Arabidopsis thaliana*, genome programs have been launched to construct physical maps of ordered, contiguous clones of chromosomal DNA covering the entire genome. Conceivably, these maps will serve as a major tool in cloning any gene of interest through map-based cloning strategies (WICKING and WILLIAMSON 1991).

Given its economical importance and favorable genetic and molecular attributes (RICK and YODER 1988; HILLE *et al.* 1989) tomato (*Lycopersicon esculentum* Mill.) is a species well suited to detailed genome analysis. A wealth of genetic information about its morphology, development, reproduction, physiology and disease resistance has been gathered over the past five decades (STEVENS and RICK 1986). A wide variety of mutants has been identified, approximately 300 of which have been mapped (see TANKSLEY 1993). In addition, thanks to the pioneering studies of Tanksley and coworkers, a molecular linkage map of over 1000 restriction fragment length polymorphism (RFLP) markers has been constructed (TANKSLEY *et al.* 1992) that provides the molecular framework for dissecting the twelve chromosomes constituting the tomato genome. Finally, yeast artificial chromosome (YAC) libraries of tomato DNA have become available that are ready to be used, in combination with the polymorphic markers, to construct detailed genetic and physical maps (MARTIN, GANAL and TANKSLEY 1992; R. VAN DAELEN and P. ZABEL, unpublished data).

To make the most of all the molecular and genetic information currently available, there is, however, one condition to be met and that concerns the correlation of the classical map with the molecular map. As both maps have been made independently with a limited number of markers in common and in different genetic backgrounds, they only partially match and provide as yet limited information about the relative positions of classical and molecular markers. Another complicating factor that contributes to the incongruity of the classical and molecular maps is the statistical

inaccuracy of the position of the markers on their respective maps. Most of the classical map positions have been calculated on the basis of linkage data collected from different and sometimes relatively small sized populations, without the use of mathematical procedures that take into account standard deviations and correct for double crossovers. Thus, the map position and relative order of various markers is questionable and in some cases already shown to be wrong (VAN DER BEEK *et al.* 1992; JONES *et al.* 1993; KOORNNEEF *et al.* 1993). Only after integrating the classical and molecular maps, can a physical/genetic map be constructed that allows one to choose the proper DNA probes for walking to a target gene.

For particular chromosomal segments, which have been introgressed from wild relatives of tomato and which are highly polymorphic at the DNA level, integration can be achieved through analysis of pairs of nearly isogenic lines only differing for the introgressed region carrying the target gene (see YOUNG *et al.* 1988; BEHARE *et al.* 1991; KLEIN LANKHORST *et al.* 1991a; MARTIN, WILLIAMS and TANKSLEY 1991; MESSEGUER *et al.* 1991; SARFATTI *et al.* 1991; HO *et al.* 1992; SEGAL *et al.* 1992; VAN DER BEEK *et al.* 1992; KOORNNEEF *et al.* 1993). This approach, however, only applies to a small proportion of all the tomato loci mapped. Most of the interesting loci affecting plant morphology, physiology, reproduction and development have been identified as mutations within the species *L. esculentum* and mapped using intraspecific crosses. As the number of DNA polymorphisms detectable between genotypes of *L. esculentum* is very low (MILLER and TANKSLEY 1990; VAN DER BEEK *et al.* 1992), construction of an integrated map of an entire chromosome is only attainable through linkage analysis in crosses that involve chromosome(s) from wild relatives of tomato and segregate for both the classical and molecular markers. Populations derived from interspecific crosses are not as suited to this as they usually segregate for many traits that affect morphology, including sterility, with the individual markers being masked by the complexity of the component phenotype.

The introgression lines developed by RICK (1969, 1972), in which only a particular chromosome of *L. esculentum* has been replaced by the homeologous chromosome from *L. pennellii*, should better serve this purpose. By analyzing crosses of the respective substitution lines to *L. esculentum* lines recessive for the corresponding marker loci, the position of the DNA markers relative to the segregating classical markers can be determined within the same population.

In this paper we describe the application of such an approach to the construction of an integrated linkage map of chromosome *6*. In addition, we present a highly improved version of the classical linkage map that is based on a combined analysis of a large number of new data and published data using the mapping program JOINMAP (STAM 1993).

MATERIALS AND METHODS

Plant material: All genotypes used in the present study are listed in Table 1 along with their main characteristics and source or reference. Genotypes developed at the Department of Genetics of the Wageningen Agricultural University (WAU) are indicated by the letter W prefixed to a number.

The chromosome *6* substitution line *L. esculentum* LA1641, developed by RICK (1969), was derived from a hybrid of *L. pennellii* LA716 and a *L. esculentum* genotype recessive for *yv*, *m-2* and *c*, that was backcrossed five times to the *L. esculentum* parent, while selecting in each backcross generation for the *L. pennellii* wildtype alleles of the chromosome *6* marker genes. A wild-type plant from the fifth backcross generation was subsequently selfed for three generations to yield LA1641.

Molecular probes: The following chromosome *6*-specific DNA probes were used in the linkage analysis: (1) tomato genomic (TG-) and cDNA (CD-) clones obtained from TANKSLEY; (2) potato genomic (GP-) and cDNA (CP-) clones that have been mapped to the homeologous chromosome *6* of potato (GEBHARDT *et al.* 1991), obtained from GEBHARDT; (3) tomato genomic clones *14#6* and *CC32*, carrying sequences flanking Ac-elements (OSBORNE *et al.* 1991), obtained from BAKER; (4) tomato cDNA (LC-) clones mapping around the root-knot nematode resistance *Mi* locus, provided by WILLIAMSON (HO *et al.* 1992); (5) tomato genomic (H-) clones from a *Hin*dIII-library in plasmid pUC18 (KLEIN LANKHORST *et al.* 1991a; HO *et al.* 1992); (6) an *Adh-2* cDNA clone (WISMAN *et al.* 1991); (7) RAPD (R-) markers (WELSH and MCCLELLAND 1990; WILLIAMS *et al.* 1990), prepared using random 10-mer oligonucleotide primers from Operon and identified as described (KLEIN LANKHORST *et al.* 1991b).

DNA methodology and acid phosphatase isozyme assay: Plant DNA was isolated from leaves as described (VAN DER BEEK *et al.* 1992). DNA was labeled with alpha ^{32}P-dATP using the random hexamer method (FEINBERG and VOGELSTEIN 1983). All other DNA methodologies were carried out according to SAMBROOK, FRITSCH and MANIATIS (1989). Acid phosphatase assays and hybridization of Southern blots were performed as described (KLEIN LANKHORST *et al.* 1991a).

Linkage analysis and map construction: Estimates of recombination frequencies were calculated from F_2 data using the RECF2 program, which produces maximum likelihood estimates and standard errors (KOORNNEEF and STAM 1992). For the construction of a classical linkage map of chromosome *6*, new linkage data collected by us were added to the literature data (Table 2) and analyzed using the mapping program JOINMAP (STAM 1993), which takes raw segregation data and/or listed pairwise recombination estimates as input and is especially suited for integrating different types of linkage data sets which have markers in common (HAUGE *et al.* 1993). To construct the genetic map, JOINMAP uses every piece of linkage information, *i.e.*, all the available pairwise estimates of recombination, weighed by the corresponding LOD scores. Thus, recombination percentages near to 50% are assigned low weights and, consequently, contribute little to the combined estimate of the distance covering such pairs. Nevertheless, recombination frequencies near to 50% are informative; knowing that two

TABLE 1

Plant material

Code	Marker genes[a]	Origin	Source/Reference
LA62	*ms-16, sp*	TGC[b]	RICK (1953)
LA543	*def*	TGC	STUBBE (1957)
LA758	*tl*	TGC	CLAYBERG *et al.* (1966)
LA802	*yv, m-2, c (ms-2)*	TGC	RICK (1988)
LA1178	*yv, coa, c*	TGC	RICK (1988)
LA1189	*yv, c*	TGC	RICK (1988)
LA1641	*yv*$^+$*/yv, ndw*$^+$*/ndw, m-2*$^+$*/m-2, B (r)*	TGC	RICK (1969)
LA1641-11	*ndw, B*	See text	This work
LA1794	*ri*	TGC	LINDSTROM (1933)
LA2486	*pds, sp (inc, u, t)*	TGC	RICK (1988)
2-511	*ms-33*	TGC	CLAYBERG *et al.* (1966)
W335	*gib-1*	EMS-treated Moneymaker	KOORNNEEF *et al.* (1990)
W601	*yv, c*	F_2 (LA802 × 83M-R)	WAU[c]
W602	*m-2, gib-1*	F_2 (W335 × W606)	WAU
W603	*Aps-1*^{1}*, m-2, c*	F_2 (LA802 × 83M-R)	WAU
W605	*yv, m-2, og, sp, c*	F_2 (W606 × *og, sp* breeding line)	WAU
W606	*yv, m-2, c*	F_2 (LA802 × 83M-R)	WAU
W610	*ms-33, m-2, c*	F_2 (2-511 × W606)	WAU
W611	*yv, ms-33*	F_2 (2-511 × W606)	WAU
W612	*ms-33, c*	F_2 (2-511 × W606)	WAU
W613	*yv, ms-33, c*	F_2 (2-511 × W606)	WAU
WSL6	*ndw, B*	See text	This work
MM-Cf2	*Aps-1*^{3}*, Cf-2*	CPRO-DLO[d]	Ho *et al.* (1992)
83M-R	*Aps-1*^{1}*, Mi*	De Ruiter Seeds	KLEIN LANKHORST *et al.* (1991a)
83M-S	*Aps-1*^{3}	De Ruiter Seeds	KLEIN LANKHORST *et al.* (1991a)

[a] Marker genes shown between brackets are not located on chromosome *6*.
[b] Tomato Genetics Cooperative Seed Stock Center, Davis.
[c] Wageningen Agricultural University.
[d] Centre for Plant Breeding and Reproduction Research—DLO, Wageningen.

markers are at a large distance is helpful in finding the best fitting order on a multilocus map.

To obtain populations segregating for both classical and molecular markers, WSL6 and LA1641-11 were crossed to a set of *L. esculentum* lines recessive for classical markers located on chromosome *6* (Table 3). Analysis of F_2 populations thus provided accurate linkage data for morphological markers in the same genetic background as needed for molecular linkage analysis. F_2 plants with a recombinant phenotype for the markers *pds/Aps-1*, *yv/m-2* and *m-2/c* were selected for RFLP and RAPD linkage analysis. To assess the genotype of both gametes, F_3 lines of these recombinants were screened for segregation of the classical markers. For each molecular marker, the distance to both flanking morphological markers was estimated directly from the number of recombinants on both sides of the molecular marker within the interval, divided by the total number of recombinants within the interval analyzed, and multiplied by the estimate of the total genetic length (in cM) of the interval. The latter was calculated on the basis of all available data, using the Kosambi mapping function. These estimates of distances were converted to recombination fraction estimates, again using the Kosambi mapping function. The recombination fraction estimates were fed to JOINMAP to construct the integrated linkage map of chromosome *6*, taking *m-2-sp-c* as a fixed order. Standard errors were based on the effective size of the populations that were screened with molecular markers.

RESULTS

Update of the classical linkage map: The most recently published classical linkage map of chromosome *6* (TANKSLEY 1993) has been assembled from linkage data that were collected over the past three to four decades and analyzed without the application of mathematical procedures that take into account the statistical accuracy of the data.

Over the past several years, we have been collecting a large number of new linkage data from crosses segregating for the markers *pds, tl, Cf-2, Aps-1, yv, ms-33, d-2, coa, ms-16, ri, m-2, B, rv-3, sp, c* and *gib-1* (Table 2). These linkage analyses involved both marker intervals that had not been studied before as well as intervals for which literature data were available. To establish more precise map positions, we have added these new linkage data to the published ones and subjected the combined set to analysis using the mapping program JOINMAP (STAM 1993). The only literature data not used were those for *d-2* (RICK *et al.* 1973), because they are internally inconsistent, as noted by the authors. The results of the exercise are shown in Figure 1B.

In broad outline the current map (Figure 1B) is similar to the most recent map published in "Genetic Maps" (Figure 1A). There are, however, some notable differences. First, markers at the revised map are found in "locus groups," with relatively large gaps between them, the largest one being at the short arm. Two-thirds of the markers are grouped in three clus-

TABLE 2

Estimates of recombination percentages between markers of chromosome 6, used for construction of the classical map

Experimental data				Literature data			
Markers	Method[a]	Recombination %	LOD	Method[a]	Recombination %	LOD	Reference[b]
Aps-1/c	C2	47.8 ± 2.5	0.17				
Aps-1/coa	C2	24.6 ± 3.0	12.11				
Aps-1/m-2	C2	26.6 ± 3.0	10.73				
Aps-1/Mi				CT	0.0 ± 0.02	1,505.15	1
Aps-1/yv	C2	0.0		CT, C2	0.0 ± 0.002	15,051.50	1
B/c	C2	4.6 ± 1.6	37.71	RT	1.0 ± 0.1	2,739.42	2
B/d-2	C2	24.2 ± 3.7	8.13				
B/m-2				RT	16.6 ± 0.5	586.01	2
B/sp				RT	0.32 ± 0.07	1,898.62	2
c/Cf-2				CT	47.0 ± 2.3	0.37	3
c/cl-2				R2	0.0 ± 6.6	4.56	4
c/coa	C2	18.8 ± 1.3	82.32	R2	32.4 ± 1.9	16.68	5
c/coa	R2	19.6 ± 5.8	4.04				
c/d-2	C2	30.3 ± 3.1	7.61				
c/gib-1	R2	11.7 ± 3.2	14.56				
c/m-2				CT	19.0 ± 0.4	864.40	2; 3; 6
c/m-2				RT	22.0 ± 1.9	34.32	7
c/m-2	C2	23.3 ± 0.9	143.99				
c/Mi				CT	46.4 ± 1.5	1.25	3; 6
c/ms-16	R2	18.7 ± 7.0	2.85				
c/ms-33	C2	44.4 ± 5.2	0.25				
c/ms-33	R2	27.8 ± 5.5	2.94				
c/pds	R2	54.9 ± 3.7	0.38	R2	49.0 ± 2.0	0.05	8
c/ri	C2	25.3 ± 2.9	12.45				
c/ri	R2	11.1 ± 4.1	8.78	R2	38.2 ± 7.2	0.56	9
c/rv-3	R2	0.0 ± 5.8	5.19	R2	0.0 ± 2.1	14.33	10; 11
c/sp				CT	0.7 ± 0.1	1,966.55	2
c/sp	C2	1.3 ± 0.9	42.91	C2	1.2 ± 0.2	808.58	12
c/sp	R2	0.0 ± 9.8	3.07				
c/tl	R2	44.4 ± 2.7	0.92				
c/ves				R2	20.1 ± 7.2	2.57	13
c/yv	C2	42.8 ± 1.1	9.14				

[a] CT, RT: results of testcrosses in coupling and repulsion phase, respectively; C2, R2: results of F_2's in coupling and repulsion phase, respectively.

[b] 1: MEDINA-FILHO (1980). 2: ITO and CURRENCE (1964). 3: KERR *et al.* (1977). 4: RICK (1963). 5: ZOBEL *et al.* (1969). 6: GILBERT (1960). 7: BURDICK (1959). 8: RICK, ZOBEL and OPEÑA (1970). 9: RICK and MARTIN (1962). 10: HANSEN, RICK and BOYNTON (1962). 11: RICK *et al.* (1974). 12: ROBINSON and SHANNON (1968). 13: BOYNTON and RICK (1965). 14: KANWAR, KERR and HARNEY (1980). 15: RICK and BOYNTON (1966). 16: GILBERT and CENTINA (1965a). 17: GILBERT and CENTINA (1965b). 18: GILBERT (1958).

ters that together comprise only 9 cM. Second, the order of some of the markers is different. For example, the *Cladosporium fulvum* resistance gene *Cf-2* appears to map above and not below *Aps-1/yv* (see also DICKINSON, JONES and JONES 1993 and JONES *et al.* 1993), *d-2* is located above *m-2* in the interval *ms-33/coa* and not below *m-2*, whereas *def*, which is allelic to *ri*, is located below *coa*. In addition, the order of the markers *c*, *sp* and *B* is reversed. The present order of *tl/Cf-2/Mi/Aps-1/yv* and *B/sp/c* within the respective gene clusters is based on the estimated recombination percentages as well as on the presence or absence of joint recombination in multiple heterozygotes. Since recombinants between the markers *coa*, *ves*, *ms-16*, *ri* and *m-2* were neither found by us nor described in literature, the mutual order within this cluster is ambiguous. Third, a number of mutations previously assigned to chromosome *6* was found to be allelic to other mutations described earlier. For example, *mu* and *rv-3*, which are morphologically very similar, did not show complementation to wild type in the F_1, indicating allelism. Complementation was neither observed between the potato leaf mutants *c* and *int*, an observation also described by KERR (1960). Similarly, as mentioned above, *def* turned out to be allelic to *ri*.

Breeding of the chromosome *6* substitution line WSL6: At present virtually all RFLP markers for chromosome *6* have been mapped in segregating F_2 populations of a *L. esculentum* × *L. pennellii* cross, without reference to classical markers. Only for the *Aps-1/Mi* region (MESSEGUER *et al.* 1991; HO *et al.* 1992; DICKINSON, JONES and JONES 1993) and the *sp*- and *B*-loci (PATERSON *et al.* 1991) integration of the classical and RFLP map has been achieved to some extent.

With the objective of constructing an integrated

TABLE 2—Continued

Experimental data				Literature data			
Markers	Method[a]	Recombination %	LOD	Method[a]	Recombination %	LOD	Reference[b]
Cf-2/coa	C2	29.2 ± 3.6	6.18				
Cf-2/m-2				CT	32.8 ± 3.4	5.00	3; 14
Cf-2/pds				CT	39.4 ± 8.5	0.32	14
Cf-2/yv	C2	3.0 ± 1.1	58.32				
cl-2/m-2				R2	27.2 ± 6.3	2.34	4
cl-2/yv				R2	48.0 ± 5.1	0.03	4
coa/ri	R2	0.8 ± 4.0	1.39				
coa/rv-3	C2	17.7 ± 2.6	21.18	R2	32.4 ± 6.3	1.52	11
coa/tl				R2	39.9 ± 4.6	1.01	5
coa/ves				R2	0.0 ± 5.3	5.68	5
coa/yv	C2	28.9 ± 1.6	32.03	R2	33.3 ± 1.9	15.69	5
gib-1/m-2	R2	33.9 ± 3.3	4.72				
gib-1/m-2	C2	29.4 ± 3.3	7.24				
gib-1/yv	R2	48.9 ± 2.9	0.03				
m-2/Mi				CT	34.2 ± 1.5	22.06	3; 6
m-2/ms-16	R2	0.0 ± 9.7	3.10				
m-2/ms-33	C2	17.5 ± 3.2	14.05				
m-2/ms-33	R2	0.0 ± 6.1	4.93				
m-2/pds	R2	36.8 ± 4.6	1.68				
m-2/sp	R2	6.6 ± 6.3	3.04	CT	16.9 ± 0.5	582.72	2
m-2/tl	R2	36.0 ± 4.4	2.05	R2	33.0 ± 3.0	6.29	15
m-2/yv	C2	26.1 ± 1.2	69.25				
Mi/ri				CT	38.4 ± 1.5	12.40	16; 17
Mi/yv				C2	0.9 ± 0.5	99.44	18
ms-16/sp	C2	19.1 ± 4.1	8.20				
ms-33/tl	R2	21.7 ± 7.0	2.56				
ms-33/yv	R2	19.4 ± 5.8	4.06				
pds/tl	R2	31.6 ± 5.1	2.50				
pds/yv	R2	36.9 ± 4.6	1.66	R2	34.8 ± 2.5	7.40	8
ri/m-2	R2	0.0 ± 5.8	5.19				
ri/yv	R2	29.2 ± 3.5	6.54	R2	19.3 ± 5.8	4.07	9
tl/yv	R2	0.0 ± 4.6	6.54	R2	10.3 ± 2.4	25.18	15
ves/yv				R2	33.0 ± 7.4	1.03	13

TABLE 3

Crosses made to construct the integrated linkage map

Cross	No. of F_2 progeny analyzed	Markers assayed
W601 × WSL6	1142	*yv, ndw, c*
W602 × WSL6	111	*m-2, ndw, gib-1*
W605 × WSL6	259	*yv, ndw, m-2, sp, c, RFLPs*
W606 × LA1641-11	143	*Aps-1, yv, ndw, m-2, c, RFLPs, RAPDs*
W606 × LA1641-11	223	*yv, ndw, m-2, c*
W610 × WSL6	194	*ms-33, ndw, m-2, c*
W611 × WSL6	211	*yv, ms-33, ndw*
W612 × WSL6	105	*ms-33, ndw, c*
W613 × WSL6	55	*yv, ms-33, ndw, c*
LA2486 × WSL6	329	*pds, Aps-1, RFLPs*

map of the entire chromosome, the chromosome *6* substitution line LA1641 carrying the wild-type (*L. pennellii* LA716) alleles yv^+, $m\text{-}2^+$ and c^+ (RICK 1969) was regarded as a useful parent in crosses to *L. esculentum* chromosome *6* marker lines. However, as the material obtained from the Tomato Genetics Stock Center appeared to segregate for the markers *yv* and *m-2*, it was not useful as such. Apart from these markers, a hitherto unrecorded semi-dwarf phenotype with epinastic leaves was observed in 9 of 21 plants screened. Because these dwarfs showed necrosis in stem and leaves, we refer to this trait as *necrotic dwarf* (*ndw*). All the ndw individuals exhibited the wild-type (*L. pennellii*) phenotype for the markers yv^+, $m\text{-}2^+$ and c^+, indicating that the *ndw* locus resides on chromosome *6* with the recessive allele on the *L. pennellii* chromosome *6* homologue. Apparently, selection for "normal" (tomato-like) plants in the selfed progeny of backcross plants had led to the maintenance of *L. esculentum*-derived ndw^+ alleles in stock LA1641. Necrotic dwarf phenotypes were also observed in independent F_2 generations derived from *L. esculentum* × *L. pennellii* LA716 crosses (data not shown), suggesting that LA716 is homozygous recessive for *ndw* alleles but that expression of the *ndw* allele is masked in a *L. pennellii* genetic background.

To select from the segregating progeny of LA1641 a line homozygous for the *L. pennellii* alleles of chro-

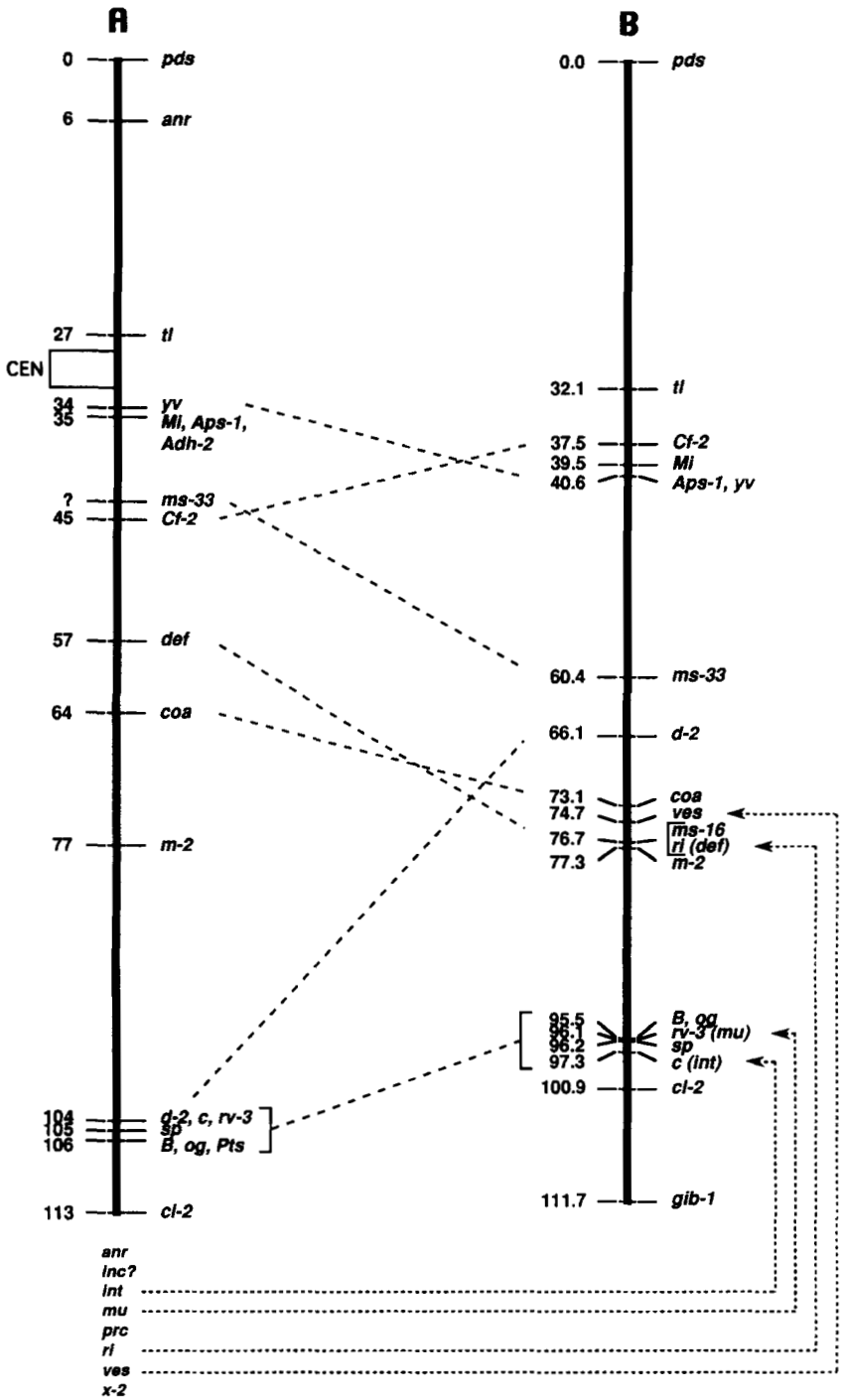

FIGURE 1.—Tomato classical linkage maps. (A) Classical map according to TANKSLEY (1993). CEN: centromere position, based on deletion mapping (KHUSH and RICK 1968). (B) Classical map according to the data of Table 2. A dashed line between the maps shows a marker whose relative position differs on the two maps. Dashed lines with arrowheads show the position of markers that could not be positioned on map A, but were found to be allelic to markers for which linkage data were available. Numbers on the left of the maps are distances from *pds* in cM; for map B they were calculated from recombination frequencies using the Kosambi mapping function.

mosome *6*, plants showing the wild-type phenotype for yv^+, $m\text{-}2^+$and c^+ were screened with the chromosome *6*-specific RFLP markers shown in Figure 2. One plant (LA1641-11), a necrotic dwarf, met this requirement except for marker *TG193*—located at the distal end of the long arm (TANKSLEY *et al.* 1992)—for which it was homozygous *L. esculentum*. To assess whether there were any *L. pennellii* sequences left on other chromosomes, LA1641-11 was screened with the RFLP markers shown in Figure 2. The only non-chromosome *6* *L. pennellii* sequences found were those corresponding to the loci *CD41* and *TG358* on chromosome *5* and the loci *TG34*, *TG354* and *TG141* on chromosome 2. To remove these residual introgressed *L. pennellii* loci, LA1641-11 was crossed with the *L. esculentum* chromosome *6* linkage tester line W606 ($Aps\text{-}1^+$, *yv*, ndw^+, *m-2*, *c*). From the resulting F_2 population, six individuals that were homozygous for the yv^+, $m\text{-}2^+$, c^+, *ndw* alleles were chosen for additional RFLP analysis. One plant, WSL6, proved to be a true substitution line in showing only *L. pennellii* alleles for chromosome *6*, except for the distal end carrying marker *TG193*.

Identification of molecular markers for chromo-

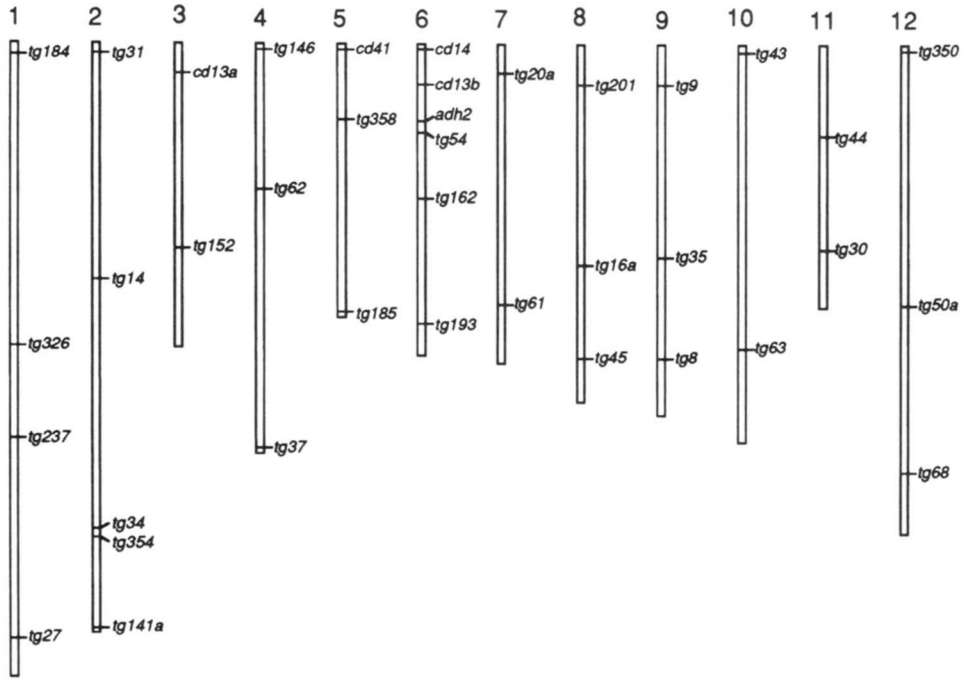

FIGURE 2.—RFLP markers used to assist the breeding of the chromosome 6 substitution line WSL6.

some 6: At the start of our study only a limited number of chromosome 6-specific RFLP markers was available to us. Therefore, a search was made for additional markers using a genomic DNA library from *L. esculentum* 83 M-R as a source of clones (KLEIN LANKHORST *et al.* 1991a). To identify RFLP markers for chromosome 6, Southern blots containing DNA from *L. esculentum*, WSL6 and *L. pennellii* LA716 were hybridized with randomly chosen single- and low-copy clones. Clones exhibiting a *L. pennellii*-specific hybridization pattern in lanes with DNA from WSL6 were regarded as chromosome 6-specific RFLP markers and used in further mapping (Figure 3). Of the 500 clones screened, 12 could thus be assigned to chromosome 6. Similarly, blots were probed with cDNA clones (*CP12*, *CP61*) and genomic clones (*GP79*, *GP89*, *GP102*, *GP136*, *GP164* and *GP202*) from potato, which had been mapped already to the homoeologous chromosome 6 of potato (GEBHARDT *et al.* 1991). Thus, clones *CP12*, *GP136*, *GP79*, *GP89*, *GP164* and *GP202* were attributed to chromosome 6 of tomato, with the first two behaving as dominant (*CP12*, *L. esculentum*-specific; *GP136*, *L. pennellii*-specific) and the latter four as codominant markers.

The collection of chromosome 6-specific RFLP markers was supplemented with chromosome 6-specific RAPD markers identified as described (KLEIN LANKHORST *et al.* 1991b). Of 60 random decamer primers tested, 16 directed the amplification of a sequence of chromosome 6.

Construction of an integrated linkage map: To obtain populations segregating for both classical and molecular markers, WSL6 and LA1641-11 were crossed to a set of *L. esculentum* lines recessive for

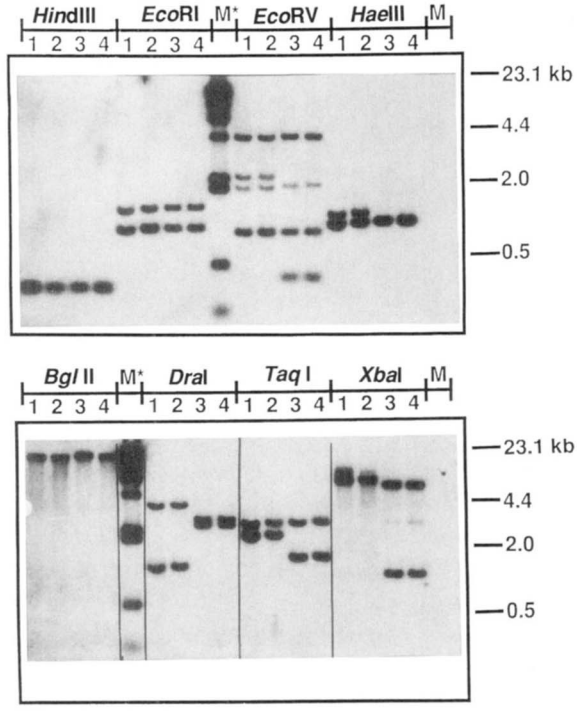

FIGURE 3.—Identification of a chromosome 6-specific RFLP marker. Total DNA (3 μg) from *L. esculentum* 83M-R (lane 1), 83M-S (lane 2), WSL6 (lane 3) and *L. pennellii* LA716 (lane 4) was digested with the restriction enzymes indicated. The restriction fragments were separated on a 1% agarose gel, transferred to a Gene Screen Plus membrane and hybridized with a clone (*H8C4*) randomly chosen from a genomic *Hin*dIII library in pUC18.

classical markers located on chromosome 6 (Table 3). Analysis of F_2 populations thus provided linkage data for the classical marker loci *pds*, *Aps-1*, *yv*, *ms-33*, *ndw*, *m-2*, *sp*, *c* and *gib-1* in the same genetic background as was needed for molecular linkage analysis (Table 4).

TABLE 4

Estimates of recombination percentages between markers of chromosome *6*, derived from crosses with chromosome *6* substitution lines LA1641-11 and WSL6

Markers	Recombination %	LOD	Markers	Recombination %	LOD
Aps-1/ndw	11.5 ± 1.9	41.18	*pds/GP164*	29.8 ± 3.7	5.57
Aps-1/yv	0		*GP164/Aps-1*	24.6 ± 2.8	13.90
Aps-1/pds	42.1 ± 3.3	1.22	*pds/GP79*	38.9 ± 3.7	1.87
c/m-2	18.5 ± 1.5	62.35	*GP79/Aps-1*	9.1 ± 1.9	38.64
c/ms-33	27.6 ± 2.9	10.73	*Aps-1/GP202*	0.7 ± 0.5	78.66
c/ndw	21.8 ± 2.6	18.49	*GP202/m-2*	12.1 ± 2.0	37.44
c/sp	7.6 ± 1.7	44.77	*Aps-1/TG232*	1.4 ± 0.7	75.79
c/yv	24.5 ± 1.2	76.08	*TG232/m-2*	11.4 ± 1.9	41.11
gib-1/m-2	47.2 ± 6.9	0.04	*Aps-1/H2D1*	2.1 ± 0.8	82.48
gib-1/ndw	28.6 ± 8.6	1.13	*H2D1/m-2*	10.8 ± 1.9	40.66
m-2/ms-33	5.0 ± 1.6	39.86	*Aps-1/TG352*	5.3 ± 1.3	62.67
m-2/ndw	0.5 ± 3.4	1.24	*TG352/m-2*	7.7 ± 1.6	50.85
m-2/ndw	1.4 ± 0.7	75.79	*TG352/TG153*	0.6 ± 0.6	47.23
m-2/sp	19.3 ± 2.8	17.48	*TG153/TG25*	2.2 ± 2.2	11.34
m-2/yv	12.8 ± 1.5	66.91	*TG25/m-2*	4.9 ± 1.3	59.59
ms-33/ndw	0.0 ± 4.2	7.17	*Aps-1/Adh-2*	8.4 ± 1.7	46.80
ms-33/yv	10.3 ± 2.0	36.27	*Adh-2/m-2*	4.6 ± 1.3	57.13
ndw/sp	20.6 ± 5.9	3.77	*Aps-1/H9A11*	12.1 ± 2.0	37.44
ndw/yv	0.0 ± 3.1	9.71	*H9A11/m-2*	0.7 ± 0.5	78.66
sp/yv	29.8 ± 3.5	6.23	*m-2/H2C1*	0	
			m-2/TG240	0.5 ± 0.5	57.18
			TG240/c	18.1 ± 2.7	19.45
			m-2/GP89	4.4 ± 1.4	47.79
			GP89/c	14.6 ± 2.4	26.08
			GP89/TG253	4.9 ± 2.9	11.97
			TG253/CC32	2.0 ± 1.8	15.63
			m-2/CC32	11.4 ± 2.1	33.66
			CC32/c	7.7 ± 1.8	40.18
			m-2/TG162	13.3 ± 2.3	28.50
			TG162/c	5.8 ± 1.6	43.72
			m-2/TG275	17.2 ± 2.6	21.42
			TG275/c	1.5 ± 0.8	61.69

Only one marker is shown for each locus. For RFLP markers (except ***TG153*** and ***TG253***), only data on recombination with flanking classical markers are shown. Segregation of *TG153* and *TG253* has been analyzed on a subset of recombinants, carrying a crossover in the vicinity of these markers; therefore, only data on recombination of these markers with adjacent (RFLP) markers are shown.

Plants with a recombinant phenotype for *pds/Aps-1*, *yv/m-2* and *m-2/c* were selected for RFLP and RAPD analysis. Using the mapping program JOINMAP, all the data were then combined to construct the integrated map of chromosome *6* as shown in Figure 4B.

In addition to the crosses shown in Table 3, *L. esculentum* W603, which carries *L. peruvianum* sequences in the *Aps-1* region, was crossed to the *thiaminless* (*tl*) *L. esculentum* markerline LA758, and F_2 plants with a crossover between *tl* and *Aps-1* were screened with RFLP markers. Although the *tl* data can not be analyzed in combination with the other data due to the different genetic backgrounds, the presence of *tltlAps-1*$^{+}$*Aps-1*^{1} recombinants that were heterozygous for the RFLP marker *GP79*, did allow us to situate *tl* on the map above *GP79*.

In comparing the integrated map with the separate maps of the classical and the molecular markers, several features emerge which are worth mentioning:

1. The order of the loci along the chromosome on the integrated map is in complete agreement with the order of the classical and molecular markers on their respective maps.

2. The total genetic lengths of the three maps are similar, but their corresponding short and long arms differ significantly. While the long arm of the integrated map comprises 44.5 cM (*yv-gib-1* interval), the corresponding arm on the classical map is 71.1 cM. Apparently, recombination in the long arm is decreased in crosses involving the substitution line carrying the introgressed chromosome *6* from *L. pennellii*. As was already found by RICK (1969), suppression of recombination is most severe in the interval *yv-m-2* proximal to the centromere (Figure 5). Unlike the long arm, the short arm of the integrated map is longer than its counterpart on the classical map, although it should be noted that this finding is solely based on the data obtained with *pds*.

3. The distal end of the long arm below *TG275/c* comprises only 13.2–14.4 cM on the integrated (Figure 4B) and the classical map (Figure 4A), respectively, but spans over 34 cM on the molecular map

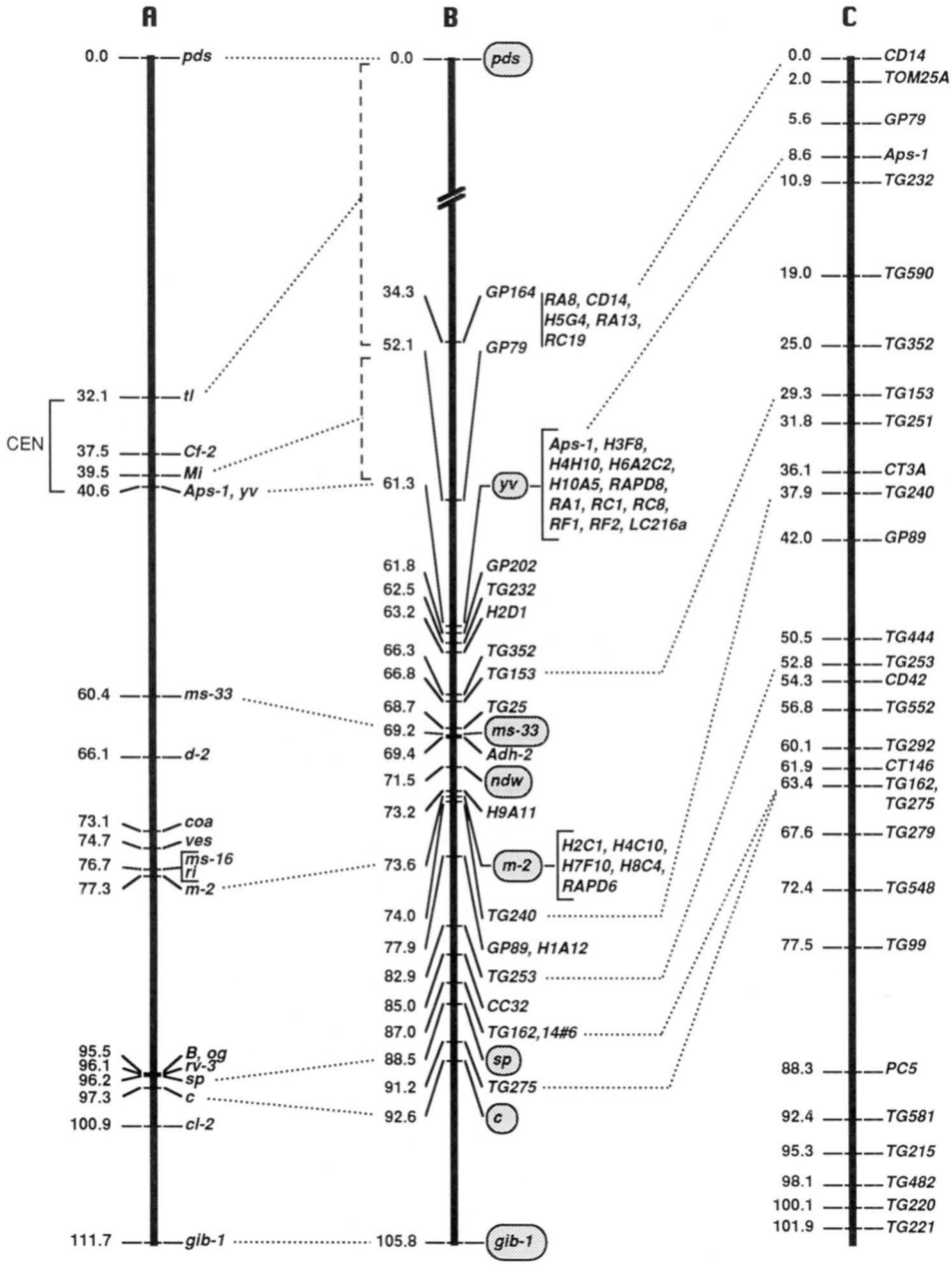

FIGURE 4.—Linkage maps of the tomato genome. (A) Classical map according to the linkage data of Table 2. CEN: centromere position, based on deletion mapping (KHUSH and RICK 1968). (B) Integrated linkage map according to the linkage data of Table 4. Names of morphological markers are shadowed. (C) RFLP map, based on the map of TANKSLEY *et al.* (1992). Only one marker is shown per locus. All distances are in cM. Positions of selected markers from the classical map and the RFLP map on the integrated map are shown by connecting lines between the maps. Approximate positions of *tl* (see RESULTS), *Mi* (KLEIN LANKHORST *et al.* 1991a) and *CD14* (HO *et al.* 1992) are not based on the data of Table 4. The linkage data from Table 4 make a position of *sp* below *c* more likely than a position of *sp* above *c*, as is found on the classical map. However, the genotype of markers flanking *sp* (placed either below or above *c*) was in some individuals inconsistent with the genotype inferred from the sp phenotype, which is probably due to misclassification of *sp*. This problem was also observed by PATERSON *et al.* (1991) and makes estimates of recombination between *sp* and other markers relatively unreliable. As the order of *sp* and *c* on the classical map has been established quite firmly, we decided to fix the position of *sp* on the integrated map above *c*, in accordance with the classical linkage data.

(Figure 4C). Conversely, over 30 cM of the short arm on the integrated map is not covered by the markers mapped on the molecular map.

DISCUSSION

The present study has generated an improved version of the classical linkage map as well as an integrated map of chromosome 6 that shows the position of molecular markers with regard to classical markers.

As to the classical linkage map, differences in relative map positions with the published map (TANKSLEY 1993) can be attributed in most cases to the limited data available previously (*e.g.*, *d-2*, *ms-33*, *def* = *ri*). The reversed order in the *c-sp* region is in fact in agreement with the accurate data of ITO and CURRENCE (1964) that apparently have not been taken into account before. Our data for the order of the markers *yv* and *Cf-2* are clearly in conflict with the order published by KERR *et al.* (1977) showing *Cf-2* to be located between *yv* and *m-2*. The present position, however, has been confirmed by RFLP mapping using an F_2 population segregating for *Cf-2* (M. F. VAN WORDRAGEN, unpublished data) and is in agreement with the data of DICKINSON, JONES and JONES (1993) and JONES *et al.* (1993), who, in addition, found that *Cf-5* is tightly linked to *Cf-2*. The arguments for the location of *Mi* in the same region above *yv*/*Aps-1* have been adduced previously (MESSEGUER *et al.* 1991; HO *et al.* 1992). Taken together, these mapping data indicate an apparent clustering of the disease resistance genes *Cf-2*, *Cf-5* and *Mi*.

A remarkable feature emerging from the revised classical map, more than from the previous version, is

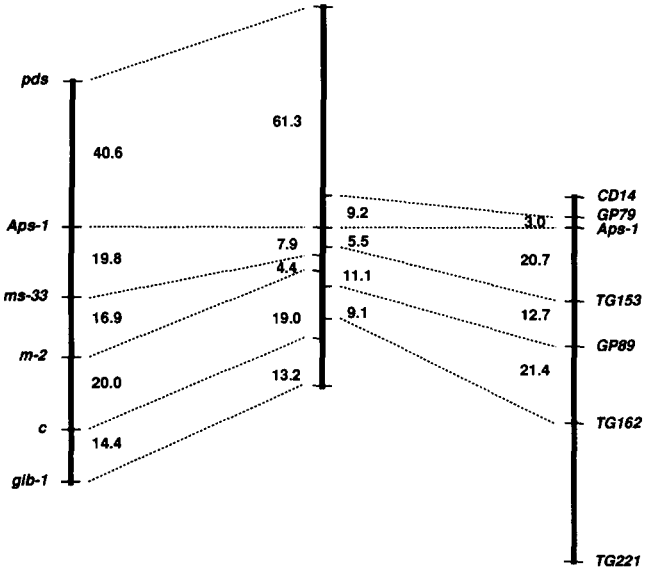

FIGURE 5.—Comparison of map distances on the classical map (left), the integrated linkage map (middle) and the RFLP map (TANKSLEY *et al.* 1992) (right). Map distances are in cM.

the clustering of most of the morphological markers into three groups around *yv*, *m-2* and *c*, respectively. Clustering was also observed for the molecular markers, but as yet mainly around *yv* and *m-2*. Regions of high marker density have been identified in all chromosomes of tomato and have been shown to correspond to centromeric areas and, in some instances, telomeric regions (TANKSLEY *et al.* 1992). As for chromosome *6*, the centromere has been located between *yv* and *tl* on the basis of irradiation-induced deletion mapping, with *yv* mapping in the pericentromeric heterochromatin on the long arm (KHUSH and RICK 1968). Thus, regarding this particular locus, the clustering of molecular markers was not unexpected (see also MESSEGUER *et al.* 1991; HO *et al.* 1992). Regarding the relative distribution of markers over the euchromatic portion of the genome, however, the situation was not as clear. Close inspection of the molecular linkage map of chromosome *6* constructed by TANKSLEY and coworkers reveals that 12 markers map at the same locus as *TG240*, which is here shown to map near the *m-2* cluster located in euchromatin and distant from the centromere and telomere. Apparently, reduced crossing over, a plausible explanation for the clustering, is not restricted to sequences in centromeric regions, but is also a feature of some regions in euchromatin. Taking into account that euchromatin can experience much higher levels of recombination as well (SEGAL *et al.* 1992), meiotic recombination is likely to vary drastically along the DNA sequence of euchromatin, which is in line with the nonrandom distribution of recombination nodules along the chromosome as recently observed by SHERMAN, HERICKHOFF and STACK (1992). Alternatively, the clustering of markers may simply reflect the presence of chromosomal regions enriched for single- and low-copy sequences.

In addition to the regional differences in recombination frequency along the chromosome, another feature emerging from the present studies deserves discussion. Unlike the RFLP map that is based on a *L. esculentum* × *L. pennellii* cross and shows little suppression of recombination when compared with the classical map (TANKSLEY *et al.* 1992), the integrated map presented here shows severe suppression of recombination, particularly with respect to the long arm. The strongest suppression of recombination in the *L. esculentum* × WSL6 crosses occurs in the interval *Aps-1/TG153* close to the centromere, but also the interval *GP89/TG162* below *m-2* is strongly reduced in recombination (Figure 5). Lack of homology between the chromosomes of the parental species—like the heteromorphisms observed in the proximal heterochromatin (KHUSH and RICK 1963)—seems to be of minor concern in this respect as in both cases crossing-overs between the *L. pennellii* and *L. esculentum* chromosome *6* are involved. Possibly, other *L. pennellii* chromosomes promote somehow pairing/recombination of chromosome *6* of *L. pennellii* with its *L. esculentum* homologue in *L. esculentum* × *L. pennellii* crosses. Alternatively, the low recombination frequencies in the *L. esculentum* × WSL6 crosses may have to do with the *L. esculentum* origin of the distal part of the long arm of chromosome *6* in WSL6 and LA1641. If chromosome pairing in *L. esculentum* × WSL6 crosses is preferentially initiated at this strongly homologous region of the component chromosomes *6*, it might impose a suppressing effect on recombination in other regions of the chromosome.

Unlike the long arm, recombination in the short arm was not reduced but rather elevated in *L. esculentum* × WSL6 crosses. In view of the weak linkage ($0.01 < P < 0.05$) found between *pds* and *Aps-1* in the cross *L. esculentum* LA2486 × WSL6 (see Table 4), it may be argued that *pds* is not at all located on chromosome *6*. However, using the same *pds* linkage tester line LA2486 in crosses with *L. esculentum* (see Table 2), we found significant linkage between *pds* and *yv* ($P < 0.01$) and between *pds* and *tl* ($P < 0.005$) respectively, while RICK, ZOBEL and OPEÑA (1970) demonstrated very significant linkage to exist between *pds* and *yv* ($P << 0.001$). A chi square test of the segregation of *pds* relative to *GP164* showed a significant deviation from independency ($P = 0.05$), providing another argument for the location of *pds* on the short arm of chromosome *6*. Currently, additional linkage data are being collected for the intervals *pds/tl* and *tl/yv* in crosses with WSL6 to further define the integrated map of the short arm. Similarly, mapping experiments are now in progress with lines carrying the region below *c* introgressed from *L. pennellii*

(ESHED *et al.* 1992) and from *L. hirsutum* so as to achieve further integration of the classical and molecular linkage maps of tomato chromosome *6*.

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