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ORIGINAL PAPER

Introgressed and endogenous *Mi-1* gene clusters in tomato differ by complex rearrangements in flanking sequences and show sequence exchange and diversifying selection among homologues

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Abstract Many plant disease resistance genes (R-genes) encode proteins characterized by the presence of a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region and occur in clusters of related genes in plant genomes. One such gene, *Mi-1*, confers isolate-specific resistance against root-knot nematodes, aphids and whiteflies in cultivated tomato, *Solanum lycopersicon*. The DNA region carrying *Mi-1* and six closely related sequences was introgressed into tomato from *Solanum peruvianum* in the 1940s. For both susceptible and resistant tomato, *Mi-1* homologues are present in two clusters with 3 and 4 copies each on the short arm of chromosome 6. Two homologues from each source are pseudogenes, and one homologue from each source encodes a truncated product. DNA sequence identity among the homologues including the truncated genes, but excluding the pseudogenes, ranges from 92.9 to 96.7%. All the non-pseudogene homologues are transcribed. Comparison of homologues suggests that extensive sequence exchange has occurred. Regions of diversifying selection are present in the ARC2 domain of the NBS region and dispersed throughout the LRR region, suggesting that these regions are possible locations of specificity determinants. Other sequences in the introgressed region have similarity to the Arabidopsis auxin-receptor protein TIR1, a jumonji-like transcription factor and a Na^+/H^+ antiporter. Analysis of sequences flanking the *Mi-1*-homologues reveals blocks of homology, but complex differences in arrangement of these blocks when susceptible and resistant genotypes are compared indicating that the region has undergone considerable rearrangement during evolution, perhaps contributing to evolution of specificity.

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Introduction

Many plant resistance (R) genes encode proteins characterized by the presence of a nucleotide-binding site (NBS) and a carboxyterminal leucine-rich repeat (LRR) region (reviewed in Dangl and Jones 2001; Takken et al. 2006). These proteins are distributed across a wide range of plant taxa and are commonly found in clustered gene families (Hulbert et al. 2001; Cannon et al. 2002; Michelmore and Meyers 1998). One function of these proteins is to serve as surveillance molecules that detect infection by specific pathogens and pests (Chisholm et al. 2006). There has been considerable speculation about whether the clustered organization contributes to sequence exchanges among R genes and whether such exchanges contribute to the evolution of resistance specificities (Kuang et al. 2004; Mondragon-Palomino and Gaut 2005; Meyers et al. 2005).

One such NBS-LRR gene, *Mi-1*, confers resistance against three species of root-knot nematodes, potato aphids and whiteflies in cultivated tomato (Milligan et al. 1998; Rossi et al. 1998; Nombela et al. 2003; Vos et al. 1998). *Mi-1*encodes a protein of 1,257 amino acids and has been mapped to the short arm of chromosome

6 (Kaloshian et al. 1998; Milligan et al. 1998). The short arm of chromosome 6 in various *Solanum* species carries an impressive collection of R-genes effective against diverse and economically important crop pathogens. *Rpi-blb2*, which confers resistance to the oomycete *Phytophtora infestans*, maps to the corresponding region in the diploid potato relative *Solanum bulbocastanum*, and the encoded protein is 82% identical in sequence to Mi-1 (van der Vossen et al. 2005). *Cf2* and *Cf5*, genes conferring resistance to the fungus *Cladosporium fulvum*, encode proteins with a signal peptide, extracellular LRR region and a C-terminal membrane anchor, and are telomeric, but tightly linked, to *Mi-1* (Dixon et al. 1995, 1998; Kaloshian et al. 1998). Several R-genes that have not yet been cloned also map to the region. An additional root-knot nematode-resistance gene, *Mi-9*, which is effective at higher temperatures than is *Mi-1*, maps to the same chromosomal interval as *Mi-1* (Ammiraju et al. 2003). *Ol-4* and *Ol-6*, genes for resistance to the fungus *Oidium neolycopersici*, and *Am*, which encodes alfalfa mosaic virus resistance, map to the short arm of chromosome 6 (Bai et al. 2005; Parella et al. 2004). Quantitative resistance traits *Ty-1* and *Bw-5*, for tomato yellow leaf curl virus and bacterial wilt, respectively, as well as traits for resistance to *Erwinia carotova* and *P. infestans* also map to this chromosomal region (Zamir et al. 1994; Thoquet et al. 1996; Gebhardt and Valkonen 2001). Remarkably, cytological studies and molecular marker analyses indicate that this chromosome arm may contain only 1.4 Mb of euchromatic DNA (Zhong et al. 1999).

Many disease resistance genes were bred into crop plants from a related wild species. *Mi-1* was introgressed into cultivated tomato, *Solanum lycopersicon* (previously *Lycopersicon esculentum*), from a nematode resistant accession of the complex species *Solanum peruvianum*, using embryo rescue to obtain a hybrid of these normally incompatible species (Smith 1944). Following extensive breeding, the introgressed region of the genome was reduced to about 650 kb on the short arm of chromosome 6. Further attempts to reduce the size of the introgression were frustrated by repressed recombination in this area (Ho et al. 1992; Liharska et al. 1996; Kaloshian et al. 1998). Introgression of desirable traits from wild relatives is a common practice, but is often accompanied by reduced recombination and maintenance of undesirable traits (Ganal and Tanksley 1996; Schulte et al. 2006; Mondragon-Palomino and Gaut 2005; Moreau et al. 1998).

NBS-LRR proteins mediate recognition of pathogen infection and initiate defense signaling leading to host resistance (Belkhadir et al. 2004). The NBS in plant R-proteins is part of a larger, highly conserved domain of approximately 320 amino acids that is characteristic of a large class of proteins widely represented in bacteria, plants and animals and generally involved in signal transduction associated with large conformational changes upon NTP hydrolysis (Leipe et al. 2004). In plant R-genes this domain is referred to as the NB-ARC, due to conservation of sequence with a group of nucleotide binding (NB) proteins including plant R-proteins, animal cell-death effectors such as the mammalian Apaf-1 and *C. elegans* CED-4 (van der Biezen and Jones 1998; Leipe et al. 2004). Several of these proteins, including Mi-1, have been shown to bind and hydrolyze ATP (Tameling et al. 2002). In vitro mutagenesis and immunoprecipitation experiments indicate that NB-ARC domain of at least some R genes is involved in intramolecular interactions with other domains of the protein, or interactions with other proteins (Mestre and Baulcombe 2006; Moffett et al. 2002). LRR regions are found in diverse proteins, and in several examples have been shown to function as sites of protein–protein interaction or protein ligand binding. LRR regions are generally the most variable regions among closely related *R*-genes. Comparison of nucleotide and codon sequences reveals that LRR regions encoded by closely-related R-genes are subject to diversifying selection, suggesting that these regions are under positive selection and may be involved in recognition of pathogen-derived or pathogen-modified ligands (Meyers et al. 1998; Dodds et al. 2006; Bergelson et al. 2001). There is also evidence that LRRs play a role in downstream signaling (Warren et al. 1998; Banerjee et al. 2001; Hwang and Williamson 2003).

NBS-LRR proteins can be divided into two groups, those that carry a Toll-interleukin (TIR) domain at their N-terminus and those that do not (Belkhadir et al. 2004). The latter class is often characterized by the presence of a predicted coiled-coil domain in the N-terminal region. Compared to a typical non-TIR NBS-LRR protein, Mi-1 carries an N-terminal extension of about 200 amino acids. In vitro mutagenesis experiments indicate that the N-terminal extension of Mi-1.2 is a negative regulator of defense signaling by this gene (Hwang and Williamson 2003; Wrobel, Telleen and Williamson, unpublished). Related extensions are present on the products of the tomato genes *Sw-5* conferring resistance to spotted wilt virus, *Hero*, which encodes resistance to cyst nematodes, and *Prf*, which is required for bacterial wilt resistance, as well as the potato genes *R1* and *Rpi-blb2*, which confer resistance to the oomycete *Phytophtora infestans* (Ernst et al. 2002; Ballvora et al. 2002; Brommonschenkel et al. 2000; Salmeron et al. 1996).

The availability of genomic clones from the corresponding regions of resistant and susceptible tomato provided an opportunity to compare the structure of the endogenous locus in susceptible tomato to that of the *S. peruvianum* introgression in resistant tomato. Here we compare the DNA sequences of the *Mi-1* homologues as well as their expression, organization, and flanking sequences. Tomato and potato belong to closely related but distinct sections (Lycopersicon and Petola, respectively) of the large genus *Solanum* (Bohs and Olmstead 1997). The availability of the sequence of the MiGH *Rpi-blb2* from a potato relative allowed a broader perspective for comparison of the region. These comparisons indicate that this region is evolutionarily very dynamic, possibly contributing to development of new resistance specificities.

Materials and methods

Sequence determination and assembly of MiGHs and flanking sequences

BAC clones *Ble 2* and *Ble 8* that span the *MiGH* Cluster 2e and 1e were previously isolated from nematode susceptible tomato Heinz 1706 (Seah et al. 2004). BAC *B170* is a subclone of YAC clone *Y54D9* (Seah et al. 2004) and contains all 4 *MiGHs* of Cluster 2p from resistant tomato. DNA was extracted from BAC clones by alkaline lysis using the CONCERT™ high purity plasmid midiprep system (Life Technologies, Rockville, Md, USA). Three strategies were utilized to obtain plasmid subclones from these BAC clones for sequencing:

1. *Shotgun cloning*. 10 µg of DNA was hydrosheared using a nebulizer (Invitrogen, Carlsbad, CA, USA) to 0.4–3 kb fragments. The sheared DNA was precipitated then resuspended in TE and size fractionated using CHROMA SPIN™-400 columns (BD Biosciences Clontech, Palo Alto, CA, USA) to remove DNA fragments smaller than 600 bp. The sheared, fractionated DNA was repaired and blund-ended using T4 DNA polymerase (Promega, Madison, WI, USA) and Klenow DNA polymerase (Promega) then purified and concentrated using the DNA Clean & Concentrator™-5 kit (Zymo Research, Orange, CA, USA). The DNA fragments were A-tailed and cloned into the pGEM®-T easy vector (Promega).
2. *Cloning of BAC DNA fragments*. BAC DNA was digested with *Bam*HI, *Bst*XI, *Eco*RI, *Hin*DIII, *Kpn*I, *Sac*I or *Xba*I and purified using the CHROMA SPIN™-100 columns (BD Biosciences Clontech). Restriction fragments were cloned into linearized and dephosphorylated pBluescript® SK+ vector (Stratagene, La Jolla, CA, USA).
3. *PCR amplification*. PCR For/Rev primer pairs from the *Mi-1* gene sequence, shown in Table 1, were used in all combinations to amplify *Mi-1* homologue sequences directly from the BAC clones. The amplified sequences were extracted from gels and purified using the CONCERT™ rapid gel extraction system (Life Technologies) and cloned into the pGEM®-T easy vector.

DNA from the subclones was sequenced in both directions using the M13 For/Rev or the T7/SP6 universal sequencing primers.

Sequences of larger inserts were completed by primer walking. Sequencing was performed using the dye-primer method (CEPRAP, University of California, Davis) or the dye terminator method (UC Davis Division of Biological Science DNA sequencing facility or CAES genomics facility). Raw DNA sequence data were visually inspected and vector sequences removed manually using the editing program Chromas 1.45 (Technelysium Pty Ltd, Tewantin, QLD, Australia). The sequence contigs for the three BAC clones were assembled using the computer program Sequencher™ 4.0.5 (Gene Codes Corporation, Ann Arbor, MI, USA).

Table 1 PCR primers used for BAC contig assembly and RT-PCR analysis

Primer[a]	Sequence (5′–3′)[b]	Location in *Mi-1*
Mi-ATG-F	ATGGAAAAACGAAAAGATAW	Translation start
Mi-PL-F	GGTTCGGTAAAACTACTTTG	Beginning of NBS region
Mi-PL-R	CAAAGTAGTTTTACCTGAACC	Beginning of NBS region
Mi-HDL-F	ATTTCCAAATTCATGATCTT	Between NBS and LRR domains
Mi-HDL-R	AAGTCATGCACAAGATCATG	Between NBS and LRR domains
Mi-STOP-R	CTACTTAAATAAGGGGATAT	Translation stop
Mi-intron1-F	GCCTSTGTRAAGGTAMCATCTT	Spans intron-1 5′ splice site
Mi-intron1-R	GATTCTACTTTTTCTCWAGACC	Spans the intron-1 3′ splice site

[a] F and R following primer name indicates priming of the sense and antisense strand, respectively

[b] Some nucleotides are degenerate W = A/T, R = A/G, S = G/C, M = A/C

RT-PCR detection of Mi-1 homologue transcripts

Leaves and roots were harvested from three plants each of 2 and 6 week-old tomato plants (cultivars Motelle and Heinz 1706) grown in sand at 19°C under a 16 h photoperiod. Total RNA was extracted from pooled, harvested leaves or roots from each growth period using the RNeasy plant mini kit (Qiagen, Valencia, CA, USA). The plant tissue was homogenized in at least 10 ml of lysis buffer (from supplier) at 450 µl buffer per 100 mg plant tissue using a rotor-stator homogenizer. Subsequent steps were as directed by the supplier. mRNA was purified from the total RNA mixture using the Biomag® SelectaPure™ mRNA Purification System (Polysciences Inc., Warrington, PA, USA). Contaminating DNA was removed by treating a 23.3 µl aliquot of the mRNA solution with 1 µl of RQ1 RNase-free Dnase (Promega) for 30 min at 37°C. The reaction was inactivated by the addition of 3 µl of 10× stop buffer (from the supplier).

Five microliter of each DNA-free mRNA sample was used for cDNA synthesis with the Superscript™ One-Step RT-PCR with Platinum® *Taq* kit (Invitrogen). Expressed *Mi-1* transcripts were subsequently amplified with the primers Mi-ATG-F and Mi-PL-R (Table 4) under PCR conditions described above. The amplified RT-PCR products were gel purified, cloned into the pGEM®-T easy vector, and DNA sequence of insert was determined.

Sequence analysis

The Wisconsin Genetics Computer Group (Madison, WI, USA) suite of programs was used for analysis of contig sequences. The BESTFIT program was used to determine sequence similarity, the PILEUP program was used for comparing multiple sequences, and Genedoc Version 2.6 was used to visualize the sequence files generated by the PILEUP program (Nicholas et al. 1997). A nucleic acid dot plot program from the website http://www.vivo.colostate.edu/molkit/dnadot was used for sequence comparisons. The Synonymous/Non-synonymous Analysis Program (SNAP) based on the method of Nei and Gojobori (1986) was used to calculate the synonymous and non-synonymous substitution rates (Korber 2000; http://www.hcv.lanl.gov/content/hcv-db/SNAP/SNAP.html).

RFLP analysis

End clones B170-F and B170-R were isolated from BAC *B170* by TAIL-PCR and used as RFLP probes as described in Seah et al. (2004).

Results

Comparison of MiGH sequences

Seven *Mi-1* gene homologues (MiGHs) are present on the short arm of chromosome 6 in both susceptible and resistant tomato (Seah et al. 2004). In resistant tomato, we refer to the homologues as *Mi-1.1* through *Mi-1.7*, with *Mi-1.2* corresponding to the functional nematode R-gene. All seven copies in resistant tomato are contained in a contiguous 650-kb region introgressed from *S. peruvianum*, and are grouped into two clusters, 1p and 2p, separated by 300 kb (Fig. 1a). The homologues in the corresponding region of susceptible tomato are referred to as *Mi-1A* through *Mi-1G* and are also organized in two clusters, 1e and 2e (Fig. 1b). Although the resistant and susceptible tomato loci carry the same number and distribution of homologues, positions of molecular markers indicate that the sequences flanked by the two clusters are inverted on the chromosome in resistant relative to susceptible tomato (Fig. 1; Seah et al. 2004).

DNA sequences that span each MiGH in Cluster 2p, Cluster 2e, and Cluster 1e were obtained from BACs *B170*, *Ble2* and *Ble8*, respectively (Seah et al. 2004), and assembled into eight contigs ranging from 13 to 28 kb (Table 2; Fig. 1). The 52 kb BAC clone, *BAC3*, which spans Cluster 1p in nematode resistant tomato, was previously sequenced (Milligan et al. 1998). Based on contig sequences, we classified four homologues, *Mi-1.3*, *Mi-1.5*, *Mi-1A* and *Mi-1D*, as pseudogenes because they carry large insertions and deletions that result in lack of a complete ORF compared to *Mi-1.2*. In all four cases parts or all of intron 1 and parts of the N-terminal region are missing. Two additional homologues, *Mi-1.6* and *Mi-1G*, carry single nucleotide changes that result in a truncated ORF. *Mi-1.6* encodes an ORF of 224 amino acids due to a frameshift mutation. This sequence diverges from that of *Mi-1.2* at position 206 and 18 non-homologous amino acids follow before the stop codon. *Mi-1G* encodes a truncated ORF of 996 amino acids due to a 1 bp substitution. For both *Mi-1.6* and *Mi-1G*, a complete ORF could be restored by a single nucleotide change. Sequence identity between the predicted MiGH-coding regions including the truncated homologues, ranges from 92.8 to 96.8% (Table 3). At the protein level, the similarity and identity range from 92.6–96.4 to 87.3–93.8%, respectively.

Comparison of the homologue sequences suggested that a patchwork of sequence exchanges had occurred. For example, short insertions or deletions (indels) of 3–20 nucleotides are present between homologues, but

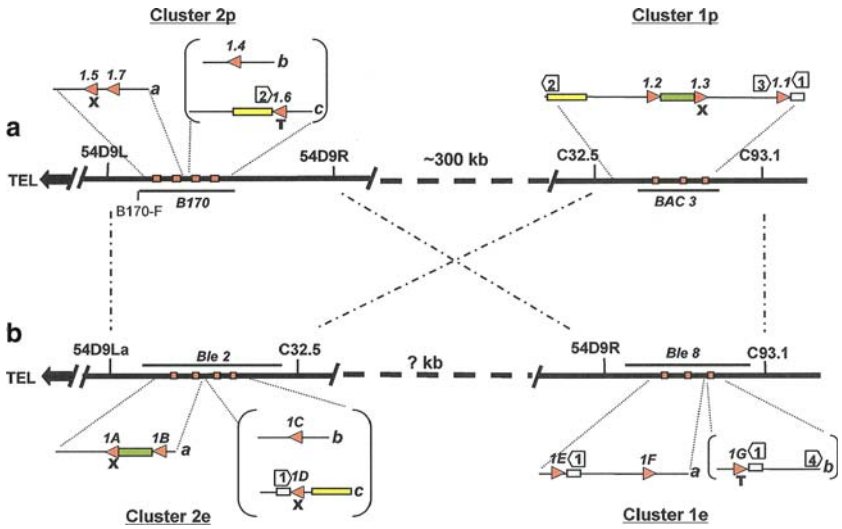

Fig. 1 Comparison of the *Mi-1* locus in resistant and susceptible tomato. *Thick horizontal lines* represent the region of the short arm chromosome 6 in nematode resistant tomato that was introgressed from *S. peruvianum* (Lp) (**a**) and the corresponding sequence from susceptible tomato (**b**). *Red boxes* indicate approximate positions of *Mi-1* homologues. Homologues are present in two clusters in both resistant tomato (Clusters 1p and 2p) and susceptible tomato (Clusters 1e and 2e). Positions of BAC clones BAC3, B170, Ble2, and Ble8 spanning the homologue clusters and of key RFLP markers are indicated. Expanded regions correspond to the sequenced contigs (Table 2). For contigs in *brackets*, the orientation of the contig on the chromosome could not be determined. The *Mi-1* homologues are shown as *red triangles* with the point directed to the 3′ end. An '**X**' is marked below *Mi-1* pseudogene sequences and truncated homologues are indicated by 'T.' The *numbers in box-arrows* correspond to the putative genes listed in Table 2 ('1' resembles *TIR-1*; '2,' jumonji family transcription factor; '3,' transposase; '4,' Na^+/H^+ transporter) with the point indicating the predicted transcript direction. *Boxed segments* of the same color are highly similar in sequence (*yellow blocks* are ~7 kb and are 94–97% identical to each other; *green blocks* are ~5 kb and are 97–99% identical; blue blocks, ~3 kb, are 94–97% identical). The green sequence between Mi-1A and Mi-1B is in opposite orientation relative to the chromosome compared to the corresponding block between Mi-1.2 and Mi-1.3 on BAC3

Table 2 MiGHs and other putative genes on contigs

Sequence contig				Other gene homologues on contig	
Name	Size (nt)	Accession no.	MiGHs	Name and accession number of related gene	*E* value
BAC3	51,953	U81378	*Mi-1.1, Mi-1.2 Mi-1.3*	Transcription factor, jumonji family Arabidopsis, NP_172338	2e-20
				Tam3 transposase *Anthirrhinum majus*, BAB20481	6e-39
				Transport inhibitor response-1 Arabidopsis, NP_567135	4e-109
B170a	14,431	DQ863286	*Mi-1.5, Mi-1.7*	None	–
B170b	13,888	DQ863287	*Mi-1.4*	None	–
B170c	21,744	DQ863288	*Mi-1.6*	Transcription factor, jumonji family Arabidopsis, NP_172338	9e-28
Ble2a	21,466	DQ863289	*Mi-1A, Mi-1B*	None	–
Ble 2b	13,249	DQ863290	*Mi-1C*	None	–
Ble 2c	17,118	DQ863291	*Mi1-D*	Transport inhibitor response-1 Arabidopsis, NP_567135	8e-96
Ble 8a	28,152	DQ863292	*Mi-1E, Mi-1F*	Transport inhibitor response-1 Arabidopsis, NP_567135	2e-107
Ble 8b	18,285	DQ863293	*Mi-1G*	Transport inhibitor response-1 Arabidopsis, NP_567135	2e-80
				Na+/H+ antiporter, isoform 1 *Lycopersicon esculentum*, CAC84522	7e-22

comparison of their locations among homologues does not reveal a clear evolutionary relationship either within or between loci (Fig. 2a). Also, the distance trees produced for N-terminus, NBS, and LRR regions using *Rpi-blb2* as an outgroup are incongruent, consistent with the occurrence of frequent sequence exchanges among the homologues (Fig. 2b). Thus, sequence comparisons could not identify orthologous relationships of homologues between genotypes or infer evolutionary relationships between paralogs in the same background.

Each intact MiGH contains two introns. Intron 1 is upstream of the start codon and ranges in length from 556 to 2,199 bp for the *S. peruvianum*-derived paralogs and from 630 to 1,287 bp for those in *S. lycopersicon*. For comparison among homologues, we divided intron

Table 3 Comparison of sequences of MiGH ORFs

	Mi-1.1	*Mi-1.2*	*Mi-1.4*	*Mi-1.6*[a]	*Mi-1.7*	*Mi-1B*	*Mi-1C*	*Mi-1E*	*Mi-1F*	*Mi-1G*[a]
Mi-1.1		95.3	94.8	94.8	94.7	94.3	95.1	94.9	93.2	95.0
Mi-1.2	95.1/91.4		95.6	95.0	95.6	95.0	95.8	96.0	92.9	96.2
Mi-1.4	93.6/90.1	95.0/91.4		95.0	96.7	95.5	96.8	96.6	93.4	96.4
Mi-1.6	94.4/90.4	95.1/90.9	94.0/90.3		95.4	94.6	94.7	95.4	93.5	95.2
Mi-1.7	94.0/90.7	95.7/91.7	96.2/93.3	95.6/91.5		95.4	96.6	96.1	94.6	96.4
Mi-1B	93.8/89.4	95.0/90.5	94.6/91.2	94.7/90.4	95.4/91.6		96.2	96.0	92.8	95.8
Mi-1C	94.9/91.4	95.8/92.1	96.2/93.7	94.5/89.9	96.4/93.4	95.5/92.7		96.3	93.4	96.2
Mi-1E	94.4/90.8	95.6/92.1	96.2/93.7	94.8/91.5	96.2/92.9	95.6/92.4	95.9/92.8		93.6	96.6
Mi-1F	92.6/88.0	93.4/87.8	93.1/88.2	93.9/88.4	94.4/90.3	92.9/87.3	93.4/88.6	93.8/89.5		93.5
Mi-1G	94.4/90.7	96.0/92.7	95.7/92.9	94.9/91.2	96.2/93.4	95.2/92.1	96.0/93.0	96.0/93.7	93.8/89.5	

[a] Percentage nucleotide sequence identity is indicated above the shaded boxes, and percentage protein sequence similarity/identity is below

[b] The *Mi-1.6* (G deletion at position 619) and *Mi-1G* (T to C at position 2989) sequence have been artificially corrected to produce complete ORFs. Pseudogenes *Mi-1.3*, *Mi-1.5*, *Mi-1A*, and *Mi-1D* are not included in this table

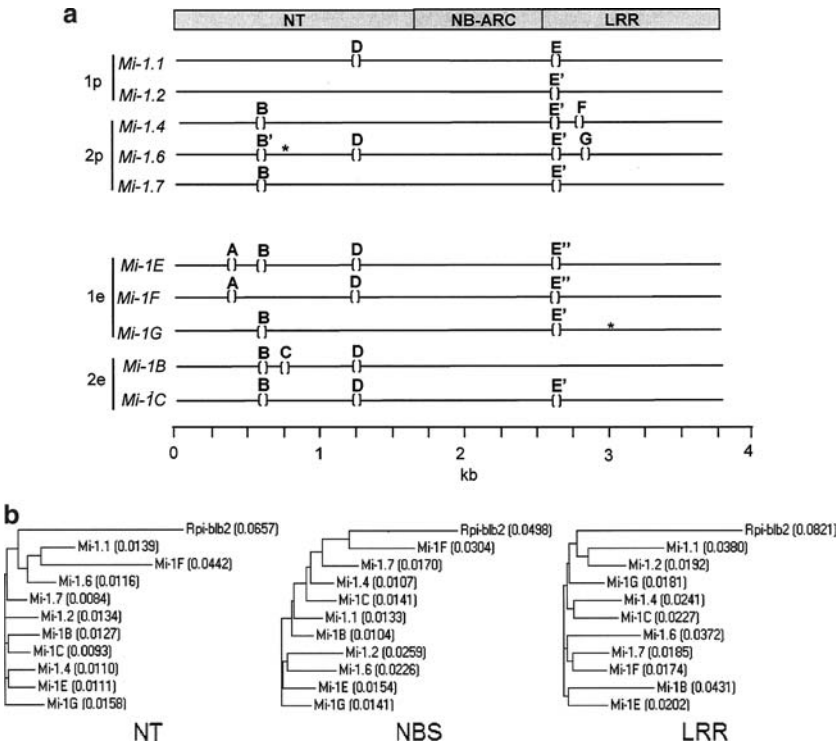

Fig. 2 Evidence for gene conversion among MiGHs. **a** Positions of indels, designated by *brackets* are compared for *Mi-1* homologues. If nucleotides are numbered based on the longest concensus sequence, starting with the start codon, the indel positions would be deletions at the following positions: *A* 320–331; *B* 603–617; *B′* 605–634; *C* 661–669; *D* 1,296–1,298; *E* 2,635–2,646; *E′* 2,635–2,643, *E′′* 2,626–2,643; *F* 2,740–2,751; *G* 2,804–2,809. *Asterisk* indicates positions of stop codons caused by deletion b' in *Mi-1.6* or single base pair substitution for *Mi-1G*. **b** Comparison of distance trees of NT, NBS and LRR regions of MiGHs. The *S. bulbocastanum* gene *Rpi-blb2* was used as an outgroup. Trees were produced with AlignX from Vector NTI Advance (Invitrogen) using the Neighbor Joining method of Saitou and Nei (1987). The *value in parentheses* is the calculated distance

1 of *Mi-1.2* into 6 segments with Segment I most distal and Segment VI most proximal to the coding region (Fig. 3). Each homologue is missing one or more of these segments compared to *Mi-1.2*. Segment VI is present in all intron 1 sequences and is highly conserved (95.1–98.4% nt identity). Segment II is also present in all the intron 1 sequences, however the sequences within this segment are more variable (68.4–92.8% nt identity). Intron 1 of *Mi-1.7* contains a large insertion within segment II that is also present in *Mi-1B* and there is a smaller insertion in region VI of the *Mi-1.6*. Intron 2, which is located within the coding

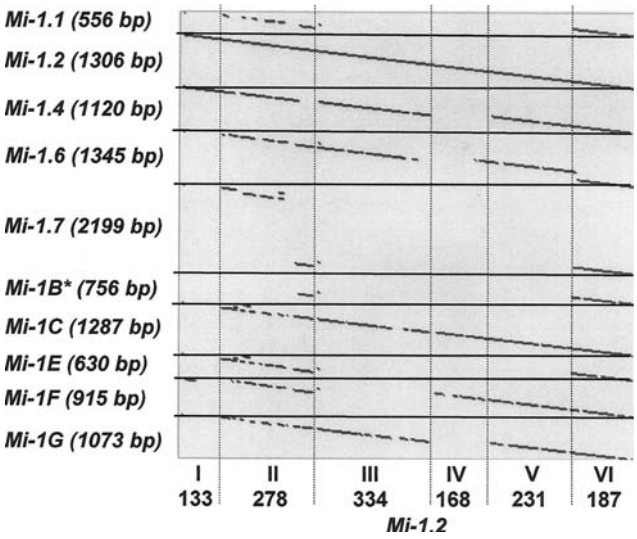

Fig. 3 Dot plot comparison of deduced intron-1 sequences of *Mi-1.2* to each of the other *Mi-1* homologue intron-1 sequences. The *numbers in parentheses* indicate the length of the intron. The *horizontal axis* represents intron 1 of *Mi-1.2* and is divided into segments I through VI, 5′ to 3′. The size of each segment in nucleotides is indicated. Window size = 15; Mismatch limit = 1. *The sequence of the upstream junction of intron-1 of *Mi-1B* was not determined

region near the N-terminus, is 75 bp in all homologues and is highly conserved in sequence. Sequence variation is limited to 8 bp with at most three differences from *Mi-1.2*. Analysis of introns also failed to reveal orthologous relationships between the homologues.

A strong preference for non-synonymous compared to synonymous codon substitutions has been noted in the LRR region of other R-gene families (Bittner-Eddy et al. 2000; Dodds et al. 2001; Meyers et al. 1998; Sun et al. 2006). To investigate whether MiGHs exhibited diversifying selection, codon-aligned nucleotide sequences of the MiGHs were compared using a SNAP (Korber 2000). A strong bias toward diversifying nucleotide changes is apparent in the LRR region and also in the 3′ region of the NB-ARC domain (Fig. 4).

The *Mi-1* homologue clusters are in inverted orientation relative to each other in both resistant and susceptible tomato

The three *Mi-1* homologues in Cluster 1p were previously determined to be in the same orientation with predicted transcription direction from telomeric to centromeric (Fig. 1) (Milligan et al. 1998). Contigs B170a, B170b, and B170c span the MiGHs in Cluster 2p. Contig B170a contains *Mi-1.7* and *Mi-1.5*, which are 2 kb apart with ORFs oriented in the same direction (Fig. 1). By probing a DNA blot of YAC clones, *Y1172*, *Y54D9*, and *Y1256*, which span the *Mi-1* locus (Vos et al. 1998; Seah et al. 2004), with BAC-end probes B170F and B170R, we determined that B170F is telomeric and B170R is centromeric to Cluster 2p on chromosome 6 (data not shown). Sequence of contig B170a places BAC end clone B170F downstream of

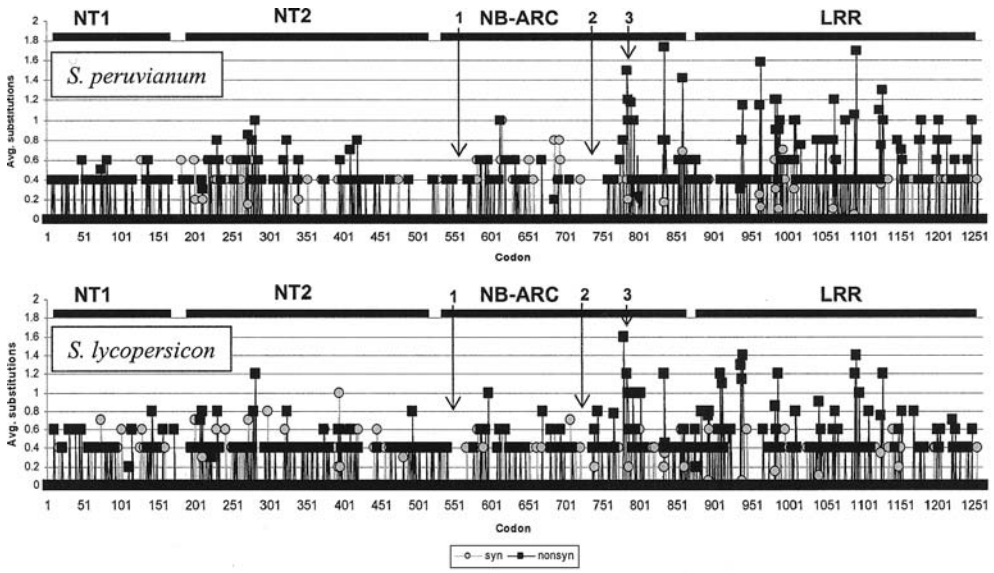

Fig. 4 Synonymous and non-synonymous nucleotide substitutions in coding regions of MiGHs. The N-Terminal (*NT*), nucleotide binding site (*NB-ARC*) and leucine-rich repeat (*LRR*) domains that span the *Mi-1* gene are depicted by *horizontal bars*. Two highly conserved regions required for nucleotide binding (*1* P-loop; *2* GxP region) and a region of ARC2 (3) displaying a high level of non-synonymous substitutions are indicted. Rates were calculated based on codon-aligned nucleotide sequences consisting of the full-length sequences and the truncated MiGHs. Homologues in *Solanum lycopersicon* and in the introgressed region from *S. peruvianum* were calculated separately

Table 4 MiGH transcripts in resistant and susceptible tomato

Cluster	Homologue	ORF	Matching transcripts[a]				
			2 week-old leaves	2-week old roots	6 week-old leaves	6 week-old roots	Expressed
1p	*Mi-1.1*	1,255 aa	0/1	1/2	0/11	0/5	Yes
1p	*Mi-1.2*	1,257 aa	0/1	0/2	3/11	1/5	Yes
2p	*Mi-1.4*	1,248 aa	1/1	0/2	3/11	1/5	Yes
2p	*Mi-1.6*	224 aa[b]	0/1	1/2	5/11	1/5	Yes
2p	*Mi-1.7*	1,252 aa	0/1	0/2	0/11	2/5	Yes
2e	*Mi-1B*	1,251 aa	0/11	1/7	0/8	1/11	Yes
2e	*Mi-1C*	1,251 aa	7/11	1/7	7/8	1/11	Yes
1e	*Mi-1E*	1,244 aa	0/11	2/7	1/8	1/11	Yes
1e	*Mi-1F*	1,249 aa	0/11	1/7	0/8	2/11	Yes
1e	*Mi-1G*	996 aa[c]	4/11	2/7	0/8	6/11	Yes

[a] Transcript matches among RT PCR products using primers Mi-ATG-F and Mi-PL-R and mRNA from resistant tomato cv. Motelle, which carries the *L. peruvianum* introgression carrying the *Mi-1* locus or from the susceptible tomato line Heinz 1906. The numerator indicates the number of times the sequence was detected and the denominator the total number of clones in each category sequenced;

[b] Due to frameshift mutation

[c] Due to 1 bp substitution

Mi-1.5; therefore, we can conclude that the ORFs of both *Mi-1.5* and *Mi-1.7* are oriented centromeric to telomeric, the opposite orientation from the *Mi-1* homologues in Cluster 1p (Fig. 1). The orientation and order of homologues *Mi-1.4* and *Mi-1.6* could not be determined from available data.

Comparison of hybridization patterns of overlapping susceptible tomato BAC clones, including *Ble2* and *Ble8*, using flanking markers allowed us to determine relative orientation and position of some paralogs (Seah et al. 2004). In Cluster 2e, *Mi-1A* is most telomeric followed by *Mi-1B*, but we could not determine the relative order of *Mi-1C* and *Mi-1D*. In Cluster 1e *Mi-1F* is centromeric to *Mi-1E* and telomeric to *Mi-1G*. *Mi-1A* and *Mi-1B* are both present on contig *Ble2a* and are in the same orientation. Likewise, *Mi-1E* and *Mi-1F* are in the same orientation on contig Ble8a. From this information, we can conclude that *Mi-1A* and *Mi-1B* are oriented in the centromeric to telomeric direction whereas *Mi-1E* and *Mi-1F* are oriented from telomeric to centromeric direction. We have not been able to deduce the orientation of *Mi-1C*, *Mi-1D* and *Mi-1G* on chromosome 6. In summary, the homologues for which it was possible to determine the orientation are present in clusters that are inverted relative to each other in both the susceptible tomato locus and the *S. peruvianum* introgression in nematode resistant tomato (Fig. 1).

Expression patterns of MiGHs

Since several of the MiGHs appear to be intact genes, it was of interest to determine whether they are expressed. We extracted RNA from leaves and roots of 2- and 6-week-old resistant and susceptible plants, then cloned and sequenced RT-PCR products using primers that span intron-2. The primers were designed to be able to amplify cDNA from all of the homologues except for the four pseudogenes, which lack the translation start codon. Sequence analysis identified products corresponding to each of the homologues, including *Mi-1.6* and *Mi-1G*, which do not contain a complete ORF, in at least one of the samples tested indicating that all are expressed (Table 4). Due to the small number of transcript clones sequenced (1–11 from each tissue and age type), the absence of sequence corresponding to a certain homologue in a particular tissue/time does not mean that that homologue is not expressed. For example, although we did not identify *Mi-1.2* transcripts in our sampling of clones from 2-week-old roots of Motelle, it is known from previous work that *Mi-1.2* is expressed in roots of this age (Goggin et al. 2004; Martinez de Ilarduya et al. 2001).

Characterization of MiGH-flanking sequences

In addition to the 14 MiGHs, other sequences with similarity to known protein-coding genes were identified on the contigs by BLASTX searches (Table 2). Most notable are several regions of sequence with similarity to the Arabidopsis transport inhibitor response-1 (*TIR-1*) gene. *TIR-1* encodes an E3 ubiquitin ligase SCF complex F-box protein in Arabidopsis and has recently been identified as the primary auxin receptor (Ruegger et al. 1998; Dharmasiri et al. 2005). *TIR-1*-related sequences are present just downstream from *Mi-1.1, Mi-1D, Mi-1E* and *Mi-1G*, and, if expressed,

would be transcribed on the opposite strand from the MiGHs (Fig. 1). Although these sequences are related to *TIR-1*, analysis of exon–intron structure and searches against solanaceous ESTs suggest that they are not the tomato *TIR-1* homologues but may encode F-box/LRR proteins with another function (data not shown). Two contigs derived from resistant tomato encode a putative protein with similarity to an Arabidopsis transcription factor (jumonji) family protein, and similarity to a tomato Na^+/H^+ antiporter was identified downstream from *Mi-1G* (Table 2; Fig. 1).

Comparison of the sequence of BAC3, which carries the functional nematode resistance gene *Mi-1.2*, to other contigs revealed blocks of synteny. For example, an ~5 kb region (yellow block in Fig. 1) is duplicated in Cluster 2p and Cluster 2e (>94% sequence identity). This block also includes a 382 nt terminal-repeat retrotransposon in miniature (TRIM) element (Witte et al. 2001) on both contigs BAC3 and B170c. A TRIM element was also identified on Ble2, but not joined to the contig Ble2C (data not shown). The ~5 kb block of non-coding sequence between *Mi-1.2* and *Mi-1.3* (green block in Fig. 1a) is >97% identical to a block of sequence between *Mi-1A* and *Mi-1B* (Fig. 1b). Additional small regions of homology to BAC3 are dispersed among the contigs, including short stretches of sequence (200–500 nt) with high similarity to *Mi-1* coding region or the 3′ end of intron 1. The microsatellite $(AT)_n$ was also found dispersed through several of the BACs.

Discussion

Comparison of MiGH sequences reveals active gene conversion among copies

Nucleotide sequence identity among the MiGHs in nematode-susceptible tomato and in the *S. peruvianum* introgression in resistant tomato lines, including the truncated genes, ranges from 92.9 to 96.7%. Two lines of evidence indicate that considerable sequence exchange has occurred between homologues. Positions of indels in the MiGHs do not reveal a simple path of evolution and distance trees derived from different domains of the coding sequences are incongruent. Similar evidence for sequence exchange has been obtained for clusters of R-genes in diverse plant species (cf., Ellis et al. 2000; Meyers et al. 1998; Kuang et al. 2004, 2005). However, other R-genes do not appear to actively exchange sequences. Based on this, R-genes have been divided into two types: fast evolving type I genes are characterized by frequent sequence exchange among members, whereas slowly evolving type II R-genes appear to have little exchange between paralogous sequences (Kuang et al. 2004, 2006). By these criteria, the MiGH families in both genotypes analyzed here would be classified as type I.

A strong preference for non-synonymous compared to synonymous nucleic acid changes has been observed in the LRR region of several NBS-LRR gene families and non-synonymous nucleotide substitutions also dominate in the LRR region of MiGHs. This preference is indicative of diversifying selection and may reflect evolution of novel interaction specificities for proteins involved in ligand binding or signaling (Holt et al. 2003; Kuang et al. 2004). However, we see no change in LRR copy number or insertions/deletions in this region in contrast to what has been observed with the nearby *Cf-2* and *Cf-5* genes (Dixon et al. 1998). Other groups have suggested that the positively selected positions in the LRR were highest in the predicted ß-strand submotif that is likely to be solvent exposed (Meyers et al. 1998; Mondragon-Palomono et al. 2002; Ayliffe et al. 2000). However, due to the degenerate nature of the LRRs in Mi-1 and its homologues, we feel that it is premature to make such a determination for the MiGH family. High non-synonymous substitution rates are also present in part of the central NB-ARC region of MiGHs (Fig. 4). Comparison to related proteins for which crystal structure has been solved suggests that the NB-ARC domain of R-proteins can be divided into three subdomains: NB, ARC1, and ARC2 (Albrecht and Takken 2006). The diversifying selection is localized to the ARC2 domain suggesting that this region is involved in specific inter- or intramolecular interactions. Positive selection in a similar region has previously been noted in a subset of groups of Arabidopsis R-gene homologues (Mondragon-Palomino et al. 2002). Interestingly, recent molecular studies with potato R-genes *Rx* and *Gpa2* have indicated that the ARC2 region may be crucial for maintaining the auto-inhibited state of NBS-LRR proteins by interaction with its cognate LRR region as well as for activation (Rairdan and Moffett 2006).

MiGHs have two introns in conserved positions, intron 1 in the 5′UTR and intron 2 in the coding region. Intron 2 is highly conserved in size and sequence among the MiGHs in resistant and susceptible tomato. Interestingly, intron 2 of *Rbi-blb2* from *S. bulbocastanum* differs from that of *Mi-1.2* by only 4 nt and 2 small insertions of 3 and 8 nt. This conservation could reflect a role in regulation of expression of the R gene. Alternatively, the sequence conservation could be due to gene conversion between homologues as has been suggested to explain homogenization of introns in

Dm3 gene family (Kuang et al. 2004). For intron 1, only the segment proximal to the gene is highly conserved among the tomato homologues. In fact, the 80 nt at the 3′ end of intron 1 of *Mi-1.2* differ from those of the potato gene *Rbi-blb2* by only 8%. In contrast, the size and sequence of the remainder of intron 1 varies among the homologues (Fig. 3) and no clear similarity was seen for *Rbi-blb2* sequences. The greater divergence in intron 1 may indicate that there is less selective pressure on this sequence, or, alternatively, that this sequence is outside the region targeted for gene conversion in the plant.

Function of MiGHs

So far the function, if any, of the sequenced MiGHs other than *Mi-1.2* and *Rpi-blb2* is not known. Sequence analysis of RT-PCR products indicates that all homologues that contain an intact N-terminus, whether with full-length or truncated coding regions, are expressed. In addition, all indels except one are in multiples of three and thus maintain the reading frame. Sequences that are required for nucleotide binding in NB-ARC proteins such as the P-loop and GxP motifs are highly conserved in MiGHs (Fig. 4) suggesting that the encoded proteins are able to bind nucleotides. Silencing using RNAi may be a useful strategy to investigate function of MiGHs, and, in tomato roots, this strategy has been demonstrated to result in loss of nematode resistance (Collier et al. 2005; Valentine et al. 2004). Silencing may allow us to determine whether other R-genes that map to this region are, in fact, encoded by MiGHs. However, for those homologues not associated with a segregating phenotype, it may be difficult to identify a clear function, and it is possible that the primary function of some homologues is not related to disease or pest resistance. In addition, the sequence similarity between homologues makes it likely that non-target homologues will also be silenced leading to ambiguity regarding the role of specific MiGHs without coupling to a forward genetic approach.

While sequence analysis indicates that two of the homologues from each background are clearly pseudogenes, one homologue from *S. peruvianum* and one from *S. lycopersicon* encode truncated reading frames of 224 and 996 amino acids, respectively. These truncated genes are expressed, and appear to be competent to produce a translation product corresponding to the N-terminal region of the protein. Previous work showed that when the N-terminal region of *Mi-1.2* was co-expressed with a constitutively lethal mutant of *Mi-1.2*, this region was able to repress the lethality (Hwang and Williamson 2003). In addition, in vitro mutagenesis has identified several single amino acid substitutions in the N-terminal 161 amino acids of Mi-1.2 that confer a lethal phenotype upon transient expression in *Nicotiana benthamiana* leaves (Wrobel, Telleen, and Williamson unpublished). These results suggest that the N-terminal regions of the truncated genes—or perhaps the full-length genes—may be translated and their products may function in trans to regulate Mi-1.2 or other R-gene products. As precedent for such a regulatory mechanism, the NBS-LRR protein Prf is required for function of the R gene product Pto and the NBS-LRR gene *NRG1* is required for resistance mediated by the tobacco mosaic virus resistance gene *N* (Salmeron et al. 1996; Peart et al. 2005). Also, an alternatively spliced, truncated transcript of the *N* gene has also been shown to be required along with the full length version for complete TMV resistance further supporting the possibility that truncated NBS-LRR gene products may play an as yet unknown role in resistance (Dinesh-Kumar and Baker 2000).

Evolutionary rearrangements in the MiGH region

In this paper, we show that MiGH-flanking sequences in both susceptible tomato and the introgression in resistant tomato include duplicated blocks of highly conserved sequences in addition to MiGHs. For example, a sequence block carrying an *MiGH* and a downstream sequence similar to Arabidopsis auxin receptor *TIR-1* (blue box in Fig. 1) appears to be duplicated in several locations in the MiGH region of *S. lycopersicon*. Blocks of highly homologous, non-coding sequences (yellow and green blocks in Fig. 1) are present in the clusters, but in relatively different positions in susceptible and resistant tomato genotypes. Smaller blocks of homology and fragments of *Mi-1*-like sequence are also scattered throughout the region. The varying positions of these sequence blocks indicate that considerable rearrangements have occurred during evolution. In both nematode-susceptible tomato and tomato with the introgressed *Mi-1* locus, MiGHs occur in two clusters in inverted repeat orientation. Intrachromosomal reciprocal recombination within inverted MiGH sequences would be predicted to lead to an inversion of the intervening sequence. By comparing markers flanking each cluster, we previously deduced that there had been an inversion of the DNA between the two clusters in *S. lycopersicon* compared to the *S. peruvianum* derived sequences at the *Mi-1* locus (Seah et al. 2004). While a single inversion between homologues could have resulted in the exchange of specific sequence blocks, the complex sequence differences between the two genotypes cannot be explained by only one inversion event.

Several events including intra- and interchromosomal recombination and/or gene conversion likely distinguish the regions possibly including inversions originating within the R-gene paralogs. The *R1* (late-blight resistance) cluster also displays rearrangements and low level of colinearity between *Solanum demissum* haplotypes (Kuang et al. 2005).

The existence of two clusters of genes in inverted orientation likely predates speciation of *S. lycopersicon* and *S. peruvianum*. In fact, a similar arrangement of homologue sequences is apparent for the corresponding region in the more distantly related species *S. bulbocastanum* although the number of copies is expanded from 7 to 15 (van der Vossen et al. 2005). A DNA blot survey of solanacous species using the Mi-1.2 NT and NBS regions as probe indicates that MiGHs are broadly present throughout this group but that the copy number varies considerably between species (Seah and Williamson, unpublished). However, DNA blots and comparison to available genomic sequence do not reveal similar genes in Arabidopsis or rice. Intrachromosomal recombination could lead to cycles of expansion and contraction of copy number as well as scrambled arrangement of flanking sequences. However, due to the complexity of the sequence order differences, understanding the evolutionary history will require extensive analysis of additional solanaceous genomes. An appropriate group for analysis may be the diverse and complex accessions of *S. peruvianum* in which many resistance traits have been mapped to the region and for which RFLPs in *Mi-1* and flanking markers are abundant (Kaloshian et al. 1998; Ammiraju et al. 2003).

There is a sequence with similarity to a transposase upsteam from *Mi-1.1* and we have noted TRIM elements in the MiGH region. Transposons have been postulated to have a role in R-gene evolution (Wei et al. 2002;Witte et al. 2001) and their presence could have played a role in MiGH evolution.

Introgressed regions and plant breeding

Recombination is severely repressed in the *Mi-1* region in crosses between susceptible tomato and lines carrying the introgressed region. By searching for recombination events using flanking markers, several recombination points were identified flanking, but not within the 650 kb region carrying the two clusters. The likely lethality of recombination events within the inverted region may be a partial explanation for this repression. However, even for intraspecific *S. peruvianum* crosses where recombination is approximately eight fold higher, events are not uniformly distributed in the region and there is repression of recombination (Kaloshian et al. 1998). The MiGH region in *S. bulbocastanum* carrying *Rpi-blb2* also displays repressed recombination in wide crosses (van der Voosen et al. 2005). The *Mi-1* region borders the centromeric heterochromatin of chromosome 6 and is part of the limited (1.4 Mb) euchromatic region on this short chromosomal arm (Zhong et al. 1999). This region is highly enriched in R genes and it is tempting to speculate that some feature of the chromosome structure may facilitate complex exchanges among paralogs.

Many R-genes or other agronomically important traits are introgressed into elite cultivars after wide crosses. For *Mi-1.2*, due to repressed recombination, at least 600 kb of continguous *S. peruvianum* sequence remains in even the most advanced resistant lines. This region could contain wild species alleles of other traits capable of affecting agronomic properties. For example, several sequences in the MiGH region bear coding regions with significant similarity to that of *TIR1*, which encodes the auxin receptor of Arabidopsis. While these sequences may not correspond to auxin receptors in tomato, members of this family of proteins interacts with specific proteins and target them for destruction by the proteosome (Gagne et al. 2002). It is conceivable that different alleles of such genes would contribute to agronomic differences between cultivars with or without the introgressed region. Alleles of the transcription factor gene and a putative Na^+/H^+ antiporter is also in the introgressed region and could also contribute to phenotype differences observed between nematode resistant and susceptible tomato. Other MiGHs in the cluster may also contribute to other subtle differences in phenotype.

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References

Albrecht M, Takken FLW (2005) Update on the domain architectures of NLRs and R proteins. Biochem Biophys Res Commun 339:459–462

Ammiraju JSS, Veremis JC, Huang X, Roberts PA, Kaloshian I (2003) The heat-stable, root-knot nematode resistance gene *Mi-9* from *Lycopersicon peruvianum* is localized on the short arm of chromosome 6. Theor Appl Genet 106:478–484

Ayliffe MA, Collins NC, Ellis JG, Pryor A. (2000) The maize *rp1* rust resistance gene identifies homologues in barley that

have been subject to diversifying selection. Theor Appl Genet 100:1144–1154

Bai Y, van der Hulst R, Bonnema G, Marcel TC, Meijer-Dekens F, Niks RE, Lindhout P (2005) Tomato defense to *Oidium neolycopersici*: dominant *Ol* genes confer isolate-dependent resistance via a different mechanism than recessive *ol-2*. Mol Plant Microbe Interact 18:354–362

Ballvora A, Ercolano MR, Weiss J, Meksem K, Bormann CA, Oberhagemann P, Salamini F, Gebhardt C (2002) The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant J 30:361–371

Banerjee D, Zhang X, Bent AF (2001) The leucine-rich repeat domain can determine effective interaction between *RPS2* and other host factors in Arabidopsis RPS2-mediated diseases resistance. Genetics 158:439–450

Belkhadir Y, Subramaniam R, Dangl JL (2004) Plant disease resistance protein signaling: NBS-LRR proteins and their partners. Curr Opin Plant Biol 7:391–399

Bergelson J, Kreitman M, Stahl EA, Tian D (2001) Evolutionary dynamics of plant *R*-genes. Science 292:2281–2285

Bittner-Eddy PD, Crute IR, Holub EB Beynon JL (2000) *RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. Plant J 21:177–188

Bohs L, Olmstead RG (1997) Phylogenetic relationships in *Solanum* (Solanaceae) based on *ndhF* sequences. Syst Bot 22:5–17

Brommonschenkel SH, Frary A, Frary A, Tanksley SD (2000) The broad-spectrum tospovirus resistance gene *Sw-5* of tomato is a homolog of the root-knot nematode resistance gene *Mi*. Mol Plant Microbe Interact 13:1130–1138

Cannon SB, Zhu HY, Baumgarten AM, Spangler R, May G, Cook DR, Young ND (2002) Diversity, distribution, and ancient taxanomic relationships within the TIR and non-TIR NBS-LRR resistance gene subfamilies. J Mol Evol 54:548–562

Chisholm S, Coaker G, Day B, Staskawicz BJ (2006) Host–microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Collier R, Fuchs B, Walter N, Lutke WK, Taylor CG (2005) Ex vitro composite plants: an inexpensive, rapid method for root biology. Plant J 43:449–457

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defense responses to infection. Nature 411:826–833

Dharmasiri N, Dhamasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445

Dinesh-Kumar SP, Baker BJ (2000) Alternatively spliced N resistance gene transcripts: their possible role in tobacco mosaic virus resistance. Proc Natl Acad Sci USA 97:1908–13

Dixon MS, Jones DA, Hatzixanthis K, Ganal MW, Tanksley SD, Jones JDG (1995) High resolution mapping of the physical location of the tomato *Cf-2* gene. Mol Plant Microbe Interact 8:200–206

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG (1998) The tomato *Cf-5* disease resistance gene and six homologues show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell 10:1915–1925

Dodds P, Lawrence G, Ellis J (2001) Six amino acid changes confined to the leucine-rich repeat beta-strand/beta-turn motif determine the difference between the *P* and *P2* rust resistance specificities in flax. Plant Cell 13:163–178

Dodds PN, Lawrence GJ, Catanzariti A, Teh T, Wang CA, Ayliffe MA, Kobe B, Ellis JG (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proc Natl Acad Sci USA 103:8888–8893

Ellis J, Dodds P, Prior T (2000) The generation of plant disease resistance gene specificities. Trends Plant Sci 5:373–379

Ernst K, Kumar A, Krisleit D, Kloos DU, Phillips MS, Ganal MW (2002) Broad-spectrum potato cyst nematode resistance gene (*Hero*) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. Plant J 31:127–136

Gagne JM, Downes BP, Shiu SH, Durski AM, Vierstra RD (2002) The F-box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc Natl Acad Sci USA 99:11519–11524

Ganal MW, Tanksley SD (1996) Recombination around the *Tm2a* and *Mi* resistance genes in different crosses of *Lycopersicon peruvianum*. Theor Appl Genet 92:101–108

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. Annu Rev Phytopathol 39:79–102

Goggin FL, Shah G, Williamso VM, Ullman DE (2004) Developmental regulation of Mi-mediated aphid resistance is independent of *Mi-1.2* transcript levels. Mol Plant Microbe Interact 17:532–536

Ho J-Y, Weide R, Ma HM, Wordragen MF, Lambert KN, Koornneef M, Zabel P, Williamson VM (1992) The root-knot nematode resistance gene (*Mi*) in tomato: Construction of a molecular linkage map and identification of dominant cDNA markers in resistant genotypes. Plant J 2:971–982

Holt BF, Hubert DA Dangl JL (2003) Resistance gene signaling in plants—complex similarities to animal innate immunity. Curr Opin Immun 15:20–25

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Ann Rev Phytopathol 39:285–312

Hwang CF, Williamson VM (2003) Leucine-rich repeat mediated intramolecular interactions in nematode recognition and cell death signaling by the tomato resistance protein *Mi*. Plant J 34:585–593

Kaloshian I, Yaghoobi J, Liharska T, Hontelez J, Hanson D, Hogan P, Jesse T, Wijbrandi J, Simons G, Vos P, Zabel P, Williamson VM (1998) Genetic and physical localization of the root-knot nematode resistance locus *Mi* in tomato. Mol Gen Genet 257:376–385

Korber B (2000) HIV signature and sequence variation analysis. In: Rodrigo AG, Learn GH (eds) Computational analysis of HIV molecular sequences. Kluwer, Dordrecht, pp 55–72

Kuang H, Woo S-S, Meyers BC, Nevo E, Michelmore RW (2004) Multiple genetic processes result in heterogeneous rates of evolution within the major cluster disease resistance genes in lettuce. Plant Cell 16:2870–2894

Kuang H, Wei F, Marano MR, Wirtz U, Wang X, Liu J, Shum WP, Zaborsky J, Tallon LJ, Rensink W, Lobst S, Zhang P, Tornqvst CE, Tek A, Bamberg J, Helgeson J, Fry W, You F, Luo M-C, Jiang J, Buell CR, Baker B (2005) The R1 resistance gene cluster contains three groups of independently evolving, type R1 homologues and shows substantial structural variation among haplotypes of *Solanum demissum*. Plant J 44:37–51

Kuang H, Ochoa OE, Nevo E, Michelmore RW (2006) The disease resistance gene *Dm3* is infrequent in natural populations of *Lactuca serriola* due to deletions and frequent gene conversions at the *RGC2* locus. Plant J 47:38–48

Leipe DD, Koonin EV, Aravind L (2004) STAND, a class of P-loop NTPases including animal and plant regulators of programmed cell death: multiple, complex domain architec-

tures, unusual phyletic patterns, and evolution by horizontal gene transfer. J Mol Biol 343:1–28

Liharska TB, Koornneef M, van Wordragen M, van Kammen A, Zabel P (1996) Tomato chromosome 6: effect of alien chromosomal segments on recombinant frequencies. Genome 39:485–491

Martinez de Ilarduya O, Kaloshian I (2001) *Mi-1.2* transcripts accumulate ubiquitously in resistant *Lycopersicon esculentum*. J Nematol 33:116–120

Mestre P, Baulcombe DC (2006) Elicitor-mediated oligomerization of the tobacco N disease resistance protein. Plant Cell 18:491–501

Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore RW (1998) Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 11:1833–1846

Meyers BC, Kaushik S, Nandety RS (2005) Evolving disease resistance genes. Curr Opin Plant Biol 8:129–134

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319

Moffett P, Farmham G, Peart JR, Baulcombe DC (2002) Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. EMBO J 21:4511–4519

Mondragon-Palomino M, Gaut BS (2005) Gene conversion and the evolution of three leucine-rich repeat gene families in *Arabidopsis thaliana*. Mol Biol Evol 22:2444–2456

Mondragon-Palomino M, Meyers BC, Michelmore RW, Gaut BS (2002) Patterns of positive selection in the complete NBS-LRR gene family of *Arabidopsis thaliana*. Genome Res 12:1305–1315

Moreau P, Thoquet P, Oivier J, Laterrot H, Grimsley N (1998) Genetic mapping of *Ph-2*, a single locus controlling partial resistance to *Phythophtora infestans* in tomato. Mol Plant Microbe Interact 11:259–269

Nei M, Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol 3:418–426

Nicholas KB, Nicholas HB Jr, Deerfield DW II (1997) GeneDoc: analysis and visualization of genetic variation. EMBNEW NEWS 4:14

Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant Microbe Interact 16:645–649

Parrella G, Moretti A, Gognalons P, Lesage M-L, Marchoux G, Gebre-Selassie K, Caranta C (2004) The *Am* gene controlling resistance to *Alfalfa mosaic virus* in tomato is located in the cluster of dominant resistance genes on chromosome 6. Phytopathol 94:345–350

Peart JR, Mestre P, Lu R, Malcuit I, Baulcombe DC (2005) NRG1, a CC-NB-LRR protein, together with N, a TIR-NB-LRR protein, mediates resistance against tobacco mosaic virus. Curr Biol 15:968–973

Rairdan GJ, Moffett P (2006) Distinct domains of the ARC region of the potato resistance protein Rx mediate LRR binding and inhibition of activation. Plant Cell 18:2082–2093

Rossi M, Goggin F, Milligan SB, Kaloshian I, Ullman D, Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95:9750–9754

Ruegger M, Dewe E, Grey WM, Hobbie L, Turner J, Estelle M (1998) The TIR1 protein of Arabidopsis functions in auxin response and is related to human SKP2 and yeast Grr1p. Genes Dev 12:198–207

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–25

Salmeron JM, Oldroyd GED, Rommens CMT, Scofield SR, Kim HS, Lavelle DT, Dahlbeck D, Staskawicz BJ (1996) Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. Cell 86:123–133

Schulte D, Cai DG, Kleine M, Fan LJ, Wang S, Jung C (2006) A complete physical map of a wild beet (*Beta procumbens*) translocation in sugar beet. Mol Gen Genomics 275:504–511

Seah S, Yaghoobi J, Rossi M, Gleason CA, Williamson VM (2004) The nematode-resistance gene, *Mi-1*, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. Theor Appl Genet 108:1635–1642

Smith PG (1944) Embryo culture of a tomato species hybrid. Proc Am Soc Hort Sci 44:413–416

Sun XL, Cao YL, Wang SP (2006) Point mutations with positive selection were a major force during the evolution of a receptor-kinase resistance gene family of rice. Plant Physiol 140:998–1008

Takken FLW, Albrecht M, Tameling WIL (2006) Resistance proteins: molecular switches of plant defence. Curr Opin Plant Biol 9:383–390

Tameling WI., Elzinga SDJ, Darmin PS, Vossen JH, Takken FLW, Haring MA, Cornelissen BJC (2002) The tomato R gene products I-2 and Mi-1 are functional ATP binding proteins with ATPase activity. Plant Cell 14:2929–2939

Thoquet P, Oliver J, Sperisen C, Rogowsky P, Laterrot H, Grimsley N (1996) Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii. Mol Plant-Microbe Interact 9:826–836

Valentine T, Shaw J, Blok VC, Phillips MS, Oparka KJ, Lacomme C (2004) Efficient virus-induced gene silencing in roots using a modified tobacco rattle virus vector. Plant Physiol 136:3999–4009

van der Biezen E, Jones J (1998) The NB-ARC domain: a novel signaling motif shared by plant resistance gene products and regulators of cell death in animals. Curr Biol 8:R226–R227

Van der Vossen EAG, Gros J, Sikkema A, Muskens M, Wouters D, Wolters P, Pereira A, Allefs S (2005) The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homologue conferring broad-spectrum late blight resistance in potato. Plant J 44:208–222

Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, de Both M, Peleman J, Liharska T, Hontelez J, Zabeau M (1998) The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. Nat Biotech 16:1365–1369

Warren RF, Henk A, Mowery P, Holub E Innes RW (1998) A mutation within the leucine-rich-repeat domain of the Arabidopsis disease resistance gene RPS5 partially suppresses multiple bacterial and downy mildew resistance genes. Plant Cell 10:1439–1452

Wei F, Wing RA, Wise RP (2002) Genome dynamics and evolution of the *Mla* (powdery mildew) resistance locus in barley. Plant Cell 14:1903–1917

Witte CP, Le QH, Bureau T, Kumar A (2001) Terminal-repeat retrotransposons in miniature (TRIM) are involved in restructuring plant genomes. Proc Natl Acad Sci USA 98:13778–13783

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, van-Oss H, Kedar N, Rabinowitch HD, Czosnek H (1994) Mapping and introgression of a *tomato yellow leaf curl virus* tolerance gene, *TY-1*. Theor Appl Genet 88:141–146

Zhong XB, Bodeau J, Fransz PF, Williamson VM, van Kammen A, de Jong JH, Zabel P (1999) FISH to meiotic pachytene chromosomes of tomato locates the root knot nematode resistance gene *Mi-1* and the acid phosphatase gene *Aps-1* near the junction of euchromatin and pericentromeric heterochromatin of chromosome arms 6S and 6L, respectively. Theor Appl Genet 98:365–370