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Rme1 is Necessary for *Mi-1*-Mediated Resistance and Acts Early in the Resistance Pathway

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The tomato gene *Mi-1* confers resistance to root-knot nematodes (*Meloidogyne* spp.), potato aphid, and whitefly. Using genetic screens, we have isolated a mutant, *rme1* (resistance to *Meloidogyne* spp.), compromised in resistance to *M. javanica* and potato aphid. Here, we show that the *rme1* mutant is also compromised in resistance to *M. incognita*, *M. arenaria*, and whitefly. In addition, using an *Agrobacterium*-mediated transient assay in leaves to express constitutive gain-of-function mutant Pto_{L205D}, we demonstrated that the *rme1* mutation is not compromised in *Pto*-mediated hypersensitive response. Moreover, the mutation in *rme1* does not result in increased virulence of pathogenic *Pseudomonas syringae* or *Mi-1*-virulent *M. incognita*. Using a chimeric *Mi-1* construct, *Mi-DS4*, which confers constitutive cell death phenotype and *A. rhizogenes* root transformation, we showed that the *Mi-1*-mediated cell death pathway is intact in this mutant. Our results indicate that *Rme1* is required for *Mi-1*-mediated resistance and acts either at the same step in the signal transduction pathway as *Mi-1* or upstream of *Mi-1*.

Resistance to biotrophic pathogens is mediated by active defense triggered in the plant by the presence of the plant resistance (*R*) gene and the corresponding avirulence (*Avr*) gene from the pathogen. The absence of either *R* or *Avr* genes will result in susceptibility (Keen 1990). Several *R* genes have been cloned, and the largest class of these genes encodes proteins with nucleotide binding domains (NB) and leucine rich repeats (LRR) (Dangl and Jones 2001). Members of this class of genes confer resistance to a diverse group of organisms, including bacteria, fungi, viruses, nematodes, and insects (Dangl and Jones 2001; Nombela et al. 2003). Although most of these genes confer resistance to a specific organism with a defined *Avr* gene, a subset recognizes more than one Avr effector protein (Grant et al. 1995; Kim et al. 2002).

The tomato (*Lycopersicon esculentum*) gene *Mi-1* confers resistance to the three most common species of root-knot nematodes, *Meloidogyne arenaria*, *M. incognita*, and *M. javanica*. *Mi-1* belongs to the NB-LRR class of *R* genes (Milligan et al. 1998). In the *Mi* locus, two transcribed genes, *Mi-1.1* and *Mi-1.2*, with 91% homology were identified. Only one of these genes, *Mi-1.2*, conferred resistance to root-knot nematodes (Milligan et al. 1998). We refer to this gene as *Mi-1*. After cloning, *Mi-1* was found to confer resistance to two additional organisms, potato aphid, *Macrosiphum euphorbiae* (Rossi et al. 1998), and the whitefly, *Bemisia tabaci* (Nombela et al. 2000, 2003). Avirulence effectors that interact with Mi-1 have not yet been identified in any of the three organisms. Most likely, *Mi-1* either recognizes more than one distinct avirulence product or recognizes perturbations in a host protein conveyed by these three organisms (Dangl and Jones 2001; Mackey et al. 2002).

There is evidence that *Mi-1*-mediated resistance to root-knot nematodes, potato aphids, and whiteflies is rather specific. *Mi-1* does not confer resistance to another economically important species, *M. hapla*. In addition, field isolates of *M. incognita* that are able to parasitize tomato with *Mi-1* have been identified (Kaloshian et al. 1996). Similarly, potato aphid populations virulent on *Mi-1*-containing tomato have been reported (Goggin et al. 2001; Rossi et al. 1998). Differences in reproduction of distinct biotypes of *B. tabaci* on tomato with *Mi-1* have also been described. Plants with *Mi-1* showed lower infestation levels of *B. tabaci* Q biotype than the B biotype (Nombela et al. 2001).

It is not clear how *Mi-1* mediates resistance to root-knot nematodes, potato aphid, and sweet potato whitefly. In addition, it is not clear whether the defense responses mediated by *Mi-1* against the three organisms are identical. The defense response mediated by *Mi-1* to root-knot nematodes includes a rapid localized cell death in root cells in which the nematodes are feeding (Dropkin 1969). Cell death limits the ability of the nematode to feed and hinders the establishment of a feeding site, which is comprised of enlarged multinucleate cells and that is essential for the survival of this obligate parasite. No cell death is observed in resistant tomato leaflets after feeding by potato aphid (Martinez de Ilarduya et al. 2003). Potato aphids feed for very limited periods on resistant tomato leaves compared with susceptible leaves. Adult aphids are able to reach the phloem and sample fluids on resistant tomato, but soon after, they stop feeding and seem to die from starvation (Kaloshian et al. 2000). In contrast, sweet potato whiteflies are unable to efficiently locate the phloem element in resistant tomato (Jiang et al. 2001). However, once they reach the phloem element, they are able to feed on the phloem sap.

The *Mi-1*-mediated resistance to root-knot nematodes and potato aphid is regulated differently during plant development. Tomato seedlings containing *Mi-1* are resistant to nematodes soon after germination. In contrast, fully expanded leaves become

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aphid resistant only when plants are four to five weeks of age (Kaloshian et al. 1995). In these plants, fully expanded leaves are resistant to aphids, irrespective of leaf position on the plant (Kaloshian et al. 1997). Similarly, *Mi-1*-mediated resistance to *B. tabaci* is observed in 8-week-old plants but not in younger plants (Pascual et al. 2000). It is not clear what controls the developmental regulation of the resistance to aphids and whiteflies. Most likely, *Mi-1* is not involved, since *Mi-1* transcripts are present in roots and leaves very early in development, and therefore, their presence is not correlated with aphid or whitefly resistance (Martinez de Ilarduya and Kaloshian 2001).

Using a genetic screen to identify suppressors of *Mi-1*, we identified a mutant, *rme1* (resistance to *Meloidogyne* spp.), compromised in resistance to *M. javanica* and to potato aphids (Martinez de Ilarduya et al. 2001). Here, we present data that *rme1* mutant plants are also compromised in resistance to *M. incognita*, *M. arenaria*, and sweet potato whitefly. We also demonstrate that mutation in *Rme1* does not result in increased virulence to *Mi-1*-virulent root-knot nematodes and to pathogenic bacteria. In addition, we show that the *rme1* mutant exhibits the cell death phenotype mediated by the tomato *R* gene *Pto*. Furthermore, we provide evidence that Rme1 acts early in the *Mi-1*- pathway, either at the same step as the *Mi-1* product or earlier in the response cascade.

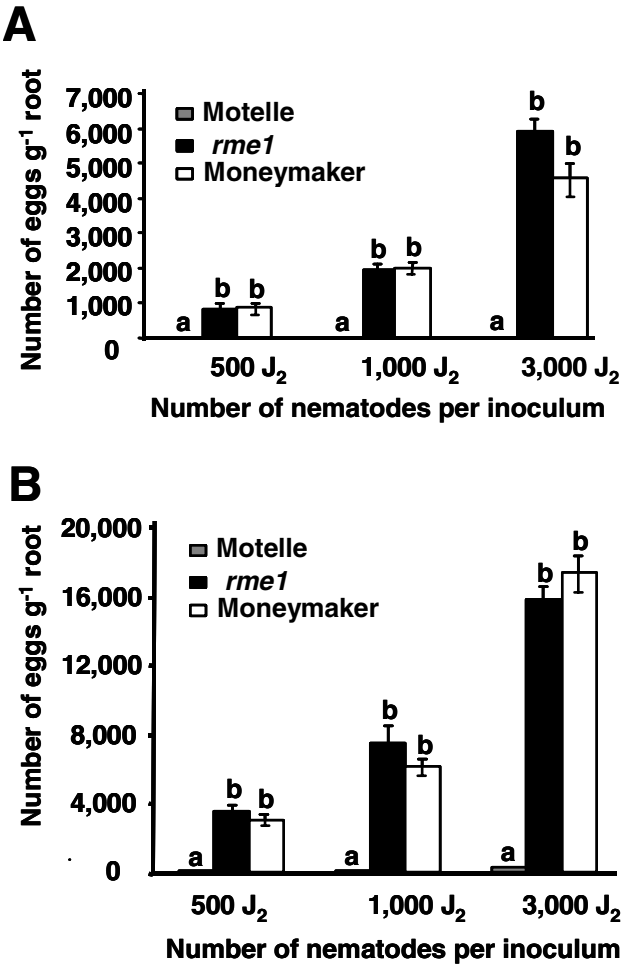

Fig. 1. Reproduction of *Mi-1*-avirulent *Meloidogyne* spp. wild-type 'Motelle' (*Mi-1/Mi-1*), *rme1* mutant, and 'Moneymaker' (*mi-1/mi-1*) on tomato plants. Plants were inoculated with three inoculum levels of nematodes and were maintained for six weeks under greenhouse conditions. **A,** *M. arenaria*; **B,** *M. incognita*. Each bar represents the mean and standard error of five replicates. Significantly different means are followed by different letters ($P < 0.05$).

RESULTS

The *rme1* mutant is compromised in resistance to three species of *Mi-1*-avirulent root-knot nematodes.

Mi-1 mediates resistance to three species of root-knot nematodes, *M. arenaria*, *M. incognita*, and *M. javanica*. Our screen to find suppressors of *Mi-1* that lead to isolation of the *rme1* mutant was based on infection with *M. javanica* (Martinez de Ilarduya et al. 2001). We tested whether mutation in *Rme1* also altered resistance to *M. arenaria* and *M. incognita*. In order to better evaluate differences in nematode reproduction, we used three different levels of nematode inoculum. Since our initial experiments were based on a single inoculum level (500 J2 per plant), we also performed these assays using *M. javanica*. Besides the wild-type parent cultivar Motelle, the near-isogenic cv. Moneymaker was used as susceptible control. The reproduction of *M. arenaria*, measured as eggs per gram of roots, was not significantly different on *rme1* mutant than on the susceptible cv. Moneymaker control at the three inocula tested (Fig. 1A). The number of eggs per gram of root was significantly higher on the *rme1* mutant than on the wild-type 'Motelle' parent at all nematode inocula tested (Fig. 1A). Similarly, significantly greater numbers of eggs per gram of root were observed with *M. incognita* and *M. javanica* on *rme1* mutant than on cv. Motelle with the three inoculum levels (Fig. 1B; data not shown). As with *M. arenaria*, the reproduction of *M. incognita* and *M. javanica* on the *rme1* mutant was not significantly different than that on 'Moneymaker' (Fig. 1B; data not shown). At all inocula levels tested, reproduction of *M. arenaria* was significantly lower than the reproduction of *M. incognita* and *M. javanica* on both *rme1* mutant and 'Moneymaker' (Fig. 1A and B; data not shown).

Mutation in *rme1* abolishes *Mi-1*-mediated resistance to *B. tabaci.*

Earlier, we showed that the *rme1* mutant was compromised in resistance to *M. javanica* and to potato aphid (Martinez de Ilarduya et al. 2001). Since *Mi-1* confers resistance to *B. tabaci* also, we analyzed the response of *rme1* mutant to the B biotype of *B. tabaci*. We used two experiments to do these evaluations. The first was based on a free-choice assay in which whiteflies were given access to 10 plants of each of the *rme1* mutant, 'Motelle', and 'Moneymaker'. The daily infestation rates of adults of the B biotype of *B. tabaci* on the *rme1* mutant plants were intermediate between those on 'Moneymaker' and 'Motelle' (Fig. 2A). However, the mean values of the number of adults per plant per day on the *rme1* mutant were similar to those on 'Moneymaker' and significantly greater than on 'Motelle' (Fig. 2B). In the no-choice experiment, a single leaflet of 11 plants from each genotype was infested with five adult female whiteflies in a clip cage. Six days after infestation, the average number of eggs observed on the *rme1* mutant plants was similar to that on 'Moneymaker' and significantly greater than that observed on 'Motelle' (Fig. 2C).

The *rme1* mutant is not compromised in *Pto*-mediated hypersensitive response.

Several mutations that abolish resistance mediated by distinct *R* genes have been identified. Some of these mutations disrupt genes that are required for resistance mediated by a single *R* gene (Dixon et al. 2000; Salmeron et al. 1994; Warren et al. 1999). Others are components of resistance signaling pathways downstream of several *R* genes (Glazebrook 2001). The tomato line Motelle does not carry the *Pto* gene that confers resistance to *Pseudomonas syringae* pv. *tomato*. Therefore, to test whether the *rme1* mutation abolishes the cell death medi-

ated by *Pto*, we used a constitutive gain-of-function mutant Pto_{L205D} in *Agrobacterium*-mediated transient expression (Rathjen et al. 1999). Equal numbers of *rme1* mutant leaflets were infiltrated with *A. tumefaciens* C58C1 containing the Pto_{L205D} construct at OD_{600} of 0.1 and 0.03. A total of 40 leaflets were inoculated in two independent experiments. At least 15 leaflets were infiltrated with *A. tumefaciens* containing empty vector in each experiment. A similar number of wild-type 'Motelle' leaflets were also infiltrated with *A. tumefaciens* containing Pto_{L205D} construct or empty vector. All *rme1* mutant and 'Motelle' leaflets infiltrated with Pto_{L205D} developed cell death, indicating that the *Pto*-mediated hypersensitive response is intact in the *rme1* mutant (Fig. 3). Initial localized cell death symptoms were observed 16 and 24 h postinfiltration at the higher and lower concentrations, respectively. Tissue collapse and cell death symptoms were complete 3 and 5 days postinfiltration at the two concentrations, respectively (Fig. 3). Leaflets of *rme1* mutant and 'Motelle' tomato infiltrated with the empty vector control at an OD_{600} of 0.03 did not show cell death symptoms (Fig. 3), while cell death was observed in 7% of *rme1* mutant leaflets and 3% of wild-type 'Motelle' leaflets infiltrated with the empty vector at OD_{600} of 0.1.

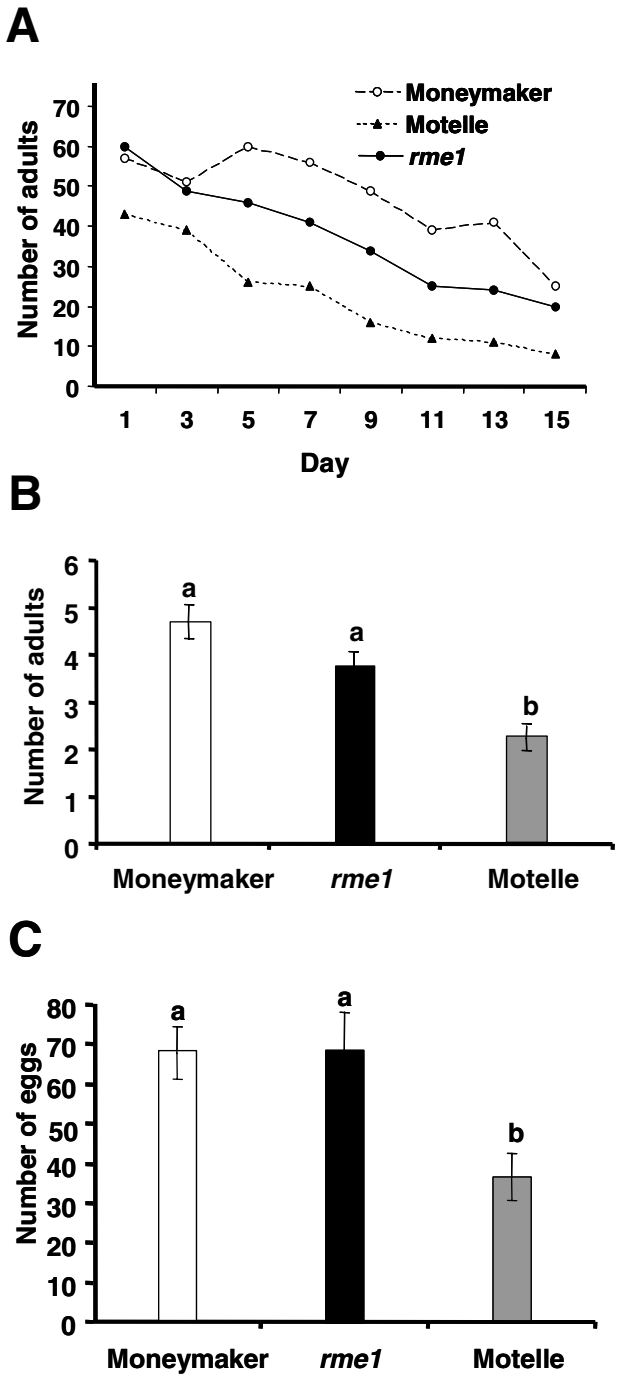

Fig. 2. Infestation of *Bemisia tabaci* B biotype on wild-type 'Motelle' (*Mi-1/Mi-1*), *rme1* mutant (*Mi-1/Mi-1*), and 'Moneymaker' (*mi-1/mi-1*) tomato plants. **A,** Daily infestation rates of adult whiteflies in the free-choice assay. Values represent the mean of 10 plants of each genotype. **B,** Average number of adult whiteflies per sampling time on each plant genotype in the free-choice assay. Sampling was done every other day. Values represent the mean and standard error of 10 plant replicates. **C,** Average number of eggs produced by *B. tabaci* B biotype on each plant genotype during the no-choice assay. Five adult female whiteflies were confined to a single leaflet per plant for six days. Each bar represents the mean and standard error of 11 replicates. Significantly different means are followed by different letters ($P < 0.05$).

Mutation in *rme1* does not result in increased susceptibility to *Mi-1*-virulent *M. incognita* or to virulent bacteria.

To determine whether *Rme1* is involved in restricting virulent root-knot nematodes, plants were infected with *Mi-1*-virulent *M. incognita* isolate W1. This *Mi-1*-virulent isolate was collected from a field where several successive tomato plantings were implemented (Kaloshian et al. 1996). Three doses of nematode inoculum were used to compare nematode reproduction on *rme1* mutant, 'Motelle', and 'Moneymaker' in two independent experiments. At inoculum levels of 500 and 1,000 J2s, no significant difference in eggs per gram of root was observed among the three tomato lines tested (Fig. 4). The reproduction of isolate W1 at 3,000 J2 inoculum level was similar on *rme1* mutant and 'Moneymaker', suggesting that *Rme1* is not involved in restricting virulent root-knot nematode. Interestingly, the number of eggs per gram of root on *rme1* mutant and 'Moneymaker' plants was significantly greater than that on 'Motelle' (Fig. 4).

Similarly, to determine whether the mutation in *rme1* alters susceptibility to virulent bacteria, plants were infiltrated with

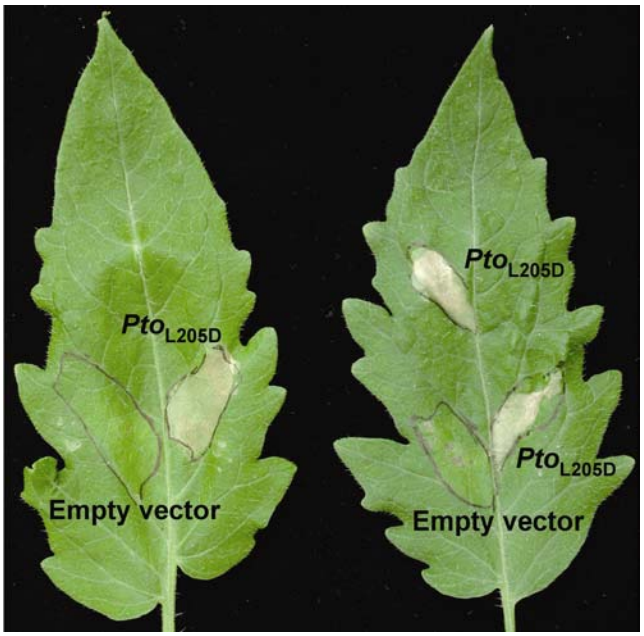

Fig. 3. Cell-death induction in wild-type 'Motelle' (left) and *rme1* mutant (right) tomato leaflets by constitutive gain-of-function mutant Pto_{L205D}. Leaflets were infected with *Agrobacterium tumefaciens* with syringe infiltration to transiently express the mutant Pto_{L205D} and empty binary vector in tomato leaflets. Leaflets at 7 days postinfiltration.

P. syringae pv. *tomato* DC3000. The bacterial number in *rme1* mutant was compared to numbers in 'Motelle' and 'Moneymaker'. Both cvs. Motelle and Moneymaker are susceptible to *P. syringae* pv. *tomato*. Similar bacterial numbers were observed in *rme1* mutant, 'Motelle', and 'Moneymaker' leaves at 2 and 4 days postinfiltration (data not shown), indicating that *Rme1* is not involved in general plant defense.

***Rme1* acts early in the *Mi-1*-mediated resistance cascade.**

The *Mi-1* locus contains two NB-LRR genes, *Mi-1.1* and *Mi-1.2*, with 91% identity (Milligan et al. 1998). *Mi-1.2* and not *Mi-1.1* confers resistance to root-knot nematodes, potato aphid, and whitefly (Milligan et al. 1998; Nombela et al. 2003; Rossi et al. 1998). A chimeric construct, *Mi-DS4*, was produced by introducing the *Mi-1.2* LRR-encoding region into *Mi-1.1* (Fig. 5A) (Hwang et al. 2000). *Mi-DS4*, when used in *A. rhizogenes*-mediated transformation of cotyledons, failed to produce transformed roots, due to lethal phenotype resulting from constitutive activation of the *Mi-1*-mediated cell death pathway (Hwang et al. 2000). We used the *Mi-DS4* construct to assess whether the *Mi-1*-mediated cell death pathway is intact in the *rme1* mutant and to determine the relative position of *Rme1* with respect to *Mi-1* in the signal transduction cascade. Cotyledons of *rme1* mutant transformed with *Mi-DS4* using *A. rhizogenes* resulted in recovery of no transformed roots (Fig. 5B). Similarly, cv. Motelle- and cv. Moneymaker-transformed cotyledons did not produce transformed roots, confirming the expression of the *Mi-DS4* construct is lethal (Fig. 5B). In contrast, transformation with *A. rhizogenes* carrying the empty binary vector resulted in hairy root production with each of the three tomato lines (Fig. 5B).

DISCUSSION

Our results here indicate that the *rme1* mutation abolishes resistance against nematodes and whiteflies mediated by *Mi-1*. Earlier, we demonstrated that the *rme1* mutation conferred full susceptibility to *M. javanica* and potato aphid. In this report, we provided additional evidence that the *rme1* mutation confers susceptibility to all known *Mi-1* avirulent nematodes and insects. The *rme1* mutant plants supported similar levels of *M. arenaria*, *M. javanica*, and *M. incognita* reproduction as that in the susceptible tomato cv. Moneymaker (Fig. 1A and B). Similarly, both free-choice and no-choice experiments indicated that resistance to the B biotype of *B. tabaci* is compromised in the *rme1* mutant (Fig. 2B and C). In addition, the cell death response mediated by the *Pto* gene is not compromised in the *rme1* mutant, further supporting a role for *Rme1* specific for the *Mi-1*-mediated resistance (Martinez de Ilarduya et al. 2001). To our knowledge, only three mutations that completely abolish a specific *R* gene-mediated resistance have been reported. Two are tomato mutants, the *prf* mutation that abolished the resistance mediated by the *Pto* gene to *P. syringae* expressing *avrPto* (Salmeron et al. 1994) and the *rcr3* mutation, which is compromised in the resistance mediated by the *Cf-2* gene to *Cladosporium fulvum* (Dixon et al. 2000). The third is the *Arabidopsis thaliana pbs1* mutant, which is compromised in resistance mediated by *RPS5* to *P. syringae* pv. *phaseolicola* expressing *avrPphB* (Warren et al. 1999). All three mutations have been cloned, and these genes along with their corresponding resistance genes represent two groups of gene pairs required for mediating specific disease resistances. *Pto* and *PBS1* encode serine-threonine kinases belonging to distinct subfamilies (Martin et al. 1993; Swiderski and Innes 2001) and require *Prf* and *RPS5*, respectively, both belonging to the NB-LRR class of resistance genes (Salmeron et al. 1996; Warren et al. 1998). *Cf-2* encodes for a transmembrane protein with an extracellualr LRR and *Rcr3*, a papain-like cysteine endoprotease (Dixon et al. 1998; Kruger et al. 2002). Although *Pto* and *PBS1* encode distantly related protein kinases and may not perform similar functions, it is formally possible that NB-LRR resistance genes require protein kinases (Swiderski and Innes 2001). Since *Mi-1* also belongs to the NB-LRR class of *R* genes, it is tempting to speculate that *Rme1* might encode a protein kinase.

The *Rcr3* gene is developmentally regulated with elevated expression detected in older plants (Kruger et al. 2002). The expression of *Rcr3* is also regulated in response to *C. fulvum* infections with higher and faster expression observed in the incompatible interaction compared with compatible interaction (Kruger et al. 2002). The *Mi-1*-mediated resistance to potato aphid and whitefly is also developmentally regulated

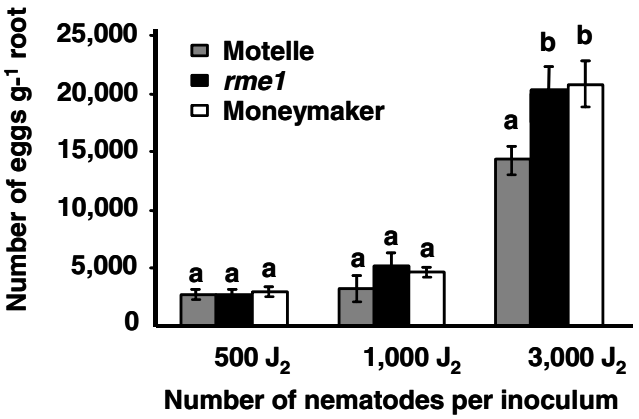

Fig. 4. Reproduction of *Mi-1*-virulent *Meloidogyne incognita* on wild-type 'Motelle' (*Mi-1/Mi-1*), *rme1* mutant (*Mi-1/Mi-1*), and 'Moneymaker' (*mi-1/mi-1*) tomato plants. Plants were infected with three inoculum levels of nematodes and maintained for six weeks under greenhouse conditions. Each bar represents the mean and standard deviation of 10 replicates. Significantly different means are followed by different letters ($P < 0.05$).

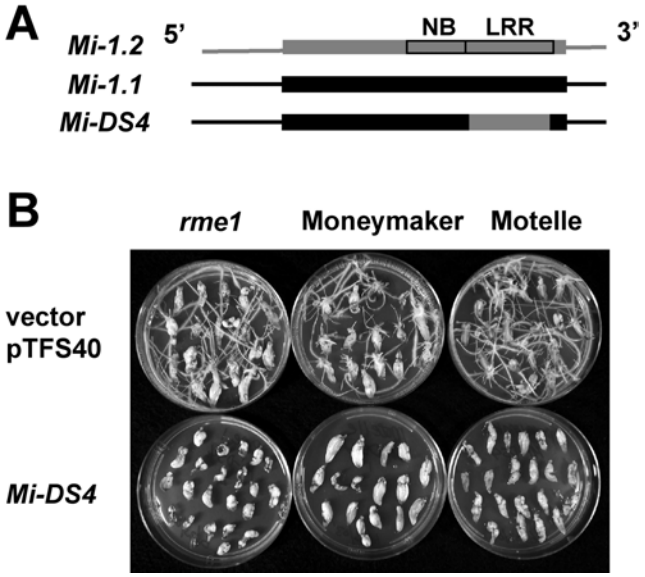

Fig. 5. *Agrobacterium rhizogenes*-mediated transformation of tomato using *Mi-DS4* construct. **A,** *Mi-1.1* and *Mi-1.2* alleles and *Mi-DS4* chimeric construct. **B,** Tomato cotyledons of wild-type 'Motelle' (*Mi-1/Mi-1*), *rme1* mutant (*Mi-1/Mi-1*), and 'Moneymaker' (*mi-1/mi-1*) were infected with *A. rhizogenes* containing *Mi-DS4* construct or empty vector. Absence of hairy roots indicates cell death phenotype.

(Kaloshian et al. 1995; Pascual et al. 2000). However, this regulation is not *Mi-1* transcription-dependent, because *Mi-1* is expressed at similar levels in leaves of young and old plants (Martinez de Ilarduya and Kaloshian 2001). It is therefore possible that, like *Rcr3*, the expression of *Rme1* is developmentally regulated in tomato leaves.

Rme1 or host defense components containing Rme1 may be the target of at least three distinct effectors from root-knot nematodes, potato aphid, and whitefly. Similarly, Pto and the *Arabidopsis* protein RIN4 with or without associated factors have been shown to be targets of two and three different bacterial effectors, respectively (Axtell and Staskawicz 2003; Kim et al. 2002; Mackey et al. 2003). Therefore, the classical meaning for "gene-for-gene" determined by the complementary pairs of R and Avr genes might not apply for these systems (Schneider 2002). To date, no *Mi-1*-effector has been conclusively isolated from plant parasitic nematodes. A *Meloidogyne avirulence* protein-1 (*map-1*) was cloned from *Mi-1*-avirulent root-knot nematode, using differential marker analysis of near-isogenic strains of root-knot nematodes that differ in virulence in the presence of *Mi-1* (Semblat et al. 2001). MAP-1 protein was localized to nematode amphidial secretions; however, no functional role of MAP-1 has yet been determined. Similarly, no Mi-1-effectors have yet been identified from aphids or whiteflies, and because of the diversity of known effectors from bacteria, fungi, and viruses (Dangl and Jones 2001), it is difficult to speculate as to the nature of these molecules. Most likely the *Mi-1*-effectors from these organisms are different. However, conserved signature motifs in the effector proteins from root-knot nematodes, potato aphid, and *B. tabaci* may interact with Rme1 singly or in association with other plant components to trigger the resistance response. Therefore, cloning *Rme1* may help in isolating *Mi-1*-effector molecules from root-knot nematodes, aphids, and whitefly.

Previous results indicated that *Rme1* is probably not involved in regulating the expression of *Mi-1*, as *Mi-1* is expressed in roots and leaves of *rme1* mutant (Martinez de Ilarduya et al. 2001). Since no Mi-1 antibody has yet been developed, it is not possible to rule out a role for *Rme1* in Mi-1 protein modification. According to the emerging "guard hypothesis," in which plant NB-LRR proteins are thought to guard the interaction of another plant protein with the pathogen or insect Avr products, Rme1 might be the Mi-1 "guardee" (Dangl and Jones 2001). In this case, Mi-1 could be monitoring changes in Rme1 brought about by the interaction with nematode, aphid, or whitefly avirulence determinants.

Our results indicate that *Rme1* acts early in the *Mi-1*-mediated resistance pathway. The mutation in *rme1* abolishes resistance to nematodes; therefore, the cell death pathway mediated by *Mi-1* is blocked in the *rme1* mutant. However, the *Mi-DS4* construct resulted in lethal phenotype in the *rme1* mutant, indicating that the cell death pathway initiated by *Mi-1* is intact in the *rme1* mutant. These results indicate that, genetically, *Rme1* is either upstream of *Mi-1* or at the same level of *Mi-1*. It has been shown that intramolecular interaction in Mi-1 regulates cell death where the LRR region seems to be involved in signaling cell death and the amino terminus, representing the first 161 amino acids, seems to negatively regulate this signal (Hwang and Williamson 2003). In addition, substituting amino acids 984 to 986 in the LRR region from the nonfunctional allele *Mi-1.1* into the functional allele *Mi-1.2* causes loss of nematode resistance, indicating that this region might be involved in perceiving nematode infection (Hwang and Williamson 2003). If Rme1 is the target of the nematode Avr factor and Mi-1 "guards" Rme1, it is intriguing to speculate that Rme1 interacts with Mi-1.2 at this specific site. It is unknown yet whether the switch in these amino acids also abolishes aphid or whitefly resistances. Therefore, direct interaction between Mi-1 and the avirulence determinant from these organisms could not be ruled out. Direct interaction of the NB-LRR type of R proteins with corresponding Avr proteins remain the exception rather then the rule (Jia et al. 2000). Recent information indicates indirect interaction of the NB-LRR type of R proteins with corresponding avr determinants (Axtell and Staskawicz 2003; Leister and Katagiri 2000; Mackey et al. 2002, 2003). Both *Arabidopsis* RPM1 and RPS2 R proteins interact with their corresponding Avr proteins through RIN4, which is the target of three different bacterial effector proteins (Mackey et al. 2002). If Rme1 is the target of the nematodes, aphid, and whitefly Avr effector proteins, cloning this gene will elucidate its role in the *Mi-1*-mediated resistance and provide further evidence for indirect interaction of NB-LRR proteins and pathogen or insect effectors to trigger the plant innate immune responses.

MATERIALS AND METHODS

Plant material.

Tomato cultivars Rio Grande 76R (*Pto/Pto*), UC82B, and Marmande *(mi-1/mi-1*), VFN (*Mi-1/Mi-1*), and a near-isogenic pair, Motelle (*Mi-1/Mi-1*) and Moneymaker (*mi-1/mi-1*), were used. In addition, a mutant in the background of the wild-type parent 'Motelle', *rme1*, was also used. Seeds for *rme1* were gathered from homozygous *rme1/rme1* plants obtained by backcrossing *rme1* twice to wild-type parent 'Motelle' and selfing. Seeds were germinated in sunshine mix or similar medium, and 4-week-old seedlings were transplanted into pots (10 cm diameter, 17 cm deep) with organic soil mix or perlite, unless otherwise stated. Plants were fertilized and maintained in either growth chambers or greenhouses.

Nematode cultures and plant inoculation.

Cultures of *Mi-1*-avirulent root-knot nematodes, *M. incognita* isolate Project 77, *M. arenaria* isolate W, and *M. javanica* isolate VW4 were maintained on tomato cv. UC82B. *Mi-1*-virulent *M. incognita* isolate W1 was maintained on tomato cv. VFN. The identity of the nematodes was confirmed using polymerase chain reaction amplification of the intergenic spacer region between the cytochrome oxidase subunit II and 16S rRNA gene, followed by restriction with *Hin*fI enzyme (Powers and Harris 1993; Williamson et al. 1994). Nematode inoculum consisting of infective-stage juveniles was obtained as described by Martinez de Ilarduya and associates (2001). Freshly emerged infective-stage juveniles (within 48 h) were used for assays. Four- to five-week-old plants in one-L pots containing steam-sterilized loamy sand were inoculated with infective-stage juveniles. Each combination of host genotype, inoculum dosage, and nematode isolate was replicated five times. After inoculation, the plants were maintained in a greenhouse at 22 to 25ºC for six weeks. Plants were then uprooted, roots were washed, and fresh weights were recorded. Eggs were extracted from individual root systems, using the bleach and blender method as described by Hussey and Barker (1973). Egg suspensions were stored at 4ºC. After appropriate dilution, eggs in two 1-ml aliquots from each sample were counted under a dissecting microscope, and the number of eggs per sample was calculated. The number of eggs per gram of root was calculated, and data were analyzed using analysis of variance (ANOVA), and means were compared by the least significance difference (LSD) test. The experiment with *Mi-1*-virulent *M. incognita* isolate W1 was repeated once, and data from both experiments were combined for statistical analysis.

Whitefly rearing and bioassay.

B. tabaci adults from the B biotype were used in the experiments. This population had been reared on tomato cv. Marmande for more than 30 generations. For the free-choice assay, 8-week-old plants were moved to an insect-free greenhouse and were placed equidistant from each other, such that leaves from a plant did not touch leaves from another plant. Greenhouse temperatures averaged 23 (day) and 18ºC (night), and a relative humidity was 46 to 69%. Plants were infested with whiteflies by releasing mature adults in the center of the greenhouse. After five days of whitefly exposure, the number of adult whiteflies was counted in situ on all leaves. Counts were repeated every other day for a period of 15 days. Counts were made early in the morning before the adults became active. Number of adults were $\log_{10}(x + 1)$-transformed and analyzed with a one-way ANOVA. When F values were significant, means were compared by the Tukey's honestly significant difference (HSD) test. Proportions (p) of *B. tabaci* daily infestation were transformed to arcsine $(p/100)^{1/2}$ before analysis (Statsoft, Tulsa, OK, U.S.A.).

For the no-choice assay, 8-week-old tomato plants were used. Adult female whiteflies were placed in a plastic clip cage and were attached to a single leaflet, such that whiteflies had access to the abaxial surface of the leaf (Nombela et al. 2001). One cage per plant was used. Plants were maintained in a growth chamber at 25ºC with a photoperiod of 16 h light and 8 h dark and a relative humidity of 68 to 75%. At the end of the experiment, the number of eggs laid on each plant was recorded. Number of eggs were $\log_{10}(x + 1)$-transformed and were compared by a one-way ANOVA and Tukey's HSD test (Statsoft).

Bacterial assay.

The *P. syringae* pv. *tomato* DC3000 was provided by D. Cooksey (University of California, Riverside). *P. syringae* pv. *tomato* was grown on MGY plate for 48 h at 28ºC (Keene et al. 1970). Confluent colonies were washed away with 10 mM $MgCl_2$ and the suspension was diluted to a concentration of 1×10^4 CFU ml^{-1}. Leaflets of 5-week-old tomato plants were infected by infiltration using a syringe. Plants were sampled at 0, 2, and 4 days after infection. For each tomato cultivar, four plants were sampled for each timepoint, using a cork borer. Leaf disks were ground, and three aliquots of each serial dilution were plated on MGY plates. Plates were incubated at 28ºC and, colony number was counted after 48 h.

Transient *Agrobacterium*-mediated expression.

Agrobacterium tumefaciens C58C1 containing binary plasmid pTFS-40 expressing a constitutive gain-of-function mutant Pto_{L205D} was transiently expressed in tomato plants (Chang et al. 2002). Bacterial inoculum was grown overnight in Luria-Bertani media, supplemented with 25 μg ml^{-1} of kanamycin plus 5 μg ml^{-1} of tetracycline per ml, at 30ºC. Overnight cultures were diluted in infiltration medium (10 mM $MgCl_2$, 10 mM MES [morpholineethanesulfonic acid], 150 μM acetosyringone) to an OD_{600} of 0.1 and 0.03, as described elsewhere (Chang et al. 2002; Rathjen et al. 1999). *A. tumefaciens* was infiltrated, using a syringe, into leaflets of seven-week-old plants. After leaf infiltration, tomato plants were maintained in the lab at low light for 5 to 7 days for symptom development. This experiment was repeated once.

Plant transformation.

The binary vector pTFS 40 containing the indicated *Mi-1* was transferred into *A. rhizogenes* A4RS by triparental mating (Bevan 1984). *A. rhizogenes*-mediated tomato transformation was carried out as previously described (Hwang et al. 2000). Briefly, individual cotyledons were excised from 8- to 10-day-old tomato seedlings and were immersed in an overnight *A. rhizogenes* suspension (OD_{600} of 0.8) for 5 min. The inoculated cotyledons were placed on Murashige-Skoog (MS) medium with 2% (wt/vol) sucrose and 0.8% (wt/vol) agar. After 3 days of preincubation, the cotyledons were transferred into MS medium supplemented with 50 μg ml^{-1} of kanamycin per ml and 250 μg ml^{-1} of cefotaxime per ml. Transformed hairy roots emerged from the surface of the cotyledons after 7 to 10 days of incubation at 25ºC in the light.

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