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Re-orientation and integration of the classical and interspecific linkage maps of the long arm of tomato chromosome 7

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Abstract To obtain reliable classical and integrated interspecies maps of the long arm of chromosome 7 of tomato, detailed mapping work was undertaken and several phenotypic and molecular markers were assigned loci on both maps to provide reliable cross-reference points. To maximise the value of the new maps, pair-wise segregation data for classical genetic markers from the literature were included, based on large segregating populations with readily scorable phenotypes. In addition, to increase confidence in these maps, introgression lines were used to confirm important map locations. The revised classical map is based on two- and three-point test-cross data from a number of F_2 and BC_1 mapping populations. The integrated interspecies map is based on F_2 mapping populations derived from crosses of *Lycopersicon esculentum* with *Lycopersicon pennellii* (LA716). The genetic analyses for both maps were performed using the computer package JoinMap. The revised composite classical map indicates that some of the map positions reported in the literature are incorrect. The linear order of the classical markers common to both the revised classical and integrated interspecies maps are in complete agreement. Production of the integrated interspecies map resulted in re-orientation of the existing molecular map.

Keywords Tomato · Chromosome 7 · Integrated genetic maps

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Introduction

Map-based cloning techniques, such as chromosome walking and targeted transposon mutagenesis, depend on reliable mapping data. For example, the *Cladosporium fulvum* resistance gene *Cf-9* of tomato was cloned by transposon mutagenesis and plasmid rescue (Jones et al. 1994) following a reassessment of the linkage distances on tomato chromosome 1 (van der Beek et al. 1992; Jones et al. 1993; Balint-Kurti et al. 1995). This demonstrated that several phenotypic markers had originally been wrongly mapped. Clearly, this is not a unique problem since other reports have revealed similar errors (Koornneef et al. 1993; Weide et al. 1993). Strong epistatic effects limit the number of phenotypic markers that can be combined into a single segregating population (Tanksley 1983). Therefore, the tomato classical map (Mutschler et al. 1987) is a composite of linkage distances derived from many separate sets of segregation data. This almost inevitably means that there will be some significant errors in the resultant map. The combination of RFLP (Tanksley 1983) CAPS (e.g. Bonnema et al. 1997) RAPD (e.g. Williams et al. 1990), AFLP (Vos et al. 1995) and microsatellite (Vosman and Arens 1997) technologies has generated large numbers of molecular markers for mapping. In principle, only one segregating population is required to generate a genetic linkage map composed of these markers (Tanksley 1983).

Computer programs that employ complex algorithms to calculate maximum-likelihood estimates of recombination frequency, such as MapMaker (Lander et al. 1987) and JoinMap, (Stam 1993), eliminate many of the errors that can be incorporated into classical linkage maps. JoinMap is an especially useful package since it is

capable of integrating different types of linkage data sets which have common markers (Hauge et al. 1993) and makes use of recombination percentages near to 50% in finding the most-likely marker order in a multi-locus map (Weide et al. 1993).

In tomato, it has not been possible to generate detailed molecular maps based on different ecotypes of *Lycopersicon esculentum* (van der Beek et al. 1992) as has been the case with Arabidopsis (Hauge et al. 1993). This has necessitated the use of different *Lycopersicon* species to introduce molecular genetic variation into mapping populations. The disadvantage of this approach is that classical and interspecies maps are not readily comparable due to the differing frequencies of expansion and contraction of recombination events that exist between them. For example, on the classical map, the chromosome-1 markers *au* and *Cf-9* are separated by 14 cM (Jones et al. 1993), whereas on an interspecies map the distance between the two markers is 3 cM (Balint-Kurti et al. 1995).

The problem of the lack of natural cultivar-specific molecular-marker alleles within tomato can be partially resolved by the use of transgenic tomato lines carrying T-DNA or heterologous transposon constructs. Assaying marker gene expression and using techniques such as inverse PCR allows these constructs to be mapped onto both classical and molecular maps (Thomas et al. 1994; Burbidge et al. 1995). Similarly, if a gene responsible for a mutant phenotype is cloned, its locus can be determined for both map types (e.g. Burbidge et al. 1999). The ability to generate interspecies populations that segregate for phenotypic markers, as well as for molecular markers, has allowed the development of integrated maps where the linkage distances and linear order of specific phenotypic loci can be compared directly with those for molecular markers (e.g. Hauge et al. 1993; Abenes et al. 1994). For example, using JoinMap (Stam 1993), Weide et al. (1993) were able to generate classical, molecular and integrated maps for tomato chromosome 6 in which the linear order of markers completely agreed.

As part of map-based cloning programmes to isolate the *notabilis*$^+$ (*not*$^+$) gene (Burbidge et al. 1997, 1999), the *lateral suppressor*$^+$ (*ls*$^+$) gene (Schumacher et al. 1995, 1999), the *flacca*$^+$ (*flc*$^+$) gene [which co-maps with *ls*$^+$, Taylor and Rossall (1982)] and the *I3* gene (Bournival et al. 1989) located on the long arm of chromosome 7 of tomato, attempts were made to integrate the *not* and *ls* mutant loci with a framework of molecular markers in an interspecies map. Mapping data that we have published previously (Burbidge et al. 1995; Schumacher et al. 1995, 1999) suggested that the classical and molecular linkage maps of the long arm of chromosome 7 (Tanksley et al. 1992) were incorrectly orientated.

In this paper we report new data which has been combined with published data to obtain an improved version of part of the classical map of chromosome 7. We have used morphological markers, together with T-DNA and transposon insertions, to provide new contact points between the classical and interspecific linkage maps of chromosome 7. These data-sets were processed using the JoinMap computer package (Stam 1993) to generate a revised classical map and an integrated interspecies map that have a number of common, co-linear markers. Finally, to increase confidence in these maps, introgression lines were used to confirm the map locations of specific phenotypic and molecular markers (Eshed and Zamir 1994). The maps show that the previously published (Tanksley et al. 1992) orientation of the classical and molecular maps is incorrect for this specific region.

Materials and methods

Plant production

Plants were grown in heated glasshouses where the minimum day and night temperatures were 25°C and 18°C respectively. Daylight was supplemented where necessary to give a 16-h day and 8-h night regime. Seedlings were germinated and grown in F2 Levington compost (Levington Horticulture Ltd., Ipswich, UK) and more mature plants transplanted into M2 Levington compost.

Assay for the presence of T-DNA

Mapping populations harbouring T-DNA constructs SLJ591 (Jones et al. 1992; Thomas et al. 1994) or PKU304 (Knapp et al. 1994) with the NPTII gene, were sprayed on 3 consecutive days with an aqueous solution of kanamycin (400 mgl^{-1}) according to the method of Weide et al. (1989). Susceptible plants developed chlorotic lesions 5–7 days after the kanamycin treatment.

Determination of phenotypes

The mutant phenotypes *ignava* (*ig*), *Lanceolate* (*La*), *notabilis* (*not*), and *rotundifolia* (*rot*) (Darby et al. 1977); *acroxantha* (*acr*) (Stubbe 1964); and *lateral suppressor* (*ls*); (Brown 1955), were all scored visually.

The generation of intraspecific test-cross populations

(1) SLJ1481J/D9/*not*/*ls*. The *Ds* element of transformant line SLJ1481J (Thomas et al. 1994, kindly provided by J. Jones, Sainsbury Laboratory, Norwich, UK) was trans-activated by crossing to a source of stabilised *Ac* (s*Ac*), (see below). The genomic tomato DNA flanking a stable transposed *Ds* element (D9) generated from this line was isolated by inverse PCR (see below) and RFLP-mapped to a position on chromosome 7. This D9 event was stabilised by selfing. Homozygous D9-carrying lines were selected following progeny testing. Fortuitously, these plants retained the T-DNA as shown by selection with kanamycin. One of these homozygous D9 plants was crossed to the *L. esculentum not not, ls ls* double mutant to produce an F_1 which was backcrossed to the homozygous double mutant to generate the multi-point test-cross seed stock.
(2) SLJ1481J/*not*/D4/*ls*. The genomic DNA flanking a *Ds* element trans-activated from line D9 by the use of a source of s*Ac* (see below) was isolated by inverse PCR and RFLP-mapped onto chromosome 7. This (D4) event was stabilised by selfing. The presence or absence of s*Ac* and/or D4 were determined by PCR assay (see below). A plant made homozygous for D4 in the absence of s*Ac* was crossed to the *L. esculentum not not, ls,ls* double mutant to produce an F_1 which was backcrossed to the homozygous double mutant to generate the multi-point test-cross seed stock.
(3) ET85/*not*/*ls*. The transgenic line ET85 (Knapp et al. 1994) was crossed to the *L. esculentum not not, ls,ls* double mutant. A kana-

mycin resistant plant from this cross was backcrossed to the homozygous double mutant to produce the BC_1 seed stock.
(4) T160/*ls*. Primary transformant line T160 was generated by transformation of a heterozygous triple mutant (*L. esculentum au +, pl+, ls+*) explant (Burbidge et al. 1995) with the T-DNA::*Ac* construct SLJ591 (Jones et al. 1992; kindly provided by J. Jones, Sainsbury Laboratory, Norwich, UK). This line's single T-DNA was found to have integrated on chromosome 7 linked to the *Ls* locus. This plant was backcrossed to homozygous *L. esculentum lsls* to produce a BC_1 seed stock.
(5) T160/acr. A kanamycin-resistant individual of an F_1 between the T160 line and *L. esculentum acr acr* was backcrossed to the homozygous *acr* mutant to produce a BC_1 seed stock.
(6) T160/ig. A kanamycin-resistant individual of an F_1 between T160 and *L. esculentum ig ig* was backcrossed to the homozygous *ig* mutant to produce a BC_1 seed stock.
(7) T160/*La*/*ls*. The T160 line [see (4) above] was selfed and an *ls* homozygote progeny plant retaining the T-DNA (determined by the kanamycin assay) selected. This plant was crossed with *L. esculentum La+* and a kanamycin-resistant progeny plant exhibiting the heterozygous *La* phenotype was backcrossed to *L. esculentum ls ls* to generate a three-point test-cross seed stock.
(8) T160/*La*/*ig*. The T160 transformant line was crossed with *L. esculentum* (*ig ig*) and a kanamycin-resistant individual backcrossed to *L. esculentum* (*ig ig*). A recombinant individual that was kanamycin-resistant and homozygous for *ig* was crossed with *L. esculentum La+*. A kanamycin-resistant plant exhibiting the heterozygous *La* phenotype was selected from the progeny and crossed to *L. esculentum* (*ig ig*) to generate a three-point test-cross seed stock.
(9) T160/*not*/*ls*. A recombinant plant retaining the T160 T-DNA and homozygous for *ls* [see (7) above] was crossed to *L. esculentum* (*not not*). A kanamycin-resistant individual was backcrossed to the *L. esculentum* (*not not, ls ls*) double mutant to generate a three-point test-cross seed stock.
(10) *ig*/*rot*/T-DNA. The T160 line was crossed to *L. esculentum* (*rot rot, ig ig*) and a kanamycin-resistant progeny plant backcrossed to *L. esculentum* (*rot rot, ig ig*) to generate a three-point test-cross seed stock.
(11) *ig*/*rot*. A plant heterozygous for *rot* and *ig* in coupling phase was backcrossed to the homozygous double mutant (*L. esculentum rot rot, ig ig*) to generate a BC_1 seed stock.

Production of recombinant plants (Rec 1 and Rec 2) from introgression line IL7–3

In an attempt to fine-map around the *not* locus to facilitate the cloning of *not* (Burbidge et al. 1999), novel recombination events were sought from plants homozygous for the *L. pennellii* (LA716) introgression IL7–3 (Eshed and Zamir 1994). These plants were crossed to *L. esculentum* (*not not*) to produce an F_1 and then backcrossed to *L. esculentum* (*not not*) to yield a BC_1 population. RFLP analyses were performed on a population of 173 BC_1 plants segregating for the *not* phenotype to identify recombination events. The *not* mutant was known from preliminary mapping research to lie between RFLP markers TG572 and TG216. Southern analysis was performed on the 173 plants with TG572 and TG216 which yielded RFLPs with the restriction enzymes *Hin*dIII and *Xba*I respectively. The plants were further analysed using the molecular markers TG639 (Tanksley et al. 1992) and AGPase (Ballvora et al. 1995), which map between TG572 and TG216 and which yield CAPS after digestion of their PCR products with *Dde*I and *Dra*I respectively. Confirmation of the CAPS analysis on putative recombinant plants was obtained by Southern analysis of *Hin*dIII-digested genomic DNA using TG639 as an RFLP marker. Two plants (Rec1 and Rec2) were identified that had novel recombination events and thus retained smaller regions of *L. pennellii*-introgressed DNA. These lines were preserved by selfing and selecting homozygous progeny plants.

Generation of F_2 mapping populations

(1) Segregating for *not* and *ls*. *L. esculentum* (*not not, ls ls*) was crossed with *L. pennellii* (LA716) to produce an F_1 generation, siblings of which were crossed to generate a segregating F_2 interspecies mapping population "A." Fifty plants expressing either or both mutant phenotypes were selected for molecular-mapping experiments.
(2) Segregating for *ls*. *L. esculentum* (*ls ls*) was crossed with *L. pennellii* (LA716) and individuals of the resulting F_1 population selfed to produce the F_2 mapping population "B" (Schumacher et al. 1995). The complete population consisting of 529 plants was analysed for the segregation of *ls* and four RFLP markers closely linked to the *ls* locus. In addition, a subset of 93 of the F_2 plants segregating for *ls* were analysed with another five RFLP markers and 12 AFLP markers. All F_2 plants of population B were selfed to generate F_3 lines that were eventually used to confirm the F_2 phenotypic analyses for *ls*.
(3) Segregation for *I-3*. A number of breeding lines containing *I-3*, a resistance gene against *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) race 3 derived from *L. pennellii* (LA716), were provided by a breeding company. An F_2 population (F_2-*I-3*) was obtained by selfing a hybrid breeding line, heterozygous for the *I-3* gene. A *Fol* race-3 resistance test was performed on 210 F_2 plants as described by Huang and Lindhout (1996). A subset of 74 F_2 plants was used for genetic analysis using AFLP and RFLP markers.

Molecular methods

Total tomato DNA was extracted for PCR reactions using the method of Thomas et al. (1994) modified by Burbidge et al. (1995). Total tomato DNA was extracted for Southern analysis using a method modified from a procedure developed by the Tanksley laboratory (Cornell University, Ithaca, N.Y.) as described in Burbidge et al. (1995). Southern analyses were performed as described in Burbidge et al. (1995) with the exceptions that probes were labelled using a Rediprime kit according to the manufacturer's instructions (Amersham) and unincorporated nucleotides were removed from the labelling mix using 'Nick' columns according to the manufacturer's instructions (Pharmacia). PCR and inverse-PCR (IPCR) amplifications were performed using either a Stratagene Robocycler (35 cycles of 99°C for 40 s, 55°C for 60 s, 72°C for 75 s) or a Techne PHC-3 (35 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 75 s). PCR reactions were assembled as described in Thomas et al., (1994) but using 1U of *Taq* DNA polymerase (Promega) in a reaction volume of 25 µl.

Generation of novel *Ds* transposition events

Novel *Ds* transposition events were generated by crossing T-DNA::*Ds* line SLJ1481J (with the *Ds* element on chromosome 7; Thomas et al., 1994) to the stabilised *Ac* (s*Ac*) line SLJ 10512. Stable transposed *Ds* elements were generated by backcrossing to *L. esculentum* cv Ailsa craig and selecting against s*Ac*. PCR was used to assay for the *Ds* excision from the donor site, the presence of a transposed *Ds* and the absence of the s*Ac* element using primer pairs: C13 (5′ ATCTCCACTGACGTAAGGGATGACG 3′) and C16 (5′ GGATGTCGGCCGGGCGTCGTTCT 3′), SPEC3 REV (5′ GATCCTGTTCAGGAACCGGATCAAAGAG 3′) and SPEC5 (5′ CCACACAGTGATATTGATTTGCTG 3′), Ac1 (5′ CTGACC-ACTATGCTTCAAAAGGGG 3′) and B42 (5′ TATGATGTCTCA-ACTAGATGGAATT 3′) respectively. One plant, D9, was selected which contained a transposed *Ds* element but did not retain the s*Ac* element. The presence of only one *Ds* element in line D9 was demonstrated by Southern analysis of *Hin*dIII-digested genomic D9 DNA hybridised with a *Ds*-specific probe. This probe was generated by PCR-amplification of part of the *Ds* element in plasmid SLJ1481 (Thomas et al. 1994, kindly provided by J. Jones, Sainsburg Laboratory, Norwich, UK) using primers SPEC5 and SPEC3REV. The absence of the s*Ac* element in line D9 was confirmed by Southern analysis of *Hin*dIII-digested DNA of line D9

probed with the central 1.6-kb *Hin*dIII fragment of *Ac*. An IPCR product was amplified from DNA flanking the 3′ end of the *Ds* element using the method described in Burbidge et al. (1995). Primers B39 (5′ TTTCGTTTCCGTCCCGCAAGTTAAATA 3′) and SPEC2 (5′ GACATTGATCTGGCTATCTTGCTG 3′) were used for the primary IPCR reaction, and primers D71 (5′ CCGTTACCGACCGTTTTCATCCCTA 3′) and SPEC3 (5′ CTCTTTGATCCGGTTCCTGAACAGGATC 3′) for the nested IPCR reaction.

Transposition events D1 to D8 were generated from line D9 by transactivating the *Ds* element with the s*Ac* source (SLJ10512). Novel *Ds* transposition events were stabilised by backcrossing to *L. esculentum* cv Ailsa craig and selecting against s*Ac*. PCR was used to assay for *Ds* excision, the presence of transposed *Ds* elements and the absence of the s*Ac* element using the primer pairs C13/C16, SPEC3 REV/SPEC5 and Ac1/B42 respectively. Southern analyses similar to those used for line D9 were used to demonstrate that the line D4 contained only one *Ds* element and did not retain the s*Ac* element. IPCR products were amplified from DNA flanking the 3′ end of *Ds* elements D1, D2, D3, D4, D6, D7 and D8 using the same procedure and primers used for the amplification of *Ds* flanking DNA in line D9. The D5 probe was generated by IPCR from the 5′ end of the *Ds* element using primers B34 (5′ ACGGTCGGTACGGGATTTTCCCAT 3′) and B35 (5′ TATCGTATAACCGATTTTGTTAGTTTTATC 3′) for the primary IPCR reaction, and D73 (5′ TTCCCATCCTACTTTCATCCCTG 3′) and E4 (5′ GGTAAACGGAAACGGAAACGGTAG 3′) for the nested IPCR reaction. IPCR fragments flanking D2 and D3 were generated from the same plant. IPCR fragments flanking D1 and D5 were both generated from another single plant.

RFLP, CAPS and AFLP analyses

The DNA probes TG166, TG210, TG149, TG217, TG128, TG572, TG639, TG216, CT54, TG199, TG438, TG218, CD30, CD65, TG499, CT114 and CD61 were kindly provided by S. Tanksley, Cornell University, Ithaca, N.Y., USA). The DNA marker AGPase was kindly provided by C. Gebhardt (MPI, Cologne, Germany). Where the RFLP-generating enzyme was unknown, RFLPs were generated as described in Burbidge et al. (1995). CAPS markers were amplified by PCR using primers specific to the markers TG639 and AGPase [TG639for: 5′ CAAATGGTATTAGAGAGAAGAGCC 3′, TG639rev: 5′ CTCATATACCTAAGTTGTCTTTTAGCCTCTAC 3′; AGPasefor: 5′ CCAATCTACACCCAACCTC 3′ and AGPaserev: 5′ AAGTATCCATCTGTTTCCCTAG 3′ (Ballvora et al. 1995)]. Amplification products were digested for 1 h, at the optimal temperature for restriction enzyme activity, with the CAPS-generating restriction enzyme in PCR buffer whose components were adjusted, following PCR reactions, to match as closely as possible the recommended restriction enzyme buffer. Digestion products were separated in 1% agarose (Flowgen) gels in 1× TBE buffer (Sambrook et al. 1989). AFLP markers were generated and analysed as described in Haanstra et al. (1999). The AFLP marker names consisted of the primer combination used, followed by the estimated size of the amplification product and an “e” for an *L. esculentum*-specific and a “p” for an *L. pennellii*-specific amplification product. Markers that were in common with the “Haanstra map” of chromosome 7 (Haanstra et al. 1999) were designated with an extra “7” at the end of the marker name.

Generation of the intraspecies map using the computer package JoinMap

The pairwise recombination data between pairwise markers of 25 mapping populations are presented in Tables 1 to 3. These data were used to generate an integrated map by using all pairwise recombination values of marker pairs with a recombination frequency of less than 0.45 and a fixed-order file that was deduced from the individual maps.

Generation of the integrated interspecies map using the computer package JoinMap

Two interspecies F_2 populations of *L.* esculentum×L. *pennellii* LA716 were used to generate an interspecific map comprising 18 RFLP, 13 AFLP, 9 *Ds* and the two morphological markers (*ls* and *not*). Firstly, two separate maps were generated by using all pairwise recombination values of marker pairs with a recombination frequency of less than 0.45. The *ls* phenotypic marker and the RFLP markers TG166, TG199, TG210 and TG499 bridged the two populations. The other markers were unique to one population; all “D” markers were unique to population “A” and all AFLP markers to population “B.” These unique markers did not give an added value to the integrated map. To generate an integrated map comprising 42 markers the software package JoinMap does not always select the most-likely map orders. Therefore, the marker orders of the separate maps was used to deduce a fixed-order sequence of markers at a distance of about 5 cM. This fixed order was helpful in generating an integrated map that showed a good fit with the dataset.

Results

The intra-species map

Reassessment of the classical linkage map

Multi-point test-cross populations were generated involving SLJ1481J T-DNA/transposed *Ds* D9/*not*/*ls* and also SLJ1481J T-DNA/transposed *Ds* D4/*not*/*ls*. A three-point test cross analysis was performed using the marker combination ET85/*not*/*ls* and two-point test crosses involving T160 T-DNA/*ls*, T160 T-DNA/*acr* and T160 T-DNA/*ig* were also carried out (Table 1). The multi-point and traditional three-point test crosses allowed the linear order of the markers to be established. Pair-wise recombination frequencies were calculated initially as simple percentage recombinations. Recombination percentages were converted to map distances using the Kosambi formula. A χ^2 analysis was performed by bulking recombinant individuals into one class and non-recombinant individuals in a second class. In this way any skewing of the data in preference for one marker type was eliminated. The null hypothesis was that there was no linkage between each pair of markers analysed, i.e. no significant variation from a 1:1 distribution of classes. All combinations of markers tested showed significant differences between the observed data and that expected on the basis of 1:1 ratios. The only exception was SLJ1481J T-DNA/D4 which gave a map distance of around 50 cM, showing that the two markers gave the appearance of being unlinked.

The data-sets summarised in Table 1, together with previously published raw data for markers believed to be on chromosome 7 (Table 2), were subjected to analysis using the JoinMap package. This analysis resulted in the revised classical map shown in Fig. 1D.

Table 1 Summary of three- and two-point test cross data used by the computer package JoinMap to generate an intra-specific map for the long arm of chromosome 7 of tomato. Cross no. identifies the intra-specific test-cross population used (see Materials and methods). * Indicates significant divergence from a random segregation pattern according to χ^2 analysis ($P<0.05$)

Cross no.	Marker pair	Phase[a]	Recombination (%)	Map distance (cM)[b]	χ^2 (1:1)	Pop[n] size
1	SLJ1481 J/*not*	C3	33.5	40.5	16.0*	182
2	SLJ1481 J/*not*	C3	38.0	49.8	11.0*	192
1	SLJ1481 J/D9	C3	14.8	15.3	90.0*	182
2	SLJ1481 J/D4	C3	42.7	63.5	2.5	192
1	D9/*not*	C3	22.0	23.6	57.2*	182
1	D9/*ls*	C3	35.5	44.4	10.1*	183
3	ET85/*not*	C3	20.5	21.8	70.8*	190
3	ET85/*ls*	C3	35.8	45.0	12.1*	190
5	T160/*acr*	CT	4.5	4.5	291.9*	353
6	T160/*ig*	CT	25.6	28.2	61.5*	258
4	T160/*ls*	RT	31.0	36.3	49.4*	342
2	*not*/D4	C3	8.3	8.4	134.3*	193
2	not/ls	C3	14.0	14.4	100.1*	193
1	*not*/*ls*	C3	19.1	20.1	69.8*	183
3	*not*/*ls*	C3	17.4	18.2	80.9*	190
2	D4/*ls*	C3	5.7	5.7	151.5*	193

[a] CT, RT: result of a two-point testcross in coupling and repulsion phase respectively, C3: results of a three-point testcross with markers in coupling phase
[b] Map distances (cM) calculated using the Kosambi mapping function

Table 2 Summary of previously reported and new F_2 mapping data used in construction of the JoinMap generated intra-specific map. The mapping distances listed were calculated for each individual population using JoinMap

Markers	Linkage (cM)	Population size	LOD	Reference
not-lg5	21.9	928	19.6	Boynton and Rick (1965)
not-lg5	20.1	164	3.8	Boynton and Rick (1965)
not-lg5	21.2	541	8.6	Boynton and Rick (1965)
not-lg5	25.9	236	1.2	Boynton and Rick (1965)
lg5-flc	40.1	376	1.4	Reeves et al. (1966)
lg5-flc	48.7	182	0.1	Reeves et al. (1966)
not-pl	0.0	434	10.2	Rick et al. (1973)
not-La	9.1	156	18.5	Boynton and Rick (1965)
not-La	7.0	324	50.5	Boynton and Rick (1965)
La-deb	7.6	172	24.0	This paper
La-ls	7.2	178	26.6	Taylor (1981)
La-flc	11.1	492	53.6	Boynton and Rick (1965)
La-flc	9.8	100	11.7	Taylor (1981)
flc-ls	0.0	549	17.3	Taylor and Rossall (1982)
flc-ls	0.0	299	10.1	Taylor and Rossall (1982)
flc-ls	0.0	621	23.5	Taylor and Rossall (1982)

Independent confirmation of the marker order of the reassessed classical map

Three separate three-point test-cross populations were generated [see Materials and methods 'Crosses for the generation of intraspecific test-cross populations' (7), (8), (9) and Table 3] as an independent check of the linear order of selected markers given by JoinMap analysis of the data in Tables 1 and 2. The advantage of three-point test crosses is that they allow the order of markers to be determined reliably because they show double recombination events. This class of events is the least frequent and indicates which of the three markers under investigation is flanked by the other two markers. The linkage distances (cM) of these markers are shown in Table 3. This test gives clear confirmation of the linear order T160 T-DNA/*not*/*La*/*ig*/*ls* as given in the revised classical map (Fig. 1D).

Table 3 Summary of three-point test-cross data used as independent checks for the JoinMap-generated intra-specific map. Cross no. identifies the intraspecific test-cross population used (see Materials and methods). * Indicates significant divergence from a random segregation pattern according to $\chi 2$ analysis ($P<0.05$)

Cross no.	Markers	Method[a]	Recombination (%)	Map distance[b] (cM)	$\chi 2$ (1:1)	Pop[n] size
7	*ls*-*La*	C3	13.5	13.8	194.4*	364
7	*La*-T160	R3	21.4	22.9	108.8*	364
7	*ls*-T160	R3	30.5	35.4	33.2*	364
8	*La*-T160	R3	24.1	26.3	67.8*	253
8	*La*-*ig*	C3	5.1	5.1	203.7*	253
8	T160-*ig*	R3	29.2	33.6	43.6*	253
9	T160-*not*	C3	16.4	17.0	179.6*	397
9	*not*-*ls*	R3	18.4	19.3	158.6*	397
9	T160-*ls*	R3	31.2	36.6	36.9*	397

[a] C3, R3: results of a three-point testcross with markers in coupling and repulsion phase, respectively
[b] Map distances (cM) calculated using the Kosambi mapping function

Table 4 Interspecies F_2 mapping populations derived from a cross between *L. pennellii* (LA716) and *L. esculentum not not, ls ls* (population "A") and between *L. pennellii* (LA716) and *L. esculentum ls ls* (population "B"). The *not*, *ls* population consisted of a selected population of 50 plants homozygous for *not* and/or *ls*. The *ls* population 'B' consisted of 529 plants. A subset of 93 plants from population "B" was used to map the AFLP markers and further RFLP markers

a		b		c	
notabilis, lateral suppressor (population "A")		*lateral suppressor* (population "B")		*lateral suppressor* (subset of 93 plants from population B)	
Marker	Map distance (cM)	Marker	Map distance (cM)	Marker	Map distance (cM)
TG166, TG210	0.0	CT30	0.0	E35M48–170p7	0.0
SLJ1481 J	5.4	CD65	8.7	E35M48–174e7, E39M50–194e,	1.0
TG217	11.1	*ls*	9.5	E39M50–359p, TG166, TG210	
D7, D9	15.4	CD61	10.2	E32M50–293p7	2.1
D8, TG149	16.5	TG499	11.3	E39M50–292e, E35M48–113e7	2.6
TG572	23.1			E32M50–142e7	4.4
not	25.2			E32M50–119e7	6.9
TG216	30.6			E35M48–129p7	16.9
D4	38.4			E32M50–106p7	17.9
TG438	39.0			ET202	18.8
D1, D6	40.2			TG128	20.4
TG199	41.1			E39M50–315p	21.0
TG218	41.9			E39M50–283e7	24.9
D2	43.0			TG199	36.6
D3	44.5			CT30	53.7
D5	45.3			CD65	58.8
ls, TG499	62.9			*ls*	59.3
				CD61	59.3
				TG499	60.0
				CT114	60.8

Linkage of T160 T-DNA to rot *and* ig

A two-point test-cross population (cross 11) of 178 plants segregating for *ig* and *rot* gave a recombination frequency of 0.48 which, according to χ^2 ($P<0.05$) analysis, did not indicate significant divergence from a random segregation pattern. This was confirmed by a three-point test-cross population (cross 10) of 118 plants, generated to test for linkage between T-160 T-DNA, *ig* and *rot*. The recombination frequency between *ig* and *rot* was >0.50 thus showing no evidence of linkage between these two markers. However, linkage was detected between T160 T-DNA and *ig* giving a map distance of 34 cM, a distance that fits well with the re-assessed classical map position for *ig* generated with data from a separate mapping population (Table 3, Fig. 1D). The lack of linkage between *rot* and either T-160 or *ig* was a clear indication that errors existed in the published classical map, and the marker *rot* does not therefore form part of our revised map of this region of chromosome 7.

The inter-species map

Development of the partially integrated interspecies map

From F_2 mapping population "A", resulting from a cross between *L. esculentum* (*not not, ls ls*) *and L. pennellii* (LA716), 50 plants that exhibited either the *not* and/or the *ls* phenotype were chosen for molecular mapping. A selected population was used to enrich for the most-informative plants in terms of identifying recombination events. RFLP analyses were carried out on DNA from the 50 plants using markers TG166, TG210, TG149, TG217, TG128, TG572, TG639, TG216, TG199, TG438, TG218, TG499 and a number of inverse PCR (IPCR) fragments generated from DNA flanking *Ds* elements, D1 to D9, transposed from T-DNA SLJ1481J (Thomas et al. 1994). These data were subjected to analysis by JoinMap (Stam 1993) to generate a map that integrated the phenotypic loci *not* and *ls* with the RFLP markers (Table 4a). A χ^2 value of 0.00 indicates a perfect map. In general, maps generated by JoinMap with a χ^2 value <1.00 are taken to have map distances with errors of $<\pm 5$ cM. Hence, a χ^2 value of 0.41showed that this integrated map was reliable. The molecular-map position of SLJ1481J is approximate since no inverse PCR product flanking this T-DNA insert was available. However, the position of this T-DNA was determined indirectly on the basis of five co-mapping IPCR fragments flanking *Ds* elements that had transposed to sites closely linked to this T-DNA. In the same F_2 mapping population as used by Thomas et al. (1994), these *Ds* elements were shown to have mapped (data not shown) to the same interval (between RFLP markers TG149 and TG166) as the SLJ1481J T-DNA (Thomas et al. 1994). The map position of one of these IPCR fragments on the *not, ls* F_2 interspecies map was used to indicate the position of the SLJ1481J T-DNA (Table 4a).

An F_2 population, totalling 527 individuals, resulting from a cross between *L. esculentum* (*ls ls*) and *L. pen-*

nellii LA716, was used to fine-map around the *ls* locus (Schumacher et al. 1995) using markers CT30, CD65, CD61, TG499 and CT114 (Table 4b). Finally, as part of a programme to generate new molecular markers for the molecular map, a number of AFLP-generated probes and RFLP markers (Table 4c) were mapped using a subset of 93 plants segregating for *ls* from population "B." The data-sets generated from these mapping experiments were integrated with the *not*/*ls* interspecies mapping data using a framework of common markers to generate a more-comprehensive map (Fig. 1C). The T-DNA marker ET85 was originally mapped (Knapp et al. 1994) between RFLP markers TG572 and TG183 on the Tanksley F_2 interspecies mapping population of 67 plants (Tanksley et al. 1992) using an inverse PCR fragment that flanked the T-DNA. The marker TG183 is tightly linked to AFLP marker E32M50–106p7 (data not shown) and the marker TG572 is common to both the Tanksley map and the integrated interspecies map. This information allowed the approximate position of ET85 to be established on the integrated interspecies map (Fig. 1C).

The F_2-*I-3* population was used to fine map around the *I-3* locus. A resistance test was performed on 210 F_2 plants showing a monogenic inheritance (data not shown). The length of the introgressed *L. pennellii* region around *I-3* was determined and spanned the RFLP markers CT226, TG128 and TG170, and the two AFLP markers E32M50–106p7 and E39M50–315p. All five molecular markers cosegregated completely in the subset of 74 F_2 plants and were 5 cM apart from the *I-3* gene (three recombinants). Since these RFLP markers were also placed on the molecular map of chromosome 7 (Tanksley et al. 1992, Fig. 1B) this, together with the AFLP markers on the interspecies map (Fig. 1C), confined *I3* to a region of 6 cM between TG183 and TG572.

Mapping the T-DNA of line T160

The inverse PCR fragment generated from DNA flanking the T-DNA insertion site of line T160 could not be mapped onto the molecular map precisely because the probe hybridised only to *L. esculentum* DNA. Using the Tanksley F_2 interspecies mapping population of 67 plants (Tanksley et al. 1992), together with the computer package MapMaker (Lander et al. 1987), an approximate position was calculated (Burbidge et al. 1995). However, it was clear that T160 T-DNA mapped between TG149 and TG572 (data not shown). The T160 inverse PCR fragment was subsequently used as an RFLP probe of tomato lines IL7–1, IL7–2, Il7–3, IL7–4 and IL7–5 in which *L. pennellii* chromosome-7 DNA had been introgressed into *L.esculentum* (Eshed and Zamir 1994). Southern analysis showed an absence of any hybridising band for IL7–2, IL7–3 and IL7–4, but a single specific band for IL7–1 and IL7–5 (data not shown). This result showed that the T160 T-DNA was contained within the *L. pennellii* introgression of IL7–3 and confirmed the earlier data which showed that the T-160 T-DNA was located between RFLP markers TG149 and TG572 (Fig. 1C).

Generating novel recombination events on IL7–3

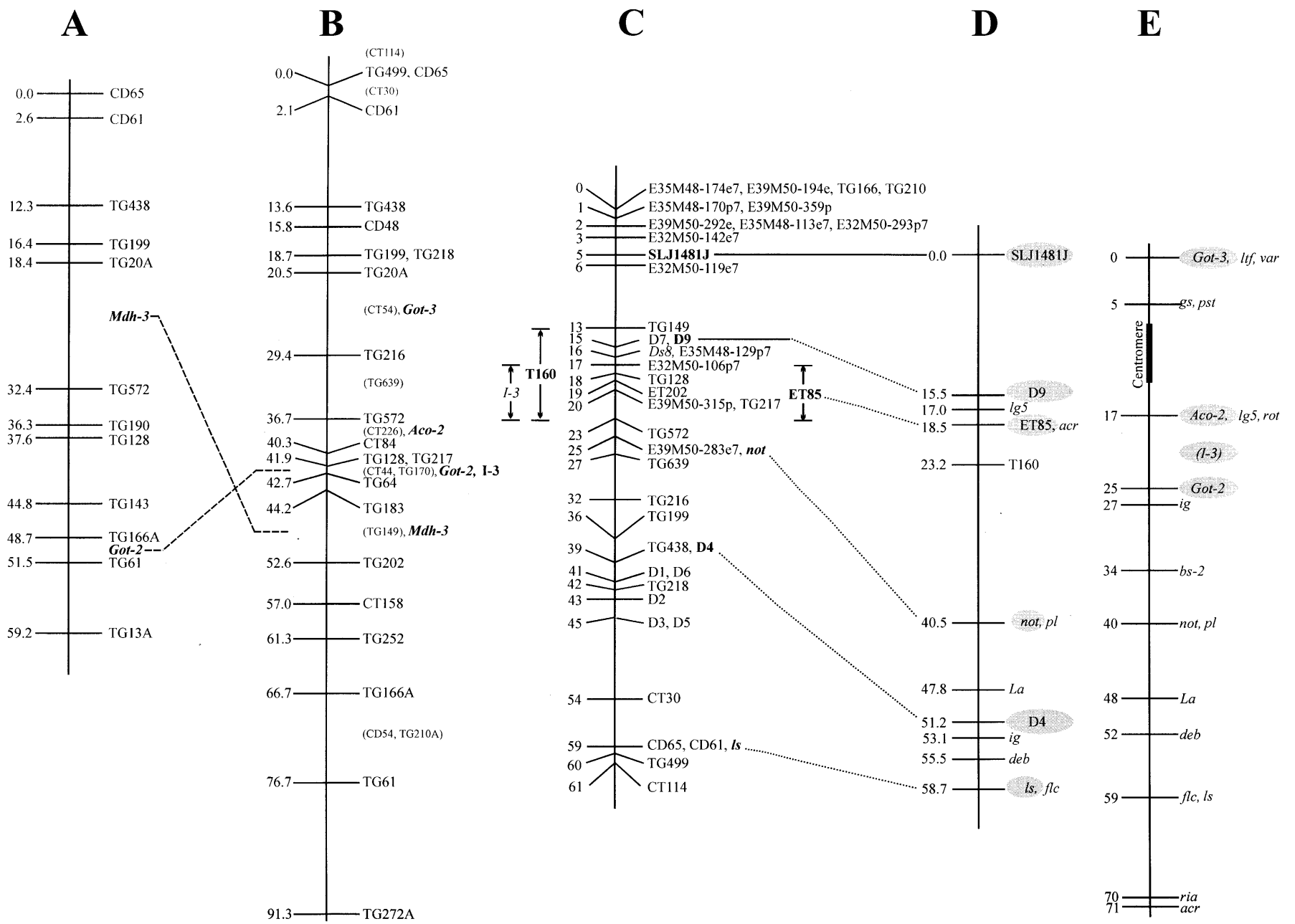
To ensure that the IL7–1 and IL7–3 seed stocks provided by the Tomato Genetics Resource Center (TGRC, Davis, California, USA) had not been outcrossed or otherwise contaminated, RFLP analyses were performed using a number of markers (Fig. 2B) to confirm the results of Eshed and Zamir (1994). This analysis showed that the seed stocks were homozygous for the *L. pennellii* introgressions and confirmed that the region believed from preliminary studies (data not shown) to contain the *not* locus (between TG216 and TG572) lay within the *L. pennellii* introgression of IL7–3.

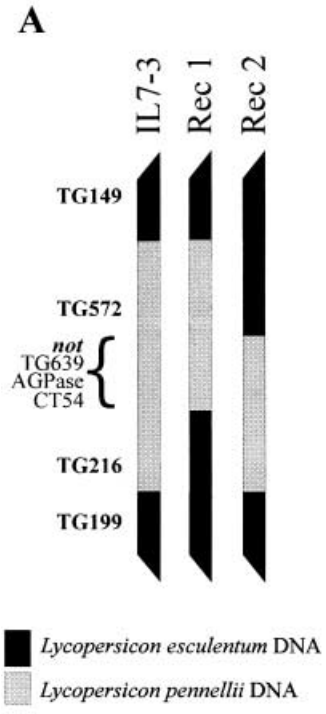
The introgression line IL7–3 was crossed to the wilty tomato mutant *not* and the resulting heterozygous F_1 was backcrossed to *L. esculentum* (*not not*) homozygotes to form a population of 173 BC_1 plants. Two BC_1 individuals, named Rec 1 and Rec 2, were identified that both had a wild-type phenotype but had either an *L. esculentum* allele for TG216 (Rec 1) or an *L. esculentum* allele for TG572 (Rec 2). This indicated that new recombination events had occurred near to the *not* locus. CAPS analysis with TG639 and AGPase, plus additional RFLP analysis with TG639, showed that these markers gave *L. pennellii* alleles in IL7–3, Rec1 and Rec2 indicating that these two markers were very close to the *not* locus. A diagrammatic representation summarising the outcome of this analysis is shown in Fig. 2A. Following isolation of the n*ot*$^+$ cDNA, now known as 9-*cis*-epoxycarotenoid-dioxygenase 1 (NCED1, Burbidge et al. 1999), the n*ot*$^+$ cDNA was used as a probe in a further RFLP analysis of recombinant plants Rec1 and Rec2. This analysis confirmed that the *not* locus was bounded by the recombination events in Rec1 and Rec2 (Fig. 2C).

Orientation of the molecular and classical maps

In order to align the revised integrated interspecies map with the existing molecular map (Tanksley et al. 1992), it was necessary to reverse the orientation that has been used previously (Tanksley et al. 1992). Once this was done, the linear order of tomato chromosome-7 molecular markers, common to the two maps, agreed well. Since the revised classical and integrated interspecies maps (Fig. 1C, D) are completely aligned, we suspect that the molecular map (Tanksley et al. 1992) was previously mis-orientated with the classical map on the basis of the limited number of common markers (see Discussion).

Several mapping experiments using a combination of multi-point test crosses, introgressed lines and molecular mapping, in conjunction with statistical analyses using JoinMap, has resulted in a series of classical, molecular and integrated maps that show no conflicting marker orders. The approach used has minimised any errors and resulted in a resource that may assist any future map-based cloning strategies on the long arm of chromosome 7 of tomato.


A
0.0 CD65
2.6 CD61
12.3 TG438
16.4 TG199
18.4 TG20A
Mdh-3
32.4 TG572
36.3 TG190
37.6 TG128
44.8 TG143
48.7 TG166A
Got-2
51.5 TG61
59.2 TG13A
B
(CT114)
0.0 TG499, CD65
(CT30)
2.1 CD61
13.6 TG438
15.8 CD48
18.7 TG199, TG218
20.5 TG20A
(CT54), Got-3
29.4 TG216
(TG639)
36.7 TG572
(CT226), Aco-2
40.3 CT84
41.9 TG128, TG217
(CT44, TG170), Got-2, I-3
42.7 TG64
44.2 TG183
(TG149), Mdh-3
52.6 TG202
57.0 CT158
61.3 TG252
66.7 TG166A
(CD54, TG210A)
76.7 TG61
91.3 TG272A
C
0 E35M48-174e7, E39M50-194e, TG166, TG210
1 E35M48-170p7, E39M50-359p
2 E39M50-292e, E35M48-113e7, E32M50-293p7
3 E32M50-142e7
5 SLJ1481J
6 E32M50-119e7
13 TG149
15 D7, D9
16 Ds8, E35M48-129p7
17 E32M50-106p7
18 TG128
19 ET202
20 E39M50-315p, TG217
I-3
T160
ET85
23 TG572
25 E39M50-283e7, not
27 TG639
32 TG216
36 TG199
39 TG438, D4
41 D1, D6
42 TG218
43 D2
45 D3, D5
54 CT30
59 CD65, CD61, ls
60 TG499
61 CT114
D
0.0 SLJ1481J
15.5 D9
17.0 lg5
18.5 ET85, acr
23.2 T160
40.5 not, pl
47.8 La
51.2 D4
53.1 ig
55.5 deb
58.7 ls, flc
E
0 Got-3, ltf, var
5 gs, pst
Centromere
17 Aco-2, lg5, rot
(I-3)
25 Got-2
27 ig
34 bs-2
40 not, pl
48 La
52 deb
59 flc, ls
70 ria
71 acr



B

RFLP marker	Restriction enzyme	Hybridising DNA species	
		IL7-1	IL7-3
TG210	*Xba*I	E	E
TG166	*Kpn*I	E	E
TG149	*Kpn*I	E	E
TG217	*Eco*RI	E	P
TG572	*Hin*dIII	E	P
TG639	*Hin*dIII	E	P
CT54	*Dra*I	E	P
TG216	*Xba*I	E	P
TG199	*Hin*dIII	E	E
TG438	*Dra*I	P	E
CD65	*Hin*cII	P	E
CD61	*Dde*I	P	E
TG499	*Eco*RI	P	E

E; *L. esculentum* allele, P; *L. pennellii* allele

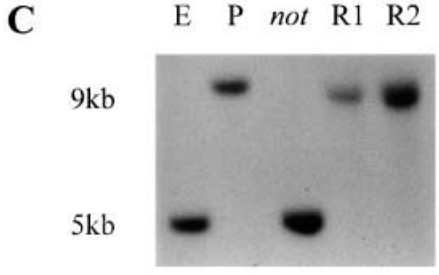


Fig. 2A–C Characterisation of introgression lines Il7–1, IL7–3 (Eshed and Zamir 1994), Rec1 and Rec2 using RFLP markers. **A** Generation of novel recombination break-points from introgression lines IL7–3. Two recombinant plants, Rec1 and Rec2, were isolated following molecular analysis of 173 (IL7–3/*not*) BC_1 plants. The four markers; TG639, AGPase, CT54 and *not* were shown to map into the small, common region of *L. pennellii* DNA in Rec1 and Rec2. **B** Summary of data obtained from RFLP analysis to confirm the recombination break points in introgression lines IL7–1 and IL7–3. **C** An RFLP for the NCED1 gene between *L. esculentum* and *L. pennellii* (LA716) genomic DNAs. *E*; *L. esculentum* genomic DNA, *P*; *L. pennellii* genomic DNA, *not*; *L. esculentum* (*not not*) genomic DNA, R1, R2; homozygous recombinant plants Rec1 and Rec2 respectively, generated from IL7–3. Both recombinant plants gave only the *L. pennellii* version of the RFLP, confirming that the NCED1 gene (*not* locus) was located between RFLP markers TG216 and TG572

Discussion

In this paper we report the integration of six marker loci from the classical map into the inter-species (molecular map). The revised classical map and the integrated map do not contain all of the known phenotypic and isozyme markers on the long arm of chromosome 7. However, even though relatively small mapping populations were used with a limited number of cross-reference markers between the two maps, the marker co-linearity is preserved and a number of independent tests confirmed the positions of selected markers. The fact that some of the dotted lines, highlighting the cross-reference points in Fig. 1C and D, are far from parallel, demonstrates differing recombination frequencies between markers in these regions of the classical and integrated interspecies maps. This highlights the problems of attempting to cross-reference directly between classical and interspecies maps and shows the value of an integrated map.

One of the strengths of this paper is that several complementary mapping strategies were used. The classical marker order generated by JoinMap was confirmed by data from independent three-point test-cross experiments. In addition, introgressed lines were used to generate novel recombination break-points to facilitate the mapping of *NCED1* (*not* locus) and confirm the order of markers in this region of chromosome 7 (Fig. 2A and B).

Of the classical markers used in this study, the positions previously reported for *lg5*, *not*, *pl*, *La*, *deb*, *ls* and *flc*, appear to be correct. The step-wise construction of the classical map linking markers on the basis of two-point test-cross data (e.g. Quiros et al. 1974) may, as these authors implied, have led to erroneous marker positions. Limitations to such published data are one reason why errors have been included in the composite classical map of Mutschler et al. (1987). Several errors on the chromosome-7 classical map have now been identified and corrected; *acr* was repositioned from the distal end

◀ **Fig. 1A–E** Genetic maps of tomato and potato chromosome 7. **A** The molecular map of potato chromosome 7 (redrawn from Tanksley et al. 1992). **B** The molecular map of tomato chromosome 7 (redrawn from Tanksley et al. 1992). **C** Interspecies map derived using data from *L. esculentum*/*L. pennellii* (LA716) *not*/*ls* F_2 mapping population "A" of 50 selected plants and *L. esculentum*/*L. pennellii* (LA716) *ls* F_2 population "B." Distances are in cM (using the Kosambi mapping function). Markers common to the interspecies map and revised classical map are emboldened. The regions into which the T160 T-DNA and *I3* marker mapped are shown to the left of the map. The approximate position of ET85 (Knapp et al. 1995), based on segregation data from the Tanksley population of 67 F_2 plants (Tanksley et al. 1992) is shown to the right of the map. **D** The revised classical map of the long arm of chromosome 7. Distances shown are cM (using the Kosambi mapping function). Markers common to the interspecies map and revised classical map are *highlighted*. **E** The classical map of tomato chromosome 7 (redrawn from Mutchler et al. 1987). Lines of cross-reference between the maps are shown by *dotted lines*. Distances shown are cM for maps **A**–**D** and percentage recombination for map E

of chromosome-7 long arm (Fig. 1E) to a position close to ET85 on the revised classical map (Fig. 1D) and *ig* was re-positioned to a more-distal position between *La* and D4 on the revised classical map. The absence of any linkage between *rot* and T160 T-DNA and between *ig* and *rot* suggests that *rot* is unlikely to be located on the long arm of chromosome 7.

The linear, but re-orientated, order of molecular markers between the Tanksley et al. (1992) map and the integrated interspecies map presented here is largely preserved, and highlights the value of being able to position many markers using one population. Only in a few cases is there any discrepancy. Where such discrepancies do occur, it is for markers assigned in the Tanksley et al. (1992) map to intervals rather than discrete loci (e.g. CT54, CT30) or where two markers are tightly linked (e.g. CD61, CD65). These order changes are likely to be due to instances where, statistically, several marker orders are equally likely due to an insufficient number of recombination events in the mapping population.

The orientation of the Tanksley et al. (1992) molecular map to the classical map conflicted with the data presented for the integrated interspecies map. The strength of the new map is that it integrates morphological markers with molecular markers, whereas the Tanksley et al. (1992) chromosome-7 molecular map does not. The integrated interspecies map provides direct cross-reference points to the classical map that are well-spaced along the length of the map. Once the Tanklsey et al. (1992) map is inverted, markers that it has in common with the integrated interspecies map cross-reference well. It is important to understand why there is a conflict between the two maps.

It appears that the Tanksley et al. (1992) molecular map of chromosome 7 was cross-referenced with the classical map (Mutschler et al. 1987) using only isozyme markers. Interestingly, none of the isozyme markers are assigned to exact loci on the Tanksley et al. (1992) molecular map, but merely to intervals where they presumably showed linkage with RFLP markers. In addition, those isozyme markers used to cross-reference the molecular and classical maps are not well-spaced along the maps; *Aco-2*, and *Got-2* cluster close to the centromere (Fig. 1B and E); only the marker *Got-3* is well-separated from the cluster. The poor spacing of these isozyme markers and their imprecise loci assignments do not make them ideal cross-reference markers.

Tanksley et al. (1992) not only reported high-density molecular linkage maps for tomato but also for potato. They noted that "the genetic content of potato chromosomes is nearly identical to that of tomato." The only differences were paracentric inversions involving entire arms of chromosomes 5, 9, 10, 11 and 12. Thus the linear order of molecular and isozyme markers between tomato and potato should be consistent for each of the other chromosomes. This is largely true, for example, on chromosome 1 where *Idh-1* maps close to TG301, while *Skdh-1* maps relatively close to TG125, and on chromosome 8, where *Got-4* maps close to TG72 in both potato and tomato. This consistency is lost on chromosome 7 where the order of the isozyme markers *Mdh-3* and *Got2* is reversed on the tomato molecular map compared with the potato molecular map and where the linkage distance between them is very different for the two maps (Fig. 1A and B). The isozyme marker *Mdh-3* maps between RFLP markers TG20 A and TG572 in potato but in a very different place in tomato; between TG183 and TG202. In addition, the isozyme marker *Got-2* maps between TG166A and TG61 in potato but between TG128 and TG64 in tomato (Fig. 1A and B). So, either very small intrachromosomal re-arrangements have occurred involving just the isozyme markers in potato compared with tomato, or the positioning of these isozyme markers in one or both species is incorrect.

The isozyme markers *Got-2* and *Got-3* were mapped in relation to *var* and *not* (Tanksley and Rick 1980). These authors reported that *Got-2* was 25 cM from *Got-3* and that *not* was linked to both *Got-2* (21 cM) and *Got-3* (27 cM); distances that do not wholly agree with the classical map (Mutschler et al. 1987) or what is now known about the position of *not* within the integrated interspecies map (Burbidge et al. 1999 and this report). The marker *I3*, which gives resistance to *Fusarium oxysporum* Race 3, was mapped in relation to *Got-2* (Bournival et al. 1989). However, these authors reported that "it was difficult to classify some BC_1 individuals for their *I3* genotype", suggesting that their map position for *I3* was only approximate. The combination of scoring errors in both the classical (Mutschler et al. 1987) and the isozyme components of the molecular (Tanksley et al. 1992) maps have undoubtedly led to incorrect positions for some markers and have resulted in the mis-orientation of the molecular and classical maps.

It is probable that one of the main reasons why there are so few integrated maps available is the research commitment required for their generation. This work represents a significant research effort by the laboratories involved. The revised maps are a significant improvement on what was previously available and provide a reliable basis for any further map-based cloning work undertaken on the long arm of chromosome 7 of tomato. However, information on the physical distances separating molecular markers either through the use of YAC (or BAC) contigs (e.g. Thorlby et al. 1997) or fluorescence in situ hybridisation (e.g. Zhong et al. 1999) would be advantageous in the further development of map-based cloning strategies.

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