MPMI Vol. 20, No. 2, 2007, pp. 159–167. DOI: 10.1094/MPMI-20-2-0159.

Characterization of the Interaction Between the Bacterial Wilt Pathogen *Ralstonia solanacearum* and the Model Legume Plant *Medicago truncatula*

Fabienne Vailleau,[1] Elodie Sartorel,[1] Marie-Françoise Jardinaud,[1] Fabien Chardon,[2] Stéphane Genin,[2] Thierry Huguet,[1] Laurent Gentzbittel,[1] and Michel Petitprez[1]

[1]INP-ENSAT, Laboratoire de Biotechnologie et Amélioration des Plantes, BP32607, 31326 Castanet-Tolosan cedex, France; [2]Laboratoire des Interactions Plantes-Microorganismes, UMR CNRS/INRA 2594, BP52627, 31326 Castanet-Tolosan cedex, France

Submitted 29 May 2006. Accepted 28 August 2006.

The soilborne pathogen *Ralstonia solanacearum* is the causal agent of bacterial wilt and attacks more than 200 plant species, including some legumes and the model legume plant *Medicago truncatula*. We have demonstrated that *M. truncatula* accessions Jemalong A17 and F83005.5 are susceptible to *R. solanacearum* and, by screening 28 *R. solanacearum* strains on the two *M. truncatula* lines, differential interactions were identified. *R. solanacearum* GMI1000 infected Jemalong A17 line, and disease symptoms were dependent upon functional *hrp* genes. An in vitro root inoculation method was employed to demonstrate that *R. solanacearum* colonized *M. truncatula* via the xylem and intercellular spaces. *R. solanacearum* multiplication was restricted by a factor greater than 1×10^5 in the resistant line F83005.5 compared with susceptible Jemalong A17. Genetic analysis of recombinant inbred lines from a cross between Jemalong A17 and F83005.5 revealed the presence of major quantitative trait loci for bacterial wilt resistance located on chromosome 5. The results indicate that the root pathosystem for *M. truncatula* will provide useful traits for molecular analyses of disease and resistance in this model plant species.

Additional keywords: QTL.

Plants have developed a wide array of defense mechanisms to defeat invading pathogenic organisms. Disease resistance can be determined by the simultaneous presence of a resistance (*R*) gene in the plant and an avirulence (*Avr*) gene in the microorganism, the so-called gene-for-gene theory (Flor 1971). In many other cases, disease resistance has been described as polygenic, genetic systems of plant defense involving multiple quantitative trait loci (QTL) (Gebhardt and Valkonen 2001; Kliebenstein et al. 2002; Koornneef et al. 2004; Wilson et al. 2001; Young 1996). Many agronomically important crop plants are legumes, and *Medicago truncatula* Gaertner has emerged as a model plant for legume genetics and genomics (Cook 1999; VandenBosch and Stacey 2003). Other plant models, including *Arabidopsis thaliana* and rice, are unable to establish nitrogen-fixing symbiosis with soil rhizobia, *Arabidopsis* also fails to form symbiotic root mycorrhizae with soil fungi (Albrecht et al. 1999; Oldroyd et al. 2005). Genetics and genomics *M. truncatula* resources are now well developed, including more than 200,000 expressed sequence tags in databases, genetic and physical maps, development of microarrays, and a BAC-by-BAC genome sequencing project (Cannon et al. 2005; Young et al. 2005). *M. truncatula* can establish symbiotic and pathogenic interactions, and the latter have focused on fungi and oomycetes to date (Kemen et al. 2005; Nyamsuren et al. 2003; Torregrosa et al. 2004). *M. truncatula* offers the opportunity to compare both symbiotic and pathogen interactions in the same plant.

Ralstonia solanacearum is the causal agent of bacterial wilt, one of the most important bacterial diseases worldwide. This soilborne pathogen attacks more than 200 plant species, including many agriculturally important crops (Hayward 1991), as well as the model plant *A. thaliana* (Deslandes et al. 1998; Yang and Ho 1998). The completion of the genome sequence of strain GMI1000 and the in-depth study of the type III secretion system (TTSS) and related pathogenicity effectors place *R. solanacearum* as one of the best characterized plant-root-pathogenic bacterium (Genin and Boucher 2004; Salanoubat et al. 2002). Although *R. solanacearum* infects a variety of legumes, including peanut (*Arachis hypogea*), winged bean (*Psophocarpus tetragonolobus*), common bean (*Phaseolus vulgaris*), and cowpea (*Vignia sinensis*), a genetic characterization of a pathosystem involving *R. solanacearum* and a leguminous host has not been described (Hayward 1994). In order to develop a pathosystem of a bacterial legume root pathogen, an in vitro root inoculation method was developed and used to examine the response of *M. truncatula* to root inoculation with *R. solanacearum*. Furthermore, a genetic analysis of recombinant inbred lines (RILs) enabled us to describe underlying QTL in this pathosystem.

RESULTS

R. solanacearum induces bacterial wilt on *M. truncatula.*

A collection of *R. solanacearum* strains, isolated from legume and nonlegume host plants from diverse worldwide locations, was used for root inoculation of two *M. truncatula* lines, Jemalong A17 (called A17 thereafter) and F83005.5 (Table 1). The 4- to 20-day-old plants were inoculated with strains of *R. solanacearum* (10^6 to 10^8 bacteria/ml) with different inoculation procedures. Compatible and incompatible interactions were

Corresponding author: Fabienne Vailleau; Telephone: (33)5.62.19.35.67; Fax: (33)5.62.19.35.81; E-mail: fabienne.vailleau@ensat.fr

Current address of F. Chardon: INRA, Unité de la Nutrition Azotée des Plantes, Route de St Cyr, 78026 Versailles, France.

identified from the different combinations in whole plant or in vitro tests. In compatible interactions, wilt symptoms (Fig. 1A) (discussed below) affecting 50 to 100% of inoculated plants were observed, depending on the bacterial inoculum concentration. The specific differential interactions were obtained regardless of the age of the plant when inoculated (4 to 20-day-old plants; data not shown). Root inoculation allowed standardized and repeatable conditions to facilitate the comparison of symptoms on the two *M. truncatula* lines and were used for further studies. Three response profiles emerged from inoculations of the 28 *R. solanacearum* strains. Profile one consisted of the F83005.5 line, which was resistant to six strains (Table 1; strains GMI1000, 006, 012A, UW502, JS763, and JS940; disease index score from 0 to 0.44). Profile two consisted of two strains, Rd15 and UW377, which produced severe symptoms on both lines (Table 1; disease index score from 1.75 to 4). Profile three consisted of 20 strains that were weakly pathogenic or caused no symptom on A17 and F83005.5 lines (Table 1; disease index score from 0 to 0.75). Among 16 strains isolated from members of the family *Leguminosae* (Table 1; strains isolated from peanut, broad bean, and wing bean), only four induced bacterial wilt symptoms on A17. Strains Rd15, JS940, and GMI1000 caused death of all A17 plants during the time of the experiment (Table 1, scores of 4; Fig. 1B), whereas inoculation with strains UW502 and UW377 resulted in intermediate symptoms, with some plants remaining healthy (Table 1, scores of 3 and 1.75; Fig. 1B).

To determine whether the *hrp* gene cluster of *R. solanacearum* was important for the pathogenicity on a *M. truncatula* strain, GMI1402, an *hrp* mutant derivative of the wild-type strain GMI1000 was used to root inoculate A17 plants. No symptoms were observed with this, indicating that *R. solanacearum* infection was dependent on the *hrp* gene cluster that encodes a TTSS (Fig. 1C).

Differential symptoms also are observed following in vitro root inoculation.

An in vitro inoculation system was set up to carry out microscopic studies of *R. solanacearum* pathogenesis in infected roots. Plants (4, 7, or 10 days old) were grown in vertically oriented petri dishes, so that the root system developed onto paper surface, and inoculated (Fig. 2A). F83005.5 plants inoculated with GMI1000 had continued growth and development of secondary roots, without wilting symptoms on cotyledons and the first leaf (Fig. 2B); they were resistant. In contrast, A17 plants showed chlorosis on cotyledons and the first leaf, and the development of secondary roots and leaves stopped (Fig. 2C). Bacterial exudates, called oozes and considered classic wilt disease symptoms, always were observed in compatible interactions on roots and hypocotyls (Fig. 2C through E). Inoculations with GMI1000 on the A17 and F83005.5 lines were consistent with the differential interactions obtained with the intact plant assay.

***R. solanacearum* colonizes *M. truncatula* xylem vessels and intercellular spaces.**

To learn more about the mode of infection and colonization of the pathogenic strain GMI1000 on A17 susceptible line, we studied whole plantlets after inoculation under in vitro conditions with cut root tips. Histological localization of *R. solanacearum* was made using strains GMI1600 and GMI1559, derivatives of strain GMI1000 expressing the

Table 1. Characteristics of the *Ralstonia solanacearum* strains used in this study and index disease obtained on two *Medicago truncatula* lines after root inoculation with these strains[a]

Strains[c]	Other designation[c]	Geographical origin	Isolated from	Source	Race	Biovar	Disease index on *M. truncatula* lines[b]	
							A17	F83005.5
	GMI1000	French Guyana	Tomato	C. Boucher	1	3	4	0
006	GMI8080	Australia, Queensland	*Xanthium pugens*	C. Boucher	1	3	4	0
012A	GMI8082	Australia, Queensland	*Rapistrum rugosum*	C. Boucher	1	3	3.25	0
UW502	…	Indonesia	Peanut	C. Allen	1	3	3	0
JS763	…	Indonesia	Peanut	J. Luisetti	1	3	4	0.33
JS940	…	Maurice	Broad bean	J. Luisetti	1	3	4	0.44
Rd15	GMI1566	Taïwan	Radish	C. Boucher	nd	3	4	3.5
UW377	…	China	Peanut	C. Allen	1	3	1.75	2.62
JS839	…	China	Peanut	J. Luisetti	1	4	0.75	0
UW369	…	China	Peanut	C. Allen	1	4	0.75	0
MOLK2	GMI8238	Indonesia	Banana	P. Prior	2	1	0	0
BA1	GMI8038	Granada	Banana	C. Boucher	2	nd	0	0
IPO1609	…	Nederlands	Potato	C. Boucher	3	2	0	0
UW82	GMI8017	Columbia	Potato	C. Boucher	3	1	0	0
UW25	K60	North Carolina	Tomato	C. Boucher	1	1	0	0
UW85	GMI8009	Ontario	Tomato	C. Boucher	1/2	nd	0	0
UW143	GMI8020	Australia, Queensland	Tomato	C. Boucher	1	3	0	0
UW203	GMI8018	North Carolina	Tobacco	C. Boucher	1	1	0	0
M41	…	Malaysia	Peanut	P. Prior	1	3	0	0
CIP418	…	Indonesia	Peanut	P. Prior	1/2	1	0	0
T1	…	Indonesia	Peanut	P. Prior	3	2	0	0
JQ116	…	La Réunion	Peanut	J. Luisetti	1	3	0	0
JT678	…	Ouganda	Peanut	J. Luisetti	1	3	0	0
UW338	…	Malaysia	Wing bean	C. Allen	nd	nd	0	0
UW368	…	China	Peanut	C. Allen	1	3	0	0
UW374	…	China	Peanut	C. Allen	1	4	0	0
UW375	…	China	Peanut	C. Allen	1	4	0	0
UW376	…	China	Peanut	C. Allen	1	4	0	0

[a] For each strain, two to three independent experiments were made; nd = not determined and 1/2 = not discriminated between race 1 or race 2.

[b] Disease index scored 24 days after *R. solanacearum* root inoculation at 10^8 bacteria/ml. Values represented the means for eight plants per line and per strain.

[c] Strain designation: GMI = C. Boucher, CNRS-INRA, LIPM, Castanet Tolosan, France; UW = C. Allen, Department of Plant Pathology, University of Wisconsin–Madison, U.S.A.; and JS, JQ, JT, M, CIP, and T = J. Luisetti and P. Prior, Laboratoire de Phytopathologie, CIRAD-FLHOR, Saint-Pierre, La Réunion, France.

green fluorescent protein (*GFP*) and β-glucuronidase (*GUS*) reporter genes, respectively, under the control of constitutive promoters (Aldon et al. 2000; Etchebar et al. 1998). First, by confocal microscopy, we recorded emitted light between 590 and 650 nm, corresponding to cell wall autofluorescence, in order to obtain general root architecture (encoded in red). Second, the GFP signal recorded between 510 and 525 nm allowed us to localize the bacteria. Bacteria appeared green or yellow when superimposed on root tissues. Confocal microscopy observations showed that *R. solanacearum* GMI1000 first colonized the intercellular spaces of the inner cortex, which appeared filled with bacteria 24 h after inoculation (Fig. 3A). Bacteria were seen outside the root, on the surface, and also inside the root, in an intercellular location between the epidermal layer and cortical cells, as well as deeper, between cortical cells.

Using bright-field microscopy, transverse sections of root revealed the presence of bacteria in the protoxylem and in the metaxylem vessels 2 to 3 days after inoculation (Fig. 3B). Intense colonization also was observed in the cortex of the primary root, at the emerging point of secondary roots (Fig. 3C). Vascular colonization progressed to aerial parts 3 to 4 days after inoculation in the case of the compatible interaction. We observed propagation of bacteria in xylem vessels of cotyledons (Fig. 3D) and of leaves (Fig. 3E), with bacteria multiplying very quickly and invading the whole aerial part

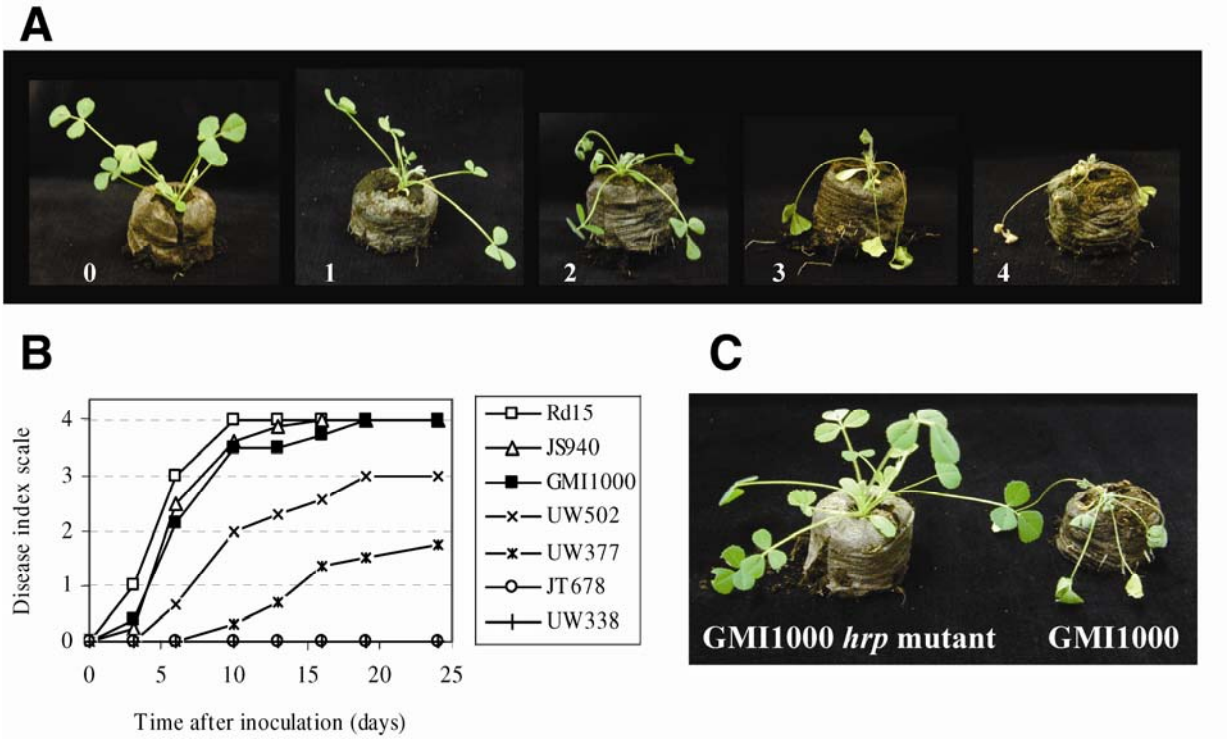

Fig. 1. *Medicago truncatula* (A17 line) symptoms after root inoculation by *Ralstonia solanacearum* strains at 10^8 bacteria/ml. **A,** Phenotypes of plants after inoculation with strain GMI1000 and disease index score associated. **B,** An example of progression of disease symptoms after inoculation with differently pathogenic strains of *R. solanacearum* in A17. Means presented were calculated for 16 plants per strain. **C,** Phenotypes of plants 10 days after the inoculation with an *hrp* mutant (left) and with the wild-type strain GMI1000 (right).

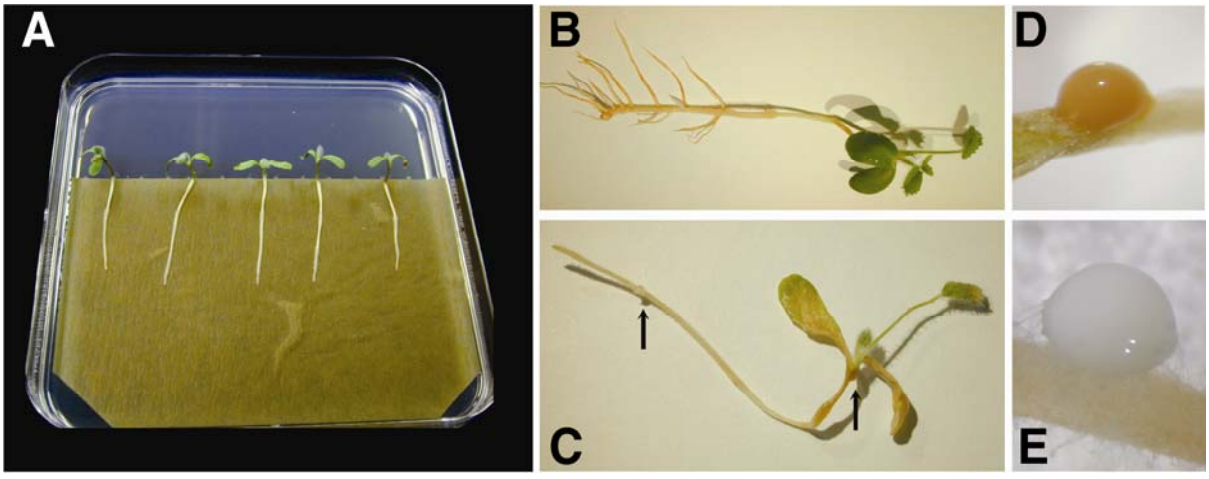

Fig. 2. Plant phenotypes after in vitro root inoculation with GMI1000 *Ralstonia solanacearum* strain. A suspension of 10^8 bacteria/ml was used for inoculation of **A** and **C** through **E,** A17 and **B,** F83005.5. Observations were made **A,** 4 and **B** through **E,** 7 days after inoculation. **A,** Presentation of the system of root inoculation in petri dishes. **B,** F83005.5 plants did not develop any symptom. **C,** A17 plants present chlorosis on aerial part and exudates (oozes) on root and hypocotyl (arrows). **D,** Ooze on hypocotyl. **E,** Ooze on root.

(Fig. 3F). When intact roots were inoculated, bacteria penetrated preferentially through the root apex. Colonization via sites of secondary root emergence also was observed (data not shown).

R. solanacearum multiplication is restricted in the F83005.5 line.

Histological localization of strain GMI1559 (GMI1000::GUS derivative) in whole plant tissues was made to estimate the propagation of the bacteria at different times after inoculation of A17 and F83005.5 lines under in vitro conditions using cut root tips. Measurement of the percentage of tissue colonized by bacteria from 1 to 7 days after inoculation highlighted differential bacterial propagation in the two lines. Whereas the whole root vascular system, hypocotyls, and cotyledons were invaded 3 days after the inoculation of A17 plants, the bacteria were visible only in roots in F83005.5 (Fig. 4A). To validate and quantify these observations, we measured the internal bacterial population in aerial parts of A17 and F83005.5 lines inoculated via roots in Jiffy pots. Bacteria were detected in the leaves 2 to 3 days post inoculation in *M. truncatula* A17 line. Six days after inoculation, bacterial concentration was at its maximum, with 10^6 to 10^7 bacteria per gram of fresh weight (Fig. 4B). The bacterial concentration plateaued until 16 days and then drastically declined. This phenomenon corresponded to the death of the plant and, consequently, to the arrest of the bacterial multiplication. In the *M. truncatula* F83005.5 line, detection of bacteria in the leaves was delayed and a highly reduced bacterial population was observed, the concentration of the strain GMI1000 being more than 10^5 times lower in the F83005.5 line than in the A17 line. Histolocalization and internal bacterial population measurements were made with the GMI1402 *hrp* mutant strain as a control. This strain is deleted from the *hrp* cluster and causes no disease symptom on inoculated tomato and *Arabidopsis* (Deslandes et al. 1998). Our results confirmed that a functional TTSS was essential for the propagation of the bacteria in the *M. truncatula* plant (Fig. 4A and B). Taken together, these results clearly indicated that the multiplication of *R. solanacearum* GMI1000 was highly restricted in the resistant F83005.5 line.

A major QTL on chromosome 5 is related to resistance of F83005.5 line.

Taking advantage of the clear phenotypical difference observed after interaction between strain GMI1000 and some *M. truncatula* lines, we conducted a QTL analysis on the average resistance to strain GMI1000 obtained on 111 F7 and 129 F8 RILs from a cross between A17 and F83005.5. The genetic analysis was done using an already existing framework genetic map (T. Huguet, *unpublished data*) comprising 105 evenly distributed simple sequence repeat (SSR) markers. Disease symptoms were scored every 2 to 3 days, from 3 to 26 days after root inoculation. Heritabilities composed between 75 and 80%, depending of the day of evaluation, indicating that more than three-quarters of the total phenotypic variation is due to the genetic variation. A major QTL was detected on chromosome 5 as early as 5 days after inoculation. This QTL reached a maximum log of the odd ratio (LOD) score value (14.0) 7 days after inoculation and could be localized between SSR markers h2-23d7 and 3B01 within a confidence interval of 5 centimorgans (cM) and account for 38.5% of the phenotypic variation (Table 2). Two minor QTL also were detected with the monitoring of the disease index score on chromosome 3, between SSR markers 2E06 and 13B3, and on chromosome 7, between the top of LG7 and SSR marker 1H02 (marker information available on the TIGR *Medicago truncatula* website). Their

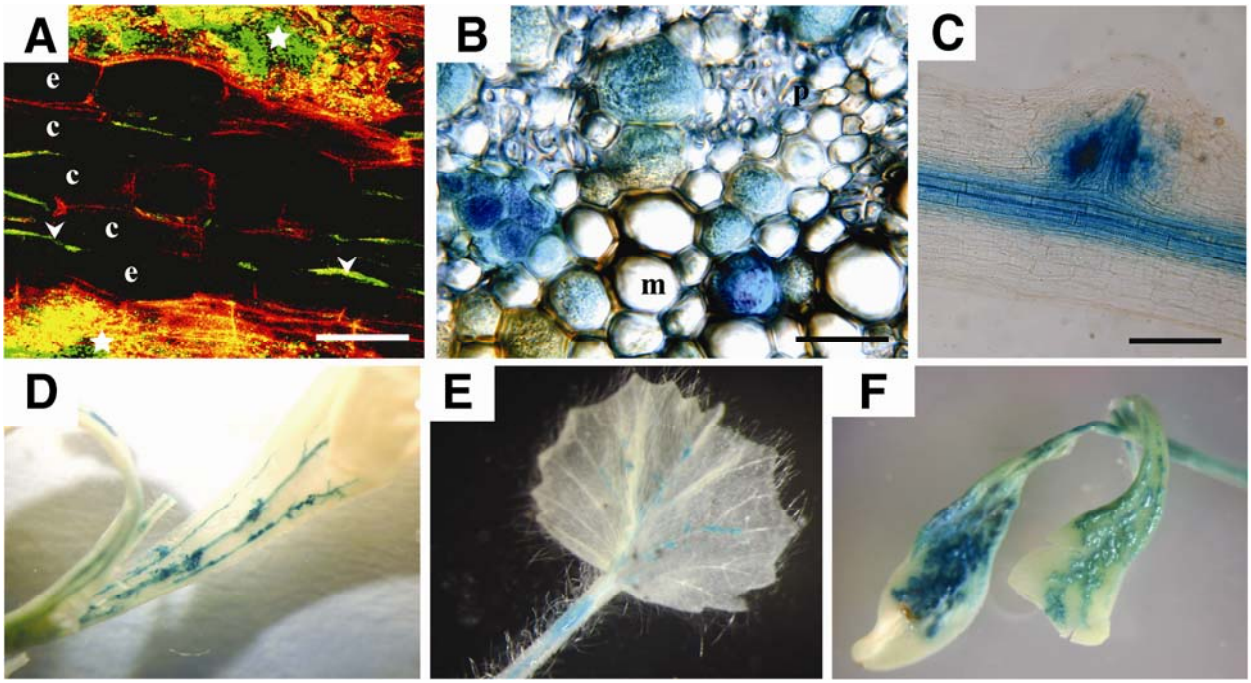

Fig. 3. Localization of *Ralstonia solanacearum* strain GMI1000 after in vitro root inoculation. A suspension of 10^8 bacteria/ml was used for inoculation of A17 plants. Observations were made **A** through **D,** 3 days after inoculation, **E,** 4 days after inoculation, and **F,** 7 days after inoculation. **A,** Confocal microscopy on roots: visualization of the colonization of the intercellular spaces with strain GMI1000::GFP in green. This view corresponds to the merging of the two wavelength images (root and bacteria detection) of a stack of four optical sections of 1 µm. In this way, we observed bacteria outside the root (green and yellow), on the surface (white stars), but also in the root (green), in an intercellular position, between the epidermal layer and cortical cells (white arrowheads), and deeper, between cortical cells; e = epidermic cell and c = cortical cell. **B** and **C,** Bright-field microscopy of infected roots. Bacteria were localized by histochemical staining of β-glucuronidase (GUS) activity. Bacteria are detected by blue staining in protoxylem (p) and metaxylem (m) vessels in **B,** transverse and **C,** longitudinal sections. **C,** Deep staining is observed at the emergence site of a secondary root. **D** to **F,** GMI1000::GUS visualization in vessels of **D,** cotyledons and **E,** first leaf and **F,** in the whole cotyledon tissues. Scale bars = **A,** 200 µm, **B,** 40 µm, and **C,** 500 µm.

LOD scores were 4.0 and 3.8, respectively, and account for 13.1 and 17.6% of the phenotypic variation (Table 2). It is noteworthy that all three QTL contribute to resistance from F83005.5 alleles. Because R^2 values of QTL are not additive, we can estimate that the three QTL together represent, at most, 45% of the phenotypic variance based on a PlabQTL multilocus model.

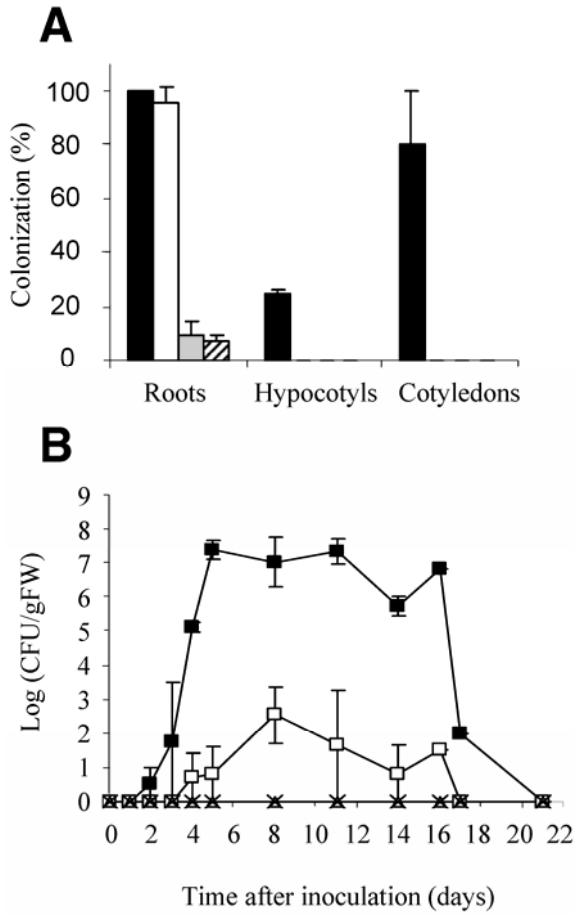

Fig. 4. Colonization and multiplication of wild-type and *hrp* mutant *Ralstonia solanacearum* strains in susceptible (A17) and resistant (F83005.5) lines of *Medicago truncatula*. **A,** Measurement of the percentage of tissue colonization by bacteria expressing the β-glucuronidase (GUS) reporter gene was made on plantlets 3 days after inoculation with A17/GMI1000 (filled column), F83005.5/GMI1000 (empty column), A17/GMI1000 *hrp* mutant (gray column), and F83005.5/GMI1000 *hrp* mutant (diagonal line column). Percent (%) represents the ratio between tissue colonized with the bacteria on the totality of the tissue of the organ observed. Means from measurements of blue staining from five plants are given. Two replicates were made. **B,** Measurement of in planta growth of *R. solanacearum* in the following interactions: A17/GMI1000 (■), F83005.5/GMI1000 (□), A17/GMI1000 *hrp* mutant (△), and F83005.5/GMI1000 *hrp* mutant (×). A suspension containing 10^7 bacteria/ml was used. Data points represent the mean of two replicate experiments and standard deviations are shown.

Search for *R. solanacearum* effectors acting as putative *Avr* genes on *M. truncatula.*

The ability of *R. solanacearum* to cause bacterial wilt on more than 200 host plants is dependent upon a large ensemble of TTSS-related effectors that presumably are injected into plant cells (Genin and Boucher 2004). Recently, 48 candidate

Table 3. *Ralstonia solanacearum* mutant strains and gene designation[a]

Mutant strains[b]	*R. solanacearum* genes
brg1	RSp0841
brg2	RSp0848
brg3	RSp0853
brg4	RSp0854
brg5	RSp0839
brg6	RSc2775
brg7	RSc0868
brg8	RSp0304
brg9	RSc3369
brg10	RSc3212
brg11	RSc1815
brg12	RSp0323
brg13	RSc3290
brg14	RSp0572
brg15	RSp1281
brg16	RSp0213
brg17	RSc1349
brg18	RSp0160
brg19	RSc1386
brg20	RSc0245
brg21	RSp0218
brg22	RSp0193
brg23	RSc0257
brg24	RSp0842
brg25	RSp0672
brg26	RSc1800
brg27	RSc1801
brg28	RSc1356
brg29	RSc1357
brg30	RSp1024
brg31	RSp0099
brg32	RSp0847
brg33	RSp0845
brg34	RSp0879
brg35	RSp0885
brg36	RSc2359
brg37	RSp1022
brg38	RSp1031
brg39	RSp0732
brg40	RSc3272
brg41	RSc1474
brg42	RSc1475
brg43	RSp1384
brg44	RSp1130
brg45	RSp1460
brg46	RSc0608
brg47	RSc3174
brg48	RSp1388

[a] F83005.5 plants were grown in Jiffy pots and were inoculated with a concentration of 10^8 bacteria/ml.
[b] More details have been described by Cunnac and associates (2004).

Table 2. Quantitative trait loci (QTL) detected in the F7 and F8 recombinant inbred line (RIL) populations derived from A17 × F83005.5

	Disease index scoring				
Chromosome	Position (cM)[a]	Confidence interval (cM)	LOD	R^{2b}	Effect[c]
3	6	0–7	4.0	13.1	–0.16
5	25	23–28	14.0	38.5	–0.34
7	3	0–10	3.8	17.6	–0.18

[a] QTL position in centimorgans (cM) at the maximum log of the odd ratio (LOD) peak.
[b] Part of the phenotypic variance explained by an individual QTL.
[c] Effect of the substituting A17 alleles for F83005.5 alleles at the LOD peak of the QTL. A negative sign indicates that the increasing resistance of the QTL alleles were contributed by the resistant parent.

type III effector genes were identified in strain GMI1000 (Cunnac et al. 2004). Therefore, we tested mutant bacterial strains to determine which candidate effectors, if any, were responsible for the resistance of F83005.5. In all, 48 mutant strains of GMI1000, each carrying an individual disruption in the candidate effector genes (Table 3), were used to inoculate the F83005.5 line. For each mutant and the wild-type strain GMI1000, no wilt symptom could be observed, suggesting that none of these putative effectors was able to act as a single determinant to induce the resistance of F83005.5.

DISCUSSION

R. solanacearum induces bacterial wilt on more than 200 different plant species, including legumes. This is the first report showing that this soilborne bacterium can infect the legume model plant *M. truncatula*. Here, we describe two genotypes of *M. truncatula* with differing responses to *R. solanacearum* which allowed us to identify and compare compatible and incompatible interactions. Interestingly, the reference A17 *M. truncatula* line was found to be susceptible to strain GMI1000, a model strain for the analysis of pathogenicity and for which many genomic resources are already available (Genin and Boucher 2004; Salanoubat et al. 2002). Strain Rd15, reported to be highly aggressive on several hosts (Yang and Ho 1998), also was found to be very aggressive on *M. truncatula*. To identify host specificity determinants, we challenged *M. truncatula* with 16 strains isolated from legumes. Nonetheless, the two lines we tested did not develop more disease symptoms with these strains compared with inoculation with strains isolated from nonlegume host plants. More *M. truncatula* genotypes and *R. solanacearum* strains will be evaluated to obtain an overview of strain–host specificity, depending or not upon host origin or the phylogenetic division.

The development of an in vitro root inoculation assay enabled us to observe root phenotypes following inoculation, especially exudates of bacteria and exopolysaccharides (EPS) in the susceptible line. The production of EPS in the xylem vessels has been proposed to contribute to wilting, leading to the death of the plant. McGarvey and associates (1999) demonstrated that the amount of EPS I produced per gram of plant tissue was inversely correlated with the level of resistance to *R. solanacearum* in tomato. It will be of interest to measure EPS production in both A17 and F83005.5 lines, or to inoculate these lines with *R. solanacearum eps* mutants affected in EPS production to learn more about the relationship.

By monitoring the constitutive expression of GFP and GUS in bacteria, different infection stages were observed. Bacteria penetrated *M. truncatula* roots by natural openings, as in tomato and eggplant, and colonized *M. truncatula* in a way similar to tomato infection (Grimault and Prior 1994; Vasse et al. 1995). In our tests, the same root infection process was observed with or without cutting root tips prior to inoculation; however, in order to standardize the inoculation method, all the experiments described here were carried out with root tips cut. Microscopic GUS activity observations showed that aerial parts of susceptible plants were invaded by *R. solanacearum* to a much greater extent than those of resistant plants. Furthermore, in planta bacterial measurements carried out on the leaves of plants revealed a highly significant difference between the two lines, the population being 10^5 times higher in the susceptible line than in the resistant one. Similar observations have been made on tomato, where ingress by *R. solanacearum* was 5- and 15-fold slower in two resistant cultivars compared with the susceptible cultivar (McGarvey et al. 1999). At this point, we cannot draw conclusions about the factors involved in limiting bacterial spread in the F83005.5 resistant line. An induced production of tyloses, a nonspecific physical barrier, was shown in some cases to occlude tomato colonized vessels, mainly contributing to resistance (Grimault et al. 1994). In addition, we demonstrated that both bacterial multiplication and development of disease symptoms were dependent upon functional *hrp* genes. The integrity of the *hrp* genes for *R. solanacearum* pathogenicity also was shown to be required in other plant–bacteria pathosystems, such as in the *Arabidopsis. thaliana–R. solanacearum* pathosystem (Deslandes et al. 1998). Taken together, these results demonstrate that *M. truncatula* is a promising model host plant for *R. solanacearum*.

To date, bacterial wilt resistance has been described as a complex trait in most hosts, with several inheritance studies reporting resistance to *R. solanacearum* demonstrating both simple and polygenic inheritance patterns. These different responses all were dependent on the host plant–*R. solanacearum* strain combination tested. In *A. thaliana*, monogenic dominant (Ho and Yang 1999) and recessive (Deslandes et al. 1998) resistances have been identified. The response of the Nd-1 accession of *A. thaliana* to strain GMI1000 permitted the identification of *RRS1-R*, the first resistance gene cloned to bacterial wilt (Deslandes et al. 2002). However, polygenic resistance response to strain 14.25 was governed by at least three loci in the Col-0 accession of *A. thaliana* (Godiard et al. 2003). Among them, the *ERECTA* gene, a developmental regulator in plants, was shown to participate in resistance. Several QTL analysis have been performed in tomato with the cross Hawaii 7996 (resistant cultivar) × WVa700 (susceptible cultivar). Inoculation of the F3 population with strain GMI8217 highlighted, among seven different loci potentially implicated, an important QTL of resistance on chromosome 6 carried by Hawaii 7996 (Thoquet et al. 1996a,b). More recent data suggested that two QTL, in fact, were present on chromosome 6, approximately 30 cM apart (Mangin et al. 1999). Other studies, with the same (Wang et al. 2000) or another cross (Danesh et al. 1994), testing other *R. solanacearum* strains, also described polygenic resistances showing that, in tomato, resistance to bacterial wilt is a complex strain-specific trait.

To unravel the genetic basis of resistance in F83005.5 to *R. solanacearum* GMI1000, we performed a preliminary QTL analysis by composite interval mapping (CIM) and identified one major locus within a confidence interval of 5 cM near the top of chromosome 5 (Choi et al. 2004). This genetic locus was identified combining two sets of experiments on 111 F7 and 129 F8 recombinant inbred lines analyzed by scoring disease index symptom progression. Two minor QTL also were detected on chromosome 3 and 7. Even if major QTL have been identified, questions remain. Are these three QTL specific to strain GMI1000 or does it confer a broad spectrum of resistance to *R. solanacearum* strains? If there is a single gene or a cluster of genes involved in resistance to *R. solanacearum* located at the locus on chromosome 5, what are the implications of the two smaller QTL in resistance to strain GMI1000? In order to answer to these questions, we currently are narrowing the confidence interval and targeting the gene or genes involved in the resistance by fine mapping new markers in the region of interest. We also are testing additional strains of *R. solanacearum* with the same *M. truncatula* parental lines to determine the spectrum of resistance controlled by these loci. Among plant–pathogen interactions developed with *M. truncatula*, few resistance loci have been reported so far. Resistance to *Colletotrichum trifolii*, the causal agent of anthracnose on legumes, has been described, probably controlled by a single dominant gene. A17 was resistant and F83005.5 susceptible (Torregrosa et al. 2004), the opposite of the responses observed in the *R. solanacearum* pathosystem here described. More recently, Klingler and associates (2005) showed that *M. truncatula* re-

sistance to aphids (*Acyrthosiphon kondoi* Shinji) in the Jester line segregated as a single dominant gene.

As a result of finding a major QTL involved in F83005.5 resistance to strain GMI1000, we evaluated the response of this line to 48 GMI1000 mutants, affected in potential effectors of TTSS. None of these mutations altered the establishment of the resistance; therefore, we can conclude that no *Avr* gene could be identified within the framework of a monogenic interaction. The screening of some *R. solanacearum* TTSS mutants conducted in the identification of PopP2 (Deslandes et al. 2003) and PopP1 (Lavie et al. 2002) in other studies led to the identification of a simple mutation in *R. solanacearum* which resulted in a loss of resistance in *Arabidopsis thaliana* and in *Petunia* spp. Following the hypothesis that some of these effectors could act as *Avr* genes on *M. truncatula*, in our study, we showed that resistance was not affected either because the avirulence determinants correspond to uncharacterized effectors not present in the set of the 48 effector genes tested, or because several effectors simultaneously are required to initiate resistance. This last point seems more plausible because we have identified three QTL involved in disease resistance. These data suggest a potentially complex mechanism, as observed in several interactions for tomato with the same pathogen.

In this work, a new pathosystem between *M. truncatula* and *R. solanacearum* was characterized. This root pathosystem constitutes a valuable tool to identify and study novel plant resistance genes and also to improve our knowledge of *R. solanacearum* type III-dependent effectors. In contrast to *A. thaliana*, where the genetics for responses to pathogen attack is better understood, modes of *M. truncatula* resistance still remain a "black box." Progress in understanding bacterial wilt resistance in *M. truncatula* will improve our knowledge of the genetics of resistance to *R. solanacearum* in legumes such as peanut as well as nonlegumes such as potato, tomato, or banana. Finally, studying defense mechanisms on the model legume plant *M. truncatula* using a root pathogen bacterium is a key step in deciphering possible relations between pathogenic and symbiotic interactions. Similar comparisons have been made previously through the study of chitinases in roots interacting with pathogenic fungi, rhizobia, and mycorrhizal fungi (Salzer et al. 2000). In alfalfa, roots were challenged by dual infections with the arbuscular mycorrhizal fungus *Glomus intraradices* and the pathogen *Rhizoctonia solani* (Guenoune et al. 2001). The development of such a pathosystem, especially under in vitro conditions, constitutes an attractive tool, representing the baseline for a system that allows us to make comparisons in the same conditions for pathogenic and symbiotic microorganisms, utilizing coinoculation experiments. Thus, we will be able to study potential crosstalk between these two forms of interaction.

MATERIALS AND METHODS

Bacterial strains.

The *Ralstonia solanacearum* strains listed in Table 1 are wild-type strains. For microscopy protocols, derivatives of the strain GMI1000 used were GMI1402 (GMI1000*hrcS::*Tn5B20) (Arlat et al. 1992), GMI1559 (GMI1000::GUS) (Etchebar et al. 1998), GMI1560 (GMI1000::GUS*hrcV*:: Ω) (C. Boucher, CNRS-INRA, LIPM, Castanet Tolosan, France), and GMI1600 (GMI1000::GFP) (Aldon et al. 2000). The GMI1000 mutant strains used (*brg*1-48) are presented in Table 3. All the strains were grown at 28ºC in BGT medium (Boucher et al. 1985).

Plant materials and growth conditions.

Seed of *M. truncatula* Gaertn. genotypes A17 (derived from cultivar Jemalong) and F83005.5 (derived from cv. Salernes) were provided by the INRA of Montpellier, SGAP laboratory, Mauguio, France. Seed were scarified with concentrated anhydrous H_2SO_4 for 7 min, washed three times in sterile water, sterilized for 2 min with a 12% sodium hypochlorite solution, and rinsed six times using sterile water. After 30 min of imbibition in sterile water, seed then were sown onto 0.8% (wt/vol) water agar in petri dishes, and germinated in the dark for 3 days at 4ºC and for 24 h at 14ºC. Germinating seed then were transferred to Jiffy pots (Jiffy France, Lyon, France) and grown for 10 days with 16 h of light at 23ºC and 8 h of darkness at 20ºC, at 170 μmol m^{-2} s^{-1}. For in vitro experiments, germinating seed were transferred on slanted agar in square petri dishes (12 by 12 cm) containing Fahraeus medium (Fahraeus 1957) adjusted to pH 7.5 before autoclaving, with $(NH_4)_2SO_2$ at 0.33 g/liter and agar at 15 g/liter. An interface of CYG seed growth paper (Mega International, St. Paul, MN, U.S.A.) was put between plantlets and Fahraeus agar medium. Plantlets were grown for 3 days with 16 h light at 23ºC at 100 μmol m^{-2} s^{-1}.

Inoculation procedures.

Plants in Jiffy pots were root inoculated by cutting approximately 1 cm from the bottom of the Jiffy pot, and the exposed roots were immersed for 20 min in a suspension containing 10^7 or 10^8 bacteria/ml. The plants then were transferred to a growth chamber at 28ºC (12 h of light) at 100 μmol m^{-2} s^{-1}. The wilt symptoms were monitored on eight plants in Jiffy pots, in two or three separate inoculation experiments. Each *M. truncatula* genotype–*R. solanacearum* strain combination was monitored by recording the wilt symptoms on 10-day-old plants in Jiffy pots inoculated with 1×10^8 bacteria/ml. A disease index scale (0 to 4) was established (Fig. 1A) and scored from 3 to 24 days after inoculation. Score 0 corresponded to plants presenting no symptom. In compatible interactions, first symptoms were visible starting 3 to 5 days after inoculation and developed as follows: leaves started to curl up on themselves (score 1), petioles collapsed gradually (score 2), and leaves became chlorotic and dried out (score 3), leading to the death of the entire plant (score 4). Death of plants occurred 10 to 24 days after inoculation, depending on the strain tested. Scores obtained at 24 days after inoculation are presented in Table 1.

With the 48 mutant strains of GMI1000, each carrying an individual disruption in a candidate effector gene (Table 3), Jiffy pots were root inoculated with a concentration of 10^8 bacteria/ml.

Plantlets in squared petri dishes were root inoculated, sectioning approximately 3 mm from the root tip with a sterile scalpel. Sectioned plants were inoculated with 300 μl of a suspension containing 10^8 bacteria/ml. Petri dishes were maintained inclined with an angle of 45º and dishes are sealed with Parafilm, with several incisions allowing gas exchange. The plants then were transferred to a growth chamber at 28ºC (12 h of light) at 100 μmol m^{-2} s^{-1}.

Microscopy protocols.

Confocal microscopy images and fluorescent signal detection to follow the strain carrying the *gfp* gene in plant tissues were acquired with a SP2 confocal laser scanning system equipped with an inverted microscope (Leica, Wetzlar, Germany) and a ×40 water immersion objective (numerical aperture 0.75). Samples were observed with the 488-nm line of an argon laser for excitation and emitted light was collected in the 510- to 525-nm spectral range. Autofluorescence was observed in the 590- to 650-nm spectral range.

For bright-field microscopy experiments with the strains carrying the *uidA* gene (GUS constructs), plants were prefixed in 0.4% paraformaldehyde and rinsed three times in 0.1 M

phosphate buffer. Plants were incubated for 24 h at 28ºC in a solution containing 0.2 M $NaPO_4$, pH 7, 0.1 M $K_3Fe(CN)_6$, 0.1 M $K_4Fe(CN)_6$, 0.5 M Na_2EDTA, and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (CAYLA, Toulouse, France) at 25 mg/ml. Plants were immersed for 1 h in 50% ethanol, 1 h in 70% ethanol, and two times 1 h in 100% ethanol. Next, a clearing was done with a mix of ethanol/benzyl benzoate in three steps of 30 min, with the ratios i) 70:30, ii) 50:50, and iii) 30:70.

In planta bacterial growth measurements.

In Jiffy pots, 10-day-old plants were root inoculated with a suspension at 10^7 bacteria/ml. Leaves were harvested and sterilized with 70% ethanol for 1 min, rinsed three times in sterile water, patted dry, weighed, crushed, and taken up in water. Bacterial concentrations were determined by dilution plating on SMSA medium (Elphinstone et al. 1996). Each datum point represents the mean and the standard deviation calculated from three replicates of three plants. The experiments were repeated twice with equivalent results.

Genetic analysis of resistance.

A RIL population was derived from the cross between A17 and F83005.5 *M. truncatula* lines. In all, 129 RILs were developed as single-seed descendants to the F7 and F8 generations. The RIL population previously was genotyped with 105 SSR markers (T. Huguet, *unpublished data*). The map covers 600 cM with an average interval between markers of 6.25 cM. Two separate inoculation experiments were conducted on 111 F7 RILs and 129 F8 RILs as two technical replicates. RILs and parental lines were planted in Jiffy pots and inoculated with strain GMI1000 as described above with a suspension at 10^8 bacteria/ml. Following inoculation, plants were cultured in a two-block design (F7 RILs experiment) and in a three-block design (F8 RILs experiment), an elementary plot consisting of four plants. The development of symptoms was monitored and disease index score was evaluated as described in Figure 1.

Statistical and QTL analysis.

Replicate and genotype effects were studied by analysis of variance with the PROC GLM procedure of the SAS Institute (1991; Carey, NC, U.S.A.) software. The genetic variance was estimated by using the PROC VARCOMP procedure of the SAS software, in which the genetic effect was assumed to be random. Heritability was estimated: $h^2 = \sigma^2_g/(\sigma^2_g + [\sigma^2_e/r])$, with σ^2_g being the genetic variance, σ^2_e the residual variance, and r the number of replicates.

QTL were detected by composite interval mapping (Jansen and Stam 1994; Zeng 1994) using the software PLABQTL V1.1 (Utz and Melchinger 1996) combining the F7 and F8 replicates. A purely additive model was employed. Individual cofactor sets were selected via stepwise regression for each trait. Final selection was for the model that minimized Akaike's information, a measure of the goodness-of-fit of the regression model (Jansen 1993). Empirical threshold value for the LOD scores were determined by computing 10,000 permutations (Churchill and Doerge 1994), using the "permute" command of the PLABQTL software. Therefore, the critical LOD score to indicate QTL significance was 2.3. QTL positions were determined at the local maxima of the LOD-curve plot in the region under consideration. Confidence intervals were set as the map interval corresponding to a 1-LOD decline on either side of the LOD peak. The proportion of phenotypic variance explained by a single QTL was obtained by the square of the partial correlation coefficient (R^2). Estimates of the additive effects of the QTL were computed by fitting a model including all putative QTL for a given trait.

ACKNOWLEDGMENTS

We thank C. Boucher and colleagues Y. Marco and A. Trigalet for stimulating discussions, and M. Rickauer and N. D. Young for critically reading the manuscript. We thank C. Allen, J. Luisetti, and P. Prior for kindly providing the legume strains of *R. solanacearum* used in this work and J. M. Prosperi for providing the A17 and F83005.5 *M. truncatula* seed. We are also grateful to M.-J. Tavella and P. Anson for technical assistance, and to M. Ghérardi for marker typing.

LITERATURE CITED

Albrecht, C., Geurts, R., and Bisseling, T. 1999. Legume nodulation and mycorrhizae formation; two extremes in host specificity meet. EMBO (Eur. Mol. Biol. Organ.) J. 18:281-288.

Aldon, D., Brito, B., Boucher, C., and Genin, S. 2000. A bacterial sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanacearum* pathogenicity genes. EMBO (Eur. Mol. Biol. Organ.) J. 19:2304-2314.

Arlat, M., Gough, C. L., Zischek, C., Barberis, P. A., Trigalet, A., and Boucher, C. A. 1992. Transcriptional organization and expression of the large *hrp* gene cluster of *Pseudomonas solanacearum*. Mol. Plant-Microbe Interact. 5:187-193.

Boucher, C., Barberis, P. A., Trigalet, A. P., and Demery, D. A. 1985. Transposon mutagenesis of *Pseudomonas solanacearum*: isolation of Tn5-induced avirulent mutants. J. Gen. Microbiol. 131:2449-2457.

Cannon, S. B., Crow, J. A., Heuer, M. L., Wang, X., Cannon, E. K., Dwan, C., Lamblin, A. F., Vasdewani, J., Mudge, J., Cook, A., Gish, J., Cheung, F., Kenton, S., Kunau, T. M., Brown, D., May, G. D., Kim, D., Cook, D. R., Roe, B. A., Town, C. D., Young, N. D., and Retzel, E. F. 2005. Databases and information integration for the *Medicago truncatula* genome and transcriptome. Plant Physiol. 138:38-46.

Choi, H. K., Kim, D., Uhm, T., Limpens, E., Lim, H., Mun, J. H., Kalo, P., Penmetsa, R. V., Seres, A., Kulikova, O., Roe, B. A., Bisseling, T., Kiss, G. B., and Cook, D. R. 2004. A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa.* Genetics 166:1463-1502.

Churchill, G. A., and Doerge, R. W. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963-971.

Cook, D. R. 1999. *Medicago truncatula*—a model in the making! Curr. Opin. Plant Biol. 2:301-304.

Cunnac, S., Occhialini, A., Barberis, P., Boucher, C., and Genin, S. 2004. Inventory and functional analysis of the large Hrp regulon in *Ralstonia solanacearum*: identification of novel effector proteins translocated to plant host cells through the type III secretion system. Mol. Microbiol. 53:115-128.

Danesh, D., Aarons, S., McGill, G. E., and Young, N. D. 1994. Genetic dissection of oligogenic resistance to bacterial wilt in tomato. Mol. Plant-Microbe Interact. 7:464-471.

Deslandes, L., Olivier, J., Peeters, N., Feng, D. X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S., and Marco, Y. 2003. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. U.S.A. 100:8024-8029.

Deslandes, L., Olivier, J., Theulieres, F., Hirsch, J., Feng, D. X., Bittner-Eddy, P., Beynon, J., and Marco, Y. 2002. Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RRS1-R* gene, a member of a novel family of resistance genes. Proc. Natl. Acad. Sci. U.S.A. 99:2404-2409.

Deslandes, L., Pileur, F., Liaubet, L., Camut, S., Can, C., Williams, K., Holub, E., Beynon, J., Arlat, M., and Marco, Y. 1998. Genetic characterization of *RRS1*, a recessive locus in *Arabidopsis thaliana* that confers resistance to the bacterial soilborne pathogen *Ralstonia solanacearum*. Mol. Plant-Microbe Interact. 11:659-667.

Elphinstone, J. G., Hennessy, J., Wilson, J. K., and Stead, D. E. 1996. Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. OEPP/EPPO 26:663-678.

Etchebar, C., Trigalet-Demery, D., van Gijsegem, F., Vasse, J., and Trigalet, A. 1998. Xylem colonization by an $HrcV^-$ mutant of *Ralstonia solanacearum* is a key factor for the efficient biological control of tomato bacterial wilt. Mol. Plant-Microbe Interact. 11:869-877.

Fahraeus, G. 1957. The infection of white clover root hairs by nodule bacteria studied by a simple slide technique. J. Genet. Microbiol. 16:374-381.

Flor, H. H. 1971. Current status of the gene for gene concept. Annu. Rev. Phytopathol. 9:275-296.

Gebhardt, C., and Valkonen, J. P. 2001. Organization of genes controlling disease resistance in the potato genome. Annu. Rev. Phytopathol. 39:79-102.

Genin, S., and Boucher, C. 2004. Lessons learned from the genome analysis of *Ralstonia solanacearum*. Annu. Rev. Phytopathol. 42:107-134.
Godiard, L., Sauviac, L., Torii, K. U., Grenon, O., Mangin, B., Grimsley, N. H., and Marco, Y. 2003. ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. Plant J. 36:353-365.
Grimault, V., Gélie, B., Lemattre, M., Prior, P., and Schimt, J. 1994. Comparative histology of resistant and susceptible tomato cultivars infected by *Pseudomonas solanacearum*. Physiol. Mol. Plant Pathol. 44:105-123.
Grimault, V., and Prior, P. 1994. Invasiveness of *Pseudomonas solanacearum* in tomato, eggplant and pepper: a comparative study. Eur. J. Plant Pathol. 100:259-267.
Guenoune, D., Galili, S., Phillips, D. A., Volpin, H., Chet, I., Okon, Y., and Kapulnik, Y. 2001. The defense response elicited by the pathogen *Rhizoctonia solani* is suppressed by colonization of the AM-fungus *Glomus intraradices*. Plant Sci. 160:925-932.
Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65-87.
Hayward, A. C. 1994 The Hosts of *Pseudomonas solanacearum*. Pages 9-24 in: Bacterial Wilt: The Disease and Its Causative Agent, *Pseudomonas solanacearum*. A. C. Hayward and G. L. Hartman, eds. CAB International, Wallingford, U.K.
Ho, G. D., and Yang, C. H. 1999. A single locus leads to resistance of *Arabidopsis thaliana* to bacterial wilt caused by *Ralstonia solanacearum* through a hypersensitive-like response. Phytopathology 89:673-678.
Jansen, R. C. 1993. Interval mapping of multiple quantitative trait loci. Genetics 135:205-211.
Jansen, R. C., and Stam, P. 1994. High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447-1455.
Kemen, E., Hahn, M., Mendgen, K., and Struck, C. 2005. Different resistance mechanisms of *Medicago truncatula* ecotypes against the rust fungus *Uromyces striatus*. Phytopathology 95:153-157.
Kliebenstein, D., Pedersen, D., Barker, B., and Mitchell-Olds, T. 2002. Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase and insect resistance in *Arabidopsis thaliana*. Genetics 161:325-332.
Klingler, J., Creasy, R., Gao, L., Nair, R. M., Calix, A. S., Jacob, H. S., Edwards, O. R., and Singh, K. B. 2005. Aphid resistance in *Medicago truncatula* involves antixenosis and phloem-specific, inducible antibiosis, and maps to a single locus flanked by NBS-LRR resistance gene analogs. Plant Physiol. 137:1445-1455.
Koornneef, M., Alonso-Blanco, C., and Vreugdenhil, D. 2004. Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu. Rev. Plant Biol. 55:141-172.
Lavie, M., Shillington, E., Eguiluz, C., Grimsley, N., and Boucher, C. 2002. PopP1, a new member of the YopJ/AvrRxv family of type III effector proteins, acts as a host-specificity factor and modulates aggressiveness of *Ralstonia solanacearum*. Mol. Plant-Microbe Interact. 15:1058-1068.
Mangin, B., Thoquet, P., Olivier, J., and Grimsley, N. H. 1999. Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci. Genetics 151:1165-1172.
McGarvey, J. A., Denny, T. P., and Schell, M. A. 1999. Spatial-temporal and quantitative of growth and EPS I production by *Ralstonia solanacearum* in resistant and susceptible tomato cultivars. Phytopathology 89:1233-1239.
Nyamsuren, O., Colditz, F., Rosendahl, S., Tamasloukht, M., Bekel, T., Meyer, F., Küster, H., Franken, P., and Krajinski, F. 2003. Transcriptional profiling of *Medicago truncatula* roots after infection with *Aphanomyces euteiches* (oomycota) identifies novel genes upregulated during this pathogenic interaction. Physiol. Mol. Plant Pathol. 63:17-26.
Oldroyd, G. E., Harrison, M. J., and Udvardi, M. 2005. Peace talks and trade deals. Keys to long-term harmony in legume-microbe symbioses. Plant Physiol. 137:1205-1210.
Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J. C., Cattolico, L., Chandler, M., Choisne, N., Claudel-Renard, C., Cunnac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Saurin, W., Schiex, T., Siguier, P., Thebault, P., Whalen, M., Wincker, P., Levy, M., Weissenbach, J., and Boucher, C. A. 2002. Genome sequence of the plant pathogen *Ralstonia solanacearum*. Nature 415:497-502.
Salzer, P., Bonanomi, A., Beyer, K., Vogeli-Lange, R., Aeschbacher, R. A., Lange, J., Wiemken, A., Kim, D., Cook, D. R., and Boller, T. 2000. Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation, and pathogen infection. Mol. Plant-Microbe Interact. 13:763-777.
Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Laterrot, H., and Grimsley, N. 1996a. Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. Mol. Plant-Microbe Interact. 9:826-836.
Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Prior, P., Anaïs, G., Mangin, B., Bazin, B., Nazer, R., and Grimsley, N. 1996b. Polygenic resistance of tomato plants to bacterial wilt in the French West Indies. Mol. Plant-Microbe Interact. 837-842.
Torregrosa, C., Cluzet, S., Fournier, J., Huguet, T., Gamas, P., Prosperi, J. M., Esquerre-Tugaye, M. T., Dumas, B., and Jacquet, C. 2004. Cytological, genetic, and molecular analysis to characterize compatible and incompatible interactions between *Medicago truncatula* and *Colletotrichum trifolii*. Mol. Plant-Microbe Interact. 17:909-920.
Utz, H. F., and Melchinger, A. E. 1996. PLABQTL: A program for composite interval mapping of QTL. J. Agric. Genomics 2:1.
VandenBosch, K. A., and Stacey, G. 2003. Summaries of legume genomics projects from around the globe. Community resources for crops and models. Plant Physiol. 131:840-865.
Vasse, J., Frey, P., and Trigalet, A. 1995. Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by *Pseudomonas solanacearum*. Mol. Plant-Microbe Interact. 8:241-251.
Wang, J. F., Olivier, J., Thoquet, P., Mangin, B., Sauviac, L., and Grimsley, N. H. 2000. Resistance of tomato line Hawaii7996 to *Ralstonia solanacearum* Pss4 in Taiwan is controlled mainly by a major strain-specific locus. Mol. Plant-Microbe Interact. 13:6-13.
Wilson, I. W., Schiff, C. L., Hughes, D. E., and Somerville, S. C. 2001. Quantitative trait loci analysis of powdery mildew disease resistance in the *Arabidopsis thaliana* accession kashmir-1. Genetics 158:1301-1309.
Yang, C. H., and Ho, G. D. 1998. Resistance and susceptibility of *Arabidopsis thaliana* to bacterial wilt caused by *Ralstonia solanacearum*. Phytopathology 88:330-334.
Young, N. D. 1996. QTL mapping and quantitative disease resistance in plants. Annu. Rev. Phytopathol. 34:479-501.
Young, N. D., Cannon, S. B., Sato, S., Kim, D., Cook, D. R., Town, C. D., Roe, B. A., and Tabata, S. 2005. Sequencing the gene spaces of *Medicago truncatula* and *Lotus japonicus*. Plant Physiol. 137:1174-1181.
Zeng, Z. B. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

AUTHOR-RECOMMENDED INTERNET RESOURCES

TIGR *Medicago truncatula* database: www.medicago.org
The Génopole Toulouse Midi-Pyrénées website: sequence.toulouse.inra.fr/R.solanacearum.html/