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ORIGINAL PAPER

High resolution genetic and physical mapping of the *I-3* region of tomato chromosome *7* reveals almost continuous microsynteny with grape chromosome *12* but interspersed microsynteny with duplications on *Arabidopsis* chromosomes *1*, *2* and *3*

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Abstract The tomato *I-3* gene introgressed from the *Lycopersicon pennellii* accession LA716 confers resistance to race 3 of the fusarium wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. We have improved the high-resolution map of the *I-3* region of tomato chromosome *7* with the development and mapping of 31 new PCR-based markers. Recombinants recovered from *L. esculentum* cv. M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 mapping populations, together with recombinants recovered from a previous M82 × IL7-3 F2 mapping population, were used to position these markers. A significantly higher recombination frequency was observed in the (IL7-2 × IL7-4) × M82 TC1F2 mapping population based on a reconstituted *L. pennellii* chromosome *7* compared to the other two mapping populations based on smaller segments of *L. pennellii* chromosome *7*. A BAC contig consisting of *L. esculentum* cv. Heinz 1706 BACs covering the *I-3* region has also been established. The new high-resolution map places the *I-3* gene within a 0.38 cM interval between the molecular markers RGA332 and bP23/gPT with an estimated physical size of 50–60 kb. The *I-3* region was found to display almost continuous microsynteny with grape chromosome *12* but interspersed microsynteny with *Arabidopsis thaliana* chromosomes *1, 2* and *3*. An S-receptor-like kinase gene family present in the *I-3* region of tomato chromosome *7* was found to be present in the microsyntenous region of grape chromosome *12* but was absent altogether from the *A. thaliana* genome.

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Introduction

Tomato fusarium wilt is caused by the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). The interaction between tomato (*Lycopersicon esculentum* syn. *Solanum lycopersicum*) and *Fol* is race-cultivar specific and three host-specific races of *Fol* have been identified. Races 1 and 2 were discovered over 50 years ago (Alexander and Tucker 1945), while race 3 was first found in Australia in 1978 (Grattidge and O'Brien 1982). Race 3 eventually became widespread in the tomato-growing area of Bowen in Queensland resulting in great yield losses by the 1980s (McGrath et al. 1987). Race 3 was later reported in the United States and Mexico (Volin and Jones 1982; Davis et al. 1988; Chellemi and Dankers 1992; Valenzuela-Ureta et al. 1996). More recently, *Fol* race 3 appeared in Brazil posing a significant threat to tomato production in the absence of race 3 resistant cultivars adapted to Brazilian conditions (Reis et al. 2005).

Wild species of *Lycopersicon* provide a source of resistance to *Fol* and dominant resistance genes controlling each of the three races have been identified. The *I* gene (conferring resistance to race 1) is located on chromosome *11* and was introduced into cultivated *L. esculentum* from the wild relative, *L. pimpinellifolium* (syn. *S. pimpinellifolium*) (Bohn and Tucker 1939; Paddock 1950). The *I-2* gene (conferring resistance to race 2) was also introduced from *L. pimpinellifolium* and is also located on chromosome *11* (Stall and Walter 1965; Cirulli and Alexander 1966; Laterrot 1976; Sarfatti et al. 1989). The *I-3* gene (conferring resistance to race 3) was introgressed from *L. pennellii* (syn. *S. pennellii*) and is located on chromosome *7* (Bournival et al. 1989). The *I-2* gene has been isolated using a map-based cloning strategy and found to be a member of the coiled coil—nucleotide-binding site—leucine-rich repeat (CC-NBS-LRR) class of resistance genes (Ori et al. 1997; Simons et al. 1998).

Map-based cloning has been widely employed to isolate various tomato disease resistance genes including the *Pto* gene for bacterial speck resistance (Martin et al. 1993), the *Ve* gene for verticillium resistance (Kawchuk et al. 2001) and the *I-2* gene for fusarium wilt resistance (Simons et al. 1998). The advantage of map-based cloning is that no prior knowledge about the gene of interest is required apart from its chromosomal location. A high-resolution genetic linkage map is needed to identify markers tightly linked to the target gene. High-density molecular-marker maps are available for tomato (Tanksley et al. 1992; Haanstra et al. 1999; Fulton et al. 2002) and combining these maps with high throughput random PCR-based marker technologies allows a target region of the tomato genome to be saturated with markers. Recombination events in this region then allow identification of markers tightly linked to the target gene. A physical map can then be constructed by screening these tightly linked markers against a genomic DNA library to isolate a clone containing the target gene (Tanksley et al. 1995).

The *I-3* gene has been described in two *L. pennellii* accessions: PI414773 (McGrath et al. 1987) and LA716 (Scott and Jones 1989). The *I-3* gene from LA716 was found to be tightly linked (about 2.5 cM) to the *Got-2* isozyme locus on chromosome *7* (Bournival et al. 1989) and to lie between RFLP markers TG216 and TG183 (Tanksley and Costello 1991). Inoculation analysis of *L. pennellii* chromosome *7* introgression lines with *Fol* race 3 showed that IL7-2, IL7-3 and IL7-4 are completely resistant (Sela-Buurlage et al. 2001; Hemming et al. 2004), thereby confirming the location of *I-3* reported by Tanksley and Costello (1991).

We are pursuing the isolation of the *I-3* gene from the *L. pennellii* accession LA716 via map-based cloning. Isolation of this gene will enhance tomato-breeding programmes either as a marker for marker-assisted breeding or as a transgene for introduction into existing elite varieties. A recombination-based high-resolution genetic linkage map around the *I-3* region was constructed previously using 28 PCR-based SCAR, CAPS and RAF markers on an F2 population segregating for *Fol* race 3 resistance (688 F2 plants from a cross between the *L. esculentum* cv. M82 and the introgression line IL7-3; Hemming et al. 2004). In that work, the *I-3* gene was placed within a 0.3 cM region flanked by the markers CT226 and TG639. As a continuation of our map-based cloning effort to isolate the *I-3* gene, our current study included the identification and mapping of new molecular markers in the *I-3* region, the screening and recovery of recombinants from two new mapping populations, the mapping of *I-3* relative to the new markers, improving the high-resolution map of the *I-3* region and construction of a BAC contig covering the *I-3* region.

Materials and methods

Plant production and fungal inoculation

Seeds were sown in seed raising mix and grown in a temperature-controlled glasshouse with a maximum day temperature of 25°C and a minimum night temperature of 18°C. Four- to five-week old plants were transferred to steam-sterilised soil mix for further growth. Inoculations with *Fusarium oxysporum* f. sp. *lycopersici* race 3 were performed at Queensland Department of Primary Industries (QDPI) facilities in Bowen or Indooroopilly, Queensland, as described by Hemming et al. (2004).

Recombinant screening

Eight recombinants obtained by Hemming et al. (2004) from an *L. esculentum* cv. M82 × IL7-3 F2 mapping population were used in this study and 31 additional recombinants from the M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 mapping populations described by Lim et al. (2006) were obtained as a part of this study. The (IL7-2 × IL7-4) × M82 TC1F2 mapping population was generated by screening the progeny of the (IL7-2 × IL7-4) × M82 cross for plants carrying a reconstituted *L. pennellii* chromosome *7* arising by recombination between the introgressions in IL7-2 and IL7-4 (Lim et al. 2006). The F2 population was, therefore, obtained from a plant heterozygous for an intact *L. pennellii* chromosome *7* and an intact *L. esculentum* chromosome *7*. Two- to three-week old seedlings of the M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 populations were screened for recombinants using CAPS markers TG128 (Hemming et al. 2004) and T1651

(developed in this study) flanking *I-3*. Total plant DNA was extracted using the method of Edwards et al. (1991) as modified by Giraudat et al. (2003). Recombinant seedlings were retained, selfed and the F3 seeds harvested for fungal inoculation analysis. Total DNA from recombinant plants was extracted according to the method described by Hemming et al. (2004).

PCR analysis

PCR was performed using a PTC-200 Peltier Thermal Cycler (MJ Research Inc, Waltham, Massachusetts). For SCAR and CAPS marker analysis, the reaction was carried out in a volume of 10–20 µL containing 10–50 ng genomic DNA, 1× PCR buffer [10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.1 mM $MgCl_2$, 0.01% (w/v) gelatin; Sigma, St Louis, Missouri], 200 µM of each dNTP, 0.5 µM of each forward and reverse oligonucleotide primer and 0.05 U REDTaq DNA Polymerase (Sigma). The PCR conditions were as follows: an initial denaturation step at 94°C for 2 min, followed by 30–35 cycles of template denaturation at 92°C for 15 s, primer annealing at 50–55°C for 30 s and product extension at 72°C allowing 1 min for every 1 kb of product expected, followed by a final extension step at 72°C for 5 min. For CAPS analysis, restriction digestions were carried out according to the manufacturer's instructions. DNA was size fractionated by agarose gel electrophoresis using 1× TAE buffer [40 mM Tris-acetate, 1 mM EDTA pH 8.0] and 0.5–3.0% (w/v) agarose as required. For SSR markers, the PCR reaction was carried out in a volume of 10 µL containing 10–50 ng genomic DNA, 1× PCR buffer [10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.1 mM $MgCl_2$, 0.01% (w/v) gelatin; Sigma], 200 µM of each dNTP, 0.2 µM of each forward and reverse oligonucleotide primer and 0.05 U REDTaq DNA Polymerase (Sigma). The PCR conditions were as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of template denaturation at 94°C for 1 min, primer annealing at 45–55°C for 1 min and extension at 72°C for 1 min, followed by a final extension step at 72°C for 10 min. SSR marker PCR products were size fractionated by electrophoresis on denaturing polyacrylamide gels [containing 2.5 M of urea, 0.1% (v/v) ammonium persulfate, 6% (w/v) acrylamide (19:1 ratio of acrylamide to bis-acrylamide) and 0.04% (v/v) TEMED] in 1× TBE buffer [90 mM Tris-borate, 2 mM EDTA pH 8.0]. TBE (1×) buffer was used as running buffer. Bands were visualised by silver staining as follows: washing of gels in dH_2O for 1 min, gel staining in 0.1% (w/v) $AgNO_3$ solution for 10 min with gentle shaking, washing in dH_2O for 1 min and then transferred into 100 mL developer solution [containing 0.025 g sodium tetraborate decahydrate, 1.5 g sodium hydroxide and 0.4 mL formaldehyde] until bands were visible.

PCR product cloning

PCR products were cloned using a TA cloning kit (Invitrogen, Carlsbad, California) according to the supplier's instructions. REDTaq Polymerase (Sigma) amplified products were ligated directly into plasmid vector pCR2.1 (Invitrogen) and transformed into competent DH5α *E. coli* cells.

DNA sequencing and sequence analysis

Cloned PCR products were sequenced with M13 (-20) forward and M13 reverse primers using the ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and ABI 377 automatic DNA sequencers at the Australian Genome Research Facility (Brisbane, Australia). Windows 32 SEQMAN and EDITSEQ 5.08 (DNASTAR Lasergene, Madison, Wisconsin) were used to edit sequence chromatograms, manipulate sequence and to assemble contigs. Windows 32 MEGALIGN 5.08 (DNASTAR Lasergene) was used to align sequences and MAPDRAW 5.08 (DNASTAR Lasergene) was used to predict restriction enzyme polymorphic sites. In some cases, BLAST2 SEQUENCES (Tatusova and Madden 1999) was used to align sequences. Sequence homology searches were performed using the BLASTN algorithm (Altschul et al. 1990) on the SOL Genomics Network (SGN, http://www.sgn.cornell.edu/, Mueller et al. 2005) unigene sequence database to retrieve homologous tomato sequences. To predict the position of introns in the tomato unigene sequence, a TBLASTX (Altschul et al. 1990) search was performed against the SGN *Arabidopsis* finished BAC sequences database or by using the SGN Intron Finder for Solanaceae ESTs tool. Gene prediction on sequenced BACs was done using GENSCAN (http://genes.mit.edu/GENSCAN, Burge and Karlin 1997), FGENESH (http://www.softberry.com/berry.phtml) and GeneMark.hmm (http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi, Borodovsky and Lukashin unpublished data, Lomsadze et al. 2005) with the *Arabidopsis* setting. The predicted gene sequences were used in BLASTX (Altschul et al. 1990) searches of the NCBI (http://www.ncbi.nlm.nih.gov/) nonredundant protein sequence database to find homologous genes.

Establishment of a BAC contig covering the *I-3* region

A BAC contig was constructed using a bioinformatic approach to identify *L. esculentum* cv. Heinz 1706 BACs

covering the *I-3* region. Sequences of four subclones from the previously identified *L. pennellii* BAC clone N9-1 (Hemming et al. 2004) and sequences of markers developed in the *I-3* region were used in BLASTN searches of the SGN tomato BAC clone end sequence database. BACs with end sequences showing >90% nucleotide identity to the query sequences were chosen for further analysis. The BACs identified in this way have either their SP6 or T7 ends anchored to the query sequences. Anchoring of BAC clones to the *I-3* region was verified by converting their opposite insert end sequences into markers and mapping them using plants recombinant in the *I-3* region. BACs from the *Hin*dIII library of tomato cv. Heinz 1706 were obtained from the Clemson University Genomics Institute (CUGI, Clemson, South Carolina) and purified using the QIAprep Spin Miniprep Kit (Qiagen, Valencia, California). The authenticity of these BACs was confirmed by PCR analysis with markers from the *I-3* region.

Microsynteny analysis

Microsynteny with regions of the *Arabidopsis thaliana* genome was initially established by identification of likely *Arabidopsis* orthologues of tomato chromosome *7* marker genes using BLASTX searches of the *Arabidopsis* proteome available through SGN (http://www.sgn.cornell.edu/tools/blast/) and correlation of their chromosomal locations in *Arabidopsis*. Similarly, BLASTX searches of the poplar and grape genomes were conducted via the poplar and grape genome BLAST search engines http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Poptr1_1&advanced=1 and http://www.genoscope.cns.fr/cgi-bin/blast_server/projet_ML/blast.pl). Following an initial correlation of orthologue locations with specific regions of the poplar and grape genomes, the gene content of these regions was examined in detail, using the poplar and grape genome browsers http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html and http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) supplemented by TBLASTN searches using specific Arabidopsis protein sequences encoded by potentially microsyntenous genes that appeared to be missing from these regions. This analysis of microsynteny was extended as sequences became available for BACs that we anchored to the *I-3* region of tomato chromosome *7* and supplied for sequencing (see http://www.sgn.cornell.edu/gbrowse/index.pl?chr=7). The predicted coding and protein sequences for some of the genes carried on these BACs are shown in Supplementary Table 1. The similarity between microsyntenous genes was examined by reciprocal BLAST searches, the results of which are shown in Supplementary Tables 2, 3 and 4 and form the basis for statements made in the "Results" and "Discussion" about the presence of orthologous genes or gene families in the regions of microsynteny or their absence from specific genomes.

Results

Generation of 31 new PCR-based markers at the *I-3* locus

We have developed 31 new PCR-based markers that map near the *I-3* gene on tomato chromosome *7*. Sequences and non-PCR based markers that were predicted to map close to *I-3,* based on the current molecular marker map of tomato, on synteny with potato and *Arabidopsis* and on positional information available in the tomato literature, were selected for PCR-marker development. The primers designed to amplify these marker sequences from genomic DNA are shown in Tables 1 and 2.

PCR amplification was performed on the susceptible backcross parent *L. esculentum* cv. M82 and the resistant introgression line IL7-3. TM18, TM23, CP56-2, bC9-SP6 and gHS primers generated co-dominant SCAR markers with different sized PCR products amplified from M82 and IL7-3 (Table 1). bO16, bO9, bP23 and P47-48 primers generated dominant SCAR markers with a PCR product amplified from only one of the two genomic templates (Table 1). PCR products of the same size amplified from both the M82 and IL7-3 templates were cloned into the vector pCR2.1 (Invitrogen) and sequenced using M13 forward and reverse primers. Comparison of the aligned *L. esculentum* and *L. pennellii* sequences identified restriction site polymorphisms that allowed the generation of co-dominant CAPS markers. The TM9B, T1651, T1756, C2_At2g42810, TG572, GP300, CP51-c, CP56-1, Got-B, bC9-T7, bP16, bL7, bA9, bB6, SLG-1, gPT, Sy-At2g42760, Sy-At2g42770, Sy-At2g42780 and Sy-At3g58790 primers all generated co-dominant CAPS markers (Table 1). SSR557 and SSR565 primers produced dominant SSR markers where heterozygotes could not be distinguished from *L. esculentum* homozygotes (Table 2). The SSRD-1 primers produced a co-dominant SSR marker (Table 2). SSR557 and two additional CAPS markers, C2_At4g15420 and C2_At2g42750, were obtained from the SOL Genomics Network (SGN) database (http://www.sgn.cornell.com) bringing the total number of new markers used for mapping *I-3* to 34 (Tables 1 and 2).

High-resolution mapping of the *I-3* locus

The M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 mapping populations were screened with the flanking CAPS markers TG128 (Hemming et al. 2004) and T1651 (developed in this study). Plants with a recombinant chromosome were homozygous at one flanking marker locus

Table 1 Oligonucleotide primer sequences for SCAR and CAPS markers in the *I-3* region

Marker source and primer sequences (5′–3′)	Restriction enzyme	Product sizes (bp)	
		L. esculentum	*L. pennellii*
Tomato EST			
TM18[a]			
(F) TTCTTGGTGATTTCAACTCC	–	427	451
(R) ATTGTTATTGTAGGACTCGT			
TM23[a]			
(F) CTGAACCAGTAGGACAAT	–	2500	2900
(R) TATCTTATTATCTCAGGATTCTTCTTC			
TM9B[a]			
(F) GGGAGGTAGAGATTTGTTCAAGTAT	*Taq*I	1500, 1000	2500
(R) TATGGTTTTTGAAATGACTCCT			
Tomato COS Markers			
T1651[a]			
(F) ATTCAACCTTCAATACACCGC	*Dde*I	416	344, 72
(R) GCACCGACAGTAATGACCCAAGG			
T1756[a]			
(F) CGAGGTTGGGTGTAGATTGG	*Sau*96I	508, 383,	699, 383
(R) AAGTTTATTCAAGCCCACAGG		191	
Tomato COSII Markers			
C2_At2g42810[b]			
(F) AAGAGAAATGTTGCGAGCACTG	*Sml*I	1801	1350, 451
(R) AAATCACCATTGAACAGATACGG			
C2_At4g15420[c]			
(F) TCTAATTTAAAGATTGGACAGCAAGC	*Taq*I	380, 170	550
(R) TGGTGTATGTCCATATCTTTCAACATAAC			
C2_At2g42750[c]			
(F) TCCAGTGCAAAGGAGAGTTTATGATG	*Taq*I	500, 150	520, 130
(R) ACTCTAGCTCTTCCAAAGTCTTCCTC			
RFLP Marker			
TG572[d]			
(F) CTGTTGATTGGATTTCTGTGACC	*Hin*dIII	900, 365	1265
(R) GCTCCACTGAATCATCAACTC			
Potato Genomic Marker			
GP300[e]			
(F) GCTTGCTGATCTCGAGTCTGCAGCTGCT	*Hpa*II	204, 111	315
(R) GTCACACGAAGTGAGCCATGAATTGTCTC			
Potato cDNA Markers			
CP51-c[e]			
(F) GCTGCTGCAATTTCGTCCA	*Psh*AI	610, 75	685
(R) GAACTAACACATCCTCCCTCATAC			
CP56-1[e]			
(F) GGTTCAACCAGGAAGGGTGTAG	*Hin*fI	400, 220,	400, 190,
(R) CTCTCATCATCCAAATCTCAATAGG		190, 50	160, 60, 50
CP56-2[e]			
(F) TGATCAAATCTGGCTTAATCTTCTC	–	623	610
(R) TTACAAATTGGCAGCAACTCG			

Table 1 continued

Marker source and primer sequences (5′–3′)	Restriction enzyme	Product sizes (bp)	
		L. esculentum	*L. pennellii*
Isozyme Marker			
Got-B[f]			
(F) AGTGGCAGTGAAAAGTCAGTTG	*Hpa*II	440, 210	650
(R) CCAAGTAACCAACATTTCCAGTAG			
Tomato BAC insert end sequences			
bO16[g]			
(F) GTAACTTAGAAATGGTCAAAATGG	–	534	–
(R) CCAAGTTAATTTCACTATTTCGGA			
bO9[i]			
(F) GGGGAGTCAACTTGAATTATAC	–	486	–
(R) ATTAGCGCAGACCCAACGTA			
bP23[g]			
(F) GCCCTTATTTTTGGTTGGAC	–	481	–
(R) CGTTGATTTCTTGTGTTTATTTC			
bC9-SP6[j]			
(F) CTGAGGAACTGCTGAATGATGT	–	480	450
(R) TACTGGATACAATGATAAGGGTC			
bA9[h]			
(F) TCAGAATACCTACAGCCAAGAAC	*Nla*III	341, 144,	485, 91,
(R) CATTGAGAGTGTTGAATTGGGC		91, 32, 21	32, 21
bB6[h]			
(F) GCCCTACAGTCCTTTTTCCT	*Alu*I	404	214, 190
(R) CGTAAGCCAAAAGGTGTGC			
bC9-T7[j]			
(F) TTGGACTGAAGTTCGTCTGAT	*Bst*NI	502	441, 61
(R) GGAATCCAGTCTCTCTACCATCT			
bL7[g]			
(F) TCTGCTTTTTCAAGAATACGG	*Nla*III	239, 200,	270, 239,
(R) TGTGGAAGTAGAGATGATAGCAAC		70, 56, 21	56, 21
bP16[h]			
(F) GGACCAGAGAGTGTTGCTTTCG	*Hph*I	194, 43	237
(R) CCCGAAATAGGCATCAGCAAT			
***L. pennellii* BAC insert end sequences**			
P47-48[k]			
(F) GGATTTTGGTGCTGTATTTGAAG	–	–	469
(R) TAGCCTGATGTTCCTCTCATTGTTC			
SLG-1[k]			
(F) CGTTCATAATCTGCTAAGTTTCTA	*Rsa*I	296, 170,	466, 68,
(R) GGTTTCTATTACTCCAGCATCCTC		68, 55, 52	55, 52
Tomato gene sequences on BAC P049P16			
gHS[l]			
(F) GAAAACTTATGAACTGGTTGATGATT			
(R) ATTGATTCTCCGCAGACGACT	–	494	450
gPT[l]			
(F) CATTATTGTGATGAGACCGACC	*Hin*fI	426, 22,	340, 86,
(R) GGAGTCGTAAAGGAGGCTGTC		4	22, 4

Table 1 continued

Marker source and primer sequences (5′–3′)	Restriction enzyme	Product sizes (bp)	
		L. esculentum	*L. pennellii*
Orthologues of *Arabidopsis* genes			
Sy-At2g42760[m]			
(F) GGTAATCCCATTTCATCTTCTTG	*Hae*III	334, 317	334, 219,
(R) TTCCGAGAAATCACAATCCC			98
Sy-At2g42770[m]			
(F) CCAAATAGTGCTAGGTCCTTCTG	*Xmn*I	495, 67	562
(R) CTTTGGACGGTAGTTACTTGCT			
Sy-At2g42780[m]			
(F) TACTGAGACATAAAAGGCGTT	*Rsa*I	290, 110	400
(R) TCACTTGCTTCATTCGCTCA			
Sy-At3g58790[m]			
(F) CATCCCTCTTGAATCATCTGCG	*Aci*I	649, 356,	838, 356,
(R) TACCTTCTTCCCTCCAACTCAG		191, 142	135

[a] Original map position and sequence from the Solanaceae Genomics Network (SGN: http://www.sgn.cornell.com)

[b] Sequence obtained from NCBI accession AY182778 (de la Fuente van Bentem et al. 2003) and marker developed prior to the publication of C2_At2g42810 by SGN

[c] Original map position and primer sequence from SGN

[d] Original map position from SGN and sequence from Steven Tanksley (Cornell University)

[e] Original map position and sequence from the Potato Maps and More database (PoMaMo; http://gabi.rzpd.de/projects/Pomamo/; Meyer et al. 2005). *L. esculentum* homologues of potato sequences were retrieved by conducting BLASTN searches against the SGN tomato unigene database. The potato CP56 sequence retrieved two homologous tomato unigenes, which were used to develop markers CP56-1 and CP56-2

[f] Got-B was developed by Wang et al. (2007) from a BAC that contained the gene corresponding to the chromosome *7* isozyme marker Got-2 (Bournival et al. 1989). Original BAC end sequences from SGN and primer sequence from Wang et al. (2007)

[g] Derived from BACs identified in Arizona Genomics Institute BAC fingerprint contig 214 (http://www.genome.arizona.edu/fpc/tomato/). Original BAC end sequence from SGN

[h] Opposite ends of BACs with end sequences anchored to *L. pennellii* BAC N9-1. Original BAC end sequence from SGN

[i] T7 end sequence of BAC P076O09 which contains GP300 at its SP6 end. Original BAC end sequence from SGN

[j] End sequences of BAC P023C09 which contains C2_At2g42810. Original BAC end sequence from SGN

[k] Original map position and sequence from Hemming et al. (2004)

[l] Partial BAC P049P16 sequence from Mondher Bouzayen (ENSAT, Toulouse)

[m] Markers developed from tomato unigenes orthologous to syntenic *Arabidopsis* genes. Original unigene sequences from SGN. Markers given the prefix Sy- for synteny

Table 2 Oligonucleotide primer sequences for SSR markers in the *I-3* region

Marker	Primer sequences (5′– 3′)	Repeat sequence	Annealing temperature (°C)
SSR557[a, b]	(F) GCCACAAGAAACATTGCTGA (R) TACGCGCACGTGCATAAATA	$(CTAT)_6$	45
SSR565[b, c]	(F) AGAACTCGCCGGAGCAACAGCA (R) ATCATCCCCATCCCCATCACCAT	$(CAG)_7$	55
SSRD-1[d]	(F) ATTGAAGTGATCTTGTTTATGAATC (R) GACAAATTAGCTAAGAGTAGCTTCAC	$(TA)_5(AT)_8$, $(AT)_8$	45

[a] Original map position and primer sequence from the Solanaceae Genomics Network (SGN: http://www.sgn.cornell.com)

[b] Polymorphic band present in *L. esculentum* but absent from *L. pennellii*

[c] Original map position and sequence from SGN. Although primer sequences for SSR565 were available from SGN, new primer sequences were designed so as to amplify a smaller PCR product for clearer visualisation on polyacrylamide gels

[d] Original map position and sequence from Burbidge et al. (1999). Polymorphic bands present in both *L. esculentum* and *L. pennellii*. This SSR is adjacent to the *NCED1* gene, which is involved in ABA biosynthesis and corresponds to the chromosome *7* morphological marker *notabilis*

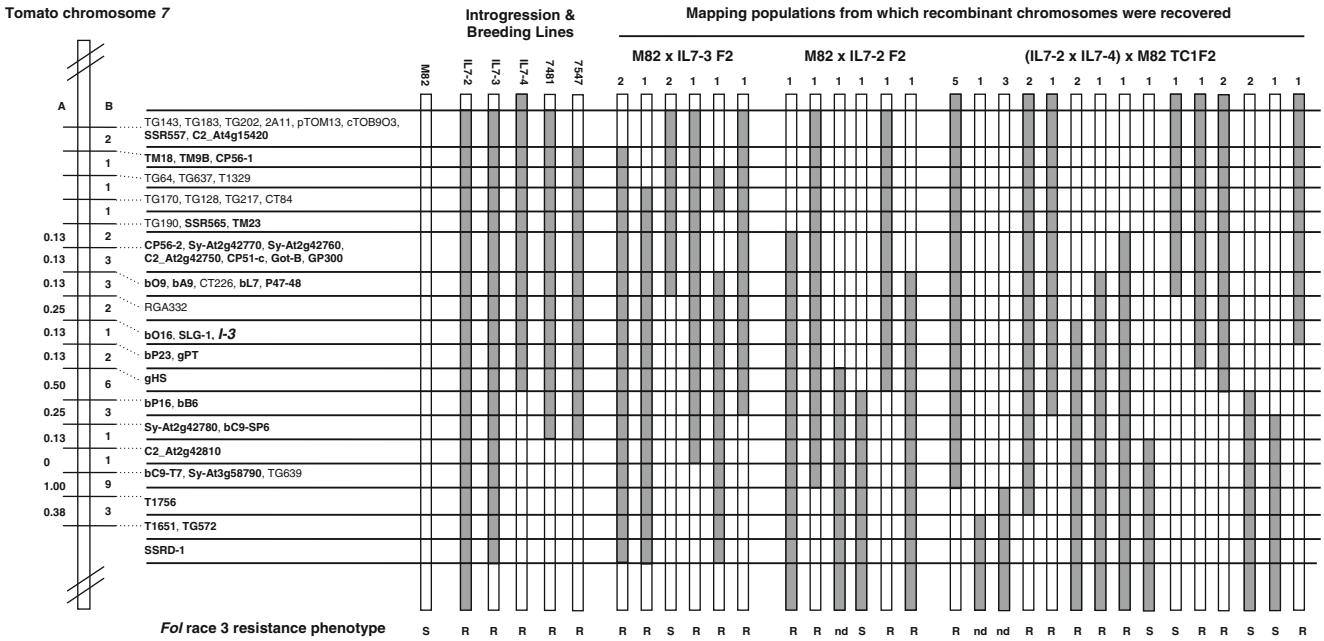


Fig. 1 An improved high-resolution genetic map of the *I-3* region of tomato chromosome *7* and diagrams of chromosome *7* showing the extent of introgressed *L. pennellii* DNA present in the IL7-2, IL7-3 and IL7-4 introgression lines, Fla. 7481 and Fla. 7547 breeding lines and recombinants recovered from the M82 × IL7-3 F2, M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 mapping populations. Introgressed segments of the *L. pennellii* genome are shaded grey. One M82 × IL7-3 F2 recombinant contains a confirmed triple recombination event. The number of recombinants recovered in each class is given above each recombinant chromosome. The first six classes of recombinant chromosome are from (Hemming et al. 2004). The 34 new markers used in this work are shown in *bold type*. Relative map positions were determined by analysis of recombinants recovered from all three mapping populations. Genetic distances determined by analysis of the recombinants recovered from the (IL7-2 × IL7-4) × M82 TC1F2 population are shown to the *left* of the map in cM (*A*). The total number of recombinants recovered for each genetic interval is given to the right of the map (*B*). The top of the map is proximal to the centromere. The *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) race 3 resistance phenotype conferred by recombinant chromosomes is indicated below each chromosome. *R* resistant, *S* susceptible, *nd* not determined

and heterozygous at the other. A total of 400 plants were screened from each of these mapping populations. Six plants with recombination events in the *I-3* region were recovered (recombination breakpoints between TG128 and T1651) from the M82 × IL7-2 F2 mapping population while 25 recombinant plants were recovered from the (IL7-2 × IL7-4) × M82 TC1F2 mapping population. All 31 recombinant plants were subsequently screened with both the current markers and the SCAR and CAPS markers developed previously by (Hemming et al. 2004), to localise the recombination breakpoints more precisely. Dominant SCAR markers were screened on marker-selected homozygous F3 recombinant plants (data not shown).

Most of these recombinants were informative in separating markers that were mapped to the same bin by Hemming et al. (2004) and hence further subdivided the *I-3* region. Based on data obtained for these recombinants and those recovered previously from the M82 × IL7-3 F2 population (Hemming et al. 2004), an improved genetic map of *I-3* was constructed (Fig. 1). Analysis of the resistance phenotypes of the F3 families obtained from recombinant F2 plants shows that the *I-3* gene is flanked by markers RGA332 and bP23/gPT within a genetic distance of 0.38 cM and co-segregates with markers bO16 and SLG-1.

Recombination frequencies between mapping populations

Recombinants from the M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 mapping populations were recovered using the markers TG128 and T1651 that flank the *I-3* region. Six and 25 recombinants were retrieved from the M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2 mapping populations, respectively. Eight recombinants were recovered from the M82 × IL7-3 F2 mapping population using the markers TG183 and TG639 (Hemming et al. 2004). In order to compare the recombination frequencies of the three mapping populations, only recombinant plants that had recombination breakpoints between the markers GP300 and C2_At2g42810 were used for calculation. The recombination frequency of the (IL7-2 × IL7-4) × M82 TC1F2 was approximately six times higher than the recombination frequencies in the M82 × IL7-3 F2 population and approximately three times higher than in the M82 × IL7-2 F2 population (Table 3).

Table 3 Comparison of recombination frequencies for a defined interval in the introgressed *I-3* region of tomato chromosome *7* for three different mapping populations

Mapping population	M82 × IL7-3 F2	M82 × IL7-2 F2	(IL7-2 × IL7-4) × M82 TC1F2
Number of plants screened	688	400	400
Number of recombinants recovered	8 (using TG183 and TG639)	6 (using TG128 and T1651)	25 (using TG128 and T1651)
Recombinants between GP300 and C2_At2g42810	4	4	13
Recombination frequency	1/172	1/100	1/31

These differences in recombination frequency are statistically significant (χ^2 with 2 degrees of freedom = 13.61, $P = 0.0011$).

Construction of a BAC contig covering the *I-3* region

Previously, we obtained BAC N9-1 carrying sequences from the *I-3* region in an *L. pennellii* cv. LA716 BiBAC library commercially available from Texas A&M University (Hemming et al. 2004). Subsequently, this library deteriorated and became unavailable, and instead new BAC resources became available as the tomato genome-sequencing project was initiated. Initially, an *L. esculentum* cv. Heinz 1706 *Hin*dIII BAC library, and later, *Eco*RI and *Mbo*I libraries, served as sources of sequencing templates for the tomato genome project. BAC end sequences were made available publicly and BACs from the *Hin*dIII library were made available commercially through the Clemson University Genomics Institute (CUGI). The availability of BAC end sequences prompted the idea to construct a BAC contig covering the *I-3* region using an in silico bioinformatics approach, which would speed up the identification of BAC clones compared to probing BAC library filters. Although *L. esculentum* BACs could not harbour *I-3*, they were seen as useful, first, in providing new sequences in the *I-3* region for further marker development and fine mapping of *I-3*, and second, as a possibe means of identifying resistance-gene-like sequences that might be alleles of *I-3* and provide a faster route to the identification of *I-3*.

Using sequences from four subclones of the BAC N9-1 (Hemming et al. 2004), 12 BACs (from the *L. esculentum* cv. Heinz 1706 *Hin*dIII, *Eco*RI and *Mbo*I libraries) were identified that had significant matches (>90% identity) in BLASTN searches of the SGN BAC end sequence database. Of these, P011O06, P166A09 and P054E13 were members of BAC fingerprint contig 214 generated by the Arizona Genomics Institute (http://www.genome.arizona. edu/fpc/tomato/). From this contig, four additional BACs were retrieved (three of which, P061L07, P137P23 and P060O16, are shown in Fig. 2) and used for marker development. A BLASTN search of the SGN BAC end sequence database with the GP300 marker sequence retrieved BAC P076O09 (>98% identity). Three BACs, P302I05, P023C09 and P214H12, identified by the overgo technique (see http://www.sgn.cornell.edu/maps/physical/overgo_process_explained.pl) using C2_At2g42810 sequence as the overgo probe (http://www.sgn.cornell.edu/search/markers/markerinfo.pl?marker_id=4003) were also retrieved for further analysis.

Nine markers (given the prefix 'b' for BAC end sequence) were developed from eight of these BAC end sequences and mapped to the *I-3* region (markers bO9, bO16, bA9, bL7, bP23, bP16, bC9-SP6, bC9-T7 and bB6). Seven BACs could not be mapped due to a lack of sequence polymorphism between corresponding sequences amplified and cloned from M82 and IL7-3. Based on this lack of polymorphism, we inferred that these BACs did not map to the *I-3* region. PCR analysis of nine BACs (BACs P166A09, P049P16, P061L07, P137P23, P133D01, P054E13, P011O06, P302I05 and P023C09) with markers in the *I-3* region allowed the position of the BACs relative to the genetic map to be determined as well as the extent of overlaps between BAC clones (Fig. 2). The BAC P166A09 (116.2 kb) extends to the left of BAC N9-1 while BAC P049P16 (102.6 kb) encompasses BAC N9-1 and has a ~40 kb overlapping region with BAC P166A09. PCR analysis with markers from the *I-3* region and *Hin*dIII restriction fingerprinting shows that BAC P302I05 encompasses BAC P023C09, lies distal to *I-3* and shows no overlap with either N9-1 or P049P16 (fingerprinting data not shown). The mapped positions of bC9-SP6 and bC9-T7 markers indicate that BAC P023C09 is oriented with the SP6 end closer to *I-3* (Fig. 2). BAC P076O09, which was retrieved by using the GP300 marker sequence, lies proximal to *I-3* and shows no overlap with the N9-1 BAC contig (Fig. 2). The marker bB6, developed from the BAC end sequence of the BAC clone SL_EcoRI026B06 (derived from the *Eco*RI library of tomato cv. Heinz 1706, which is not yet publicly available), mapped to the region between bP16 and Sy-At2g42780. The estimated physical size of this BAC was not known, but PCR analysis showed that the T7 end mapped between BACs P049P16 and P302I05. The BAC P054E13, which has the T7 end anchored to BAC N9-1, is estimated to be 91.5 kb but PCR analysis showed that the bL7 marker was present in BAC P054E13 suggesting that the SP6 end should map between CT226 and bL7. The SP6

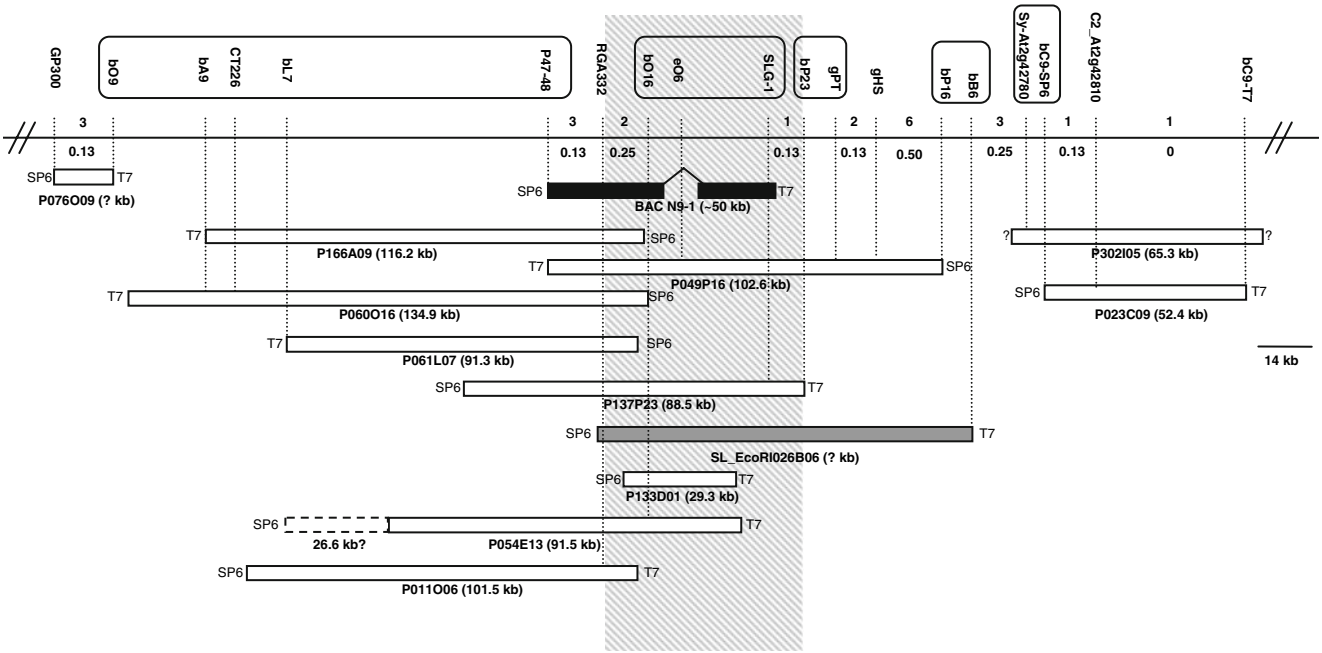


Fig. 2 Alignment of the genetic and physical maps of the *I-3* region. A linkage map of the *I-3* region is shown above a BAC contig of the region. The *left* of the map is proximal to the centromere. Markers that co-segregate are *boxed*. Genetic distances determined by analysis of the recombinants recovered from the (IL7-2 × IL7-4) × M82 TC1F2 population are shown in cM *below* the *horizontal line* and the total number of recombination events between adjacent markers is shown *above* the *horizontal line*. The genetic distances between markers are not drawn to scale. The *white bars* indicate BACs from the *L. esculentum* cv. Heinz 1706 *Hin*dIII library, the *grey bar* indicates the BAC from the *L. esculentum* cv. Heinz 1706 *Eco*RI library and the *black bar* indicates the *L. pennellii* BAC N9-1 obtained by Hemming et al. (2004). The size of each BAC, if known, is indicated in kb. The *bars* representing the BACs are drawn to scale. The *vertical lines* indicate markers shown by PCR analysis to be present on the BACs. BACs P076O09, P060O16 and SL_EcoRI026B06 were not obtained for PCR analysis but were included in this figure to show the markers developed. The marker eO6 sequence was positioned via a BLASTN analysis of the P049P16 BAC sequence. The *grey hatched area* indicates the interval containing the *I-3* gene

end of BAC P054E13 also corresponds to a putative retrotransposon-like sequence on BAC P166A09 between CT226 and bL7. Therefore, it is possible that the physical size of BAC P054E13 has been wrongly estimated.

Relative to the *L. esculentum* BAC clones, in particular BAC P133D01, BAC N9-1 appears to contain a ~10 kb deletion between bO16 and SLG-1. Work is currently being carried out to define this missing region more precisely. Based on the *Fol* race 3 resistance phenotypes conferred by recombinant chromosomes recovered from the (IL7-2 × IL7-4) × M82 TC1F2 mapping population, *I-3* is located in a 0.38 cM interval flanked by the markers RGA332 and bP23/gPT. The BAC contig suggests that this genetic interval corresponds to a physical interval of 50–60 kb.

The *I-3* region displays microsynteny with *Arabidopsis thaliana* chromosome *2,* poplar linkage group XIV and grape chromosome *12*

During the marker development process, a subset of the markers that were generated and mapped to the *I-3* region were also found to display microsynteny with chromosome *2* of *Arabidopsis thaliana*. Markers GP300, CP51-c, C2_At2g42750, bC9-SP6 and C2_At2g42810 were found to correspond to *Arabidopsis* genes At2g42600, At2g42740, At2g42750, At2g42790 and At2g42810, respectively, while markers CP51-c and bC9-SP6 also correspond to At3g58700 and At3g58740/At3g58750, respectively, consistent with the duplication of a large segment of *Arabidopsis* chromosome *2* on *Arabidopsis* chromosome *3* (Ku et al. 2000).

Other potentially microsyntenous *Arabidopsis* gene sequences were used to develop new markers in the *I-3* region and to further explore the microsynteny between tomato and *Arabidopsis*. The SGN annotation search (http://www.sgn.cornell.edu/search/direct_search.pl?search=annotations) incorporating automatic BLAST search functions (BLASTX search against the genbank/nr database and BLASTX search against the *Arabidopsis*/peptide database) was used to retrieve tomato unigenes likely to be orthologous to the *Arabidopsis* genes At2g42760, At2g42770 and At2g42780. New markers (given the prefix 'Sy-' for synteny) were developed from these unigenes (Table 1) and mapped to the *I-3* region using the recombinants recovered from all three mapping populations. Tables 4 and 5 show the relationship between chromosome *7* markers/genes and

Table 4 Relationship between chromosome *7* markers and their corresponding tomato unigenes and syntenic *Arabidopsis* genes

Marker mapping to the *I-3* region of tomato chromosome *7*	Sequence used for marker development	Tomato orthologue (SGN Unigene)	Microsyntenic *Arabidopsis* chromosome *2* gene[a]	BLASTX (E value)	Microsyntenic *Arabidopsis* chromosome *1* and chromosome *3* genes[a]	BLASTX (E value)	Predicted gene
Got-B	Tomato EST	U314712	**At2g30970**	0	At5g11520 At5g19550 At1g62800 At4g31990	1e-109 1e-108 1e-105 1e-104	Glutamate oxaloacetate transaminase
bP16	P049P16 SP6 BAC end	P049P16 strictosidine synthase gene1	**At2g41290** **At2g41300**	5e-70[b] 1e-67	**At3g57030** At5g22020 **At1g08470** **At3g57020** At3g59530 **At3g57010**	1e-117[b] 1e-84 1e-83 6e-72 3e-69 2e-67	Strictosidine synthase
GP300	Tomato EST	U316114	**At2g42600**	0	At1g53310 **At3g14940**[c] At1g68750	0 0 1e-154	Phosphoenolpyruvate carboxylase
CP51-c	Tomato EST	U312892	**At2g42740**	2e-97	**At3g58700** At4g18730 At5g45775	2e-97 2e-97 2e-97	60S ribosomal protein L11
C2_At2g42750	SGN CAPS marker	U321457	**At2g42750**	1e-136			DnaJ protein
Sy-At2g42760	Tomato EST	U323422	**At2g42760**	3e-28			Expressed protein
Sy-At2g42770	Tomato EST	U320912	**At2g42770**	1e-78			Peroxisomal membrane protein
Sy-At2g42780	Tomato EST	U318941	**At2g42780**	5e-51			Expressed protein
bC9-SP6	P023C09 SP6 BAC end	U321404	**At2g42790**	1e-127	**At3g58740** **At3g58750**	1e-110 1e-126	Citrate synthase
C2_At2g42810	AY182778	U317380 U336066	**At2g42810**	0			Type 5 serine/threonine protein phosphatase
Sy-At3g58790	Tomato EST	U322143			**At3g58790** At5g15470 At3g01040 At5g54690	1e-167 1e-126 1e-124 1e-109	Glycosyl transferase

[a] Syntenous genes are highlighted in bold font

[b] BLAST scores based on full coding region deduced from genomic sequence

[c] These genes may comprise a second more-distant region of microsynteny with *Arabidopsis* chromosome *3*

Table 5 Relationship between genes on BACs anchored to the *I-3* region of tomato chromosome *7* and their syntenic *Arabidopsis* genes

BAC anchored to the *I-3* region of tomato chromosome *7*[a]	Orthologous tomato gene	Source of predicted coding sequence	Microsyntenic *Arabidopsis* chromosome *2* gene[b]	BLASTX (E value)	Microsyntenic *Arabidopsis* chromosome *1* and chromosome *3* genes[b]	BLASTX (E value)	Predicted gene
P023C09	Gene similar to At3g58760	No unigene. Coding sequence predicted from P023C09	**At2g31800** **At2g43850**	1e-106[c] 1e-105	**At3g58760** At4g18950 **At3g59830**	1e-162[c] 1e-154 1e-102	Ankyrin protein kinase
	Gene similar to AtCOQ3	U318698	**At2g30920**	1e-118			Mitochondrial hexaprenyldihydroxybenzoate methyltransferase
P049P16	Strictosidine synthase gene 2	U323940 and P049P16 genomic sequence	**At2g41290** **At2g41300**	4e-73[c] 1e-72	**At3g57030** At5g22020 **At1g08470** **At3g57020** **At3g57010** At3g59530	1e-111[c] 1e-86 3e-82 1e-72 9e-72 4e-67	Strictosidine synthase
	Heat shock gene	U314912 and U314913 (both chimeric) and P049P16 genomic sequence			**At1g06460**	2e-42[b]	Alpha-crystallin-type heat shock protein
	Phosphate translocator-related gene = gPT	U345304 and P049P16 genomic sequence			**At1g06470**	1e-151[c]	Phosphate translocator-related protein
	Gene similar to *Arabidopsis* At2g30900	No unigene. Coding sequence predicted from P049P16 genomic sequence	**At2g30900** **At2g31110** At2g34070 **At2g42570** At2g30010	1e-108[c] 2e-94 6e-94 7e-91 1e-74	**At3g14850**[d] At1g29050 At1g78710 At5g58600 At3g54260	7e-95 2e-93 1e-91 9e-79 4e-70	Unknown protein similar to At2g30900
P166A09	Cytochrome P450 gene = CT226	U339829 and U337867 (both partial) and P166A09 genomic sequence			**At3g14650**[d] **At3g14690** **At3g14660** **At3g14610** **At3g14680** **At3g14640** **At3g14620** **At3g14630**	1e-170 1e-169 1e-168 1e-167 1e-164 1e-163 1e-163 1e-160	Cytochrome P450

[a] These BACs were supplied to the French tomato chromosome *7* sequencing project for sequencing

[b] Syntenous genes are highlighted in bold font

[c] BLAST scores based on full coding region deduced from genomic sequence

[d] These genes may comprise a second more-distant region of microsynteny with *Arabidopsis* chromosome *3*

their corresponding tomato unigenes on the one hand and syntenic genes on *Arabidopsis* chromosomes *2* and *3* on the other.

A tomato EST corresponding to At2g42800 (encoding a LRR protein) could not be found, so primers (forward 5′ to 3′: CATCTTGATTTAAGCTACAAC and reverse 5′ to 3′: TTTCTACTCAAATTCAAGTG) were instead based on the likely potato orthologue U298450. Although PCR products were successfully amplified from tomato DNA templates, no PCR products could be amplified from BAC clones in the *I-3* region, including BAC P023C09 which contains sequences orthologous to the flanking At2g42790 and At2g42810 genes, indicating that the potential At2g42800 orthologue most likely lies outside the *I-3* region or in the gap between BACs P049P16 and P302I05.

GP300 (At2g42600), CP51-c (At2g42740), C2_At2g42750, Sy-At2g42760 and Sy-At2g42770 mapped within the same bin just proximal to *I-3*, but Sy-At2g42780, bC9-SP6 (At2g42790) and C2_At2g42810 mapped to bins just distal to *I-3* (Fig. 1). This microsynteny was disrupted by phosphate translocator-like (gPT), heat-shock transcription factor (gHS), strictosidine synthase (bP16), cytochrome P450 (CT226), S-locus glycoprotein (SLG-1) and S-locus receptor-like kinase (SRLK) genes, which are orthologues/homologues of genes from other regions of the *Arabidopsis* genome.

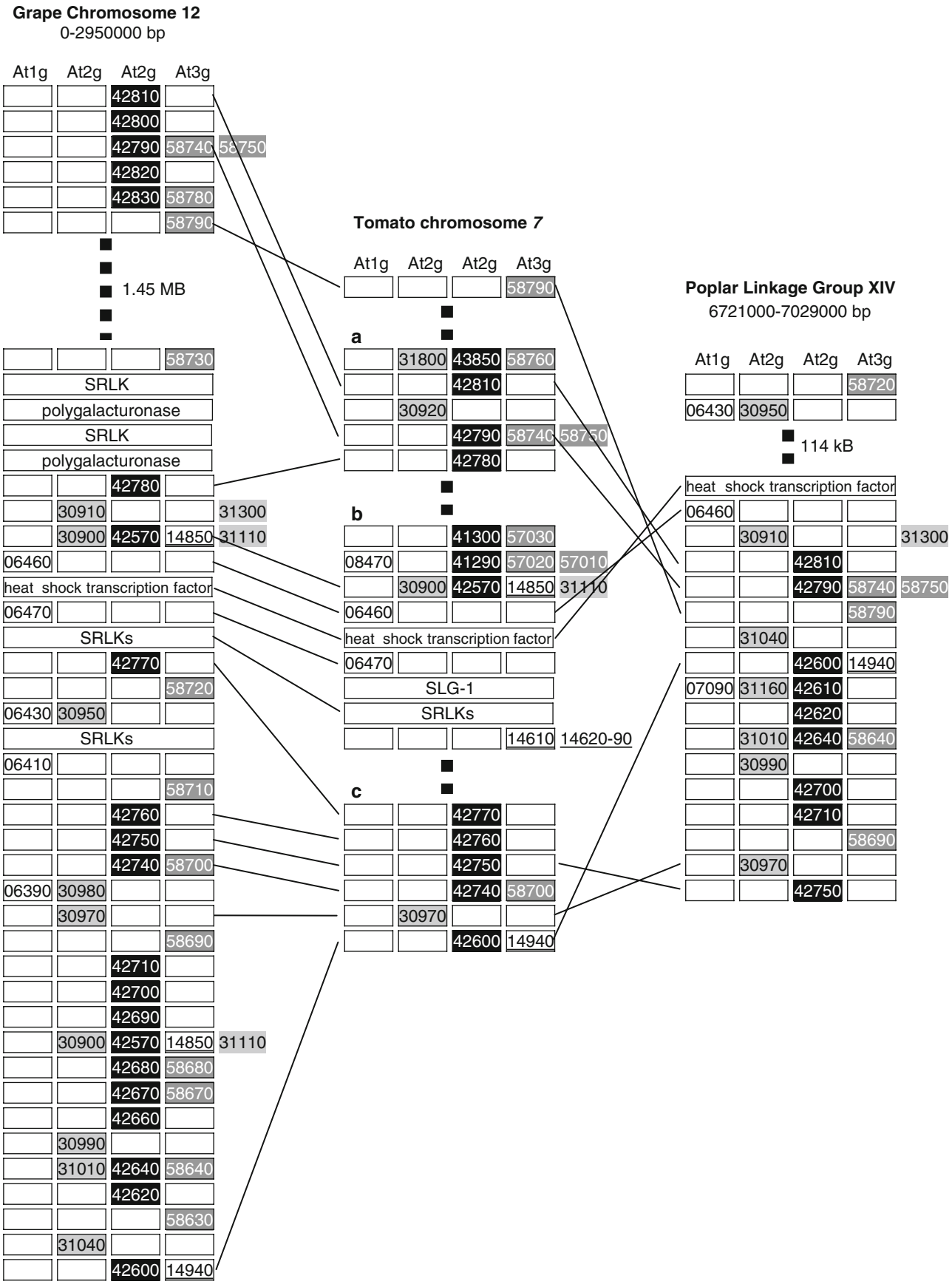
The recently sequenced poplar (*Populus trichocarpa*) and grape (*Vitis vinifera*) genomes (Tuskan et al. 2006; Jaillon et al. 2007) were also analysed for microsynteny with the tomato *I-3* region and the corresponding region of *Arabidopsis* chromosome *2*. *Arabidopsis* and poplar represent divergent lineages within the rosid subclass of the core dicotyledons, with poplar assigned to eurosids I and *Arabidopsis* to eurosids II, whereas tomato is in the asterid subclass. The position of grape in this hierarchy remains unclear with the order Vitales, which contains grape, forming an unassigned subclass within the core dicotyledons. Grape being neither rosid nor asterid, therefore, provided another potentially useful comparison with tomato because it should in theory be at least as divergent from tomato as it is from *Arabidopsis*. From these comparisons, microsynteny was found in a region of poplar linkage group XIV that showed a similar clustering of *Arabidopsis* orthologues to that found on *Arabidopsis* chromosome *2* and to a lesser extent the *I-3* region of tomato (Fig. 3). Interestingly, orthologues of the At2g30910–At2g31040 cluster of genes were found interspersed with orthologues of the At2g42600–At2g42810 cluster of genes. This included an orthologue of the *Asp1* gene (At2g30970) corresponding to the tomato *Got-2* gene (Wang et al. 2007), which also maps to the *I-3* region. Orthologues of the At3g58690, At3g58720 and At3g58790 genes were also distributed through this region. Homologues of these genes are absent from *Arabidopsis* chromosome *2*, but are present in the segment of chromosome *2* duplicated on *Arabidopsis* chromosome *3*. The possible presence of an At3g58790 orthologue in the *I-3* region of tomato chromosome *7* was tested. The tomato unigene U322143 retrieved using At3g58790 was developed into a marker (Sy-At3g58790; Table 1) and was found to map to the same bin as bC9-T7 and TG639 (Fig. 1), distal to C2_At2g42810 (Fig. 1). However, BAC P023C09, which contains C2_At2g42810, did not contain Sy-At3g58790 indicating that it lies distal to bC9-T7.

Microsynteny was also found on grape chromosome *12* (Fig. 3). Similar to poplar linkage group XIV, grape chromosome *12* contained orthologues of genes from the At2g30900–At2g31040 and At3g58630–At3g58790 clusters of genes interspersed with orthologues of genes from the At2g42600–At2g42810 cluster of genes. Surprisingly, orthologues of genes from the At1g06390–At1g06470 cluster of genes were also found interspersed with these genes. At1g06470 corresponds to the gPT marker, which maps close to *I-3* in tomato. Interestingly, a cluster of SRLK genes orthologous to that in the *I-3* region of tomato was found adjacent to the At1g06470 orthologue in grape, but orthologues of these SRLK genes were found to be entirely absent from the *Arabidopsis* genome (based on the results of reciprocal BLAST searches) and to be represented by only a single orthologue on linkage group XII in poplar (see Supplementary Table 3).

Discussion

High-resolution mapping of *I-3*

In this study, we report the high-resolution genetic and physical mapping of the tomato *I-3* gene for fusarium wilt resistance. Our work focused on the generation and mapping of new markers in the *I-3* region, the screening and recovery of novel recombinants from two new mapping populations and the construction of a BAC contig covering the *I-3* region, as a follow-up to the initial work by Hemming et al. (2004). New sequences were identified for conversion into PCR-based markers that map to the *I-3* region. Recombinants from three mapping populations, M82 × IL7-3 F2, M82 × IL7-2 F2 and (IL7-2 × IL7-4) × M82 TC1F2, were used to position these markers. Using these new markers and recombinants, we have shown that the *I-3* gene is contained within a 0.38 cM interval between markers RGA332 and bP23/gPT, and co-segregates with the markers bO16 and SLG-1 (Figs. 1, 2). The BAC P049P16 (102.6 kb) spans the RGA332 and bP23/gPT interval and may therefore contain an *L. esculentum* allele of the *L. pennellii I-3* gene.


Grape Chromosome 12
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42800
42790 58740 58750
42820
42830 58780
58790
1.45 MB
58730
SRLK
polygalacturonase
SRLK
polygalacturonase
42780
30910 31300
30900 42570 14850 31110
06460
heat shock transcription factor
06470
SRLKs
42770
58720
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42690
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42660
30990
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58630
31040
42600 14940
Tomato chromosome 7
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42810
30920
42790 58740 58750
42780
b
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58720
06430 30950
114 kB
heat shock transcription factor
06460
30910 31300
42810
42790 58740 58750
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30970
42750

◀ **Fig. 3** Microsynteny between the tomato *I-3* region (*centre*) and grape chromosome *12* (*left*) and poplar linkage group XIV (*right*). Numbers corresponding to *Arabidopsis* chromosome *1, 2* or *3* gene identities (At1g, At2g or At3g) are shown for orthologues/homologues on grape chromosome *12*, tomato chromosome *7* and poplar linkage group XIV. Genes on tomato chromosome *7* with homologues in other regions of the *Arabidopsis* genome are shown using the name of the protein encoded. Grape and poplar genes with orthologues/homologues in other regions of the *Arabidopsis* genome are not shown except for those corresponding to genes on tomato chromosome *7*. Connecting lines show the syntenic relationship between tomato genes identified in the *I-3* region and orthologues/homologues on the other two chromosomes. The *bottom* of the map of tomato chromosome *7* is proximal to the centromere. **a** This group of adjacent genes on BACs P023C09 (Genbank Accession AC210349.1) and P302I05 corresponds to markers C2_At2g42810, bC9-SP6 (At2g42790) and Sy-At2g42780. **b** This group of adjacent genes on BACs P049P16 (Genbank Accession AC212619.1) and P166A09 (Genbank Accession AC210359.1) corresponds to markers bP16 (At2g41300/At3g57030), gHS (heat shock transcription factor), gPT (At1g06470), SLG-1 and CT226 (At3g14610-90). **c** This group of genes corresponds to co-segregating markers Sy-At2g42770, Sy-At2g42760, C2_At2g42750, CP51-c, Got-B and GP300 (from top to bottom) whose order is unknown but is inferred from the order of the corresponding orthologues/homologues on *Arabidopsis* chromosome *2* and grape chromosome *12*. A second more-distant region of microsynteny with *Arabidopsis* chromosome *3* is suggested by a small number of matches to genes with the prefix At3g14 (*underlined*)

While in general agreement with older maps, our genetic map of the *I-3* region of tomato chromosome *7* shows some striking differences to the most recent SGN map based on an *L. esculentum* × *L. pennelii* F2 population. The SSR565, C2_At2g42750, TM18, C2_At4g15420, TG143 and SSR557 markers have all been mapped distal to TG639 in the SGN *L. esculentum* LA925 × *L. pennellii* LA716 F2 2000 map (http://www.sgn.cornell.edu, January 2008), whereas our data place these markers at various positions proximal to TG639. Apart from TG143, these markers have not previously been mapped using other mapping populations, whereas TG143 has been mapped proximal to TG639 on other occasions (Sarfatti et al. 1991; Tanksley et al. 1992). Given that TG143 appears to have been misplaced on the SGN *L. esculentum* LA925 × *L. pennellii* LA716 F2 2000 map, it is possible that the other markers in question have also been misplaced.

Recombination frequencies

Although the recombination frequency was a little greater in the IL7-2 cross compared to the IL7-3 cross reported by Hemming et al. (2004), suppression of recombination was still apparent in the region of chromosome *7* heterozygous for *L. pennellii* and *L. esculentum*. In contrast, the recombination frequency in the (IL7-2 × IL7-4) × M82 cross which generated a reconstituted *L. pennellii* chromosome *7* (Lim et al. 2006) was significantly higher, presumably due to a more random distribution of recombination events along the length of chromosome *7*. The observed higher recombination in the reconstituted *L. pennellii* chromosome *7* mapping population may be attributed to a lack of competition by homologous recombination (as opposed to homoeologous recombination). Similar suppression of recombination has been observed for *Solanum lycopersicoides* introgression lines in tomato and this has also been ascribed to a preference for recombination in regions of homologous pairing (Canady et al. 2006). Consistent with our results, Canady et al. (2006) also found that increasing the length of the *S. lycopersicoides* introgressions increased the recombination frequency in introgressed regions and the highest frequency of recombination was observed with the longest introgressions or with intact *S. lycopersicoides* chromosomes.

The relationship between genetic and physical distance in the *I-3* region

A comparison between the genetic map of the *I-3* region and the physical size of the corresponding BAC contig reveals considerable disparity between genetic and physical distances in the *I-3* region. The markers bO9 and P47-48 are separated by a physical distance of approximately 100 kb yet have co-segregated, whereas 14 recombination events were recovered from the adjacent interval of approximately 100 kb between P47-48 and bP16. Even within this region, six recombination events occurred in the 9–15 kb interval between gHS and bP16, whereas no recombination events were recovered from the approximately 25 kb interval between bO16 and SLG-1. A possible explanation for the latter is provided by the 10 kb size difference in this region between *L. esculentum* and *L. pennellii*, which may result in poor meiotic pairing. It is possible that small structural heterogeneities between *L. esculentum* and *L. pennellii* chromosomes will contribute to the apparent suppression of recombination events in some intervals. This interpretation is supported by an analysis of the *sun* locus in tomato conducted by van der Knapp et al. (2004). The *sun* locus is contained within a 68 kb region on the short arm of *L. esculentum* chromosome *7*, but this region is inverted on *L. pennellii* chromosome *7*, so suppressing recombination near the *sun* locus in *L. esculentum* × *L. pennellii* hybrids and preventing the use of *L. pennellii* as a fine mapping tool in this region. Similarly, Bonnema et al. (1997) ascribed the lack of recombination between a cluster of markers on the short arm of chromosome *1* in an *L. esculentum* × *L. pennellii* mapping population to small inversions or deletions in one homoeologue relative to the other. A cytological analysis of the pairing between *L. esculentum* chromosome *7* and *L. pennellii* chromosome *7* reported by Khush and

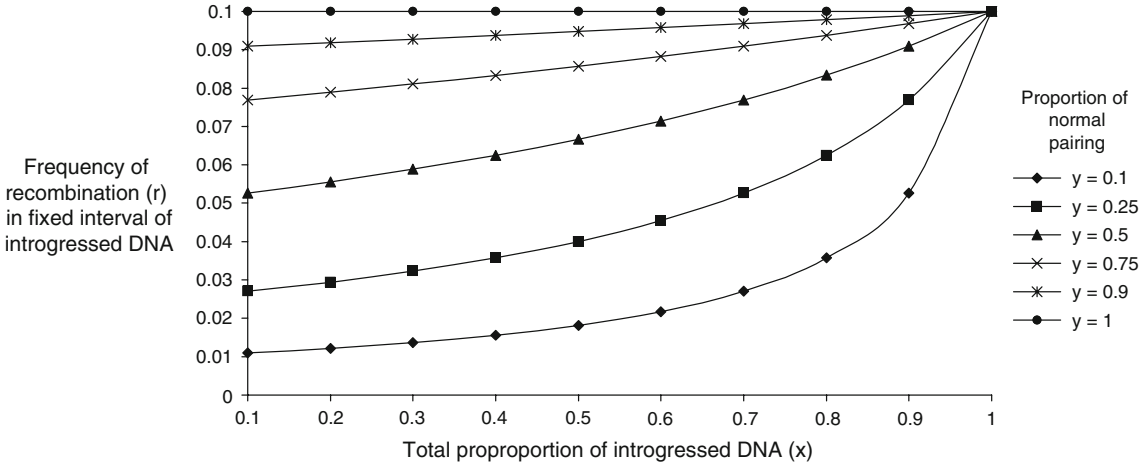

Fig. 4 Plots of the theoretical variation in frequency of recombination (r) in a fixed portion of an introgressed segment as the total length of the introgressed segment increases and for various levels of reduced pairing in the introgressed segment. The plots graph the function $r = zy/[1 - x(1 - y)]$ where x is the length of the introgressed segment as a proportion of the entire chromosome with $0 \leq x \leq 1$, z is the fixed portion of the introgressed segment over which recombination is being measured with $0 \leq z \leq x$, and y is the proportion of normal pairing occurring in the introgressed region with $0 \leq y \leq 1$. In these plots, z was given an arbitrary value of 0.1, a realistic value for the proportion of a chromosome over which recombination might be measured, and y was given values of 0.1, 0.25, 0.5, 0.75, 0.9 and 1 to cover effects on pairing in regions heterozygous for the introgression ranging from extreme to no effect. Over small intervals, the recombination frequency (r) was assumed to be proportional to the physical distance (z) over which recombination is being measured. For convenience, the maximum recombination frequency possible over the interval $z = 0.1$ was also arbitrarily set at 0.1, so that no conversion factor (k) was required to convert physical distances into recombination frequencies (i.e. $r_{max} = kz$ becomes $r_{max} = z$ and $r = kzy/[1 - x(1 - y)]$ becomes $r = zy/[1 - x(1 - y)]$)

Rick (1963) revealed no major pairing anomalies such as inversion loops. However, the greater number of chromomeres observed on the long arm of *L. pennellii* chromosome *7* than the long arm of *L. esculentum* chromosome *7*, the presence of a chromatic bulge in the long arm of the hybrid chromosome *7* bivalent and the greater length of the long arm in *L. pennellii* than *L. esculentum*, together with the resulting stretching of the *L. esculentum* chromatin in the hybrid bivalent, suggest that small structural differences exist that would affect local pairing between the two chromosomes. If pairing in regions heterozygous for the *L. pennellii* introgression is reduced because of a mosaic of small structural differences then this might explain some of the marked clustering of markers that we have observed. A mosaic of pairing might also explain the overall reduction of recombination in the introgressed region purely on a statistical sampling basis. We have modelled this scenario in Fig. 4, which shows the relationship between introgression length and recombination for various levels of reduced pairing. This figure shows that for relatively low levels of reduced pairing ($y \geq 0.5$) increasing the length of the introgressed segment would have an approximately linear effect on recombination, but for higher levels of reduced pairing ($y \leq 0.25$) increasing the length of the introgressed segment would have only a small effect on recombination until the length of the introgression became a major portion of the entire length of the chromosome at which point there would be an almost exponential increase in recombination back towards normal levels. The recombination data we obtained for our three mapping populations are consistent with the latter scenario.

These observations and the hypothesis postulated to explain them have potentially significant practical consequences for the marker-assisted elimination of linkage drag during introgression of a desirable trait from a wild relative of a crop plant into the domesticated species. Accordingly, we have formulated and begun testing a three-step strategy for the marker-assisted breeding of a minimal introgression containing a trait of interest (Lim et al. 2006), in this case *I-3*, which we hope will dramatically improve the efficiency of the process.

Arabidopsis, poplar and grape microsynteny with the tomato *I-3* region

Comparative mapping, based on microsynteny between *Arabidopsis* and tomato, has been used successfully to fine map regions of interest in the tomato genome such as the *Diageotropica* (*Dgt*) locus on chromosome *1* (Oh et al. 2002), the *Ovate* locus on chromosome *2* (Ku et al. 2000) and the *Lateral Suppressor* locus on chromosome *7* (Rossberg et al. 2001). In the case of *Dgt*, new markers developed from the microsynteny-based comparative mapping successfully narrowed down the *Dgt* region from 0.8 to 0.15 cM (Oh et al. 2002). Although the development of the four *Arabidopsis* orthologous markers (Sy-At2g42760, Sy-

At2g42770, Sy-At2g42780 and Sy-At3g58790) did not physically reduce the *I-3* region, the results obtained based on the conservation of gene order have shed light on the microsyntenic relationship between the *I-3* region on tomato chromosome *7* and corresponding regions on grape chromosome *12*, poplar linkage group XIV and *Arabidopsis* chromosomes *1, 2* and *3* (see Fig. 3).

The comparison between these microsyntenous regions of the grape, poplar and tomato genomes revealed that orthologues of genes from the At1g06390–At1g06470, At2g30900–At2g31040, At2g42600–At2g42810 and At3g58630–At3g58790 clusters of genes are interspersed with one another (Fig. 3). These patterns of gene organisation suggest inter- and intra-chromosomal duplication followed by differential gene loss in *Arabidopsis* of an ancestral gene arrangement that has been retained to various extents in grape, poplar and tomato. The extent to which such an arrangement may have been retained in tomato has not been fully assessed in this study because no attempt was made to develop and map markers from tomato unigenes orthologous to At1g06390–At1g06450, At2g30930–At2g30960, At2g30980–At2g31040, At2g42610–At2g42730 or At3g58630–58690, given that these were considered likely be outside the interval containing *I-3*.

In hindsight, the almost continuous microsynteny between the *I-3* region of tomato chromosome *7* and the corresponding region of grape chromosome *12* suggests that our map-based cloning project would have been better informed if it had been based on grape microsynteny (had the grape genome sequence been available at the time) than on *Arabidopsis* microsynteny. Alternatively, a greater awareness of the chromosomal regions related by duplication and subsequent differential gene loss in *Arabidopsis* might also have better informed our mapping efforts. The lessons learnt here might be useful for other map-based cloning projects based on identification of markers by microsynteny.

These genomic comparisons also reveal interesting differences in the representation of SRLK sequences across the four genomes. In grape, there are three clusters of intact SRLK genes in the microsyntenous region of chromosome *12*; a cluster of two comprising a duplicated pair of SRLK and polygacturonase genes, a cluster of six and a cluster of two (Fig. 3). In tomato, there is a cluster of five intact SRLK genes in the *I-3* region of chromosome *7*, but poplar and *Arabidopsis* have no SRLK genes in the corresponding regions of microsynteny with the *I-3* region and, although poplar has an intact SRLK orthologue on linkage group XII, the orthologous SRLK genes appear to have been lost altogether from *Arabidopsis*. This is reminiscent of the elaboration of resistance gene clusters in species where they play an active role in disease resistance but their reduction or loss in species where they no longer play a role in disease resistance. An example comes from the *I-3* region itself with the elaboration of the *Gro1* gene family for nematode resistance in the *I-3* region of potato chromosome VII as opposed to the reduction to a single pseudogene in the *I-3* region of *L. pennellii* and two pseudogenes in *L. esculentum* (Hemming et al. 2004).

BAC contig covering the *I-3* region

A BAC contig covering the *I-3* region was constructed based on BAC clones from the *L. esculentum* cv. Heinz 1706 BAC library and BAC N9-1 from an *L. pennellii* LA716 BAC library. We have shown that the *L. esculentum* allele of the *I-3* gene must be physically contained within the BAC clone P049P16 containing the flanking RGA332 and bP23/gPT markers. This clone has been almost completely sequenced, but genes resembling one of the five major classes of plant disease resistance genes have not been found, apart from a non-functional TIR-NBS-LRR pseudogene identified by Hemming et al. (2004). P049P16 contains a number of genes that are unlikely to function as resistance genes such as phosphate translocator-related, heat-shock transcription factor and strictosidine synthase genes (see Supplementary Table 1). This, therefore, shifts our focus to the unsequenced regions of *L. pennellii* BAC N9-1 and the segment of uncloned *L. pennellii* DNA between SLG-1 and bP23 as the potential repository of a resistance gene that may be present in *L. pennellii* but absent from *L. esculentum*. The *Arabidopsis RPM1* resistance gene provides a precedent for a gene present in resistant plants but absent from susceptible plants (Grant et al. 1998). Work is currently in progress to sequence the remainder of BAC N9-1 and to isolate and sequence the segment of *L. pennellii* DNA between SLG-1 and bP23. These findings also shift our focus to the SRLK and SLG-1 genes as potential candidates for *I-3*. A recently isolated rice gene for resistance to the rice blast fungal pathogen *Magnaporthe grisea*, *Pi-d2*, encodes a predicted B-lectin SRLK protein (Chen et al. 2006). Work is also in progress to further characterise and test the function of the SRLK and SLG-1 genes within the *I-3* region and we will report the findings in the near future.

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