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ORIGINAL PAPER

A complex genetic network involving a broad-spectrum locus and strain-specific loci controls resistance to different pathotypes of *Aphanomyces euteiches* in *Medicago truncatula*

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Abstract A higher understanding of genetic and genomic bases of partial resistance in plants and their diversity regarding pathogen variability is required for a more durable management of resistance genetic factors in sustainable cropping systems. In this study, we investigated the diversity of genetic factors involved in partial resistance to *Aphanomyces euteiches*, a very damaging pathogen on pea and alfalfa, in *Medicago truncatula*. A mapping population of 178 recombinant inbred lines, from the cross F83005.5 (susceptible) and DZA045.5 (resistant), was used to identify quantitative trait loci for resistance to four *A. euteiches* reference strains belonging to the four main pathotypes currently known on pea and alfalfa. A major broad-spectrum genomic region, previously named *AER1*, was localized to a reduced 440 kb interval on chromosome 3 and was involved in complete or partial resistance, depending on the *A. euteiches* strain. We also identified 21 additive and/or epistatic genomic regions specific to one or two strains, several of them being anchored to the *M. truncatula* physical map. These results show that, in *M. truncatula*, a complex network of genetic loci controls partial resistance to different pea and alfalfa pathotypes of *A. euteiches*, suggesting a diversity of molecular mechanisms underlying partial resistance.

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Introduction

Plants are exposed to numerous invading pathogenic organisms and have developed a wide array of active or passive defense mechanisms to defeat them, leading to complete or partial resistance. While the molecular bases of complete resistance conferred by resistance genes with qualitative effects are increasingly well-understood (Glazebrook 2005; Jones and Dangl 2006; Bent and Mackey 2007), knowledge concerning the molecular mechanisms underlying partial resistance, often controlled by multiple genes with small effects, remains limited. Complex genetic networks of additive and epistatic-effect loci controlling partial resistance have been identified in cereals and Brassicaceae (Li et al. 2006; Ma et al. 2006; Rowe and Kliebenstein 2008). To date, different hypotheses about the genes underlying partial resistance loci have been proposed. Poland et al. (2009) hypothesized that partial resistance loci could correspond to (a) "defeated" genes controlling complete resistance, (b) genes involved in basal defense, defense signal transduction, plant architecture or development, (c) components of chemical warfare or (d) genes previously unidentified. The spectrum of action of partial resistance loci toward pathogen races also remains unclear. Partial resistance was often thought to be non-race-specific but several studies have provided

evidence that these loci can be race-specific or effective against a subset of pathogen races, especially in the case of resistance to biotrophic pathogens (Calenge et al. 2004; Rocherieux et al. 2004; Wisser et al. 2005).

Model species, such as *Arabidopsis thaliana*, rice and tomato, are of important interest for deciphering the genetic and molecular bases of partial resistance. They provide useful tools, including large genomic resources generated from the complete sequencing of their genome, for identifying and cloning plant disease resistance genes. Natural genetic variation toward pathogen resistance was observed within model species germplasm collections, allowing genetic studies of resistance to various pathogens to be carried out (Rose et al. 2007; Shindo et al. 2007). Studies to elucidate the genetic mechanisms controlling partial resistance in *A. thaliana* (Perchepied et al. 2006; Jubault et al. 2008; Rowe and Kliebenstein 2008), rice (Ramalingam et al. 2003; Carrillo et al. 2005; Yu et al. 2006; Hu et al. 2008) and tomato (Smart et al. 2003; Brouwer et al. 2004; Finkers et al. 2007) have led to progress in unraveling genes underlying partial resistance loci (Poland et al. 2009).

In 1990, *Medicago truncatula* was proposed as a model legume for the analysis of rhizobia–legume symbiosis (Barker et al. 1990), and then emerged as a model species for studying plant–pathogen interactions (Rose 2008). In particular, the model legume offers an original opportunity for gaining knowledge about the molecular bases of partial resistance, by comparison with increasingly well-understood symbiosis mechanisms. In particular, the molecular pathways leading to plant symbiosis were shown to share components in common with those involved in defense responses to pathogens (Samac and Graham 2007). Various levels of resistance toward legume pathogens were identified within *M. truncatula* germplasm collections, facilitating genetic studies to identify and isolate resistance genes (Tivoli et al. 2006). Genes or QTL have been identified in *M. truncatula* for resistance to root rot nematode (Dhandaydham et al. 2008), aphids (Klingler et al. 2005, 2007), *Phoma medicaginis* (Kamphuis et al. 2008), *Colletotrichum trifolii* and *Erysiphe pisi* (Ameline-Torregrosa et al. 2008a; Yang et al. 2008), *Ralstonia solanacearum* (Vailleau et al. 2007) and recently *Aphanomyces euteiches* (Djebali et al. 2009; Pilet-Nayel et al. 2009). In these studies, specific or broad-spectrum resistance to the pathogens studied mainly involved major genes and few quantitative trait loci.

A. euteiches Drechsler is a major soil borne oomycete pathogen, considered as biotrophic, which causes economically important damage to various legume species. *A. euteiches* was isolated from a number of legume species, including pea (Wicker and Rouxel 2001; Levenfors et al. 2003) and alfalfa (Malvick and Grau 2001). Studies on the pathogenic variability of *A. euteiches* were conducted on pea and alfalfa. On pea, Wicker and Rouxel (2001) identified two main pathotypes of *A. euteiches*, based on reactions of six pea genotypes: a predominant pathotype, present in France, other European countries and the USA, and a more specific pathotype, including only strains from the USA. On alfalfa, Malvick and Grau (2001) identified two races of *A. euteiches*, races 1 and 2, based on the reactions of three alfalfa populations (Saranac, WAPH-1 and WAPH-5). *M. truncatula* is a host for *A. euteiches* and shows genetic variability for resistance to the pea and alfalfa strains of *A. euteiches* (Vandemark and Grunwald 2004; Moussart et al. 2007). To date, no data are available regarding the pathogenic variability of *A. euteiches* on *M. truncatula* differential genotypes. Complete and partial levels of resistance have been identified in *M. truncatula*, which depend on host genotypes and *A. euteiches* pathotypes (Vandemark and Grunwald 2004; Moussart et al. 2007). The *M. truncatula/A. euteiches* pathosystem is therefore a good model to investigate the genetic bases of partial resistance and their relation with resistance genes, regarding natural variation in the host and pathogen.

Previously, genetic analysis of resistance to *A. euteiches* strains from the predominant pea pathotype was carried out from two different RIL populations of *M. truncatula*, derived from crosses involving the susceptible line F83005.5 and two different sources of resistance, DZA045.5 and A17, expressing high and partial levels of resistance, respectively. A monogenic control of the resistance was identified in both studies and major loci, named *AER1* and *prAe1*, expressing high dominant and partial recessive genetic effects on resistance to the main pea pathotype of *A. euteiches*, respectively, were mapped to the distal part of chromosome 3 (Djebali et al. 2009; Pilet-Nayel et al. 2009). Partial levels of resistance to the specific American pea pathotype and the two alfalfa races described were identified in DZA045.5 (unpublished data). Till date, no loci were identified in *M. truncatula* for resistance to those pathotypes. The aim of this study was to analyze the diversity of genetic factors involved in resistance of *M. truncatula* DZA045.5 to the main pea and alfalfa pathotypes of *A. euteiches*. By doing this, a more extensive knowledge of the genetic architecture of resistance to *A. euteiches* in *M. truncatula* could be obtained, which is potentially transferable to leguminous crops, especially pea and alfalfa, because of the high levels of synteny between the model and cultivated legumes (Choi et al. 2004). In this study, we mapped, throughout the whole genome, additive and epistatic loci associated with resistance to four reference strains of *A. euteiches* belonging to the main pathotypes currently known on pea and alfalfa. We used a F5:6 recombinant inbred lines (RIL) population derived from a cross between the two lines

mentioned above, F83005.5 and DZA045.5. We analyzed the specificity of the identified loci toward the strains tested and compared them to those previously identified (Djebali et al. 2009; Pilet-Nayel et al. 2009). We anchored several of the identified regions to the physical map of *M. truncatula* for future prospects of identifying candidate genes underlying the resistance.

Materials and methods

Pathogen material

Two pea-infecting strains of *A. euteiches* were used in disease resistance tests, namely RB84, isolated from an infested field at Riec-sur-Belon (Finistère, France) (Moussart et al. 2007) and Ae109 (Wicker and Rouxel 2001), isolated from an infested field in Winconsin (USA) and referred to as strain Ae467 in Malvick et al. (1998) and Malvick and Percich (1999). The RB84 and Ae109 strains belong to the main French pea pathotype of *A. euteiches* and the minor pea pathotype from the USA, respectively, identified by Wicker and Rouxel (2001). RB84 strain has a broad host-spectrum within legumes since it has been reported pathogenic to pea, alfalfa and *M. truncatula*, bean, lentil and vetch (Moussart et al. 2008). Ae109 is pathogenic to pea and alfalfa (Malvick et al. 1998). The RB84 strain was previously used in Pilet-Nayel et al. (2009). Two alfalfa-infecting strains of *A. euteiches* were also used in this study, namely MF-1 and NC-1, isolated in the USA (Wisconsin and North Carolina, respectively). MF-1 and NC-1 belong to race 1 (R1) and race 2 (R2) of *A. euteiches*, respectively, identified by Malvick and Grau (2001). These two strains have a narrow host-range, since they were reported only pathogenic to alfalfa, but not to pea, bean or red-clover (Malvick et al. 1998).

Plant material

A population of 178 F5:6-derived RILs, produced by single seed descent from a cross between two *M. truncatula* inbred line accessions, F83005.5 (female parent, originating from southern France) and DZA045.5 (male parent, originating from Algeria), was used in this study. Previously, the F5 RIL generation was used for genetic analysis of the resistance (Pilet-Nayel et al. 2009) and for construction of a genetic map (Avia et al., submitted). In this study, the F6 RIL generation was used for resistance evaluation in disease screening tests. The two parental lines, F83005.5 and DZA045.5, are included in the smallest core-collection set (eight lines) built by Ronfort et al. (2006) (*M. truncatula* stock center, INRA, France; http://www.montpellier.inra.fr/BRC-MTR/). F83005.5 and DZA045.5 were shown to be susceptible and resistant to the RB84 French strain, respectively (Moussart et al. 2007). Next, these lines were also observed to express contrasted responses when inoculated with Ae109, MF-1 and NC-1 (unpublished data). Two *Pisum sativum* genotypes, Baccara (F. Desprez, France) and PI180693 (USDA Plant Introduction Station, USA), susceptible and partially resistant to *A. euteiches*, respectively (Wicker et al. 2003), were used as controls in all disease-resistance experiments. One additional pea genotype, MN313 (Davis et al. 1995), resistant to Ae109 and susceptible to RB84 was included in the disease-resistance tests using the Ae109 strain. Three *M. sativa* cultivars, Saranac (susceptible to R1 and R2 strains of *A. euteiches*), WAPH-1 (resistant to R1 strains and susceptible to R2 strains) and WAPH-5 (resistant to R1 and R2 strains), were added as controls in the disease-resistance tests using the two alfalfa MF-1 and NC-1 strains (Malvick and Grau 2001).

Inoculation procedures and resistance evaluation

All tests to screen for resistance to *A. euteiches* were performed as described by Pilet-Nayel et al. (2009). Each RIL test was conducted using a randomized complete block design with two blocks. In each block, five plants per recombinant line, parent and control line were grown in a pot, in a moistened vermiculite substrate and were then inoculated with one strain and evaluated. Each RIL test was repeated twice for each of the four strains. The screening tests were conducted in a growth chamber at 25°C for 16 h day and 23°C for 8 h night. Twelve days after sowing, seedlings were inoculated with a suspension of zoospores at a concentration adjusted to 2×10^3 zoospores/ml for the pea strains and 10^2 zoospores/ml for the alfalfa strains. The method for producing the inoculum was described by Moussart et al. (2001). Seedlings were inoculated by applying 25 ml of inoculum suspension per pot. Vermiculite was saturated with water after inoculation to favor disease development. Fourteen days after inoculation, the plants were uprooted and disease severity was scored on each individual plant using the 0-5 disease scoring scale described in Moussart et al. (2007) and modified in Pilet-Nayel et al. (2009). A root rot index (RRI) ranging from 0 to 5 was then calculated as the mean disease score on all plants in a pot.

Statistical analyses of the disease resistance data

The phenotypic data obtained from each pair of resistance tests per *A. euteiches* strain were statistically analyzed using a generalized linear model [(PROC GLM of Statistical Analysis System (SAS) software (SAS Institute Inc., 2000)], as followed: $Pijk = \mu + Li + Tj + Bk/$

$j + (Li \times Tj) + eijk$, where $Pijk$ is the mean disease score of the ith RIL located in the kth block of the jth test, μ is the mean of all the data, Li is the RIL i effect, Tj is the test j effect, Bk/j is the block k effect in the jth test, $Li \times Tj$ is the RIL $i \times$ test j interaction effect, and $eijk$ is the residual. Normality of residues and homogeneity of variances were checked using Skewness, Kurtosis and Shapiro–Wilk ($P \geq 0.05$) statistics and Bartlett's test ($P > 0.05$), respectively. Broad sense heritability (h^2) was estimated from ANOVA using the formula $h^2 = \sigma^2 g/[\sigma^2 g + (\sigma^2 e/n)]$, where $\sigma^2 g$ is the genetic variance, $\sigma^2 e$ the residual variance and n is the number of replicates per line. As missing data were observed from our experiments, RIL least-square means were estimated from ANOVA and used for linkage analysis. Spearman correlation coefficients (r^2) were calculated from RRI adjusted means obtained between the four strains and between the two RIL generations tested for RB84 strain (F5 in Pilet-Nayel et al. 2009 and F6 in this study), using the PROC CORR procedure of the SAS software.

Molecular markers and genetic linkage analysis

A framework genetic map, mainly based on SSR markers, was developed from the F83005.5 × DZA045.5 F5-RIL population to identify QTL for freezing tolerance (Avia et al., submitted). This genetic map included 178 markers, distributed over eight linkage groups (LG) and covered 1,197 cM Kosambi (≈1,317 cM Haldane). In this study, we genotyped the RIL population with additional SSR markers designed from *M. truncatula* BAC accessions, anchored to the integrated genetic-physical map (http://www.medicago.org/genome/map.php, Univ. Minn. 2006). Information on SSR primer sequences and BAC anchoring is described in Mun et al. (2006), on the Medicago website (http://www.medicago.org/genome/genetic_map_table.html) and in Djebali et al. (2009), except for the SSR MTIC1179 (marker information: BAC accession CR940308; forward primer: 5′-AGTGTGATTTTTACAC CAAAGA-3′; reverse primer: 5′-TGTCAAGCTTCAG TTTTTCC-3′; motif: $(TA)_6$; C. Jacquet, personal communication). SSR markers from Mun et al. (2006) are named "MtB" followed by the SSR number and SSR markers from Djebali et al. (2009) are designated "MTIC" followed by the SSR number. SSR amplification reactions were carried out using the procedure described for pea by Loridon et al. (2005). Mendelian segregation of parental alleles in the RIL population (1:1 ratio) was tested for each marker by a Chi-square test ($\alpha = 0.01$). Additional SSR markers were positioned within the existing framework map (Avia et al., submitted), using the "assign" and "try" commands of MAPMAKER/EXP version 3.0b (Lincoln et al. 1992). All genetic distances were expressed in centimorgan values, calculated with the Haldane mapping function.

Additive-effect QTL analysis

For QTL analysis, composite interval mapping (CIM, Zeng 1994) was performed using Windows QTL Cartographer 2.5 software (Wang et al. 2005). We used the standard model 6 of the program with ten cofactors selected by forward–backward regression ($P < 0.05$) and a window size of 10 cM. Walk speed was set at 2.0 cM to scan the entire genome. This procedure estimated the log-likelihood (LOD) score, additive effect and percentage of phenotypic variance (R^2) every 2.0 cM along each chromosome. A LOD threshold of 2.9 was defined for QTL detection ($\alpha = 0.05$) with Ae109, MF-1 and NC-1 and a LOD threshold of 3.0 was defined for QTL detection ($\alpha = 0.05$) with the RB84 strain, using permutation tests ($n = 1{,}000$) according to the method of Churchill and Doerge (1994). Two-LOD support intervals were established as >95% QTL confidence intervals (Van Ooijen 1992) from the CIM module. QTL were considered to co-localize if their 2-LOD confidence intervals overlap. Additive-effect QTL were named "*Ae-Mt*" (for *Aphanomyces euteiches Medicago truncatula*) followed by the LG number and the QTL number within the LG.

Epistatic-effect QTL analysis

We searched for pairwise epistatic interactions between all possible marker couples of the genetic map, using a two-way ANOVA model with an interaction component, implemented in SAS software (SAS/IML, SAS 1989), as followed : $Pijk = \mu + Xi + Yj + (Xi \times Yj) + eijk$ where $Pijk$ is the least-square mean disease score of the kth RIL within the population, μ the mean of all the data, Xi the ith genotype effect at the X locus, Yj the jth genotype effect at the Y locus, $Xi \times Yj$ the interaction effect between the ith genotype at the X locus and the jth genotype at the Y locus, and $eijk$ is the residual. The search for pairwise epistatic interactions was conducted using a SAS program adapted from the EPISTACY program (Holland 1998). To survey the whole genome for pairwise epistatic effects, we evaluated 20,100 $[n(n-1)/2]$ possible interactions for a map of $n = 201$ markers. The threshold to claim a statistically significant interaction was set at $P < 2.5 \times 10^{-4}$ and $R^2 < 5\%$. The threshold ($P < 2.5 \times 10^{-4}$) corresponded to the number of false positive interactions expected, set at five in this study, divided by the total number of marker pairs tested (Holland 1998). This threshold calculation method has been used in different studies (Jubault et al. 2008; Liu et al. 2006; Manzanares-Dauleux et al. 2000). We preferred this liberal but reasonable threshold rather

Table 1 Main results of analysis of variance obtained from RRI adjusted means with the four strains of *A. euteiches* in the F83005.5 × DZA045.5 F6-RIL population of *M. truncatula*

A. euteiches strain	R^2	Variation coefficient	Effects (*P*)			
			Genotype	Test	Block (test)	Genotype × test
RB84	0.89	19.9	<0.0001	0.0042	0.16	0.4228
Ae109	0.73	15.5	<0.0001	0.0003	0.0003	0.0978
MF-1	0.65	24	<0.0001	<0.0001	<0.0001	0.0814
NC-1	0.73	22.6	<0.0001	<0.0001	<0.0001	0.0201

than the Bonferroni correction that is considered to be overly conservative when a large number of tests is performed (Ungerer and Rieseberg 2003). The percentage of phenotypic variation explained by all the QTL detected for each strain (R^2) was estimated with a full ANOVA model including all additive and pairwise epistatic regions detected. Epistatic-effect QTL were named "*Ae-MtE*" (for *Aphanomyces euteiches Medicago truncatula Epistasis*) followed by the epistatic interaction number.

Identification of physical genomic regions associated with QTL

The list of BAC accessions included within the support interval of the *A. euteiches* resistance QTL identified was established from the position of the upper and lower bound markers, using the v2.0 of the draft genome assembly generated by the MGSC (http://www.medicago.org/genome/), with gene prediction from the International Medicago Genome Annotation Group (IMGAG). Genes predicted on these BAC accessions were examined to identify those known to be involved in disease resistance in plants.

Results

Phenotyping of the RIL population

The mean adjusted RRI scores obtained on pea controls inoculated with RB84, Ae109, MF-1, and NC-1 were 4.93, 4.51, 0.10 and 0.29, respectively, for Baccara and 3.57, 3.95, 0.12, and 0.36, respectively, for PI180693. These results confirmed that the alfalfa strains, MF-1 and NC-1, are not pathogenic to pea. In the Ae109 disease-resistance test, MN313 was more resistant than PI180693 (RRI = 3.04 and 3.95, respectively) confirming that Ae109 belongs to the US-specific pathotype, as described by Wicker and Rouxel (2001). The mean adjusted RRI scores obtained on alfalfa controls inoculated with MF-1 and NC-1 were 2.63 and 2.61, respectively, for Saranac, 1.78 and 2.86, respectively, for WAPH-1 and 1.36 and 2.05, respectively, for WAPH-5. These results confirmed that the MF-1 and NC-1 strains belong to race 1 (R1) and race 2 (R2) of *A. euteiches*, respectively, as previously described (Malvick et al. 2009).

The main results of the variance analysis (ANOVA) obtained from RRI scores with the four strains are summarized in Table 1. For the four strains, ANOVA showed highly significant genotypic and disease test effects ($P < 0.005$) on the phenotypic variation observed. No significant genotype × test interaction effects were identified ($P > 0.05$), except with the NC-1 strain ($P = 0.02$).

Mean-based heritabilities were very high in the RB84 and Ae109 strain tests ($h^2 = 0.93$ and 0.73, respectively). They were lower in the MF-1 and NC-1 strain tests ($h^2 = 0.45$ and 0.39, respectively) in accordance with smaller phenotypic ranges obtained with both these strains. Error variances obtained in the variance analysis for the MF-1 and NC-1 strains were higher than those obtained for RB84 and Ae109 (0.39, 0.33, 0.27, 0.19 for MF-1, NC-1, RB84, and Ae109, respectively) suggesting that the results obtained with the alfalfa strains were less reproducible than with the pea strains.

Distribution of RRI adjusted means in the F6-RIL with RB84 strain showed a bimodal curve (Fig. 1a), as previously observed in the F5-RIL (Pilet-Nayel et al. 2009) ($r^2_{\text{F5-F6RILs}} = 0.89$). The RRI adjusted means obtained with Ae109, MF-1, and NC-1 showed normal distributions, with disease severity median values of 2.83, 2.59 and 2.53, respectively (Fig. 1b–d). The mean adjusted RRI scores obtained for parental lines inoculated with the RB84, Ae109, MF-1, and NC-1 strains were 3.75, 3.4, 2.82, and 3.26, respectively, for F83005.5 and 1.23, 2.03, 2.2, and 2.14, respectively, for DZA045.5. In all experiments, transgressive segregations were observed, with lines that were more resistant and/or susceptible than the parents (Fig. 1).

Spearman correlation coefficients were highly significant between RRI adjusted means from resistance tests using strains RB84, Ae109, and MF-1 (Table 2). MF-1 RRI adjusted means were moderately correlated to NC-1 RRI scores. The RRI scores obtained for NC-1 were not significantly correlated with those observed for RB84 and Ae109.

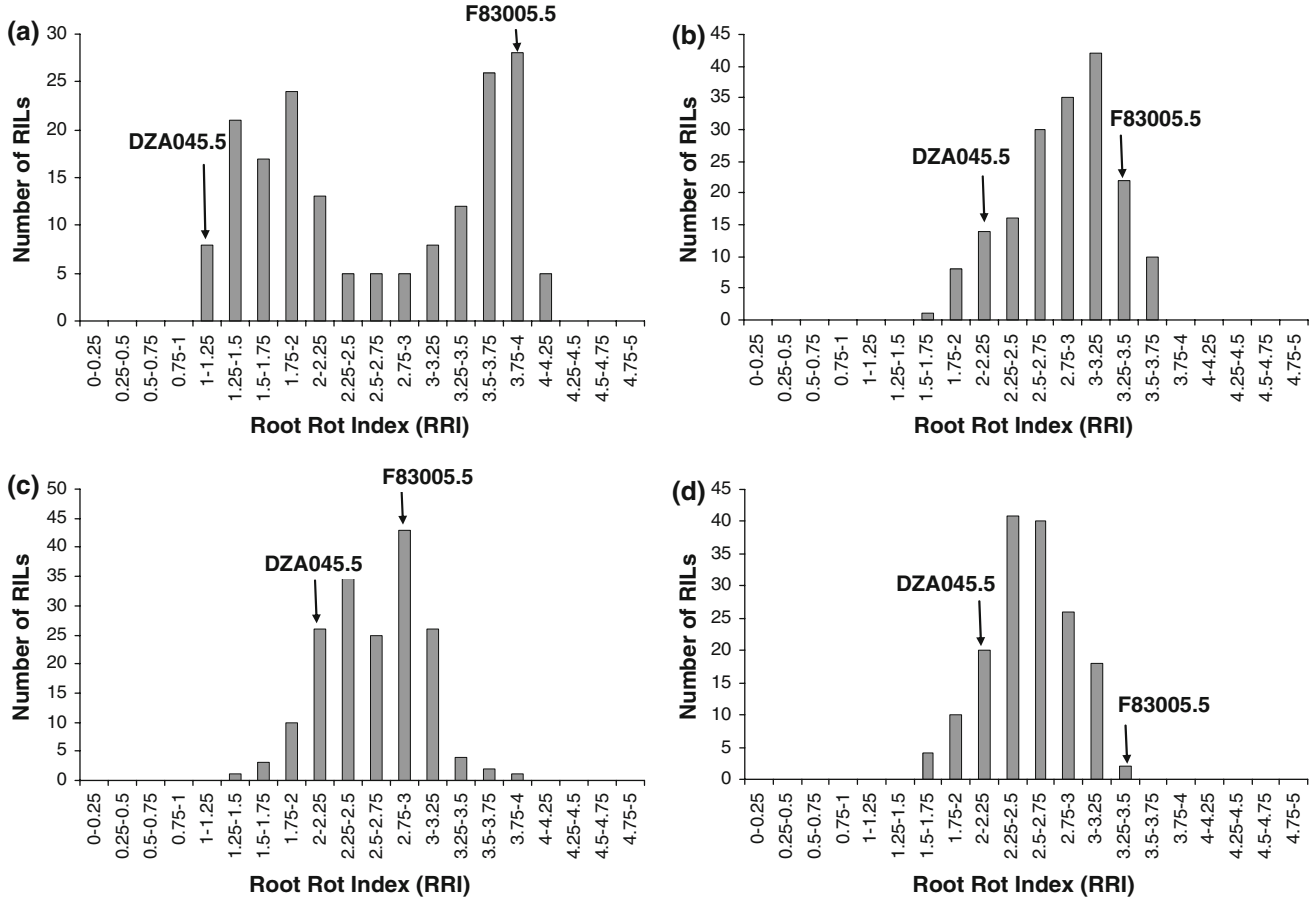


Fig. 1 Frequency distribution of root rot index scores for resistance to four strains of *A. euteiches*, in the *M. truncatula* F83005.5 × DZA045.5 F6-RIL population ($n = 178$ RILs). Values of the two parental lines are shown by *arrows*. **a** RB84 strain, **b** Ae109 strain, **c** MF-1 strain, and **d** NC-1 strain

Genetic mapping

QTL analysis was conducted using a genetic map comprising 201 markers, distributed over eight LG, and covering 1,434 cM Haldane (≈1,308 cM Kosambi) (Fig. 2). Compared to the framework genetic map described by Avia et al. (submitted), this updated genetic map included 23 additional markers, including (a) 21 SSR markers anchored to the integrated genetic-physical map (Mun et al. 2006) (MtB2, MtB205, MtB45, and MtB304 on LG2; MtB319, MtB141, MtB76, MtB6, MtB122, MtB172, and h2_11A20a on LG3; MtB253, MtB34, MtB66, MtB306, MtB124, and MtB289 on LG7; MtB311, MtB247, MtB225, and MtB3 on LG8) and (b) two SSR markers designed from the *M. truncatula* BAC accessions AC135103 and CR940308, containing the *prAe1* recessive QTL previously associated with resistance of A17 to *A. euteiches* on LG3 (MTIC742, Djebali et al. 2009), MTIC1179, C. Jacquet, personal communication. Out of the 23 additional markers mapped, 12 markers did not segregate according to expected Mendelian ratios at $\alpha = 1\%$. Among these 12 markers, one marker (MtB122) favored a higher frequency of F83005.5 alleles and 11 markers favored the DZA045.5 parental alleles (MtB2, MtB45, and MtB304 on LG2; MtB253, MtB34, MtB66, MtB306, MtB306, MtB124, and MtB289 on LG7; MtB225 and MtB3 on LG8). All the markers were mapped as expected, according to their position on the integrated genetic-physical map.

Additive-effect QTL mapping

From the F83005.5 × DZA045.5 F5:6-RIL population, a total of 11 additive-effect QTL and one major-effect locus associated with resistance to the four strains of *A. euteiches* were detected over seven LG of the *M. truncatula* genetic map (Fig. 2a). QTL results are detailed in Table 3.

As expected, the major dominant *AER1* locus, previously identified from the F83005.5 × DZA045.5 F5-RIL population for resistance to RB84 (Pilet-Nayel et al. 2009), was detected again from the F6-RIL population with the same

strain using quantitative disease scores. We mapped additional markers to gain more precise information about its position at the top of LG3 and estimated the confidence interval at 2.6 cM Haldane (≈2.1 cM Kosambi). The closest SSR marker to *AER1* was MtB199, which was anchored to the same *M. truncatula* BAC accession as the MTIC742 SSR marker. MTIC742 was closely linked to the *prAe1* locus detected from a A17 × F85005.5 RIL population when evaluated for resistance in in vitro infection assays (Djebali et al. 2009) using the ATCC201684 strain, belonging to the same pathotype as RB84 (data not shown). At the LOD score peak, *AER1* explained 78.9% of the total phenotypic variation observed for resistance to RB84. Genomic localization of *AER1* was also identified using qualitative disease scores (resistant and susceptible) obtained in the RIL population for resistance to RB84. Using qualitative scores, the *AER1* locus was localized on LG3 between the MTIC1179 and MTIC742 markers, at estimated genetic distances of 0.9 and 0.7 cM from the closest markers MTIC1179 and MTIC742, respectively (Fig. 2b). A QTL for resistance to Ae109 was also identified in the same genomic region, close to the MtB319 marker. At the LOD score peak, it explained 25.5% of the total phenotypic variation observed for resistance to Ae109. In this region, the DZA045.5 alleles contributed to resistance to both strains. The *AER1* genomic region was anchored by four markers (MtB199, MTIC742, MTIC1179, and MtB319) to contig 953 of the *M. truncatula* physical map.

On LG1 and LG8, two genomic regions, *Ae-Mt1.1* and *Ae-Mt8.1*, were identified with both the Ae109 and MF-1 strains, which individually explained 4–10.5% of the phenotypic variation, depending on the QTL and the strain. At these two regions, the DZA045.5 alleles contributed to resistance to the two strains. The *Ae-Mt8.1* genomic region was anchored by the MtB311 marker to contig 214 of the *M. truncatula* physical map.

Nine QTL were identified with a single *A. euteiches* strain, individually accounting for 4.5–14.5% of the phenotypic variation: on LG3 (*Ae-Mt3.2*) with Ae109; on LG2 (*Ae-Mt2.1*), LG3 (*Ae-Mt3.1*), LG4 (*Ae-Mt4.1*) and LG7 (*Ae-Mt7.1* and *Ae-Mt7.2*) with MF-1; on LG2 (*Ae-Mt2.2*), LG5 (*Ae-Mt5.1*) and LG8 (*Ae-Mt8.2*) with NC-1. For three of these QTL (*Ae-Mt2.1*, *Ae-Mt2.2*, and *Ae-Mt4.1*), resistant alleles were contributed by the F83005.5 susceptible parent line. Six of these QTL (*Ae-Mt2.1, Ae-Mt3.1, Ae-Mt3.2, Ae-Mt5.1, Ae-Mt7.2*, and *Ae-Mt8.2*) were anchored to the *M. truncatula* physical map by the closest markers indicated in Table 3.

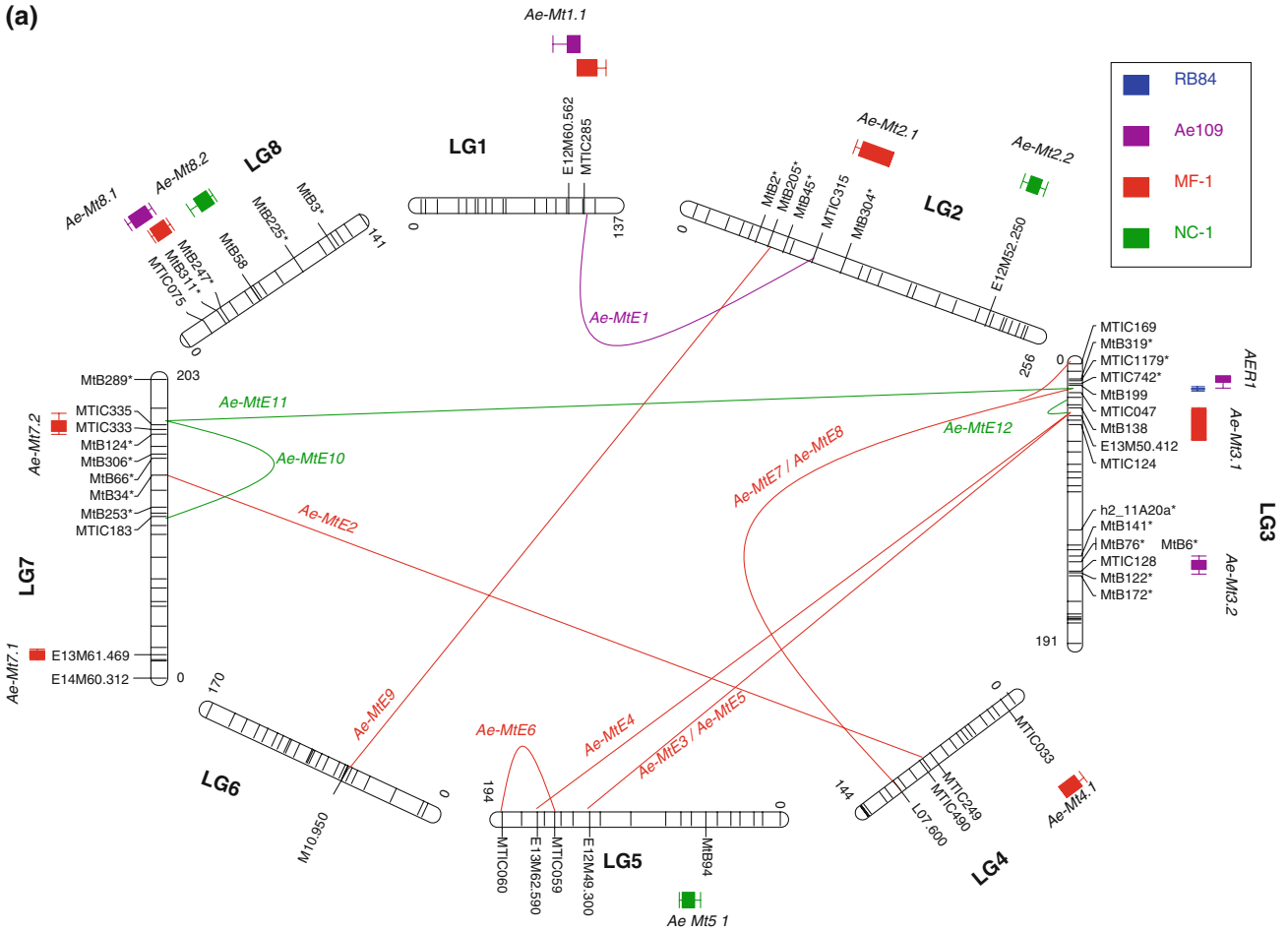
Epistatic-effect QTL mapping

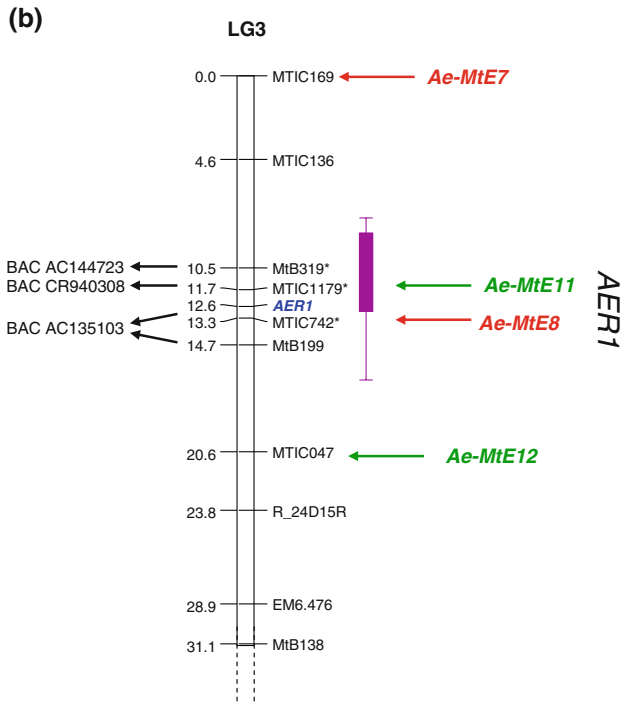
A total of 12 significant pairwise epistatic interactions was identified among all marker pairs of the genetic map, for resistance to the four strains studied, that individually accounted for up to 11.5% of the phenotypic variation (Fig. 2a; Table 4). Most of the significant digenic interactions (11 out of 12) were identified with the alfalfa strains, especially MF-1. One significant interaction, contributing to resistance to the NC-1 strain (*Ae-MtE11*), was found between the major additive effect genomic region *AER1* (detected with RB84 and Ae109) and a genomic region that shows a significant additive effect on resistance to the MF-1 strain (QTL *Ae-Mt7.2*). The two genomic regions implicated in the *Ae-MtE11* epistatic interaction were anchored to the *M. truncatula* physical map (Fig. 2b). Seven significant interactions (*Ae-MtE1, Ae-MtE3, Ae-MtE4, Ae-MtE5, Ae-MtE8, Ae-MtE10*, and *Ae-MtE12*) were found between a genomic region with a significant additive effect on resistance (including the *AER1* genomic region, QTL *Ae-Mt1.1*, *Ae-Mt3.1*, and *Ae-Mt7.2*) and a genomic region that did not have an additive effect on resistance. These epistatic interactions could contribute to resistance to different strains from those for which additive-effect loci involved in the interactions were detected. Particularly, the *AER1* genomic region was involved in the *Ae-MtE8* interaction contributing to resistance to MF-1. Four significant interactions (*Ae-MtE2, Ae-MtE6, Ae-MtE7,* and *Ae-MtE9*) were identified between genomic regions that did not have an additive effect on resistance to *A. euteiches*. For five of the total significant epistatic interactions identified (*Ae-MtE2*, *Ae-MtE6*, *Ae-MtE*, *Ae-MtE10*, and *Ae-MtE12*), F83005.5 alleles contributed to increase levels of resistance to the alfalfa strains, at loci that did not have an additive effect on resistance to those particular strains. Seven markers out of the 24 markers implicated in the significant pairwise epistatic interactions were shown to have individual significant effect on the variable tested.

For each of the four strains, the part of phenotypic variation explained by all the additive-effect QTL detected was higher than that explained by all the epistatic-effect QTL, especially for the pea strains. In the full ANOVA model including the additive and epistatic QTL detected, the overall additive and epistatic R^2 values were 78.2 and 2.9%, respectively, for resistance to RB84, 31.2 and 4.0%, respectively, for resistance to Ae109, 13.8 and 10.9%, respectively, for resistance to MF-1 and 25.7 and 11.0%, respectively, for resistance to NC-1.

Physical genomic regions underlying *Aphanomyces* resistance loci

The *AER1* genomic region, identified for the four strains with an additive (RB84 and Ae109) or epistatic effect (MF-1 and NC-1), was anchored to three *M. truncatula* BAC accessions, including the same BAC accessions, AC135103 and CR940308, as the region *prAe1* (Djebali


(a)
RB84
Ae109
MF-1
NC-1
LG1
LG2
LG3
LG4
LG5
LG6
LG7
LG8
Ae-Mt1.1
Ae-Mt2.1
Ae-Mt2.2
Ae-Mt8.1
Ae-Mt8.2
Ae-Mt7.2
Ae-Mt7.1
Ae-Mt3.1
Ae-Mt3.2
Ae-Mt4.1
Ae Mt5 1
AER1
Ae-MtE1
Ae-MtE2
Ae-MtE4
Ae-MtE6
Ae-MtE7 / Ae-MtE8
Ae-MtE9
Ae-MtE10
Ae-MtE11
Ae-MtE12
Ae-MtE3 / Ae-MtE5
E12M60.562
MTIC285
MtB2*
MtB205*
MtB45*
MTIC315
MtB304*
E12M52.250
MtB3*
MtB225*
MtB58
MtB247*
MtB311*
MTIC075
MtB289*
MTIC335
MTIC333
MtB124*
MtB306*
MtB66*
MtB34*
MtB253*
MTIC183
E13M61.469
E14M60.312
MTIC169
MtB319*
MTIC1179*
MTIC742*
MtB199
MTIC047
MtB138
E13M50.412
MTIC124
h2_11A20a*
MtB141*
MtB76*
MtB6*
MTIC128
MtB122*
MtB172*
MTIC033
MTIC249
MTIC490
L07.600
MTIC060
E13M62.590
MTIC059
E12M49.300
MtB94
M10.950


(b)
LG3
0.0 MTIC169
4.6 MTIC136
10.5 MtB319*
11.7 MTIC1179*
12.6 AER1
13.3 MTIC742*
14.7 MtB199
20.6 MTIC047
23.8 R_24D15R
28.9 EM6.476
31.1 MtB138
BAC AC144723
BAC CR940308
BAC AC135103
Ae-MtE7
Ae-MtE11
Ae-MtE8
Ae-MtE12
AER1

◀ **Fig. 2** **a** Genomic localization, on the eight linkage groups (LG) of *M. truncatula*, of QTL detected for resistance to the RB84, Ae109, MF-1 and NC-1 strains of *A. euteiches* from the F83005.5 × DZA045.5 F5:6-RIL population. Additive and epistatic-effect QTL are referenced to the Tables 3 and 4, respectively, and are colored in *blue*, *purple*, *red* and *green* for RB84, Ae109, MF-1 and NC-1, respectively. Lengths of additive-effect QTL boxes correspond to the LOD-1 support interval (from the peak marker) and QTL lines are extended to the LOD-2 support interval. Markers associated with additive and epistatic-effect QTL are indicated. The 23 additional markers, compared to the framework genetic map described by Avia et al. (submitted) are indicated by one *asterisk*. SSR markers are coded "MtB" (Mun et al. 2006), "MTIC" (Djebali et al. 2009; C. Jacquet, personal communication) or h2 11A20a (http://www.medicago.org/genome/map.php. Univ. Minn. 2006). AFLP markers are coded "E12M", "E13M" or "E14M" and RAPD markers are "L07.600" and "M10.950" (Avia et al., submitted). The size of each LG is given in cM Haldane. **b** The *AER1* genomic region. The length of the additive-effect QTL box for resistance to Ae109 strain correspond to the LOD-1 support interval (from the peak marker) and QTL lines are extended to the LOD-2 support interval. Markers involved in epistatic interactions in the *AER1* genomic region involved in resistance to MF-1 and NC-1 strains are indicated by *red* and *green* arrows, respectively. Genetic distances between markers are indicated on the left of the linkage group, in centimorgans Haldane (cM). The highest probable position of *AER1* obtained for resistance to RB84 strain using qualitative disease scores is indicated in *blue*. The name of BAC accessions included within the *AER1* genomic region are indicated by *black arrows*

Table 2 Spearman phenotypic correlation coefficients between RRI adjusted means obtained in the F83005.5 × DZA045.5 F6-RIL population of *M. truncatula*, for resistance to the RB84, Ae109, MF-1 and NC-1 strains of *A. euteiches*

	RB84	Ae109	MF-1
Ae109	0.64***		
MF-1	0.25***	0.40***	
NC-1	0.03	0.15	0.22**

** $P < 0.01$, *** $P < 0.001$

et al. 2009) and the BAC accession AC144723 (Fig. 2b). It covered a region of 440 kb, including 89 annotated genes (BAC AC135103, CR940308, CT030192, CT963080, and AC144723), among which: (a) the 13 candidate genes identified by Djebali et al. (2009) in a 135 kb region [nine genes encoding putative cyclin-like F-box proteins, a ubiquitin-associated enzyme, a cinnamyl alcohol gene (CAD), one vegetative cell wall protein gp1 precursor and a gene with homology to ethylene insensitive 3], (b) three genes encoding disease-resistance proteins, including a RPM1-like protein (BAC AC135103), (c) nine other genes encoding putative cyclin-like F-box proteins (BAC CT030192 and CT963080), and (d) two genes encoding late nodulins (BAC CT030192).

One additive-effect genomic region identified with both the Ae109 and MF-1 strains (*Ae-Mt8.1*) and six additive and/or epistatic-effect QTL detected with one strain (*Ae-Mt2.1, Ae-Mt3.1, Ae-Mt3.2, Ae-Mt5.1, Ae-Mt7.2*, and Ae-*Mt8.2*) were also anchored to the *M. truncatula* physical map (Table 3). A total of 315 annotated genes were identified on 18 BAC accessions covering the seven genomic regions associated with resistance to one or two strains of *A. euteiches*. Among these genes, 36% were identified as of hypothetical proteins. For each genomic region, BAC identification and prediction of a selection of genes known to be involved in plant disease resistance are summarized in Table 5.

Discussion

A complex network of loci controls resistance to different pathotypes of *A. euteiches* in *M. truncatula*

This study is the first report evaluating the diversity of genetic factors involved in resistance of *M. truncatula*, toward the pathogenic variability of *A. euteiches* described on pea and alfalfa. A total of 12 additive and 12 epistatic genetic factors, distributed over the eight *M. truncatula* chromosomes, were identified for resistance to *A. euteiches*, using two pea and two alfalfa-infecting strains from France and the USA (Fig. 2).

AER1, a major broad-spectrum genomic region

The *AER1*/*prAe1* genomic region was detected on LG3 for different sources of resistance (DZA045.5 and A17, respectively) expressing different genetic effects (dominant and recessive, respectively) leading to high or partial levels of resistance in the DZA045.5 and A17 lines, respectively (Djebali et al. 2009; Pilet-Nayel et al. 2009). In this region, the *AER1* locus confidence interval, detected for resistance to RB84, spans the same two BAC accessions as the *prAe1* locus confidence interval. The *AER1* and *prAe1* loci may therefore correspond to different alleles of a same locus or to different closely linked loci. In this study, the *AER1* region had a high or reduced additive effect on resistance to the RB84 and Ae109 strains, respectively, and an epistatic effect on resistance to MF-1 and NC-1. The *AER1* region therefore has a broad-spectrum of action on pea and alfalfa-infecting *A. euteiches* strains, acting either alone or by interacting with other genes for conferring expression of resistance. These results suggest that the *AER1* genomic region contains a resistance gene or multiple closely linked genes contributing to high levels of resistance in *M. truncatula* against RB84-pathotype strains occurring in Europe and the USA. In the case of the existence of a resistance gene underlying the *AER1* genomic region, the resistance gene may have evolved under selection pressure from other pathotypes of *A. euteiches* occurring in the USA, resulting in its residual additive or epistatic expression against those

Table 3 Significant additive-effect QTL controlling resistance to four strains of *A. euteiches*, identified from the F83005.5 × DZA045.5 F5:6-RIL population of *M. truncatula*

A. euteiches strain	QTL name	LG	Position (cM)[a]	Left marker[b]	LOD[c]	LOD-2 support interval (cM)[d]	R^2 (%)[e]	Additive effect[f]	*Mtr* contig[g]
RB84	*AER1*	3	16.4	MtB199	65.7	15.9–18.5	78.9	0.9	953
Ae109	*AER1*	3	10.6	MtB319	19.1	7.8–16.7	25.5	0.23	953
	Ae-Mt8.1	8	10	MTIC075	4.1	0.4–21.0	6.6	0.12	214
	Ae-Mt3.2	3	135.5	MtB6	4.6	131.4–143.9	5	0.1	962
	Ae-Mt1.1	1	108.6	E12M60.562	3.2	95.6–113.2	4	0.09	–
MF-1	*Ae-Mt8.1*	8	16.6	MTIC075	6.2	9.1–24.7	10.5	0.14	214
	Ae-Mt2.1	2	119	MtB304	4	98.0–125.0	6.9	−0.12	1,004
	Ae-Mt4.1	4	14.6	MTIC033	3.9	1.9–19.2	6.3	−0.11	–
	Ae-Mt7.1	7	14.4	E13M61.469	3.5	11.0–19.0	4.9	0.13	–
	Ae-Mt3.1	3	41.9	MTIC124	3.2	30.5–51.9	4.5	0.09	99
	Ae-Mt7.2	7	169.1	MTIC333	3.2	166.2–181.5	4.5	0.11	979
	Ae-Mt1.1	1	113.1	MTIC285	3	106.6–125.7	4.3	0.09	–
NC-1	*Ae-Mt5.1*	5	58.1	MtB94	6.5	52.1–66.9	14.5	0.15	972
	Ae-Mt8.2	8	54.1	MtB58	5.3	41.7–60.3	9.8	0.12	1,018
	Ae-Mt2.2	2	221.1	E12M52.250	3.8	212.9–229.8	8.9	−0.18	–

QTL are ordered by strain and decreasing R^2 values

[a] QTL position from the first marker of the linkage group (in centimorgans Haldane)

[b] Nearest marker from the LOD score peak of the QTL

[c] Log of likelihood ratio (LOD) value at the position of the LOD score peak for the QTL

[d] Confidence interval in centimorgans Haldane

[e] Percentage of phenotypic variance explained by an individual QTL

[f] Effect of substituting "DZA045.5" alleles for "F83005.5" alleles at the QTL. A positive sign indicates that QTL alleles increasing the resistance are contributed by the resistant parent "DZA045.5", whereas a negative sign means that resistant alleles are brought by the susceptible parent "F83005.5"

[g] BAC contig associated with the closest anchored marker from the LOD peak

pathotypes. However, it is also possible that the *AER1* genomic region includes different closely linked genes, contributing to the resistance to different *A. euteiches* pathotypes, as observed in other pathosystems (Huang et al. 2004).

Ae-Mt8.1 and Ae-Mt1.1, two genomic regions associated with resistance to two A. euteiches strains isolated from different hosts

The *Ae-Mt1.1* and *Ae-Mt8.1* genomic regions, with additive and/or epistatic effects on resistance to *A. euteiches*, were common to the pea-infecting Ae109 and alfalfa-infecting MF-1 strains. A third genomic region, *Ae-Mt7.1*, detected for significant resistance to MF-1, was also associated with resistance to Ae109 with a LOD score close but lower than the LOD threshold defined (LOD = 2.7 and R^2 = 3%, data not shown). These results suggest that some common genes in *M. truncatula* could control resistance to both the Ae109 and MF1 strains, which may interact with common pathogenicity factors to the two *A. euteiches* strains, despite their different host-specificities (MF-1 is pathogenic on alfalfa only, while Ae109 is able to infect both pea and alfalfa; Malvick et al. 1998). Ae109 and MF-1 were both isolated in Wisconsin (USA) from pea and alfalfa, respectively (Malvick and Percich 1999; Malvick and Grau 2001), where *A. euteiches* was first described as an important pathogen of pea in 1925, and was associated with root rot in alfalfa in 1927 (Jones and Drechsler 1925; Linford 1927). Peas have been grown intensively in Wisconsin for the last century and the central USA also has fields with a history of alfalfa production (Malvick et al. 2009). Holub et al. (1991) showed that 97% of isolates from pea fields in Wisconsin were able to infect alfalfa. Recently, reactions of the three reference alfalfa populations (Saranac, WAPH-1 and WAPH-5) to infection by Ae109 were found to be typical of responses observed with race 1 strains (Malvick et al. 2009) (data not shown). Genotypic relationships, based on RAPD, between 62 strains of *A. euteiches*, showed that Ae109 and MF-1 are

Table 4 Pairwise epistatic interactions associated with resistance to four strains of *A. euteiches*, identified from the F83005.5 × DZA045.5 F5:6-RIL population of *M. truncatula* ($P < 2.5 \times 10^{-4}$ and $R^2 > 5\%$)

A. euteiches strain	QTL name	Markers in interaction[a]	P[b]	R^2 (%)[c]	Phenotypic RRI adjusted means for each genotypic class[d]			
					DD	DF	FD	FF
Ae109	*Ae-MtE1*	***Ae-Mt1.1*** (MTIC285) × MTIC315	1.5×10^{-4}	9.2	**2.64**	3.02	2.97	2.78
MF-1	*Ae-MtE2*	MTIC490 × MtB34	0.4×10^{-4}	10.6	2.53	3.10	2.60	**2.44**
	Ae-MtE6	MTIC059 × MTIC060	1.2×10^{-4}	9.4	2.67	**2.34**	**2.34**	2.67
	Ae-MtE3	E12M49.300 × ***Ae-Mt3.1*** (MTIC124)	0.6×10^{-4}	9.3	**2.35**	2.74	2.69	2.54
	Ae-MtE4	E13M62.590 × ***Ae-Mt3.1*** (MTIC124)	0.6×10^{-4}	9.2	**2.37**	2.77	2.62	2.48
	Ae-MtE7	L07.600 × MTIC169	1.8×10^{-4}	8.5	**2.43**	2.73	2.67	2.46
	Ae-MtE5	E12M49.300 × ***Ae-Mt3.1*** (E13M50.412)	1.2×10^{-4}	8.0	**2.39**	2.76	2.70	2.56
	Ae-MtE9	M10.950 × MtB205	2.0×10^{-4}	8.0	2.79	**2.39**	2.57	2.69
	Ae-MtE8	L07.600 × ***AER1*** (MTIC742)	1.8×10^{-4}	8.4	**2.43**	2.76	2.61	2.55
NC1	*Ae-MtE10*	***Ae-Mt7.2*** (MTIC335) × MTIC183	0.3×10^{-4}	11.5	2.53	**2.28**	2.39	2.85
	Ae-MtE11	***Ae-Mt7.2*** (MTIC335) × ***AER1*** (MTIC1179)	0.3×10^{-4}	11.5	**2.49**	2.50	2.61	2.67
	Ae-MtE12	MTIC047 × ***Ae-Mt3.1*** (MtB138)	1.9×10^{-4}	8.9	2.55	2.52	**1.65**	2.57

QTL are ordered by strain and decreasing R^2 values. Bold values indicate the genotypic classes displaying the lower phenotypic RRI adjusted means values

[a] The name of additive-effect QTL involved in epistatic interactions are indicated in bold

[b] Significance level of each pairwise epistatic interaction obtained with the two-way ANOVA model

[c] Percentage of phenotypic variation explained by an individual QTL

[d] RRI adjusted means for the four genotypic classes defined by each marker pair: (DD) and (FF) DZA045.5 and F83005.5 alleles at the two markers, respectively; (DF) DZA045.5 allele at the first marker, F83005.5 allele at the second marker, and conversely (FD)

close genetically and are closer than MF-1 and NC-1 (Malvick et al. 1998). These findings suggest that Ae109 and MF-1 may be derived from each other or from a common ancestral isolate, and evolved through their adaptation to different hosts (pea and/or alfalfa). Consequently, these strains could share common pathogenicity genes which may interact with common genes involved in resistance in the host *M. truncatula* plant.

Ae-Mt3.1 and Ae-Mt7.2, two genomic regions associated with resistance to two strains isolated from a same host

Two other genomic regions (*Ae-Mt3.1* and *Ae-Mt7.2*) were identified with additive or epistatic effects on resistance to the two alfalfa strains. In addition, two minor additive-effect QTL (data not shown) were identified on LG2 (near the marker MtB2, LOD = 2.7 and $R^2 = 5\%$) and LG4 (near the marker MTIC249, LOD = 2.6 and $R^2 = 5\%$) for resistance to NC-1, with a LOD score close to but lower than the defined LOD threshold, close to markers having a significant epistatic effect on resistance to MF-1. These results suggest that common or linked genes may control resistance to strains from different pathotypes isolated from a same host that would share genetic similarities. However, seven additive-effect QTL and 12 loci involved in epistatic interactions were also significantly detected for a single strain, mainly MF-1, which indicates that there are also specific genes controlling resistance to the different pathotypes described on pea and alfalfa.

At present, knowledge of *A. euteiches* genetic diversity is limited (Malvick et al. 1998; Grunwald and Hoheisel 2006; Akamatsu et al. 2007). Further studies and tools would be valuable to investigate genetic relationships among strains and the diversity of genetic factors controlling pathogenicity in *A. euteiches*, leading to a better understanding of the diversity of genetic loci involved in resistance to *A. euteiches* pathotypes in *M. truncatula*. Recently, two cDNA libraries were constructed (Gaulin et al. 2008), to generate a unigene set of about 8,000 sequences that was organized in a database named AphanoDB (Madoui et al. 2007). This genomic resource would be useful for developing new molecular markers to elucidate *A. euteiches* genetic diversity and study pathogen gene expression during the *M. truncatula*/*A. euteiches* interaction.

Table 5 Selection of gene reported to be involved in disease resistance in plants, included within the support interval of seven additive and/or epistatic-effect QTL, detected for resistance to one or two strains of *A. euteiches*, from the F83005.5 × DZA045.5 F5:6-RIL population of *M. truncatula*

QTL name	Mtr contig[a]	BAC accession[b]	Genes associated with plant disease resistance	References
Ae-Mt2.1	1,004	AC139356	2 Cyclin-like F-box	Lechner et al. (2006)
			1 Hydroxyproline-rich glycoprotein family	Esquerré-Tugayé et al. (1999)
Ae-Mt3.1/Ae-MtE12	99	AC135161	1 WRKY transcription factor	Ulker and Somssich (2004)
			1 Cyclin-like F-box	Lechner et al. (2006)
Ae-Mt3.2	962	CT954236	4 Serine/threonine protein kinases	Hardie (1999)
		CT573365	2 Trehalose-phosphate synthases	
		CU062477	3 Cytochromes P450	
		AC147877		
		AC140849		
Ae-Mt5.1	972	AC126785	2 NBS-LRR genes	Ameline-Torregrosa et al. (2008b)
			1 Serine/threonine protein kinase	Hardie (1999)
Ae-Mt7.2/Ae-MtE10/ Ae-MtE11	979	AC145329	3 Heat shock proteins	Colditz et al. (2004)
		AC126016		
Ae-Mt8.1	214	AC149580	9 Lipoxygenases	Prost et al. (2005)
		AC140032	1 TIR-NBS-LRR	Ameline-Torregrosa et al. (2008b)
		AC150203	1 Cyclin-like F-box	Lechner et al. (2006)
		AC148398	5 Ankyrin related	Yan et al. (2002)
Ae-Mt8.2	1,018	AC151816	3 Serine/threonine protein kinases	Hardie (1999)
		AC121241	2 Pathogenesis-related proteins	Colditz et al. (2007)
		AC135229	7 NBS-LRR genes	Ameline-Torregrosa et al. (2008b)
		AC148525		

[a] BAC contig associated with the closest anchored marker from the LOD peak

[b] Completely sequenced BAC accession (phase 3)

Genomic bases of partial resistance to *A. euteiches* in *M. truncatula*

Molecular mechanisms and genes involved in partial resistance are currently poorly understood. Hypothesis about function of genes underlying partial resistance loci was recently reviewed in Poland et al. (2009). Based on our results, we hypothesize that partial resistance is controlled by a diversity of mechanisms which depend on pathogen and plant genotypes, have some overlap with complete resistance and involve a network of genes expressing different genetic effects. A few hypotheses about function of genes underlying genetic factors identified for resistance to *A. euteiches* in *M. truncatula* can be discussed.

The *AER1/prAe1* genomic region, conferring resistance against a broad-spectrum of *A. euteiches* pathotypes, is involved in complete or partial resistance, depending on the strain and the plant genotype. Similar results were previously observed in other pathosystems, leading to the hypothesis that resistance QTL may be weak forms of R-genes, such as in apple (Soufflet-Freslon et al. 2008), *Brassicaceae* (Manzanares-Dauleux et al. 2000; Rocherieux et al. 2004) or pepper (Caranta et al. 1997). Li et al. (1999) also suggested that "defeated" major resistance genes can have residual effects on different races of the same pathogen or different pathogens. However, the first gene cloned associated with partial resistance, namely *pi21* for blast resistance in rice, is not a classical RGA (Fukuoka et al. 2009). In our study, the *AER1/prAe1* genomic region, anchored to *M. truncatula* BAC contig 953 at one end of LG3, was localized to the same region as a supercluster of nucleotide binding site leucine-rich repeat (NBS-LRR) genes, encoding approximately 40% of all coiled-coil-NBS-LRRs, including three coiled-coil-NBS-LRRs from the BAC AC135103 (Ameline-Torregrosa et al. 2008b). However, Djebali et al. (2009) fine mapped the *prAe1* locus to a 135 kb sequence interval, within which no RGA was identified. In this study, we showed that the 440 kb broad-spectrum *AER1* genomic region overlaps with this same *prAe1* 135 kb interval and particularly includes (a) genes encoding proteins associated with the proteasome (cyclin-like F-box proteins, ubiquitin-associated enzyme) and defense responses (hydroxyproline-rich glycoprotein family, cinnamyl alcohol dehydrogenase) (Djebali et al. 2009), (b) three genes encoding disease-resistance proteins, including a RPM1-like protein, that was shown, in

A. thaliana, to confer dual specificity to pathogens expressing either of two unrelated *Pseudomonas syringae* avr genes (Grant et al. 1995), and (c) two genes encoding late nodulins. In legumes, Graham et al. (2004) showed that nodule-specific genes encoded cystein-cluster proteins (CCPs), such as late nodulins, have strong similarities to plant defensins and proposed that nodule-specific CCPs are induced as a secondary defense to protect the nodule from pathogenic organism, while allowing the symbiosis to continue.

Among the genes known to be involved in disease-resistance plant underlying the four genomic regions associated with resistance to two strains anchored to the physical map, the most striking features were (a) the presence of a cluster of nine lipoxygenase encoding genes at *Ae-Mt8.1*, (b) genes involved in defense signal transduction, such as a WRKY transcription factor, serine/threonine protein kinases and cyclin-like F-box proteins at *Ae-Mt3.1* and (c) a cluster of three heat shock proteins at *Ae-Mt7.2*. Lipoxygenases play a role in signal transduction and are toxic to microbes (Rosahl 1996). In *A. thaliana*, Hubert et al. (2003) showed that mutations in a heat shock protein can modulate RPM1 function. Interestingly, the *Ae-Mt7.2* QTL, containing a cluster of heat shock proteins, was shown to interact with the *AER1* genomic region, including a RPM1-like protein, for contributing to resistance.

Among the three other QTL anchored to the *M. truncatula* physical map, associated with resistance to one strain, two QTL (*Ae-Mt5.1* and *Ae-Mt8.2*) particularly contain NBS-LRR genes, which may be in accordance with the narrow-specificity of these resistance QTL (Poland et al. 2009). We also identified genes involved in defense responses, such as a hydroxyproline-rich glycoprotein family, at *Ae-Mt2.1*.

Among the total eight genomic regions anchored to the physical map, one striking feature is the presence of genes encoding cyclin-like F-box proteins underlying four genomic regions (*AER1*, *Ae-Mt2.1*, *Ae-Mt3.1*, and *Ae-Mt8.1*), suggesting another hypothesis that these genes may correspond to paralogs playing a role in resistance to *A. euteiches*. In *A. thaliana*, Bikard et al. (2009) suggested that epistatic interactions between loci controlling a recessive embryo lethality could be explained by divergent evolution occurring among paralogs of an essential duplicate gene. In the *M. truncatula* genome, duplication events remain poorly known.

Many other genes, not or less known to be involved in disease resistance in plants, have been identified underlying confidence intervals of the identified QTL. Further fine mapping and functional validation studies will be necessary to conclude on the function(s) of genes underlying *A. euteiches* resistance in *M. truncatula*.

Conclusion

This study provides insight into the complex architecture of genetic factors involved in the resistance of *M. truncatula*, toward the pathogenic variability of *A. euteiches* described on pea and alfalfa. We identified a major broad-spectrum genomic region, namely *AER1*, four additive and/or epistatic genomic regions associated with resistance to two strains and several additive or epistatic-effect loci associated with resistance to a specific strain.

It will be of significant interest to isolate the gene(s) underlying the *AER1* region associated with broad-spectrum resistance to *A. euteiches* in order to study its (their) regulation and regulated molecular pathways leading to complete or partial resistance. Consequently, the gene functions underlying a part of the genetic network controlling resistance to *A. euteiches* may be elucidated.

Furthermore, transferring knowledge on the genetics and genomics of resistance to *A. euteiches* from the model legume to cultivated leguminous crops infected by *A. euteiches* (especially pea and alfalfa), in which few (Pilet-Nayel et al. 2005) or no genetic mapping studies of the resistance have been developed, would also be of great interest for optimizing breeding strategies for resistance to *A. euteiches* in legumes.

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