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Integration of the classical and RFLP linkage maps of the short arm of tomato chromosome 1

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Abstract The classical map of the short arm of chromosome 1 of tomato (*Lycopersicon esculentum*) has been shown to contain inaccuracies while the RFLP map of this region is known to be generally accurate. Molecular analysis of populations derived from crosses between *L. esculentum* lines carrying chromosome 1 classical markers and *L. pennellii* has enabled us to produce an integrated classical and RFLP marker map of this region. New data concerning the linkage relationships between classical markers have also been combined with previous data to produce a new classical map of the short arm of chromosome 1. The orders of the classical markers on these two new maps are in almost complete agreement and are very different to that shown on the previous classical map.

Key words Tomato (*Lycopersicon esculentum*) · Genetic map · RFLP · Integrated map

Introduction

The classical map of tomato has been constructed over a number of years using data from many different crosses, often involving relatively small populations. Markers were frequently positioned relative to each other on the basis of their genetic distances from a third marker. It is likely that, although the map might be very broadly correct, significant inaccuracies will be discovered if a region is studied in detail. This has been the case with chromosomes 3 (Koornneef et al. 1993), 6 (Weide et al. 1993) and 7 (I. Taylor, personal communication).

Cf-4 and *Cf-9* are tomato genes conferring resistance to specific races of *Cladosporium fulvum*, the causal agent of tomato leaf mould. Previous studies (Kerr and Bailey 1964; Jones et al. 1993; Balint-Kurti et al. 1994) have shown that *Cf-4* and *Cf-9* are very closely linked to one another and to another *C. fulvum* resistance gene, *Cf-1*, on the short arm of chromosome 1. Linkages of classical markers to the *Cf-4* and *Cf-9* genes have been investigated (this report and Jones et al. 1993). During these investigations it became clear that there were substantial inaccuracies in the classical map of the short arm of chromosome 1 (for the most recently published report see Tanksley et al. 1992). *Cf-4* was originally mapped to chromosome 1 (Kerr and Bailey 1964) due to its loose linkage with the chromosome 1 markers *brachytic* (*br*) and lacking *yellow* fruit epidermis (*y*). However, it had clearly been placed in an erroneous position relative to other chromosome 1 markers (Jones et al. 1993). *Cf-9* had been mapped to chromosome 10 (Kanwar et al. 1980) due to flawed linkage data (Jones et al. 1993). It was also suspected that many of the classical markers were wrongly positioned relative to each other (Jones et al. 1993).

For these reasons, several of the classical markers known, or thought, to map to the short arm of chromosome 1 were mapped relative to RFLP markers. As the tomato RFLP map was constructed by mapping all the markers relative to each other using the same population of 67 F_2 plants from a *L. esculentum* × *L. pennellii* cross (Tanksley et al. 1992), the RFLP marker order is generally reliable. Thus, by mapping chromosome 1 classical markers to intervals on the RFLP map, the two maps could be integrated and the order of the classical markers corrected. In addition, new data concerning the linkage relationships of chromosome 1 classical markers were collected. These, together with data available in the literature, facilitated the construc-

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tion of a new classical map of the short arm of chromosome 1.

Materials and methods

Plant material and segregating populations

Table 1 lists the sources of all the stocks used in this work

Testing for resistance to *Cladosporium fulvum* conferred by the genes *Cf-4* and *Cf-9*

Populations segregating for *Cf-4* were scored for resistance or susceptibility by inoculation with *C. fulvum* race 5 as described in Jones et al. (1993) and Balint-Kurti et al. (1994). Populations segregating for *Cf-9* were scored using intercellular fluid from infected plants as described in de Wit and Spikman (1982) and Balint-Kurti et al. (1994).

DNA extraction, Southern and PCR-based analysis

Genetic analysis using molecular markers was performed as detailed in Balint-Kurti et al. (1994).

RFLP linkage analysis and morphological marker linkage analysis

The JoinMap program (Stam 1993) was employed for the construction of the integrated and new classical maps (Figs. 1 and 2) presented here. The Kosambi mapping function (Kosambi 1944) was used to estimate map distances from recombination frequencies.

To detect linkage between *Cf*-genes and morphological markers, or between morphological markers, joint segregations were tested pairwise for departures from independent assortment by carrying out χ^2 tests for association on 2×2 contingency tables. For F_2 data, recombination values and standard errors were estimated using the maximum likelihood method (Mather 1951). For recombination values of zero the upper limit of recombination at $P = 0.05$ for n gametes screened was calculated according to the formula $1 - p^{1/n}$ (see Table VIII, Fisher and Yates 1963).

Results

Mapping of classical markers on the tomato RFLP map

Crosses were made between eight *L. esculentum* lines carrying different classical markers on chromosome 1 and *L. pennellii*. From the resulting F_2 or test cross populations, it was possible to map six of the classical markers (*au*, *ms-32*, *bs*, *imb*, *Lpg* and *ses*) relative to RFLP markers on the short arm of chromosome 1 (Table 2). The PCR-based mapping procedure described in Balint-Kurti et al. (1994) was employed in the analysis of these populations. The segregations of two markers, *br* and *com*, were impossible to score in these crosses and, consequently, these markers could not be assigned to an RFLP interval. Details of the populations generated are reported below. When gene symbols are used in the crosses described below, the reader should infer that the *L. esculentum* line carrying the relevant mutation(s) (as shown in Table 1) was used.

Mapping of *aurea* (*au*)

Test-cross progeny from the cross *au* × (*au* × *L. pennellii*) were analysed. These progeny segregated 1:1 au: wild-type, the au phenotype (yellow foliage) being easily scorable. Thirty-five au individuals and 12 wild-type individuals from this population were used for RFLP analysis (as reported in Jones et al. 1993). In addition, individuals from *au* × (*Lpg au* × *L. pennellii*) populations and au segregants from *Lpg au* × *L. pennellii* F_2 populations were analysed. TG236 was found to completely co-segregate with *au*. No recombination events between *au* and TG236 (see Table 6) have been identified from 98 meioses (0% recombination with an upper limit of 3.3%, $P = 0.05$). This places *au* in the "TG236 cluster" in which

Table 1 A list of all the stocks used in this study. Those prefixed LA or 2- originated from C. Rick at the Tomato Genetics Cooperative Stock Centre and, except for *L. pennellii* LA716, are mentioned in Rick (1990). Those prefixed GCR originate from the Glasshouse Crops Research Institute Littlehampton (GCRI, now called Horticultural Research International). The imbecilla line is reported in Maxon-Smith and Ritchie (1983), while the existence of the other GCR lines used was communicated to us by J. Maxon-Smith

Stock (gene symbol)	Stock number	Source
Lycopersicon pennellii	LA716	C. Rick
aurea (*au*)	2-655A	C. Rick
aurea Tm-2^2 (*au Tm-2^2*)	2-655A × GCR758 F_3	D. Jones
aurea scurfy invalida diageotropica (*au, scf, inv, dgt*)	LA1186	C. Rick
brachytic (*br*)	LA2069	C. Rick
brown seed (*bs*)	LA2935	C. Rick
complicata (*com*)	LA664	C. Rick
eramosa (*era*)	LA880	C. Rick
imbecilla (*imb*)	GCR362	GCRI
irregularis (*irr*)	LA613	C. Rick
Lapageria (*Lpg*)	2-561 (+/*Lpg* F_2)	C. Rick
Lapageria aurea (*Lpg au*)	GCR705	GCRI
male sterile 32 (*ms-32*)	LA359 (*ms-32*/+ F_2)	C. Rick
propellor (*pr*)	LA326	C. Rick
semisterilis (*ses*)	LA826 (*ses*/+ F_2)	C. Rick
terminating flower (*tmf*)	LA2462	C. Rick
umbrosa (*um*)	LA630	C. Rick
villous (*vi*)	LA759	C. Rick
yellow fruit epidermis (*y*)	GCR442	GCRI

Table 2 RFLP and PCR analysis of populations from crosses between *L. pennellii* and *L. esculentum* lines carrying morphological markers. Only data from the crosses in which the morphological marker could be easily scored in the resulting segregating population are shown. In each case E = homozygous for *L. esculentum* RFLP; H = heterozygous for *L. esculentum* and *L. pennellii* RFLPs; P = homozygous for *L. pennellii* RFLP; - = not scorable or not analysed. Data shown in bold represent instances where there was no recombination event between the morphological marker and the RFLP marker. Data not shown in bold represent instances where such a recombination event has occurred. Probes are listed in order of their location on the short arm chromosome 1 with the most distal probe on the left and the most proximal on the right (see Fig. 2C). The phenotype (pheno.) of each group of plants is indicated as mutant or wild-type (*W.t.*). By progeny testing the populations segregating for *imbecilla* (*imb*) and *brown seed* (*bs*), all three genotypes (geno.) were distinguished [i.e., homozygous for the mutation (*imb/imb* or *bs/bs*), heterozygous (*imb/+* or *bs/+*), and homozygous wild-type (+/+)]. The restriction enzymes used to distinguish the RFLP for each RFLP marker are as shown in Balint-Kurti et al. (1994) for the *L. pennellii*/Cf9 polymorphism except for TG310 (*Eco*RI), TG224 (*Eco*RI)TG83 (*Eco*RI), TG71 (*Eco*RV) and TG59 (*Eco*RV)

Gene	Cross	Pheno. or Geno.	RFLP marker										No. of plants
			CT233	TG301	CP46	TG236	TG51	TG310	TG224	TG59	TG71	TG83	
au	Test	au	**E**	**E**	-	**E**	**E**						14
			E	**E**	-	**E**	H						1
			H	H	-	**E**	**E**						2
			H	**E**	-	E	E						2
			-	-	-	**E**	-						21
		W.t.	**H**	**H**	-	**H**	**H**						8
			H	**H**	-	**H**	E						1
			E	E	-	**H**	**H**						1
			-	-		**H**	-						18
	F_2	au	-	-	-	**E**	-						10
ses	F_2	ses			**E**	**E**	**E**	**E**					10
					H	**E**	**E**	**E**					1
					E	**E**	**E**	H					10
					H	**E**	**E**	H					2
					H	H	**E**	**E**					1
					H	H	**E**	H					1
					E	**E**	H	H					6
bs	F_2	*bs/bs*				**E**	**E**	**E**					2
						E	**E**	H					1
						H	**E**	H					2
		bs/+				**H**	**H**	**H**					5
						E	**H**	**H**					1
						P	**H**	P					1
						H	**H**	P					1
		+/+				**P**	**P**	**P**					2
ms-32	Test	ms				**E**	**E**	**E**	**E**	**E**			18
						E	**E**	**E**	**E**	H			4
						H	**E**	**E**	**E**	**E**			1
						H	H	**E**	**E**	**E**			1
						H	**E**	**E**	**E**	H			1
						E	**E**	**E**	H	H			2
						H	H	H	H	**E**			1?
		W.t.				**H**	**H**	**H**	**H**	**H**			15
						H	**H**	E	E	E			1
						E	E	-	**H**	**H**			1
						E	E	E	E	E			1?
Lpg	Test	Lpg				**E**	**E**	**E**	**E**	-	**E**		3
						E	**E**	**E**	**E**	-	H		1
						H	H	**E**	**E**	-	**E**		2
						H	**E**	**E**	**E**	-	**E**		2
		W.t.				**H**	**H**	**H**	**H**	-	**H**		9
						H	E	**H**	**H**	-	**H**		2
						E	E	H	**H**	-	**H**		2
						E	-	H	**H**	-	**H**		2
						H	**H**	**H**	E	-	E		1
imb	F_2	*imb/imb*					**E**	**E**	-	-	**E**	**E**	2
							E	**E**	-	-	**E**	H	2
							H	-	-	-	**E**	**E**	2
							H	H	-	-	**E**	P	1
		imb/+					**H**	**H**	-	-	**H**	**H**	3
							P	P	-	-	**H**	**H**	1
							E	-	-	-	**H**	**H**	1
		+/+					**P**	**P**	-	-	**P**	**P**	4
							P	**P**	-	-	**P**	H	1
							H	**P**	-	-	**P**	H	2
							H	H	-	-	-	**P**	1

12 RFLP markers have been shon to cosegregate in populations derived from *L. esculentum* × *L. pennellii* crosses (Tanksley et al. 1992; Balint-Kurti et al. 1994). Other information (Balint-Kurti et al. 1994) suggests that *au* is proximal to FT33, another marker in the TG236 cluster.

Mapping of *male sterile-32* (*ms-32*)

Seeds from the cross *ms-32* × (*ms-32* × *L. pennellii*) were obtained from R. Jorgensen. Forty-eight of these seeds were grown to flowering (the stage when the ms-32 phenotype – shrunken anthers and exposed stigmas – could be determined). The ms-32 phenotype was, in most cases, clearly scorable. In a few cases there was some difficulty in scoring due to the segregation of *L. pennellii* factors affecting flower morphology. The population segregated 29:19 ms-32: wild-type (not significantly different from 1:1). RFLP analysis of this population indicated that *ms-32* was probably located between the markers TG51 and TG310. Two segregants yielded data inconsistent with this conclusion (shown with question marks in Table 2). The probable cause of this was misscoring of the plants for the ms-32 phenotype. The apparent inconsistency might also be the result of a double recombination event within a single RFLP interval. Information from these two segregants was not included amongst the data used to produce the integrated map of chromosome 1.

Mapping of *Lapageria* (*Lpg*)

Rick (1964) reported *Lapageria* (*Lpg*) as an incompletely-dominant morphological marker causing, among other things, reduced trichome size and number, and reduced viability. An *L. esculentum* plant heterozygous for *Lpg* was crossed to *L. pennellii*. Of the resulting 30 F_1 progeny none showed any features associated with the Lpg phenotype. Three of these F_1 plants were crossed back to *L. esculentum* plants wild-type at the *Lpg* locus. The progeny of one cross segregated 8:16 Lpg: wild-type (fitting a 1:1 ratio). None of the progeny from the other two crosses displayed the Lpg phenotype. F_2 progenies from these same three F_1 plants were also examined. The same plant that gave Lpg progeny when test-crossed, produced 13:83 Lpg:wild-type progeny in the F_2. The low number of Lpg individuals here may be due to segregation distortion in this region as previously observed among F_2 progeny from *L. esculentum* × *L. pennellii* crosses (Chetelat and de Verna 1991). The other two F_1 plants gave no Lpg F_2 progeny out of a total 51 plants.

RFLP analysis of the 24 test-cross plants segregating for *Lpg* (Table 2) showed that *Lpg* cosegregated with the RFLP marker TG310 located on chromosome 1 (Tanksley et al. 1992). The fact that no plants gave RFLP data inconsistent with their phenotypes, and the absence of a modified segregation ratio in the test cross, suggests that the locus in *L. pennellii* responsible for masking the Lpg phenotype in the F_1 is at, or near, the *Lpg* locus. However, RFLP analysis of the 13 Lpg plants from the F_2 population showed that, while five individuals were homozygous for the *L. esculentum* allele of TG310, eight individuals were heterozygous for this marker and flanking loci. This implies that, in certain individuals from the F_2 population, a phenotype associated with *Lpg* can be seen although they are only heterozygous for *Lpg*. The fact that no Lpg phenotype was observable in the original F_1 progeny (see above) suggests that the appearance of the Lpg phenotype in those F_2 individuals heterozygous for *Lpg* depends upon the segregation of other unlinked genes.

It seems probable that the wild-type *L. pennellii* allele of *Lpg* is usually dominant over the *L. esculentum* mutant allele, but that the segregation of other genes in the F_2 population from the *Lpg* × *L. pennellii* cross has an effect on the dominance relationship of these alleles.

Mapping of *semisterilis* (*ses*)

An F_2 population from the cross *ses* × *L. pennellii* was generated. The segregation of the ses phenotype (very obvious sectors of rough-surfaced tissue on the cotyledons and true leaves) was easily scorable in this population. The segregation observed was 262:31 wild-type: ses (a significant difference from 3:1). The deviation from a 3:1 ratio in this population was presumably due to the segregation distortion observed in the *L. esculentum* × *L. pennellii* crosses mentioned above. The 31 ses plants were subjected to RFLP analysis. This analysis placed *ses* in the interval defined by the RFLP markers TG236 and TG51 (Table 2).

Mapping of *imbecilla* (*imb*)

An F_2 population from the cross *imb* × *L. pennellii* was generated. Scoring of the imb phenotype (yellowish leaves, sectoring of rough-surfaced tissue on cotyledons) was difficult in this population, due, presumably, to the segregation of *L. pennellii*-derived features. However 20 plants were selected as conforming most closely to the expected imb phenotype. RFLP analysis of these 20 plants indicated that they could not all be *imb* homozygotes as there was no interval in which all 20 plants were homozygous for *L. esculentum*-derived DNA. To determine the true *imb* genotype of these plants, they were all back-crossed to an *imb* homozygous *L. esculentum* mother. A minimum of two successful crosses was performed for each of the 20 plants. The segregation of *imb* was quite clear in the F_2 backcross progeny. This meant that the *imb* genotype of all 20 of the original F_2 plants could be determined. TG71 was found to cosegregate with *imb* (0 recombination events from 40 meioses – see Table 2).

Mapping of *brown seed* (*bs*)

An F_2 population from the cross *bs* × *L. pennellii* was generated. Reliable scoring of the bs phenotype (dark-

brown seed) was not possible in this population (note that the brown colouring of the endosperm is a reflection of the genotype of the embryo rather than of the parent plant). Therefore 20 plants from this population were backcrossed to a *bs* homozygous *L. esculentum* mother. Examination of the seeds from this backcross enabled the *bs* genotype of the original F_2 parents to be unambiguously determined. RFLP analysis of the F_2 parents (Table 2) showed that TG51 cosegregated with *bs* (0 recombinants from 30 meioses).

Construction of a new classical map and an integrated RFLP/classical map of the short arm of chromosome 1

JoinMap (Stam 1993) is a computer program designed to calculate the most-likely combined map given segregation data from different crosses, involving different subsets of markers. The crosses need not be of the same type (e.g., in this case we had backcross and F_2 data). This program was used to construct a new classical map of the short arm of chromosome 1. This was achieved using all the linkage data for classical markers around *Cf-4/9* obtained in this and other studies. These data are shown in Tables 3–6. The closest linkage found between *Cf-4/9* and a classical marker was 14.4 cM between *Cf-9* and *au* (reported in Jones et al. 1993). There is very little evidence for the linkage of *inv* to other markers on the short arm of chromosome 1, so it was omitted from the new map. The markers *pr*, *tmf*, *vi* and *era* have previously been mapped to chromosome 1 (Tanksley et al. 1992), but data presented in Table 3 show that they are not linked to *Cf-4*. For the purposes of this new map, recombination distances between *Cf-4* or *Cf-1* and other classical markers were interpreted as distances between

Table 3 Linkage of *Cf-9* and *Cf-4* to classical markers on chromosome 1. F_2 progeny segregating for *Cf-9* were scored by injection with either race 4 or race 5 apoplastic fluid, and those segregating for *Cf-4* by inoculation with *C. fulvum* race 5. C = coupling, R = repulsion. The segregant classes are as follows: double dominant-A: one dominant phenotype and one recessive phenotype-B,C; both phenotypes recessive-D. F_2 populations from the crosses *um* × Cf4 and *fla* × Cf4 were also generated but it was impossible to score these two mutant phenotypes reliably. * = significant $P = 0.05$; ** = significant at $P = 0.001$; a, Jones et al. (1993)

Marker	*Cf-*	Phase	A	B	C	D	χ^2	Rec	Reference
au[tl]	*9*	C	265	32	24	83	172.4**	14.4 ± 1.9%	a
scf	*9*	C	220	76	66	37	3.95*	43.2 ± 3.5%	a
ses	*9*	C	717	90	87	154	289.1**	19.3 ± 1.4%	a
ms-32[a]	*9*	C	152	25	23	27	30.8**	25.4 ± 3.4%	a
imb	*9*	C	120	32	32	8	0.02	50.0 ± 5.4%	a
br	*9*	C	35	15	17	5	0.40	54.7 ± 9.3%	a
pr	*4*	C	105	35	---51---		0	50%	This study
era	*4*	C	38	12	12	8	1.8	39.5 ± 7.9%	This study
vi	*4*	C	75	34	21	11	0.001	48.6 ± 6.2%	This study
tmf	*4*	C	47	17	9	7	0.01	40.9 ± 7.5%	This study
bs	*9*	C	67	6	12	11	18.8**	22.9 ± 5.0%	This study
com	*9*	C	188	53	53	34	9.5*	38.7 ± 3.6%	a
Lpg	*9*	R	32	18	17	25	5.07*	38.0 ± 5.1%	a
au	*9*	C	43	9	6	34	41.6**	16.3 ± 3.9%	a
irr	*9*	C	117	26	31	21	10.3*	35.2 ± 4.4%	a
y	*9*	C	67	25	19	10	0.57	45.4 ± 6.8%	This study

[a] The linkage value between *ms-32* and *Cf-9* contradicts the lack of linkage previously reported by Jones et al. (1993). The difference in the two linkage values is believed to be due to initial erroneous scoring of the *Cf-9* segregation by PBK

Table 4 Previously reported data on the linkage of chromosome 1 classical markers to *Cf-1* and *Cf-4*. Kanwar et al. (1980) present the only linkage data previously reported for *Cf-9*, which placed *Cf-9* on chromosome 10. These were not included as they are clearly wrong (as shown in Jones et al. 1993). The crosses performed are indicated: backcross (BC), F_2 in coupling phase (F_2C), or F_2 in repulsion (F_2R). Recombination distances (rec) are shown. * = significant at $P = 0.05$; ** = significant at $P = 0.001$. The segregant classes are as in Table 3. The results of chi-square tests for association are shown. a, Kerr and Bailey (1964); b, Kanwar et al. (1980); c, Langford (1937)

Cf-	marker	Cross	A	B	C	D	χ^2	Rec	Reference
4	*br*	BC	542	368	330	429	44.7**	41.8%	a
4	*y*	BC	475	333	301	447	53.3**	40.7%	a
4	*y*	F_2C	497	140	140	74	13.6**	42%	a
4	*y*	BC	8	5	8	10	0.8	42 ± 9%	b
4	*imb*	BC	9	3	1	13	12.5**	15 ± 7%	b
1	*y*	F_2C	148	27	48	38	29.9**	30.9 ± 3.6%	c
1	*y*	BC	61	40	35	51	7.3*	40.1 ± 3.6%	c
1	*br*	BC	45	48	46	47	0.1	50.5 ± 3.7%	c
1	*y*	BC	11	8	8	9	0.4	44 ± 8%	b
1	*imb*	BC	14	4	3	15	13.4**	19 ± 7%	b
Cf-4/Cf-1		F_2R	---225---		67	0(1?)	17.5**	0?	a

Table 5 New data on linkage between classical markers on chromosome 1. Symbols are as used in Tables 3 and 4

Markers	Cross	Phase	A	B	C	D	χ^2	Rec
com-au	*com* × *au* $Tm2^2F_2$	R	109	41	34	3	4.7*	31.1 ± 6.5%
imb-au	*imb* × *au* $Tm2^2F_2$	R	58	23	30	3	5.3*	31.1 ± 8.3%
imb-com	*imb* × *com* F_2	R	125	58	48	4	11.7**	27.0 ± 6.0%
au-bs	*au* × *bs* F_2	R	–	–	85	3	21.9**	18.1 ± 4.0%
Lpg-bs	*Lpg* × *bs* F_2	C	–	–	14	64	135.4**	9.4 ± 3.3%
Lpg-au	*imb* × *Lpg au* F_2	C[a]	318	57	47	57	62.5**	26.1 ± 2.4%
Lpg-imb	*imb* × *Lpg au* F_2	C	306	48	63	62	65.4**	27.0 ± 2.4%
au-imb	*imb* × *Lpg au* F_2	R	284	91	97	13	6.1*	38.4 ± 3.8%
Lpg-au	*ses* × *Lpg au* F_2	R[b]	100	46	60	2	21.4**	18.2 ± 6.7%
Lpg-ses	*ses* × *Lpg au* F_2	C	133	13	17	45	107.2**	14.6 ± 2.7%
au-ses	*ses* × *Lpg au* F_2	R	105	45	55	3	15.1**	23.5 ± 6.5%
au-scf	Cf9 × LA1186 F_2	C	227	67	69	36	5.7*	42.1 ± 3.4%
au-ms-32	*au ms-32* × (*au* × *ms-32*)	–	37	132	154	61	92.0**	25.5 ± 2.2%
au-ms-32	*au* × *ms-32* F_2	R	---342[c]---		44	1	12.5**	14.9 ± 14.5%
imb-ms-32	*imb* × *ms-32* F_2	R	---216---		67	3	16.0**	20.7 ± 11.3%
au-imb	(*au* × *imb*) × *au imb*	–	38	48	38	35	1.1	45.9 ± 4%

[a] *Lpg* is a partially dominant marker, so a cross involving this marker is effectively in coupling either way round

[b] In this case *Lpg* was scored as a dominant marker

[c] The excess of non-aurea plants was thought to be due to delayed germination and damping off of *au* seedlings

Cf-9 and these other genes. This was thought to be valid as the tight linkage of *Cf-1*, *-4* and *-9* has been clearly demonstrated (Kerr and Bailey 1964; Jones et al. 1993). The resulting new classical map, shown in Fig. 1, differs considerably from the old classical map.

The linkage data used to generate the new classical map were obtained from intraspecific crosses between different *L. esculentum* lines. Linkage data for the isozyme markers *Skdh-1*, *Idh-1* and *Prx-1*, which appear on the old classical map, were obtained from interspecific crosses. It was thought inappropriate to combine these two sets of data as the patterns of recombination are different between inter- and intra-specific crosses involving *L. esculentum* (see Rick 1969; Balint-Kurti et al. 1994). For this reason it was decided to omit the isozyme markers from the new classical map and instead to include them on the integrated map which is based on interspecific crosses. Although *Prx-1* and *Skdh-1* have been RFLP mapped (Chetelat and De Verna 1991; Eshed et al. 1992; Tanksley et al. 1992), the raw linkage data, necessary to integrate them in this study, are unpublished. Nevertheless, their integration was possible because appropriate linkage data have been published with respect to *aurea*, one of the classical markers integrated in this study (Table 6b). *Idh-1* has been mapped relative to *Prx-1* and *Skdh-1*, but only in the F_2 of a cross between *L. hirsutum* and *L. pennellii*. The linkage between *Prx-1* and *Skdh-1* in this cross was so similar to that for *L. esculentum* and *L. pennellii* (see Table 6b) that it was considered legitimate to use data from this cross to help generate the integrated map. The isozyme *Skdh-1* has been mapped to the interval between TG310 and TG51 by Tanksley et al. (1992) and by R. Chetelat (personal communication) and Y. Eshed (personal communication). This information was used to orient the isozyme linkages on the integrated map.

JoinMap was used to analyse the RFLP data presented in Table 2 and the isozyme data shown in Table 6b, together with other data (not shown), to construct an integrated map of the short arm of chromosome 1, involving six classical markers, the RFLP markers used to map them (Table 2), and four isozyme markers (Table 6b). *Cf-9* was also included on this map by incorporating the TG236-*Cf-9*, CP46-*Cf-9* and TG301-*Cf-9* recombination distances previously noted (Balint-Kurti et al. 1994). The resulting map is shown in Fig. 2b. The order of the classical markers in the integrated map (Fig. 2b) is the same as their order in the new classical map (Fig. 1b), apart from the order of *Lpg* and *ms-32* which is reversed. The RFLP markers are in the same order and separated by approximately the same genetic distances as shown in the RFLP map of Tanksley et al. (1992). Several pairs of markers cosegregated but were separated by JoinMap due to extra data concerning one of them. These pairs are indicated on Fig. 2b.

Discussion

This paper details the construction of a new, more accurate, classical map and an integrated RFLP/classical map of the short arm of chromosome 1 of tomato. The generation of a considerable amount of new data, involving the measurement of linkage distances between several previously unanalysed marker pairs, combined with the availability of the JoinMap program, has helped to produce these new maps. The almost complete correspondence between marker order in the integrated map and the new classical map attests to the accuracy of both, especially as different sets of data were used to generate each map. The only difference in gene order between the two is the inverted order of *Lpg* and *ms-32* (see Figs. 1b and 2b). In this study, *ms-32* was localised

Table 6a–c **a** Previously reported linkage data between classical markers on chromosome 1 used in construction of the new classical map. **b** Previously reported linkage data between isozyme and classical markers on chromosome 1 used in construction of the integrated map. **c** Previously reported linkage data between chromosome 1 classical and isozyme markers not used in the construction of the new classical map. Symbols are as used in Tables 3 and 4 except where stated. Blank spaces indicate data that were not available. a, Koornneef et al. 1990; b, Tanksley and Rick 1980; c, MacArthur 1934

	Markers	Phase	A	B	C	D	χ^2	Linkage	Reference[a]
a	*sit-au*							0.0 ± 6.5%	a
	sit-gib-2							28.4 ± 3.5%	a
	au-ses	R	476	213	199	1	76.8**	7.5 ± 3.3%	18:34
	au-bs							15.2 ± 3.0%	23:28
	au-ms-32							15.2 ± 4.4%	21:36
	au-Lpg	C	278	24	40	74	151.0**	16.7 ± 2%	14:25
	au-gib-2							25.1 ± 3.7%	a
	au-com	R	707	178	190	16	16.6**	39.0 ± 2.5%	23:32
	au-imb	R	673	205	258	20	27.6**	36.9 ± 2.5%	13:42
	au-br	R	519	254	152	20	31.6**	31.4 ± 2.9%	8:12
	au-scf							41.8 ± 2.8%	a
	au-scf	C					4.17*	46.4 ± 1.6%	22:31
	ses-scf	R	675	230	248	43	13.5**	40.5 ± 2.4%	18:34
	bs-ms-32							19.8 ± 5.1%	21:34
	bs-imb							38.0 ± 3.2%	23:28
	bs-scf							39.8 ± 4.2%	23:28
	ms-32-imb							18.9 ± 7.6%	23:28
	ms-32-y							15.5 ± 5.1%	23:28
	ms-32-scf							23.6 ± 6.1%	23:28
	irr-y	C	56	5	2	10	23.8**	11.3 ± 4.0%	14:16
	Lpg-y	C	37	21	8	8	3.9*	15.0 ± 28.9%[b]	16:30
	Lpg-scf	C	151	36	46	17	35.7*	33.5 ± 23.4%[b]	16:30
	y-br	R	892	399	397	34	91.3**	28.0 ± 1.5%	c
	y-scf	R			84	6	16.1**	25.8 ± 9.7%	15:52
	gib-2-scf							20.1 ± 5.1%	a
	com-scf	R	675	201	203	4	46.8**	20.8 ± 7.4%	23:32
b	[c] *Idh-1-Prx-1*							7.1 ± 1.9%	35:20
	[c] *Idh-1-Skdh-1*							33.3 ± 4.5%	35:20
	Prx-1-au	C					214.83**	7.1 ± 2.1%	b
	Prx-1-au	C					99.1**	9.0 ± 2.2%	b
	Prx-1-Bnag-1		25	13	4	35	25.3**	22.1 ± 4.7%	38:11
	Prx-1-Skdh-1	C					44.6**	25.7 ± 2.8%	b
	[c] *Prx-1-Skdh-1*							28.0 ± 4.5%	35:20
	au-Skdh-1	C					56.0**	17.1 ± 4.7%	b
	Bnag-1-Skdh-1		38	1	0	38	73.1**	1.3 ± 1.3%	38:11
c	[d] *Prx-1-ms-32*	C	1	65	69	5	118.9**	4.3 ± 1.7%	31:18
	Prx-1-scf	C					4.27	Unlinked	b
	sit-au	R	250	116	83	1?	28.2**	11.4 ± 4.6%	11:23
	[e] *au-y*	R	63	38	20	0	11.3**	0.0 ± 9.1%	9:47
	[f] *au-scf*	C	239	42	41	12	1.95	38.8 ± 3.6%	b
	au-inv	R	303	98	104	12	4.6*	36.4 ± 3.8%	13:42
	au-pr	R	597	114	153	32	0.87	Unlinked	8:12
	au-dgt							Unlinked	a
	ses-inv	R	724	181	245	46	1.8	Unlinked	18:34
	bs-era							Unlinked	23:28
	bs-pr							Unlinked	23:28
	[d] *Skdh-1-ms-32*	C	0	63	74	0	137**	0 ± 1.1%	31:18
	ms-32-pr							Unlinked	23:28
	ms-32-era							39.5 ± 4.9%	23:28
	[e] *irr-y*	R	54	34	18	4	4.4*	34.5 ± 8.3%	14:16
	Lpg-inv	C	140	31	51	28	12.5**	38.5 ± 23.1%[b]	16:30
	y-fla	R	68	32	23	2	5.5*	27.4 ± 8.2%	8:15–17
	com-inv	R	693	183	171	36	1.1	Unlinked	23:32
	scf-inv	R	128	44	52	7	4.0*	37.9 ± 5.6%	15:52

[a] Unless otherwise stated all the references refer to the volume and page number in Reports of the Tomato Genetics Cooperative

[b] The recombination frequency was calculated from the two non-*Lpg* classes due to a deficiency of *Lpg* plants

[c] These data were generated from an *L. hirsutum* × *L. pennellii* F_2

[d] These data were generated from the testcross of a line of *L. esculentum* carrying an introgressed segment of chromosome 1 from *L. pennellii* and were not used to help generate the integrated map because recombination was severely reduced

[e] Data not used because they were contradicted by other, more reliable data or because one of the markers involved could not be mapped accurately.

[f] These data were generated from an *L. esculentum* × *L. pennelli* F_2 and so were not used to help generate the new classical map, which is based entirely on data generated from *L. esculentum* crosses

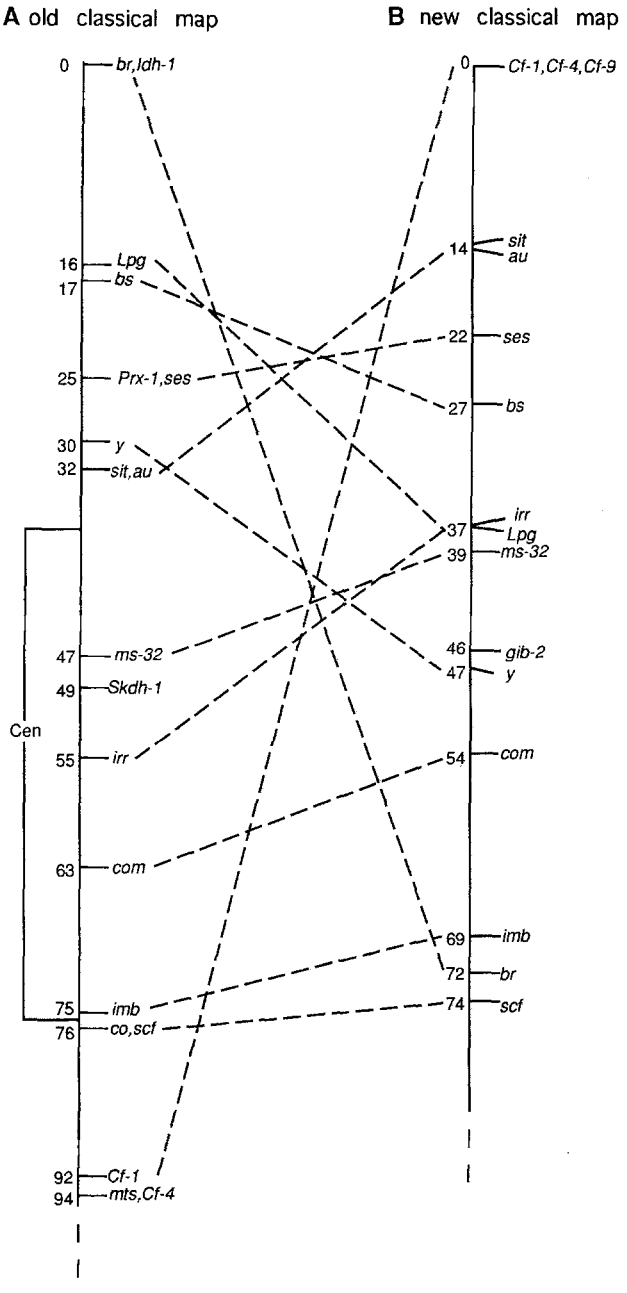

Fig. 1A, B **A** The classical map of the short arm of tomato chromosome 1 as it appears in Tanksley et al. (1992). **B** A new classical map of the short arm of chromosome 1 generated with the JoinMap computer program (Stam 1993) and the data shown in Tables 3–6. Dashed lines indicate the relative positions of markers on the new classical map compared to the old classical map

to the interval between TG310 and TG51 while *Lpg* cosegregated with TG310. In fact, these data do not allow the relative positions of the two genes to be determined. More data are needed to determine their relative positions with confidence.

The positions of the isozyme markers *Prx-1* and *Skdh-1* on the integrated map should not be regarded to be as reliable as the positions of the classical markers, as they have not been positioned directly relative to RFLP markers, but indirectly via *au*. In spite of this, the position of *Skdh-1* on the integrated map is consistent with it's RFLP map location (Tanksley et al. 1992). The positions of *Idh-1* and *Bnag-1* should be regarded as even less reliable because they have in turn been positioned via *Prx-1* and *Skdh-1*. In addition, the *Idh-1* data was generated from a different interspecific cross than the other markers. Nevertheless, the integrated map should provide a useful guide to the general positions of these four isozyme markers.

The combining of data derived from F_2 and backcross populations to produce these maps is not strictly valid, as recombination frequencies have been shown to vary between male and female gametes (de Vicente and Tanksley 1991). Recombination events in both male and female gametes are detectable in F_2 populations, but only those in male gametes are detectable in backcross populations. The map distances shown on these maps are therefore not completely valid for either cross. The combining of F_2 and backcross data also provides another source of error in the integrated map, because segregations of markers on the short arm of chromosome 1 are skewed in *L. esculentum* × *L. pennelli* F_2 progeny, but not apparently in progeny of the interspecific F_1 backcrossed to *L. esculentum*. This appears to be due to unilateral incompatibility determined by a *L. pennellii* gene located on the short arm of chromosome 1 (Chetelat and De Verna 1991). Another possible source of error includes the different *L. esculentum* lines used in the various crosses (e.g, the *ms-32* mutation was carried in a *L. esculentum* var. *cerasiforme* line). These lines may differ slightly in their recombination frequencies over the same intervals. Finally, the varied conditions in which the crosses were performed may have caused differences in recombination (Griffing and Langridge 1963). It is, the therefore, important to bear in mind that the new maps only represent the most likely order of markers given the available data. However, the close correspondence between the RFLP marker distances on the integrated map and the RFLP map (generated from *L. esculentum* × *L. pennellii* F_2 data) shows that any distortion of map distances caused by the combination of these data cannot have been extreme. On the other hand, it is not suprising that the map distances in the integrated map are quite different to those in the new classical map (some intervals are contracted, others are expanded), which further validates the decision not to mix data arising from intra- and inter-specific crosses to generate these maps.

More important than marker distances are the marker orders of these new maps, especially because they reveal a gene order remarkably different to that of the old classical map of the short arm of chromosome 1. The inaccuracies in the previous classical map can be ascribed mainly to lack of data and to the way in which the map was assembled, as discussed in Jones et al. (1993). Koornneef et al. (1993) and Weide et al. (1993) have integrated the classical and RFLP maps of chromosomes 3 and 6 of tomato, respectively, and likewise

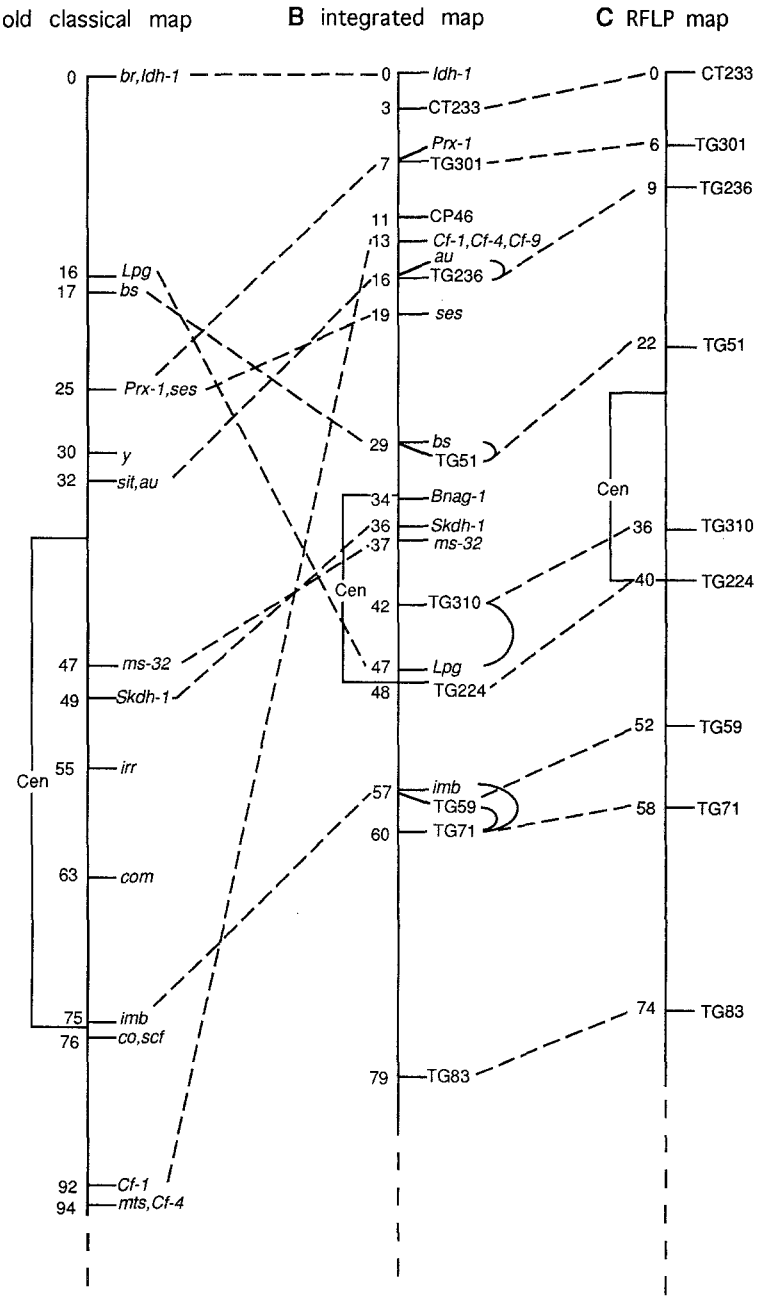

Fig. 2A–C **A** The classical map of the short arm of tomato chromosome 1 as it appears in Tanksley et al. (1992). **B** The integrated map of the short arm of chromosome 1 of tomato, including ten RFLP markers, seven classical and four isozyme markers. This map was generated using the JoinMap computer program (Stam 1993) with the data shown in Tables 2 and 6c, data for *Cf-9* linkages reported in Balint-Kurti et al. (1994), and some data not shown including data from the analysis of the *br* × *L. pennellii* F_2 population and data from further analysis of the populations detailed in Table 2. Markers which cosegregate have been joined by curved lines to the right of the figure. Dashed lines indicate the relative positions of markers on the integrated map compared to the old classical map or the RFLP map. The region delimiting the location of the centromere (Cen) is the same as that on the RFLP map. **C** The RFLP map of the short arm of chromosome 1 of tomato showing a subset of the markers mapped by Tanksley et al. (1992). The region delimiting the location of the centromere (Cen) is based on the data of Lapitan et al. (1991) and Tanksley et al. (1992)

revealed significant inaccuracies in the previous classical map. In the case of chromosome 6, this was achieved in a similar way to the work presented here, but using crosses with a *L. esculentum* line in which most of chromosome 6 had been substituted by the *L. pennellii* homoeologue, instead of *L. esculentum* × *L. pennellii* crosses. Progeny from crosses between this line and *L. esculentum* lines carrying chromosome 6 classical markers were analysed with chromosome 6 RFLP markers. However, the scoring of classical markers was more clearcut because the segregation of *L. pennellii* characteristics was limited to *L. pennellii* genes on chromosome 6. If this approach had been used for the present study, it may well have obviated the need to backcross individuals to establish their genotype as was required for the populations segregating for *imb* and *bs*. Eshed et al. (1992) have produced *L. esculentum* lines carrying small *L. pennellii* introgressions, including some with suitable introgressions of chromosome 1 which could have been used for this purpose.

It must not be assumed that the marker orders in the maps presented here are completely accurate, especially with regard to the isozyme markers. They are, however, a better representation of the positions of classical markers on the short arm of chromosome 1 than was previously available. It is likely that future work will lead to the integration of the molecular and classical maps over most of the tomato genome. This should facilitate the positional cloning of many important and interesting genes.

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References

Balint-Kurti PJ, Dixon MS, Jones DA, Norcott KA, Jones JDG (1994) RFLP linkage analysis of the *Cf-4* and *Cf-9* genes for resistance to *Cladosporium fulvum* in tomato. Theor Appl Genet 88:691–700

Chetelat RT, de Verna JW (1991) Expression of unilateral incompatibility in pollen of *Lycopersicon pennellii* is determined by major loci on chromosomes 1, 6 and 10. Theor Appl Genet 82:704–712

Eshed Y, Abu-Abied M, Saranga Y, Zamir D (1992) *Lycopersicon esculentum* lines containing small overlapping introgressions from *L. pennellii*. Theor Appl Genet 83:1027–1024

Fisher RA, Yates F (1963) Statistical tables for biological, medical and agricultural research, Oliver and Boyd, Edinburgh

Griffing B, Langridge J (1963) Factors affecting crossing over in tomato. Aust J Biol Sci 16:826–837

Jones DA, Dickinson MJ, Balint-Kurti PJ, Dixon MS, Jones JDG (1993) Two complex resistance loci revealed in tomato by classical and RFLP mapping of the *Cf-2*, *Cf-4*, *Cf-5* and *Cf-9* genes for resistance to *Cladosporium fulvum*. Mol Plant-Microbe Interact 6:348–357

Kanwar JS, Kerr EA, Harney PM (1980) Linkage of the *Cf-1* to *Cf-11* genes for resistance to tomato leaf mould, *Cladosporium fulvum* Cke. Rep Tomato Genet Coop 30:20–21

Kerr EA, Bailey DL (1964) Resistance to *Cladosporium fulvum* Cke obtained from wild species of tomato. Can J Bot 42: 1541–1554

Koornneef M, Bosma TDG, Hanhart CJ, van der Veen JH, Zeevaart JAD (1990) The isolation and characterisation of gibberellin-deficient mutants in tomato. Theor Appl Genet 80:852–857

Koornneef M, Bade J, Hanhart C, Horsman K, Schel J, Soppe W, Verkerk R, Zabel P (1993) Characterisation and mapping of a gene controlling shoot regeneration in tomato. Plant J 3: 131–141

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Langford AN (1937) The parasitism of *Cladosporium fulvum* Cooke and the genetics of resistance to it. Can J Res 15:108–128

Lapitan NLV, Ganal MW, Tanksley SD (1991) Organization of the 5S ribosomal RNA genes in the genome of tomato. Genome 34:509–514

MacArthur JW (1934) Linkage groups in the tomato. J Genet 29: 123–133

Mather K (1951) The measurement of linkage in heredity. Methuen, London

Maxon Smith JW, Ritchie DB (1983) A collection of near-isogenic lines of tomato: research tool of the future? Plant Mol Biol Rep 11:41–45

Rick CM (1964) Inheritance and linkage relations of *Lapageria* (*Lpg*) Rep Tomato Genet Coop 14:24–25

Rick CM (1969) Controlled introgression of chromosomes of *Solanum pennellii* into *Lycopersicon esculentum*: segregation and recombination. Genetics 62:753–768

Rick CM (1990) TGSC Stocklists. Rep Tomato Genet Coop 40: 44–64

Stam P (1993) Construction of integrated genetic maps by means of a new computer package: JoinMap. Plant J 3:739–744

Tanksley SD, Rick CM (1980) Isozymic gene linkage map of the tomato: applications in genetics and breeding. Theor Appl Genet 57:161–170

Tanksley SD, Ganal MW, Prince JP, de Vincente MC, Bonierbale MW, Broun P, Fulton TM, Giovanonni JJ, Grandillo GB, Martin, GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Vicente MC de, Tanksley SD (1991) Genome-wide reduction in recombination of backcross progeny derived from male versus female gametes in an interspecific cross in tomato. Theor Appl Genet 83:173–178

Weide R, van Wordragen MF, Klein Lankhorst R, Verkerk R, Hanhart C, Liharska T, Pap E, Stam P, Zabel P, Koornneef M (1993) Integration of the classical and molecular linkage maps of tomato chromosome 6. Genetics 135:1175–1186

Wit PJGM de, Spikman G (1982) Evidence for the occurrence of race and cultivar-specific elicitors of necrosis in intercellular fluids of compatible interactions of *Cladosporium fulvum* and tomato. Physiol Plant Pathol 21:1–11