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Characterization of disease resistance gene-like sequences in near-isogenic lines of tomato

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Abstract We examined near-isogenic lines (NILs) carrying either of the tomato mosaic virus (ToMV) resistance genes *Tm-1* and *Tm-2* for sequences homologous to the isolated disease-resistance genes. DNA fragments were amplified from the genomic DNA of the NILs by the polymerase chain reaction (PCR) using primers designed on the basis of sequences of certain domains conserved among some disease-resistance genes. Of ten PCR products cloned, five were identified as having homology to either of the two classes of disease-resistance genes. The first class encoded proteins containing leucine-rich repeats (LRRs) and a nucleotide-binding site (NBS), such as the *RPS2* gene in *Arabidopsis* and the *N* gene in tobacco. The second class encoded proteins containing a C-terminal membrane anchor but no NBS, such as the *Cf2* and *Cf9* genes in tomato. In Southern hybridization of the genomic DNAs of the NILs carrying either *Tm-1* or *Tm-2* and their parental NIL carrying neither of these resistance genes, multiple bands could be detected with most of the clones used as probes. This suggests that the genomes of the NILs contain multiple copies of sequences homologous to some of the known disease-resistance genes. No evidence was obtained to show that the *Tm-1* and/or *Tm-2* loci encode either class of protein, since no polymorphic band patterns between the NILs were detected by Southern hybridization.

Key words Disease-resistance gene · *Lycopersicon esculentum* · Near-isogenic line (NIL) · Polymorphism · Tomato mosaic virus (ToMV)

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Introduction

The genes *Tm-1* and *Tm-2*, conferring resistance to tomato mosaic virus (ToMV) in the tomato, *Lycopersicon esculentum*, originated from the wild relatives of tomato, *L. hirsutum* and *L. peruvianum*, respectively. Using near-isogenic lines (NILs) of tomato, we have mapped both gene loci with a number of random amplified polymorphic DNA (RAPD) markers and sequence-characterized amplified-region (SCAR) markers converted from the RAPD markers (Ohmori et al. 1995 a, b, 1996; Motoyoshi et al. 1996). We have been examining whether the NILs with *Tm-1*, *Tm-2* or without ToMV-resistance genes carry the sequences similar to those of disease-resistance genes already isolated and characterized.

Recently, a number of genes involved in defense responses to specific pathogens or specific races of pathogens have been isolated by positional cloning, transposon-tagging, or based on sequence homologies to other reported genes. Proteins encoded by these genes are categorized into three distinct groups: those containing both leucine-rich repeats (LRRs) and a nucleotide-binding site (NBS) (group 1); those having a C-terminal membrane anchor but no NBS (group 2); and those having a serine-threonine protein-kinase domain, some of which also contain LRRs (group 3).

The genes encoding the group-1 proteins are *RPS2* (Bent et al. 1994; Mindrinos et al. 1994) and *RPM1* (Grant et al. 1995), conferring race-specific resistance to *Pseudomonas syringae* pv *maculicola* and pv *tomato*, respectively, in *Arabidopsis thaliana*; *N* to tobacco mosaic virus (TMV) in tobacco (Whitham et al. 1994; Dinesh-Kumer et al. 1995); *L6* (Lawrence et al. 1995) and *M* (Anderson et al. 1997) to *Melampsora lini* in flax; and *Prf* (Salmerson et al. 1996) and *I2C-1* (Ori et al. 1997) to *P. syringae* pv *tomato* and *Fusarium oxysporium* f. sp. *lycopersici* race2, respectively, in tomato. The genes encoding the group-2 proteins are *Cf2* and

Cf9 conferring resistance to specific races of *Cladosporium fulvum* (Jones et al. 1994; Dixon et al. 1996). The protein encoded by $Hs1^{pro-1}$ conferring nematode (*Heterodera schachtii*) resistance in sugar beet, may also be a member of group 2, because it carries LRRs and a putative membrane-spanning segment (Cai et al. 1997). The genes encoding the group-3 proteins are *Pto* conferring resistance to *P. syringae* pv *tomato* in tomato (Martin et al. 1993), *Xa21* to *Xanthomonas oryzae* in rice (Song et al. 1995), and *Lr10* to leaf rust in wheat (Feuillet et al. 1997). However, Pto protein lacks LRRs (Martin et al. 1993), whereas Xa21 and Lr10 proteins have no extracellular LRRs (Song et al. 1995; Feuillet et al. 1997).

Although the overall DNA sequences of these resistance genes are highly divergent, significant similarity can often be identified between the deduced amino-acid sequences in limited regions (Grant et al. 1995; Staskawicz et al. 1995). Sequences homologous to known resistance genes were identified in a number of products amplified by the polymerase chain reaction (PCR) from the genomic DNAs of potato (Leister et al. 1996) and soybean (Kanazin et al. 1996; Yu et al. 1996) using the primers based on the sequences highly conserved between *PRS2* and *N*.

In the present study, amino-acid sequences with a consensus to certain domains in the group-1 and -2 protein genes described above were employed to design PCR primers for amplifying genomic DNA fragments from the NILs. Some amplified DNA fragments showed high or moderate similarities to the sequences of the resistance genes. Here, we characterize those PCR products, and also discuss their relationship to the *Tm-1* and *Tm-2* genes.

Materials and methods

Plant material

Three near-isogenic lines (NILs) were used. GCR26 is a tomato variety 'Craigella' which is susceptible to tomato mosaic virus (ToMV). GCR236 and GCR237 have a common genetic background of 'Craigella', but the former carries *Tm-2*, and the latter *Tm-1*. These lines, originally bred at the Glasshouse Crops Research Institute, Littlehampton, UK (Smith and Ritchie 1983), have been maintained through selfing those used in previous studies (Motoyoshi and Oshima 1975, 1977).

PCR analysis

Total genomic DNA (100 ng) extracted from young leaves and purified by CsCl gradient-centrifugation (Ohmori et al. 1996) was amplified with 1.25 U of *Taq* DNA polymerase (Perkin Elmer Cetus) in 25 μl of 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.001% (w/v) gelatin, 0.2 mM dNTPs and 1 μM of each primer. The primer sequences are listed in Table 1. Primers were used in combinations of S1 and AS1 (I), S2 and AS2 (II) and S3 and AS3 (III). Combinations I and III were used for PCR of the genomic DNA from GCR236 (*Tm-2*/*Tm-2*), and II and III for PCR of the genomic DNA from GCR237 (*Tm-1*/*Tm-1*). Annealing temperatures were 60°C for I and 45°C for II. In combination III, the genomic DNA from GCR236 (*Tm-2*/*Tm-2*) was annealed at 58°C and the genomic DNA from GCR237 (*Tm-1*/*Tm-1*) at 45°C. PCRs were repeated for 30 cycles of 1-min denaturation at 94°C, 1-min annealing at the temperatures as above, and 2 min elongation at 72°C. The products were separated by electrophoresis in a 1.4% agarose gel, and stained with ethidium bromide.

Table 1 Primers designed for PCR on the basis of amino-acid sequences conserved between the proteins encoded by genes *RPS2* in *A. thaliana* and *N* in tobacco, and those between genes *Cf2* and *Cf9* in tomato

Primers to detect sequences homologous to *RPS2* and *N*[a]									
		N-terminal							
Amino-acid sequence		G	P	G	G	V	G	K	T	T
Sense-primer	S1	5′-GGA	CCT	GGT	GGG	GTT	GGG	AAG	ACA	AC-3′
	S2			5′-GGI	GGI	GTI	GGI	AA(A/G)	ACI	AC-3′
		C-terminal								
Amino-acid sequence		G	L	P	L	A	L			
Antisense-primer	AS1	3′-CCT	AAC	GGT	GAT	CGC	AAC-5′			
	AS2	3′-CCI	(G/A)AI	GGI	(G/A)AI	CGI	(G/A)A-5′			
Primers to detect sequences homologous to *Cf2* and *Cf9*[a]									
		N-terminal							
Amino-acid sequence		S	N	K	L	H	G	P	I	
Sense-primer	S3	5′-(A/T)(G/C)I	AA(C/T)	AA(A/G)	(C/T)TI	CA(C/T)	GGI	CCI	AT-3′	
		C-terminal								
Amino-acid sequence		G	E	I	P	Q	Q	L	A	
Antisense-primer	AS3	3′-CCI	CT(T/C)	TAI	GGI	(T/G)CT	GT(T/C)	(G/A)AI	CG-5′	

[a] Primer combination I, S1 and AS1; II, S2 and AS2; III, S3 and AS3

Cloning and sequencing of amplification products

PCR products were extracted with phenol/chloroform, and precipitated with ethanol. The PCR-product termini were filled with T4 polynucleotide polymerase, and phosphorylated with T4 polynucleotide kinase according to Sambrook et al. (1989). The PCR products with blunt-ends were ligated into the *Sma*I site of a pBluescript II KS vector (Stratagene). Nucleotide sequences of the clones were determined using a Dye Deoxy Terminator Cycle Sequenceing Kit (Applied Biosystems) and an automated DNA sequencer (373A, Applied Biosystems). Nucleotide and amino-acid sequences were analyzed using the GENETYX software system (Software Development). Updated versions of the sequence databases Genbank and EMBL were searched using the BLAST algorithm of Altschul et al. (1990).

Southern-hybridization analysis

DNA samples from GCR236 (*Tm-2*/*Tm-2*), GCR237 (*Tm-1*/*Tm-1*) and GCR26 (+/+) were digested with *Dra*I, *Eco*RI, *Eco*RV or *Hin*dIII, electrophoresed in 1.0% agarose gels, and transferred to nylon membranes (Hybond-N, Amersham) as described by Kreike et al. (1990). Hybridization was performed with digoxigenin (DIG)-labelled probes (Boehringer Mannheim) and chemical luminescent signals were detected according to the manufacturer's instruction. The probes were labelled by PCR as described previously (Ohmori et al. 1996).

Results

Tomato PCR products homologous to the disease-resistance genes encoding proteins with LRRs and an NBS

We designed two combinations of primers (I and II in Table 1) corresponding to two conserved amino-acid sequences, GGVGKTT and GLPLAL, between products of the tobacco *N* gene (Whitham et al. 1994; Dinesh-Kumer et al. 1995) and the *A. thaliana RPS2* gene (Bent et al. 1994; Mindrinos et al. 1994). The amino-acid sequences GGVGKTT contain the kinase-1a or P-loop motif (Saraste et al. 1990) in the NBS, and GLPLAL is located approximately 160 amino acids further downstream (Mindorinos et al. 1994). Primers S1 and AS1 in combination I were designed directly based on conserved sequences of *RPS2*, while the primers S2 and AS2 in II contained degenerate nucleotides. Primer combination I was used for PCR of the genomic DNA of GCR236 (*Tm-2*/*Tm-2*), and primer combination II was used for that of GCR237 (*Tm-1*/*Tm-1*). By using primer combination I, we detected a single band of about 500 bp and unclear bands of less than 500 bp (Fig. 1). From these bands, three fragments of 512 (TC10.1), 362 (TC10.2) and 198 bp (TC10.3) were cloned. With primer combination II we detected six amplification products, ranging in size from 0.3 to 1.2 kilobases (kb) (Fig. 1), and cloned five products of 522 (TC11.1), 511 (TC11.2), 466 (TC11.3), 335 (TC11.4) and 289 bp (TC11.5).

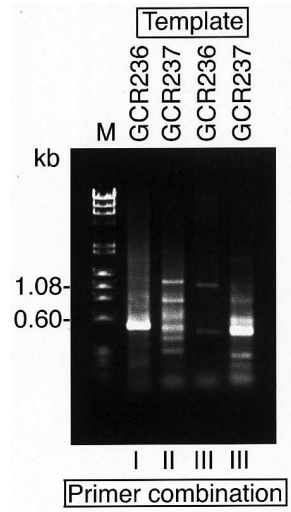

Fig. 1 PCR products amplified from genomic DNAs of near-isogenic lines (NILs) of tomato using primer combinations I (S1 + AS1), II (S2 + AS2) and III (S3 + AS3), respectively. Primer sequences are listed in Table 1. *M*, DNA size markers (λ/*Hin*dIII + ϕX174/*Hae*III digests)

The three clones derived from GCR236 (*Tm-2*/*Tm-2*) and the five clones from GCR237 (*Tm-1*/*Tm-1*) were sequenced, and the amino-acid sequences encoded by the cloned fragments were deduced. Four clones (TC10.1, 10.3, 11.1, 11.2) had possible frames encoding polypeptides uninterrupted by stop codons, but the other four (TC10.2, 11.3, 11.4, 11.5) contained multiple stop codons. A database search showed that clones TC10.1, TC11.1 and TC11.2 have significant sequence similarities to *RPS2*, *N*, *RPM1*, *L6*, *M*, *Prf* and *IC2-1*, all of which are members of plant resistance genes encoding the group-1 proteins as mentioned previously (Table 2). These three clones contained the kinase-2 and/or kinase-3a motifs (Traut 1994), NBS motifs conserved among these resistance genes (Fig. 2). Moreover, the deduced amino-acid sequences of clones TC10.1 and TC11.2 showed homologies as high as 98.7% to potato clone St124 and 93.9% to another potato clone St332 (Leister et al. 1996). The deduced amino-acid sequence of clone TC10.3 was not homologous to any of the known resistance genes, and this clone was shown to be a part of the repetitive sequences in tomato as estimated by genomic Southern hybridization (data not shown).

Tomato PCR products homologous to *Cf9* and *Cf2* genes

We designed a primer pair (III) corresponding to two conserved amino-acid sequences, SNKLHGP and GEIPQQLA, between products of the *Cf9* and *Cf2* genes in tomato, and used for PCR-amplification of tomato genomic DNA (Table 1). The amino-acid sequences SNKLHGP and GEIPQQLA are present in domain C containing LRRs, while GEIPQQLA is located approximately 140 amino acids downstream from the sequence SNKLHGP (Dixon et al. 1996). The primers in III (S3 and AS3; Table 1) were designed as

Table 2 PCR-amplified fragments cloned from genomic DNAs of near-isogenic lines (NILS) of tomato

Clone no.	Insert size (bp)	NILs used for PCR	Gene having similarity to the clone[a]
TC10.1	512	GCR236	*N* (69), *RPS2* (73), *RPM1* (65), *L6* (66), *M* (69) *Prf* (71), *12C-1* (71)
TC10.3	198	GCR236	Unknown
TC11.1	522	GCR237	*N* (71), *RPS2* (70), *RPM1* (74), *L6* (68), *M* (70) *Prf* (76), *12C-1* (75)
TC11.2	511	GCR237	*N* (78), *RPS2* (65), *RPM1* (67), *L6* (76), *M* (82) *Prf* (66), *12C-1* (70)
TC20.1	461	GCR236	*Cf9* (84), *Cf2* (81)
TC21.1	461	GCR237	*Cf9* (83), *Cf2* (81)
TC21.2	465	GCR237	*Cf9* (98), *Cf2* (82)

[a] Similarities (%) in amino-acid sequences are shown in parentheses

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TC10.1   LAEKIRHKAIQER----LFDDIVMVTVSQQP----NLKGIQGEIAGGLGLKLEGDN-FWS   51
TC11.1   LAKKLYNCPDIAS----SFPTRAWICVSQEYNTMDLLKTIIKSIQGRTMETLDLLERMTE   56
TC11.2   LARVIYDNLRSR------FQGACFLHEVRDRSAKQGLEHLQEILLSEILVVKKLRI-NDS   53
     N   IARAIFDTLLGRMDSSYQFDGACFLKDIKEN--KRGMHSLQNALLSELLR-EKANY-NNE   56
  RPS2   LMQSINNELITKG---HQYDVLIWVQMSREF----GECTIQQAVGARLGLSWDEKE-TGE   52
         .   .             .     .   .          .   .

TC10.1   RGDQLHTRLMDQNRRTLVILDDVWEALHDLEKLGIPSGSNHNHRCKVTLTTRIRDVCEAM  111
TC11.1   GDIEIYLRDLLKERKFLVAVDDVWQKEA-WESLKRAFPDSKNG-NRVIITTRKEDVAERA  114
TC11.2   FEGANMQKQRLQYKKVLLVLDDVDHIDQ-LDALAGKREWFGDG-SRIIITTKDKHLLVKY  111
     N   EDGKHQMASRLRSKKVLIVLDDIDNKDHYLEYLAGDLDWFGNG-SRIIITTRDKHLIEK-  114
  RPS2   NR-ALKIYRALRQKRFLLLLDDVWEEID-LEKTGVPRPDRENK-CKVMFTTRSIALCNNM  109
                 . .. *. .**.       .              ..  **.   .

TC10.1   G-AQKIMEVGTLPEEEAWILFKEKVGNLADD--PSLLDVVKDVAKECK              156
TC11.1   DDRGFVHKLRFLSQEESWDLFCRKLLDVRAM-VPEMEGLAKDMVEKCR              161
TC11.2   E-TEKIYRMKTLDKYESLRLFKQHAFKKNYP-TKEFEDLISSSDRAYW              157
     N   --NDIIYEVTALPDHESIQLFKQHAFGKEVP-NENFEKLSLEVVNYAK              159
  RPS2   G-AEYKLRVEFLEKKHAWELFCSKVWRKDLLESSSIRRLAEIIVSKCG              156
                .  *    .  **  .              .
```

Fig. 2 Alignment of the deduced amino-acid sequences encoded by three clones of the PCR-amplified fragments (TC10.1, 11.1 and 11.2) to those of *RPS2* in *A. thaliana* and *N* in tobacco. Gaps represented by *dashes* are introduced to maximize the homology. Kinase-2 motifs are shown in *black*, and kinase-3a motifs in *gray*. *Asterisks and dots* at the bottom indicate conserved amino-acid residues and similar amino-acid residues, respectively, among the proteins shown here

degenerate oligonucleotides, and used for PCR of genomic DNAs from GCR236 (*Tm-2*/*Tm-2*) and GCR237 (*Tm-1*/*Tm-1*). Two DNA bands of approximately 1100 and 500 bp in length were detected in the electrophoresed PCR products from genomic DNA of GCR236 (*Tm-2*/*Tm-2*) (Fig. 1). From a 500-bp band, which was presumed to be derived from the region between the two sequence motifs, a PCR fragment of 461 bp (TC20.1) has been cloned. From genomic DNA of GCR237 (*Tm-1*/*Tm-1*) we detected five amplification products, ranging in size from 0.2 to 1.0 kb. From these bands, two PCR products of 461 (TC21.1) and 465 bp (TC21.2) were successfully cloned.

The nucleic-acid sequences of clones TC20.1 and TC21.1 showed 99% identity, indicating that these sequences were common between GCR236 (*Tm-2*/*Tm-2*) and GCR237 (*Tm-1*/*Tm-1*). Each of the TC20.1 and TC21.2 sequences appeared to encode part of a protein, and a homology search in the databases showed that both sequences have significant similarities to both resistance genes *Cf9* and *Cf2* (Table 2). The deduced amino-acid sequences of clones TC20.1 and TC21.2 showed 61.9% and 91.4% identities to that of *Cf9*, respectively, and 53.5% and 46.0% identities to *Cf2*, respectively. Moreover, these two clones contained the LRRs conserved in both *Cf9* and *Cf2*, and two potential N-linked glycosylation sites in the same positions as *Cf9* (Fig. 3). However, clone TC20.1 contained an Arg at residue 74 in place of Val which is conserved between the LRRs of *Cf9* and *Cf2* (Fig. 3).

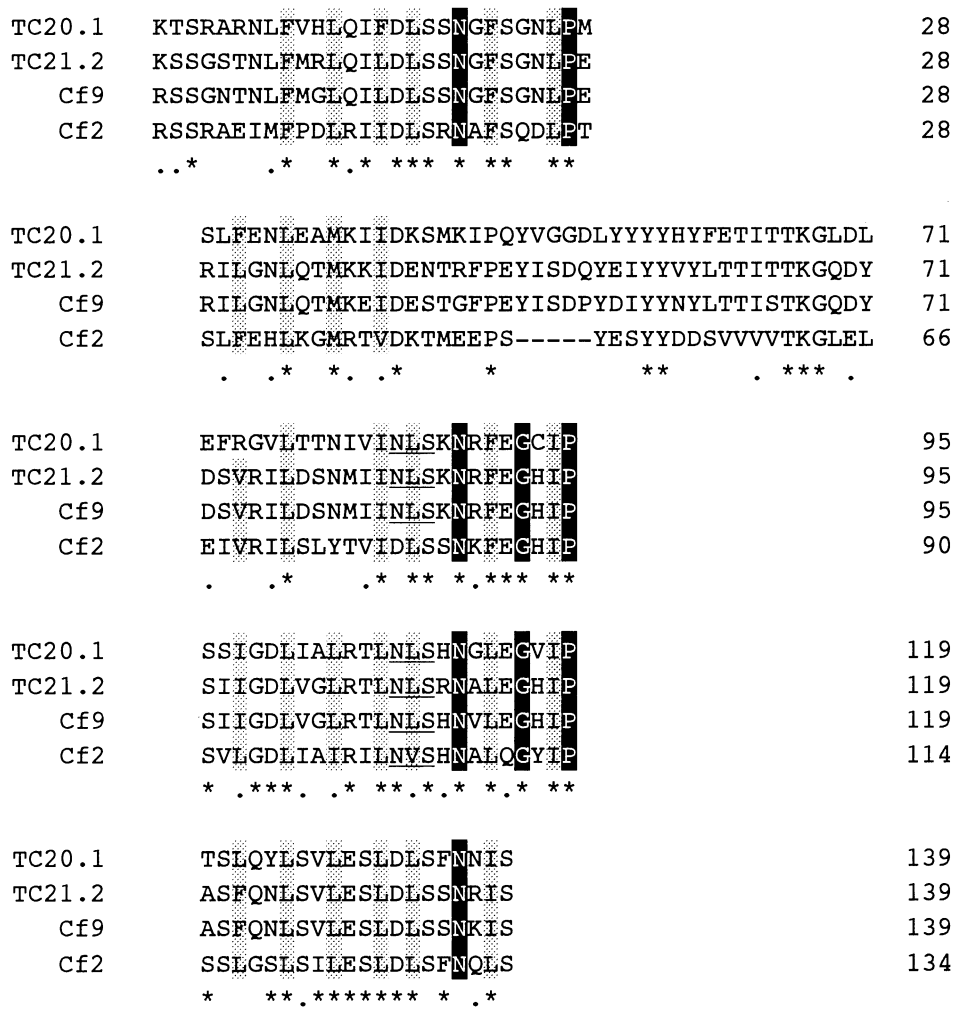

Fig. 3 Alignment of the deduced amino-acid sequences encoded by two clones of the PCR-amplified fragments (TC20.1 and 21.2) to those of *Cf9* and *Cf2* in tomato. Gaps represented by *dashes* are introduced to maximize the homology. Amino-acid residues conserved among the four sequences are shown in *black*. Residues where the conserved L is replaced by V, F, I, or M are shown in *gray*. Putative N-linked glycosylation sites are *underlined*. *Asterisks and dots* at the bottom indicate conserved amino-acid residues and similar amino-acid residues, respectively, among the proteins shown here

Southern-hybridization analysis of tomato PCR fragments

To examine the relationship between the five clones having homology to the disease-resistance genes and ToMV-resistance genes *Tm-2* or *Tm-1*, and to estimate the numbers of sequences homologous to each clone, Southern hybridization was performed with the five clones as probes. Among the clones related to the genes encoding proteins with LRRs and an NBS, clone TC10.1 derived from GCR236 (*Tm-2*/*Tm-2*) hybridized to several DNA bands common to both GCR26 (+/+) and GCR236 (*Tm-2*/*Tm-2*) (Fig. 4A). Both TC11.1 and TC11.2 were derived from GCR237 (*Tm-1*/*Tm-1*). The former hybridized to more than ten bands in each lane of GCR26 (+/+) and GCR237 (*Tm-1*/*Tm-1*), but the latter to only a few bands in each lane. In either case, however, no band pattern polymorphisms were found (Fig. 4B, C). These results suggest that the NILs have multiple copies of the sequences related to the first class of genes, such as *RPS2* and *N*.

Southern hybridization patterns with the clones related to the second class of genes encoding proteins with a C-terminal membrane anchor, such as *Cf2* and *Cf9*, are shown in Fig. 4 D and E. Hybridization of genomic DNAs with clone TC20.1 as a probe showed two fragment bands of 4 kb and 7 kb in both GCR236 (*Tm-2*/*Tm-2*) and GCR26 (+/+) when the DNAs were digested with *Hin*dIII. Since all other restriction enzymes (*Dra*I, *Eco*RI and *Eco*RV) generated the single same-sized band in both lines, the sequence was estimated to exist as a single copy. Clone TC21.2 hybridized to several bands, but no polymorphisms between the NILs were detected.

Discussion

From genomic DNAs of two tomato lines carrying either of the ToMV-resistance genes, *Tm-1* and *Tm-2*, we cloned three PCR-amplified fragments (TC10.1, 11.1 and 11.2), which are homologous to the first class of disease resistance genes including tobacco *N*, *A. thaliana RPS2* and *RPM1*, flax *L6* and *M* and tomato *Prf* and *I2C-1*. Similarly, we have cloned two amplified

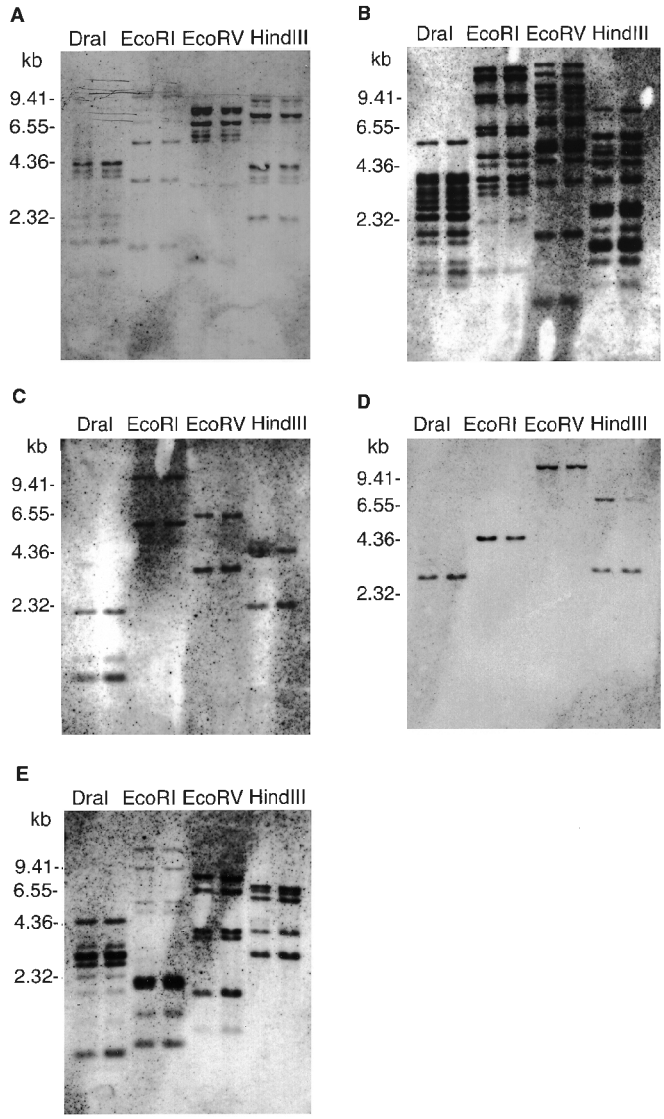

Fig. 4A–E Southern hybridization of DNAs from GCR236 (*Tm-2/Tm-2*) (*left lane* for each pair in **A, D**) or GCR237 (*Tm-1/Tm-1*) (*left lane* for each pair in **B, C and E**) and GCR26 (+ / +) (*right lane* for each pair in **A, B, C, D and E**) with TC10.1 (**A**), TC11.1 (**B**), TC11.2 (**C**), TC20.1 (**D**) and TC21.2 clones (**E**). The restriction endonucleases used are indicated above the lanes

fragments (TC20.1 and 21.2) homologous to the second class of genes such as *Cf9* and *Cf2*. The deduced amino-acid sequences of clones TC10.1 and TC11.2 are 98.7% and 93.9% homologous to those of St124 and St332, respectively (Leister et al. 1996). St124 and St332 were clones of potato genomic DNA fragments amplified by PCR using primers based on the sequences highly conserved between *RPS2* and *N*. The St332 locus has been found to be very closely linked to *Gro1*, conferring resistance to the root cyst nematode *Globodera rostochiensis*, in chromosome VII in potato (Ballvore et al. 1995), suggesting that St332 might encode the *Gro1* resistance allele (Leister et al. 1996). Therefore, the clone TC11.2 might be derived from a region of a gene having a function similar to *Gro1*.

The interaction of *N* gene with TMV has been investigated as a typical instance of the hypersensitive responses induced by genes in viruses. In plants, resistance expression is dependent on temperature conditions in both tomato carrying *Tm-2* and tobacco carrying *N*. Usually at a higher temperature than the suitable range, viruses become spread systemically irrespective of the presence of *Tm-2* or *N*, and sometimes cause systemic necrosis in response to these resistance genes (Cirulli and Ciccarese 1975; Shimomura and Ohashi 1977). The question of whether *Tm-2* is homologous to *N* or not emerges from such similarities between *Tm-2* and *N*. Symptomatically, however, there is a distinct difference between *Tm-2* and *N*; the former does not induce necrosis lesions in inoculated leaves, while the latter induces them as a result of a hypersensitive response to primarily multiplying virus. In the inoculated leaves of tobbaco plants carrying *N*, the virus spreads to and multiples in the cells surrounding the necrotic spots and induces further necrosis in these cells, which leads to an enlargement of the necrotic spots. By contrast, in the presence of *Tm-2* in tomato, the virus is hardly able to spread from the primarily infected cell to neighboring cells (Nishiguchi and Motoyoshi 1987). Moreover, the fact that *N* causes a necrotic response to TMV in transgenic tomato (Whitham et al. 1996) suggests that *Tm-2* and *N* are not equivalent in function. Since the mutants of ToMV which can overcome the effect of *Tm-2* to multiply in cells have amino-acid substitutions in its 30-kDa movement protein, the product of *Tm-2* is presumed to interact directly or indirectly with the movement protein (Meshi et al. 1989). The sequences of clones TC10.1, TC11.1 and TC11.2 have 70% or more similarity to the corresponding region of the *N* gene, but genomic Southern hybridization with these clones as probes exhibited no polymorphisms in restricted fragment bands between genomic DNAs of GCR236 (*Tm-2/Tm-2*) and GCR26 (+/+). This suggests that these clones originated from regions other than the *Tm-2* locus. Recently, Leister et al. (1996) assigned *Tm-2* to a different linkage group from *N* in their potato RFLP map. Based on these aspects, *Tm-2* may be a type of gene different from *N* in structure and function.

Thirteen different genes related to the responses to pathogens have so far been isolated and their molecular structures analyzed (Martin et al. 1993; Jones et al. 1994; Mindorinos et al. 1994; Whitham et al. 1994; Grant et al. 1995; Lawrence et al. 1995; Song et al. 1995; Dixon et al. 1996; Salmerson et al. 1996; Anderson et al. 1997; Cai et al. 1997; Feuillet et al. 1997; Ori et al. 1997). Among them, seven (*RPS2*, *RPM1*, *N*, *L6*, *M*, *Prf* and *I2C-1*) are genes which encode the proteins with LRRs and an NBS (group 1), three (*Cf2*, *Cf9* and $Hs1^{pro-1}$) are genes which encode the proteins with a C-terminal membrane anchor but no NBS (group 2), and the other

three (*Pto*, *Xa21* and *Lr10*) are genes which encode serine-threonine protein kinase (group 3). In the present study, we looked for the presence of sequences related to the genes encoding the proteins of groups 1 and 2. The Southern-hybridization analysis for the NILs revealed significantly more sequences related to the group-1 protein genes than to the group-2 protein genes. It is thus likely that the family of the group-1 protein genes is significantly larger than that of the group-2 protein genes in the NILs. We have no evidence that *Tm-1* and/or *Tm-2* are related to any of the group-1 or -2 protein genes, since the Southern hybridizations have not detected any polymorphisms between the NILs. We will also examine whether or not the two genes are related to the genes encoding group-3 protein genes which have a serine-threonine protein kinase domain.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Anderson PA, Lawrence GJ, Morrish BC, Ayliffe MA, Finnegan EJ, Ellis JG (1997) Inactivation of the flax rust resistance gene *M* associated with loss of a repeated unit within the leucine-rich repeat coding region. Plant Cell 9:641–651

Ballvore AJ, Hesselbach J, Niewöhner J, Leister D, Salamini F, Gebhardt C (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harboring the nematode resistance gene *Gro*1. Mol Gen Genet 249:82–90

Bent A, Kunkel B, Dahlbeck D, Brown K, Schmid R, Giraudat J, Leung J, Staskawicz B (1994) *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science 265:1856–1860

Cai D, Kleine M, Kifle S, Harloff H-J, Sandal NN, Marcker KA, Klein-Lankhorst RM, Salentijn EM, Lange W, Stiekema WJ, Wyss U, Grundler FMW, Jung C (1997) Positional cloning of a gene for nematode resistance in sugar beet. Science 275:832–834

Cirulli M, Ciccarese F (1975) Interaction between TMV isolates, temperature, allelic conditions and combination of the *Tm* resistance gene in tomato. Phytopathol Meditter 14:100–105

Dinesh-Kumar S, Whitham S, Choi D, Hehl R, Corr C, Baker B (1995) Transposon tagging of tobacco mosaic virus resistance gene *N*: its possible role in the TMV-N-mediated signal transduction pathway. Proc Natl Acad Sci USA 92:4175–4180

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JD (1996) The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat protein. Cell 84:451–459

Feuillet C, Schachermayer G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at *Lr10* disease resistance locus of wheat. Plant J 11:45–52

Grant M, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A, Innes R, Dangl J (1995) Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. Science 269:843–846

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JDG (1994) Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789–793

Kanazin V, Marker LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93:11746–11750

Kreike CM, de Koning JRA, Krens FA (1990) Non-radioactive detection of single-copy DNA-DNA hybrids. Plant Mol Biol Rep 8:172–179

Lawrence G, Finnegan EJ, Ayliffe M, Eillis J (1995) The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. Plant Cell 7:1195–1206

Leister D, Ballvore A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genet 14:421–429

Martin GB, Brommonschenkel SH, Chunwongse J, Fray A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Meshi T, Motoyoshi F, Maeda T, Yoshikawa S, Watanabe Y, Okada Y (1989) Mutations in the tobacco mosaic virus 30-kDa protein gene overcome *Tm-2* resistance in tomato. Plant Cell 1:515–522

Mindrinos M, Katagiri F, Yu G-L, Ausbel F (1994) The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78:1089–1099

Motoyoshi F, Oshima N (1975) Infection with tobacco mosaic virus of leaf mesophyll protoplasts from susceptible and resistant lines of tomato. J Gen Virol 29:81–89

Motoyoshi F, Oshima N (1977) Expression of genetically controlled resistance to tobacco mosaic virus infection in isolated tomato leaf mesophyll protoplasts. J Gen Virol 34:499–506

Motoyoshi F, Ohmori T, Murata M (1996) Molecular characterization of heterochromatic regions around the *Tm-2* locus in chromosome 9 of tomato. In: Heslop-Harrison JS (ed) Unifying plant genomes. SEB Symposium no. 50. The Company of Biologists Limited, Cambridge, pp 65–70

Nishiguchi M, Motoyoshi F (1987) Resistance mechanisms of tobacco mosaic virus strains in tomato and tobacco. In: Evered D, Harnett S (eds) Plant resistance to viruses. John Wiley and Sons, Chichester, UK, pp 38–46

Ohmori T, Murata M, Motoyoshi F (1995 a) Identification of RAPD markers linked to the *Tm-2* locus in tomato. Theor Appl Genet 90:307–311

Ohmori T, Murata M, Motoyoshi F (1995 b) RAPD markers linked to the tomato mosaic virus resistance gene, *Tm-1*, in tomato. Jpn J Genet 70:179–184

Ohmori T, Murata M, Motoyoshi F (1996) Molecular characterization of RAPD and SCAR markers linked to the *Tm-1* locus in tomato. Theor Appl Genet 92:151–156

Ori N, Eshed Y, Paran I, Presting G, Aviv D, Tanksley S, Zamir D, Fluhr R (1997) The *I2C* family from the wilt disease resistance locus *I2* belongs to the nucleotide-binding, leucine-rich repeat superfamily of plant resistance genes. Plant Cell 9:521–532

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

Sambrook F, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold spring Harbor Laboratory, Cold Spring Harbor, New York

Saraste M, Sibbald PR, Wittinghofer A (1990) The P-loop – a common motif in ATP- and GTP-binding proteins. Trends Biochem Sci 15:430–434

Shimomura T, Ohashi Y (1977) Tobacco mosaic virus multiplication and necrotization in local lesion hosts. Ann Phytopathol Soc Japan 43 : 224–227

Smith JWM, Ritchie DB (1983) A collection of near-isogenic lines of tomato: research tool of the future? Plant Mol Biol Rep 1 : 41–45

Song W-Y, Wang G-L, Chen L-L, Kim H-S, Pi L-Y, Holsten T, Gardner J, Wang B, Zhai W-X, Zhu L-H, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270 : 1804–1806

Staskawicz BJ, Ausubel FM, Baker BJ, Eills JG, Jones JDG (1995) Moleculer genetics of plant disease resistance. Science 268 : 661–667

Traut TW (1994) The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide-binding sites. Eur J Biochem 222 : 9–19

Whitham S, Dinesh-Kunmar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene *N*: similarity to toll and interleukin-1 receptor. Cell 78 : 1101–1115

Whitham S, McCormick S, Baker B (1996) The *N* gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc Natl Acad Sci USA 93 : 8776–8781

Yu YG, Buss GR, Maroof MAS (1996) Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93 : 11751–11756