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Fine genetic mapping of *RXopJ4*, a bacterial spot disease resistance locus from *Solanum pennellii* LA716

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Abstract The *RXopJ4* resistance locus from the wild accession *Solanum pennellii* (*Sp*) LA716 confers resistance to bacterial spot disease of tomato (*S. lycopersicum*, *Sl*) caused by *Xanthomonas perforans* (*Xp*). *RXopJ4* resistance depends on recognition of the pathogen type III effector protein XopJ4. We used a collection of *Sp* introgression lines (ILs) to narrow the *RXopJ4* locus to a 4.2-Mb segment on the long arm of chromosome 6, encompassed by the ILs 6-2 and 6-2-2. We then adapted or developed a collection of 14 molecular markers to map on a segregating F_2 population from a cross between the susceptible parent *Sl* FL8000 and the resistant parent *RXopJ4* 8000 OC_7. In the F_2 population, a 190-kb segment between the markers J350 and J352 cosegregated with resistance. This fine mapping will enable both the identification of candidate genes and the detection of resistant plants using cosegregating markers. The *RXopJ4* resistance gene(s), in combination with other recently characterized genes and a quantitative trait locus (QTL) for bacterial spot disease resistance, will likely be an effective tool for the development of durable resistance in cultivated tomato.

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Introduction

Bacterial spot is among the most significant diseases limiting tomato production throughout tropical and subtropical regions (Jones et al. 2005). Attempts to control the disease have included seed decontamination as well as the application of bactericides such as streptomycin and copper-based compounds. However, streptomycin has been ineffective since the 1960s, and copper resistance has also become nearly ubiquitous among pathogen strains in the field (Obradovic et al. 2008; Stall et al. 2009). While new strategies employing systemic acquired resistance and biocontrol have shown promise (Obradovic et al. 2008), most recent efforts are focused on genetic resistance as a sustainable path to the control of bacterial spot disease.

The causative agent of bacterial spot of both tomato and pepper was once considered a single species, *Xanthomonas campestris* pv. *vesicatoria*. Jones and colleagues (2004)

used DNA:DNA hybridization to reveal four distinct species: *X. euvesicatoria*, *X. vesicatoria*, *X. gardneri*, and *X. perforans*, which differ in their distribution, metabolic properties, and effector repertoires (Potnis et al. 2011). In the southeastern United States, where nearly half of all fresh market tomatoes in the USA are grown (Glades Crop Care 1999) and where bacterial spot disease is a major problem due to warm, humid conditions, *X. euvesicatoria* was long the predominant species. Since the mid-1990s, however, it has been replaced by *X. perforans* (Jones et al. 1998); the competitive advantage of *Xp* is likely due to its production of bacteriocins that antagonize *Xe* strains (Hert et al. 2005). The virulence of xanthomonads and other bacterial pathogens depends on a type III secretion system, which delivers an arsenal of effector proteins directly into plant cells. These effectors function to suppress components of the plant immune system. Some plants, however, have evolved resistance proteins that can directly or indirectly recognize pathogen effectors and activate a more robust defense response known as effector-triggered immunity; this recognition is specific, in that a given resistance protein is triggered by only one or two particular effectors (Jones and Dangl 2006).

Cultivated tomato (*Solanum lycopersicum*) lacks effective genetic resistance to bacterial spot disease, and breeding efforts have employed related wild species as sources of resistance. These have included both quantitative resistance, which is present in the accession *S. lycopersicum* var. *cerasiformae* PI 114490 (Hutton et al. 2010), and effector-triggered immunity conferred by single dominant loci, such as *Xv3*, found in the breeding line *S. lycopersicum* Hawaii 7981 and in at least two *S. pimpinellifolium* accessions (Wang et al. 2011). Unfortunately, *Xv3* resistance cannot be effectively used in the field in Florida, since many pathogen strains isolated over the past decade do not contain the recognized *avrXv3* effector gene. This may be due to selective pressure conferred by grape tomatoes, which likely originated from *S. pimpinellifolium* (Stall et al. 2009). The *avrBs2* effector gene, however, is highly conserved among diverse xanthomonads (Kearney and Staskawicz 1990) and has been shown to play a role in virulence on tomato (Zhao et al. 2011). The *Bs2* resistance gene from the pepper species *Capsicum chacoense* is effective against bacterial spot in transgenic tomato plants (Tai et al. 1999).

A durable disease-resistance strategy will ideally combine quantitative sources of resistance with effector-triggered immunity. A key approach to achieving durability will be the use of multiple resistance genes recognizing conserved pathogen effectors that play a role in virulence, in order to minimize the possibility for pathogen strains to mutate the recognized effector with no cost to fitness. Previous work has shown that the *Xp* effector XopJ4

[formerly designated AvrXv4 (Potnis et al. 2011)], a putative SUMO protease, plays a role in pathogen virulence (Roden et al. 2004). A preliminary survey of *Xp* field isolates from throughout the state of Florida suggests that the *xopJ4* effector gene is conserved in the pathogen, making it a promising target for durable resistance. The XopJ4 effector is recognized by a dominant resistance locus, *RXopJ4*, in the wild accession *Solanum pennellii* LA716. The locus was previously referred to as *Xv4* and was reported to map to a ∼20-cM segment on chromosome 3 (Astua-Monge et al. 2000). Since the taxonomy of the *Xanthomonas* genus continues to evolve, and there is overlap among type III effector repertoires of different species, we have chosen to base the name of this resistance locus, *RXopJ4*, on the recognized pathogen effector (XopJ4), rather than on the pathogen species.

In this study, we sought to further refine the mapping of the *RXopJ4* locus to identify cosegregating markers that would aid in the cloning of the resistance gene or genes. Initially, using a collection of introgression lines of *S. pennellii* in *S. lycopersicum*, we determined that the resistance locus lies within a 4.2-Mb region on the long arm of chromosome 6. We developed or adapted 14 molecular markers across this region and used these for fine mapping on an F_2 population. Analysis of recombinant F_3 plants showed that a 190-kb genomic region cosegregates with resistance. Our markers can be used for the detection of resistant recombinants and further fine mapping, and for the identification of candidate genes with the eventual goal of durable resistance to bacterial spot disease of tomato.

Materials and methods

Plant materials and DNA extraction

Solanum pennellii LA716 was obtained from Charles M. Rick at UC Davis. Jay W. Scott at the Gulf Coast Research and Education Center of the University of Florida provided the *S. lycopersicum* line FL8000, and the M82 line was received from Roger T. Chetelat at UC Davis. *S. pennellii* introgression lines were obtained from the C.M. Rick Tomato Genetics Resource Center at UC Davis, except IL 6-1, which was provided by Zachary Lippman at Cold Spring Harbor Laboratory. The *RXopJ4* 8000 OC$_7$ resistant parent was produced from an initial cross between *Sp* LA716 and *Sl* H7998 (Astua-Monge et al. 2000), followed by five generations of outcrossing resistant F_2 plants to *Sl* FL216 (a seventh backcross line from the cross between *Sl* FL7060 and *S. pimpinellifolium* PI 128216 containing *Xv3* resistance), and then two outcrosses to FL8000, which also contains *Xv3* resistance. The eighth F_1 generation and F_2 mapping population were generated from another outcross

to FL8000; 910 plants from this F_2 population were used for fine mapping of the *RXopJ4* resistance locus. Seeds were sown directly in soil and allowed to germinate and grow for 2 weeks in a growth chamber at 24 °C with 12 h light/dark cycle. Seedlings were then transferred to a greenhouse with supplemental lighting.

For DNA extraction, ∼60 mg young leaf tissue was collected per plant. The extraction protocol was adapted from one provided by Lauren Headland at UC Davis. Briefly, tissue was frozen at −80 °C overnight. Tissue was then disrupted in a BeadBeater (BioSpec Products, Bartlesville, OK, USA) with a 3-mm glass bead and extraction buffer containing 200 mM Tris–HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, and 14 µg/ml RNase A. Samples were then treated with SDS followed by extraction with phenol:chloroform:isoamyl alcohol (25:24:1). After phase extraction, DNA was precipitated with isopropanol, washed with 70 % ethanol, and resuspended in 1 mM Tris pH 8.

Bacterial strains, inoculum preparation and inoculation

Bacterial strains used in this study are described in Table 1. The *Xp* 4B∆*xopJ4* strain was constructed using the suicide vector pLVC18 containing an upstream fragment spanning 1,054 bp of the *xopJ4* promoter plus 33 bp of its open reading frame (ORF), and a 1,108-bp downstream fragment beginning at 7 bp past the stop codon; a *Bam*HI restriction site was introduced between the two fragments. A double homologous recombination event resulted in deletion of the *xopJ4* ORF, which was confirmed by PCR and Sanger sequencing. The 4B∆*xopJ4* deletion strain was complemented by selecting for a single crossover with pLVC18 containing 846 bp of the *xopJ4* promoter plus the ORF.

For inoculation, bacteria were grown on nutrient yeast glycerol agar (NYGA) supplemented, as appropriate, with 100 µg/ml rifampicin, 25 µg/ml kanamycin, and 10 µg/ml

Table 1 Bacterial strains used in this study

Strain	Relevant characteristics
Xanthomonas euvesicatoria 69-1	Rif^R
X. euvesicatoria 69-1 (EV)	Rif^R, Tc^R; contains the empty vector pLAFR6
X. euvesicatoria 69-1 (*xopJ4*)	Rif^R, Tc^R; pLAFR6 with *xopJ4* subclone including native promoter
X. perforans 4B-WT	Rif^R
X. perforans 4B∆*xopJ4*	Rif^R; *xopJ4* deletion mutant
X. perforans 4B∆*xopJ4*/comp	Rif^R, Km^R; *xopJ4* deletion mutant complemented with pLVC18 *xopJ4* including native promoter

Rif rifampicin, *Tc* tetracycline, *Km* kanamycin

tetracycline, and were incubated at 28 °C for 48–72 h. Cells were then washed from agar plates with 1 mM $MgCl_2$ and concentration was adjusted as necessary. Inoculation was performed by leaf infiltration using a 1-ml needleless syringe. For HR, two sections of each leaflet were infiltrated with suspensions of *Xe* 69-1 containing pLAFR6 or pLAFR6 *xopJ4*, both at 3×10^8 cfu/ml ($OD_{600} = 0.3$). For disease assays, whole leaflets were infiltrated with *Xp* 4B-WT, 4B∆*xopJ4*, or 4B∆*xopJ4*/comp at ∼10^4 cfu/ml. For disease assays, inoculated plants were placed in a growth chamber at 24 °C with 12 h light/dark cycle. For HR assays, inoculated plants were placed either in a growth chamber or in a greenhouse. HR was assessed 24–48 h after inoculation, while disease was assessed 10–14 days after inoculation. For the growth assay, four to six 0.8-cm^2 punches were taken from leaves of each genotype at each time point and homogenized in 1 mM $MgCl_2$ in a BeadBeater; appropriate dilutions were plated on NYGA supplemented with 50 µg/ml rifampicin and 50 µg/ml cycloheximide (with 12.5 µg/ml kanamycin for the complemented mutant strain).

Marker development and PCR conditions

For mapping of the *RXopJ4* resistance locus, we used a total of 14 molecular markers spanning the ∼27 cM or 7.3 Mb from TG118 to CT204 (Table 2; Figs. 2, 5) on the long arm of chromosome 6. Thirteen of these markers are cleaved amplified polymorphic sequence (CAPS) markers, for which a short (<1,000 bp) sequence is amplified by polymerase chain reaction (PCR) and then digested with a restriction enzyme to yield product sizes that differ between the two parent genotypes—in this case, *Sl* and *Sp* (Fig. S2). One marker, SLM 6-15, is a simple sequence repeat (SSR) marker; it spans a region of tandem TA repeats that is 39 nucleotides longer in *Sl* than in *Sp* and can thus be visualized directly by agarose gel electrophoresis without restriction digestion (Fig. S2).

The markers TG118, CT83, C2_At1g21640, TG164, TG352, and CT204 were derived from RFLP or CAPS markers used in previous mapping studies (Fig. 5). Sequences, and in some cases CAPS protocols, for these markers were obtained from the Sol Genomics Network (Bombarely et al. 2011). Primers were optimized for PCR and CAPS visualization and are listed in Table 2. 06g060670 is derived from a predicted gene model in the International Tomato Annotation Group (ITAG) 2.3 genome annotation release (Sato et al. 2012). J350, J351, J352, J353, J366, and J385 markers were adapted from CAPS marker predictions based on preliminary *Sl* and *Sp* mRNA sequence information (manuscript in preparation). The SLM 6-15 SSR marker protocol was taken directly from Geethanjali et al. (2010). *S. pennellii* genome assemblies

Table 2 Molecular marker information for mapping of the *RXopJ4* resistance locus in *Solanum pennellii* LA716

Marker name	Genome position[a]	Marker type	Primer sequences (5′–3′)	Annealing temperature (°C)	Restriction enzyme	Product size (bp)		References
						Sl FL8000	*Sp* LA716	
TG118	32556111..32556711	CAPS	f: TACTGTCCATCCCTCATT r: GATATGGAAGTAGATGTGTC	50	*Sal*I	330/271	601	Bombarely et al. 2011, This study
SLM 6-15	33442726..33442965	SSR	f: GGATTTCAGCTGCCTACTGAG r: TTCGGAGAACATAATAGGGGTTT	55	–	240	201	Geethanjali et al. 2010
CT83	33748762..33749158	CAPS	f: GACTGCAGAGGTTAACTGAC r: CGAGGCTTCCTCTCTGATG	60	*Apo*I	240/157	397	Bombarely et al. 2011, This study
C2_At1g21640	34364263..34364672	CAPS	f: AGAAAAGTCATCCATGGAAACAACAC r: TGGCCACAATGACACCATCACCTTG	55	*Apo*I	290/120	410	Bombarely et al. 2011, This study
J350	35036517..35036818	CAPS	f: CTGCTGGAATTGTAATTATTCACG r: TTGAGCATAAACAGTCCAAAACAT	55	*Hin*fI	302	159/143	This study
06g060670	35078590..35079503	CAPS	f: CACCGGACACGAAGTATAAGACA r: CCAAACCACCAACCCAAA	53	*Hind*III	538/376	911	Bombarely et al. 2011, This study
J351	35127552..35127921	CAPS	f: CACCCTGAAAATCATCCAAAGCAC r: ACCCACACCCTCTATATAAA	55	*Hin*fI	317/53	268/53/49	This study
J352	35241322..35241736	CAPS	f: AAAATTGGAGGGACAACAAATAAA r: AAGGAAGTTCAAGAGCTAAGGTCA	55	*Hinc*II	415	283/132	This study
J353	35359974..35360331	CAPS	f: GAGAGAAAATGGGATAACCTTCAA r: CACTTATTATCAGGAGGAGGAGGA	50	*Hin*fI	268/90	358	This study
TG164	36270932..36271174	CAPS	f: GGTATAGTTTCATGCACATC r: CCTGTAGGTTTCTTTGATTC	50	*Apo*I	127/116	243	Bombarely et al. 2011, This study
J366	36676727..36677181	CAPS	f: CTTCACTTCTTGCCTGAGATGTTA r: AGATCAATTACCGATATCACCCAT	55	*Hin*fI	360/95	455	This study
TG352	36837890..36838115	CAPS	f: GAGGGATGTCTCATGTAG r: CATATGTCCTGCTAAAAGAAG	50	*Tsp*509I	149/77	226	Bombarely et al. 2011, This study
J385	38589739..38590229	CAPS	f: GGCAAGTGAAGGTGTAAAAGTTCT r: GTTCAAAATTAAACAGAACGTCCC	55	*Hin*fI	340/151	237/151/103	This study
CT204	39834480..39834861	CAPS	f: TCCCTCCTCAGATTTAGCTT r: TTGGTGCCCTCTTCTTTTT	50	*Bam*HI	382	196/186	Bombarely et al. 2011, This study

[a] Coordinates on the SL2.40ch06 genome assembly (Sato et al. 2012)
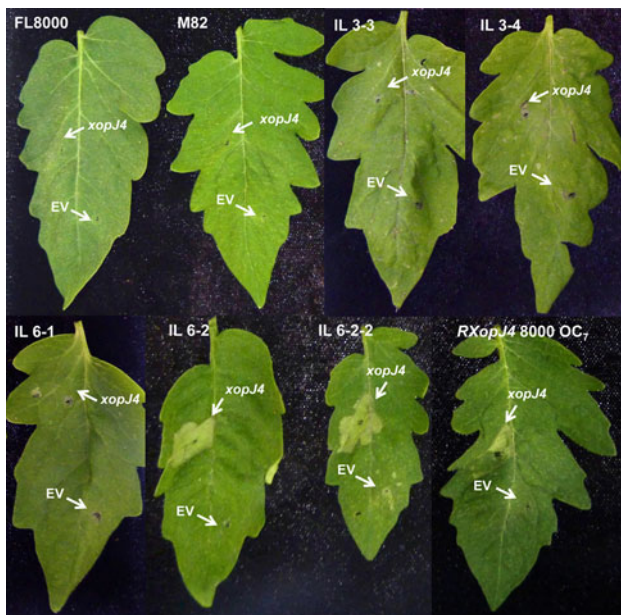
Fig. 1 The effector XopJ4 elicits a hypersensitive response in the *S. pennellii* introgression lines (ILs) 6-2 and 6-2-2 and *RXopJ4* 8000 OC₇. Plants were inoculated with the compatible strain *Xe* 69-1 carrying pLAFR6 empty vector (EV) or pLAFR6 *xopJ4* at a concentration of $\sim 3 \times 10^8$ cfu/ml. Photographs were taken at 24 h post inoculation

were provided by Anthony Bolger, Alisdair Fernie, and Björn Usadel at the Max Planck Institute for Molecular Plant Physiology, Golm, Germany.

Primers were designed using Amplify (Bill Engels, University of Wisconsin, USA) and CLC Main Workbench (CLC bio, Aarhus, Denmark) and were obtained from Integrated DNA Technologies (Coralville, IA, USA). PCR was conducted using Klentaq LA (DNA Polymerase Technology, St. Louis, MO, USA) according to the manufacturer's protocol. Initial denaturation was at 94 °C for 5 min, followed by 35 cycles of 5 s at 94 °C, 30 s at 50–60 °C (Table 2), and 1 min at 68 °C, and a final extension at 68 °C for 7 min. Restriction digestion was

carried out for 2 h at the appropriate temperature with enzymes and buffers purchased from New England Biolabs (Ipswich, MA, USA). PCR and restriction digestion were performed in a programmable thermal controller (PTC-100; MJ Research, Inc., Watertown, MA, USA). PCR/restriction products were separated on 3 % (SLM 6-15 and J351) or 2.5 % agarose gels (all other markers) and stained with ethidium bromide (SLM 6-15 and J351) or GelRed (Phenix Research Products, Candler, NC, USA) (all other markers; GelRed stain was included in the loading dye).

Results

### Phenotypic analysis of XopJ4-dependent hypersensitive response

In order to determine the chromosomal location of the *RXopJ4* resistance locus from *S. pennellii*, we employed a collection of 50 introgression lines (ILs) from the cross between *S. pennellii* LA716 and *S. lycopersicum* M82 (Eshed and Zamir 1994). Each line contains a homozygous introgression of *S. pennellii* over one segment of one chromosome, while the rest of its genome is *S. lycopersicum*; introgressed segments are overlapping. Each IL was inoculated with the compatible strain *Xe* 69-1 containing the empty vector pLAFR6 or pLAFR6 *xopJ4* (Fig. 1). IL 6-2 and its sub-line IL 6-2-2 exhibited a XopJ4-dependent hypersensitive response (HR), a rapid cell death associated with pathogen resistance (Jones and Dangl 2006). However, IL 6-1, which contains a *S. pennellii* introgression that partially overlaps with those of ILs 6-2 and 6-2-2 (Fig. 2), did not exhibit HR. Furthermore, ILs 3-3 and 3-4, which Astua-Monge et al. (2000) identified as containing the *RXopJ4* locus, did not exhibit XopJ4-dependent HR. The XopJ4-dependent HR of ILs 6-2 and 6-2-2, but not IL 6-1, indicated that the *RXopJ4* locus was within a 4.2-Mb region on the long arm of chromosome 6 (Fig. 2).
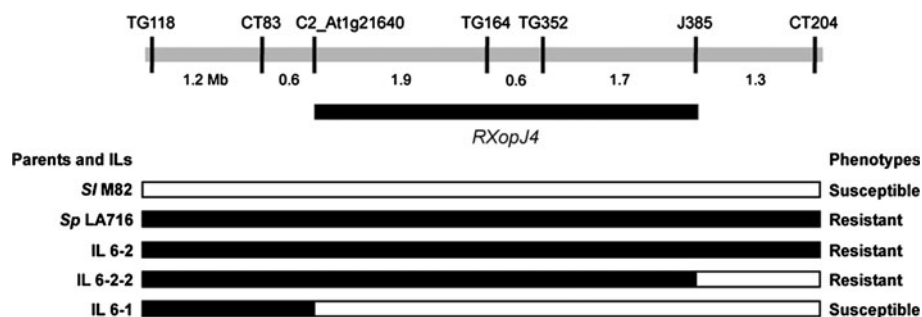
Fig. 2 Initial mapping of the *RXopJ4* resistance locus using *S. pennellii* introgression lines (ILs), showing molecular marker genotypes and phenotypes of parent lines and ILs. *White bars* indicate the marker genotype of *S. lycopersicum* M82 and *black bars* indicate *S. pennellii* LA716. Distances between markers are not drawn to scale but are indicated in megabases (Mb). A *black bar* indicates the 4.2-Mb region containing the *RXopJ4* locus
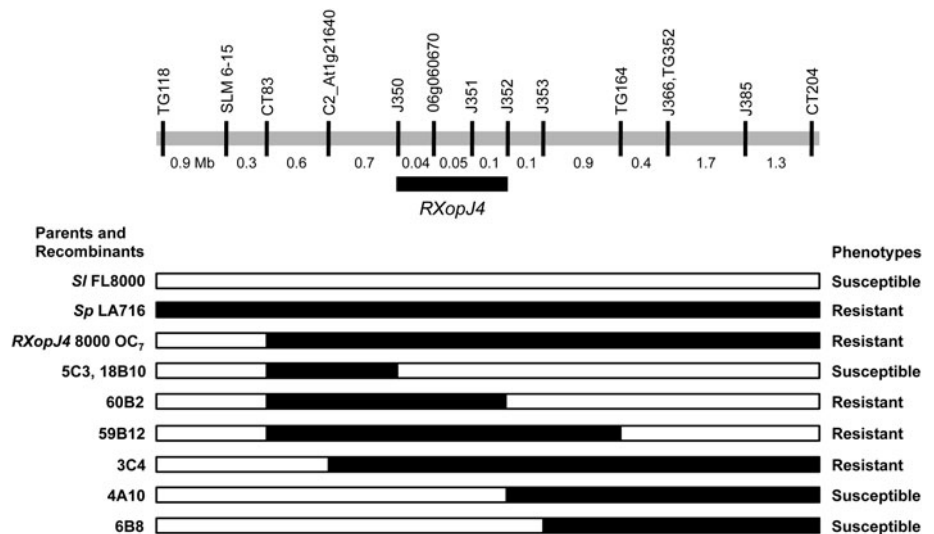
Cosegregation of HR and resistance with *Sp* introgression in an F$_2$ mapping population

The ILs 6-2 and 6-2-2 proved undesirable as resistant parents of an F$_2$ mapping population due to a linkage drag that conferred poor fertility and germination, small leaves, and necrosis of leaves upon exposure to cold or low light intensity, as well as an autogenous necrosis as the plants grew older. Instead, a resistant seventh outcross line from crosses between various commercial tomato lines and *Sp* LA716 was used (see "Materials and methods"). This line displayed a XopJ4-dependent HR (Fig. 1) but was missing part of the *S. pennellii* introgression present in ILs 6-1, 6-2, and 6-2-2 (Figs. 2, 3) and, importantly, also lacked the necrotic phenotype of ILs 6-2 and 6-2-2. A mapping F$_2$ population was generated from a cross between FL8000 and this outcrossed line (designated *RXopJ4* 8000 OC$_7$). To assess the segregation of HR in this mapping population, we inoculated a collection of 17 F$_2$ plants with *Xe* 69-1 containing pLAFR6 or pLAFR6 *xopJ4*, and found that XopJ4-dependent HR segregated as a single locus (Fig. S1). Plants homozygous for *Sp* LA716 at the *RXopJ4* locus exhibited strong HR, while plants homozygous for FL8000 did not exhibit HR. Heterozygous plants occasionally displayed a strong HR, but most produced weak or no HR; only some heterozygous plants showed disease resistance (data not shown), suggesting that *RXopJ4* resistance is semi-dominant.

To verify the disease resistance of the *RXopJ4* 8000 OC$_7$ parent line, we conducted a bacterial growth assay on this line, the susceptible parent FL8000, and *Sp* LA716. In order to assess the XopJ4 dependence of the resistance, we inoculated each line with the bacterial strains *Xp* 4B-WT, 4BΔxopJ4, and 4BΔxopJ4/comp. *Sp* LA716 and *RXopJ4* 8000 OC$_7$ restricted the growth of *Xp* 4B-WT (Fig. 4). This
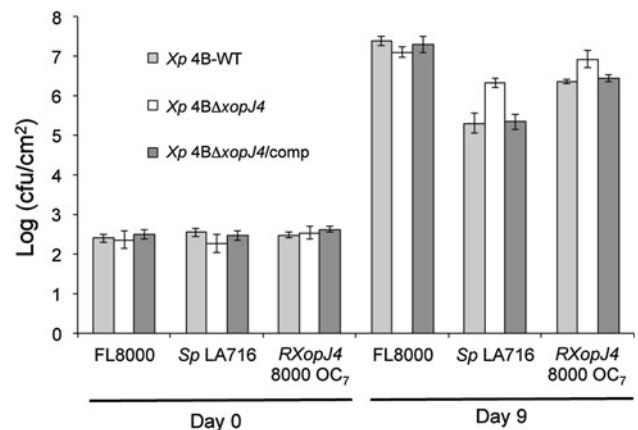

Fig. 4 *Solanum pennellii* LA716 and *RXopJ4* 8000 OC$_7$ display XopJ4-dependent resistance to *X. perforans* infection. Whole leaflets of *Sl* FL8000, *Sp* LA716, or *RXopJ4* 8000 OC$_7$ plants were infiltrated with *Xp* 4B-WT (*light gray bars*), 4BΔxopJ4 (*white bars*), or 4BΔxopJ4/comp (*dark gray bars*). Bacteria were syringe-infiltrated into leaves at 10^4 cfu/ml. Bacterial counts were determined on the day of infiltration (Day 0) and 9 days post infiltration (Day 9). *Error bars* indicate the standard deviation from the mean of 4–6 samples

resistance was dependent on XopJ4 recognition, since these lines were susceptible to the 4BΔxopJ4 deletion strain; complementation of this strain restored recognition by the resistant lines. The *Sl* FL8000 line, however, was susceptible to *Xp* 4B-WT, 4BΔxopJ4, and 4BΔxopJ4/comp strains. The overall lower levels of bacterial growth seen in *Sp* LA716 are likely due to factors other than the *RXopJ4* resistance.

Mapping of the *RXopJ4* resistance locus

Initially, F$_2$ plants from a cross between *Sl* FL7060 and IL 6-2-2 were scored at the genetic markers TG352, CT83, TG164, and CT204. This analysis revealed that the marker
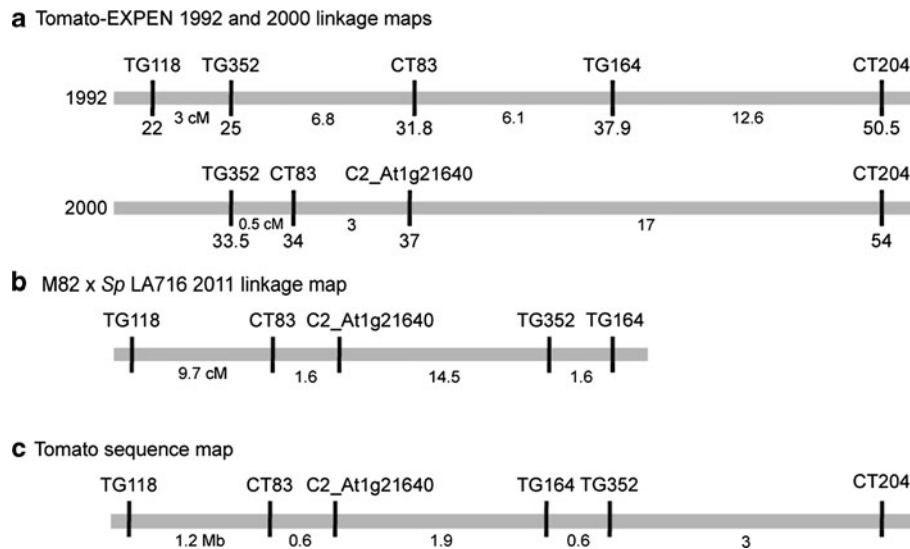
Fig. 5 Maps of the *RXopJ4* region on tomato chromosome 6. Marker names are on the *top* with map positions and distances in centimorgans (cM, **a**, **b**) or megabases (Mb, **c**) between each marker on the *bottom*. Map distances are not to scale. **a** Linkage maps based on F$_2$ individuals from a cross between *S. lycopersicum* cv. VF36 and *S. pennellii* LA716. The Tomato-EXPEN 1992 map is based on RFLP markers and 67 F$_2$ individuals, while the 2000 map is based on RFLP and CAPS markers and 80 F$_2$ individuals; the 2000 map also includes conserved ortholog set (COS) markers such as C2_At1g21640 (Tanksley et al. 1992; Fulton et al. 2002). **b** Linkage map from this study based on CAPS markers and 62 F$_2$ individuals from a cross between *S. lycopersicum* cv. M82 and *S. pennellii* LA716. **c** Sequence map based on tomato genome sequence release SL2.40 (Sato et al. 2012)

positions were not as previously mapped; for instance, among 347 F$_2$ individuals, no recombinants were found between TG352 and TG164, which are 12.9 cM apart on the Tomato-EXPEN 1992 map (Fig. 5a). This could be partially explained by a suppressed homeologous recombination rate between FL7060 and the relatively short *Sp* introgression in IL 6-2-2 (Canady et al. 2006), but could also be due to the difficulty of accurately genotyping with RFLP markers. To remedy the discrepancy between the 1992 linkage map and the observed recombination rate, and to avoid the possible suppressed recombination resulting from a short *Sp* introgression, we mapped five CAPS markers—TG118, CT83, C2_At1g21640, TG352, and TG164—on a *Sl* M82 × *Sp* LA716 F$_2$ population. This revealed, in particular, a location for the marker TG352 that differed from the EXPEN-1992 and 2000 linkage maps (Fig. 5b). At the same time, a fully assembled version of the tomato genome sequence became available (Bombarely et al. 2011; Sato et al. 2012). This provided a more accurate picture of the distances between markers, and also showed that TG164 and TG352 were in the opposite order from the M82 × *Sp* LA716 linkage map—this order was based on a single recombinant (Fig. 5b, c).

Subsequently, the markers CT83, TG352, and J385 were scored on 910 individuals from an FL8000 × *RXopJ4* 8000 OC$_7$ F$_2$ population; 27 recombinant plants were identified—a recombination rate of ~3 % across the 4.8 Mb spanned by these three markers. All F$_2$ plants were recombinant only on one copy of chromosome 6, and thus were heterozygous on one side of the recombination breakpoint. Since, as previously discussed, the resistance phenotype was inconsistent in heterozygotes, the F$_3$ progeny of these plants were collected and homozygous recombinants were scored for both HR and disease resistance. The genotypes and phenotypes of key recombinant individuals are shown in Fig. 3. The left boundary of the *RXopJ4* resistance locus is defined by the recombinants 5C3 and 18B10, which have a crossover from the *Sp* genotype to the *Sl* genotype between J350 and 06g060670, and are susceptible. The right boundary is defined by the recombinant 60B2, which has a crossover from the *Sp* to the *Sl* genotype between J351 and J352 and is resistant. The *Sp* introgressions in these recombinants indicate that the *RXopJ4* locus lies within a 190-kb region between the markers J350 and J352.

Discussion

In the present study, we have defined the *RXopJ4* resistance locus from *S. pennellii* LA716 to a 190-kb segment on the long arm of chromosome 6. We first narrowed the resistance to a 4.2-Mb region within the *Sp* introgressions of ILs 6-2 and 6-2-2. Then, we used a combination of previous linkage maps, our own linkage map of the *RXopJ4* region, and new molecular markers to map the resistance on a segregating FL8000 × *RXopJ4* 8000 OC$_7$ F$_2$ population; this enabled fine mapping to a region of 190 kb. Although

this resistance locus was originally reported to map to chromosome 3 (Astua-Monge et al. 2000), upon re-characterization of the *S. pennellii* ILs, we were unable to reproduce this result (Fig. 1).

The emergence of an annotated genome sequence for *S. lycopersicum* and preliminary genome and mRNA sequence data for *S. pennellii* greatly facilitated the development of precise molecular markers for fine mapping. Recently, the mapping of both a bacterial spot resistance quantitative trait locus (QTL) from *S. lycopersicum* var. *cerasiformae* PI 114490 (Hutton et al. 2010) and a major resistance gene, *Rx4*, from *S. pimpinellifolium* PI 128216 (Pei et al. 2011) was also aided by genome sequence data. The sequencing of more *Solanum* accessions and the identification of SNPs from cultivated varieties will continue to accelerate the process of marker development and thus the mapping of disease resistance and other desirable traits in the future.

Linkage drag is a common problem resulting from the introgression of disease resistance into crop plants by conventional breeding, and has been characterized by Lewis and colleagues (2007) in backcrossed tobacco lines containing the *N* gene, which confers resistance to tobacco mosaic virus. Notably, transgenic lines expressing the *N* gene exhibited significant increases in yield and cash return compared to backcrossed resistant lines. Similarly, the *S. pennellii* LA716 introgression lines 6-2 and 6-2-2 contain the *RXopJ4* resistance locus, but suffer from a linkage drag that confers low fruit yield, small fruit, and an autogenous leaf necrosis. A seventh outcross resistant line showed improved traits and was suitable for mapping of the *RXopJ4* locus, but may still have a disadvantage in the field when compared to the parent line FL8000. Transgenic approaches to disease resistance may mitigate the effects of linkage drag seen in backcrossed lines; alternatively, because of barriers to the acceptance of transgenic crops, backcrossed resistant lines can be developed that contain a minimal genomic region from the wild accession. We are currently working to identify such a recombinant individual containing *RXopJ4* resistance.

Another common feature of disease-resistance genes is incomplete dominance. This has been observed for the *Xv3* and *Rx4* resistance genes, which were identified in different accessions but map to the same region of chromosome 11. Based on allelism tests that failed to identify susceptible F$_2$ plants from a cross between *Xv3* and *Rx4* resistant parents, the genes are either closely linked or are alleles of the same gene (Wang et al. 2011). While *Xv3* heterozygous plants developed HR more slowly than homozygous resistant plants (Wang et al. 2011), *Rx4* heterozygotes showed similar disease ratings to homozygous plants under field conditions (Robbins et al. 2009). *RXopJ4* heterozygotes exhibited slow, weak, or nonexistent HR, and also showed inconsistent phenotypes in disease assays. This could be problematic in the field, since hybrids are usually grown for their increased vigor. However, the heterozygous resistance phenotype may be improved in transgenic plants or in a different genetic background; moreover, the additive effect of multiple resistance genes in a single line may outweigh any reduced resistance due to heterozygosity.

Our current efforts are focused on finding new resistant recombinant plants from the F$_2$ population and employing both the tomato genome sequence and preliminary *S. pennellii* sequence to identify and test candidate resistance genes within the region we have defined here. Once the *RXopJ4* gene has been identified, it can be combined with *Bs2*, perhaps in an elite line containing the bacterial spot resistance QTL identified by Hutton et al. (2010), to produce a line that we expect will possess durable resistance to *X. perforans* in the southeastern United States and other regions where similar strains predominate. Next-generation sequencing now makes it possible to survey pathogen strains from the field for their type III effector repertoires and other virulence components. This approach will aid the development of durable resistance, as the search for new sources of resistance can be targeted to resistance genes that recognize the most conserved pathogen effectors (Bart et al. 2012). Surveying pathogen populations from different geographic areas could also guide strategies for both broadly applicable and region-specific disease resistance. Genetic resistance to bacterial spot disease has heretofore met with limited success in the field. Now, though, the use of genome sequence data for both pathogen and host will enable targeted resistance through precise marker-assisted selection and gene identification. We anticipate that these developments will help to accelerate the process of breeding and gene discovery to produce durably resistant varieties.

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