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ORIGINAL PAPER

Genetic mapping of a major dominant gene for resistance to *Ralstonia solanacearum* in eggplant

A. Lebeau · M. Gouy · M. C. Daunay · E. Wicker · F. Chiroleu · P. Prior · A. Frary · J. Dintinger

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Abstract Resistance of eggplant against *Ralstonia solanacearum* phylotype I strains was assessed in a F_6 population of recombinant inbred lines (RILs) derived from a intra-specific cross between *S. melongena* MM738 (susceptible) and AG91-25 (resistant). Resistance traits were determined as disease score, percentage of wilted plants, and stem-based bacterial colonization index, as assessed in greenhouse experiments conducted in Réunion Island, France. The AG91-25 resistance was highly efficient toward strains CMR134, PSS366 and GMI1000, but only partial toward the highly virulent strain PSS4. The partial resistance found against PSS4 was overcome under high inoculation pressure, with heritability estimates from 0.28 to 0.53, depending on the traits and season. A genetic map was built with 119 AFLP, SSR and SRAP markers positioned on 18 linkage groups (LG), for a total length of 884 cM, and used for quantitative trait loci (QTL) analysis. A major dominant gene, named *ERs1*, controlled the resistance to strains CMR134, PSS366, and GMI1000. Against strain PSS4, this gene was not detected, but a significant QTL involved in delay of disease progress was detected on another LG. The possible use of the major resistance gene *ERs1* in marker-assisted selection and the prospects offered for academic studies of a possible gene for gene system controlling resistance to bacterial wilt in solanaceous plants are discussed.

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A. Lebeau · M. Gouy · E. Wicker (✉) · F. Chiroleu · J. Dintinger (✉)
CIRAD, UMR Peuplements végétaux et Bioagresseurs en Milieu Tropical (PVBMT), 7 chemin de l'IRAT, 97410 Saint Pierre, La Réunion, France
e-mail: emmanuel.wicker@cirad.fr

J. Dintinger
e-mail: jacques.dintinger@cirad.fr

M. C. Daunay
Unité de Génétique et Amélioration des Fruits et Légumes, Institut National de la Recherche Agronomique (INRA), Centre d'Avignon, UR1052, Domaine St Maurice, BP94, 84143 Montfavet, France

P. Prior
INRA/CIRAD, UMR PVBMT, 7 Chemin de l'IRAT, 97410 Saint-Pierre Cedex, La Réunion, France

A. Frary
Department of Molecular Biology and Genetics, Izmir Institute of Technology, Urla, Izmir 35430, Turkey

Introduction

The causal agent of bacterial wilt disease (BW), *Ralstonia solanacearum*, ranks among the most devastating pathogens in important agricultural solanaceous crops such as potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*) and tobacco (*Nicotiana* spp.) (Food and Agricultural Organization FAO, http://faostat.fao.org/faostat). This bacterium has a huge host range encompassing more than 200 monocot and dicot plant species and has spread worldwide due to its capacity to adapt to tropical, subtropical and temperate regions (Denny 2006; Elphinstone 2005; Hayward 1991, 1994; Kelman 1998). This soil-borne bacterium penetrates through the root system and proliferates in xylem tissue. Irreversible foliar wilting generally develops quickly, resulting in plant death. Historically,

R. solanacearum has been classified into five races and six biovars according to host range and trophic traits, respectively (Buddenhagen et al. 1962; Hayward 1964; He et al. 1983; Pegg and Moffett 1971). More recently, phylogenetic analysis described four distinctive phylotypes that are related to the geographical origin of the strains: phylotype I (Asia), phylotype II (America), phylotype III (Africa) and phylotype IV (Indonesia) (Cook et al. 1989; Cook and Sequeira 1994; Fegan and Prior 2005). BW resistance is a key method for controlling the disease, together with agronomic practices such as crop rotation and fallowing. Resistance is strongly affected by environmental factors, and more importantly, by the strain pathoprofile, which can vary among and within the different phylotypes of the *R. solanacearum* species complex (Lebeau et al. 2011).

So far, most studies of inheritance of BW resistance have been performed in tomato and pepper, two species which display close syntenic relationships with eggplant (Doganlar et al. 2002). Resistance in tomato was described as monogenic or polygenic with recessive to dominant effects, depending on the genetic material used and environmental conditions (Gonzalez and Summers 1995; Grimault et al. 1995; Hanson et al. 1996; Mohamed et al. 1997; Scott et al. 1988). Mapping studies have shown the involvement of generalist as well as strain-specific quantitative trait loci (QTLs) in *S. lycopersicum* Hawaii 7996 (Carmeille et al. 2006; Mangin et al. 1999; Thoquet et al. 1996; Wang et al. 2000). In addition, bulked segregant analysis has shown the presence of two incompletely dominant genes associated with resistance in tomato cultivar T51A (Miao et al. 2009). In pepper, the inheritance of BW resistance in a double haploid population derived from the cross *C. annuum* var. Yolo Wonder × *C. annuum* var. PM687 was found to be polygenic (Lafortune et al. 2005). More recently, mapping studies using the same double haploid population and a recombinant inbred lines population derived from the cross Yolo Wonder × CM334, have shown the involvement of three to six QTLs with additive effects and digenic interactions (Mahbou Somo Toukam 2010).

BW-resistant eggplant material has been identified in several countries, including India, Taiwan, and Japan (Chen et al. 1997; Hanudin and Hanafiah Gaos 1993; Li et al. 1988; Mochizuki and Yamakawa 1979; Ponnuswami et al. 1996; Rao et al. 1976; Sakata et al. 1996; Sitaramaiah et al. 1985; Wang et al. 1998). Commercial resistant cultivars have been released, but have mostly been used on a local scale, such as the F_1 Kalenda in the French West Indies (Daly 1973). The genetic control of eggplant resistance to BW was described as variable among the varieties studied, but few studies involving molecular markers have been carried out to date. Nunome et al. (1998) were the first to identify two QTLs involved in BW resistance, in an intraspecific F_2 population derived from a cross between the Indian resistant accession WCGR112-8 and a breeding line EPL1. More recently, two AFLP markers linked to a single recessive gene originating from the susceptible parent 5810 (Sun et al. 2008); an AFLP marker linked to a major resistance gene from the Indonesian accession S69 (Li et al. 2006); and a RAPD marker linked to a single dominant gene from the Chinese accession E31 (Cao et al. 2009) were obtained using bulked segregant analysis and were converted into sequence characterized amplified region (SCAR) markers. So far, few segregating populations have been used for tagging and mapping BW-resistance genes or QTLs in eggplant (Fukuoka et al. 2010; Nunome et al. 1998). Moreover, genes or QTLs associated with BW-resistance were reported against non-characterized *R. solanacearum* strains, whereas strain- and phylotype-specific QTLs were reported for tomato. Among solanaceous-infecting *R. solanacearum* populations, phylotype I strains are the most prevalent clade found in most Asian eggplant production areas (Horita and Tsuchiya 2001; Ivey et al. 2007; Jaunet and Wang 1999; Xu et al. 2009) as well as in Africa (Mahbou Somo Toukam et al. 2009), America (Ji et al. 2007; Norman et al. 2009; Sanchez Perez et al. 2008), and the Caribbean (Wicker et al. 2009). In a previous study, we showed that phylotype I strains display different virulence patterns, called pathoprofiles, on a core collection of tomato, pepper and eggplant representative of the genetic diversity for resistance in these species (Lebeau et al. 2011).

The low molecular polymorphism present within eggplant germplasm (Nunome et al. 2001) has been a major problem for building genetic maps based on intraspecific segregating populations. Amplified fragment length polymorphism (AFLP) is a good technique for genotyping in such cases, because it generates a large number of genome-wide polymorphic markers. Simple sequence repeat (SSR) markers from eggplant genic and genomic libraries are also described as polymorphic within eggplant germplasm (Nunome et al. 2009; Nunome et al. 2003a, b). These markers present the advantage of often being transferable between related species. Therefore, they can provide anchoring points for comparing genetic maps of eggplant and tomato.

The objectives of the present study were (1) to determine the genetic control of resistance to phylotype I *R. solanacearum* strains in an segregating population of recombinant inbred lines (RILs) derived from an intraspecific cross between resistant (AG91-25) and susceptible (MM738) eggplant lines, (2) to map the genes or QTL controlling this resistance and (3) to evaluate their strain-specificity for phylogenetically close strains displaying different virulence patterns on the eggplant line AG91-25.

Materials and methods

Plant material

The resistant parent AG91-25 (MM960), a *S. melongena* commercial-type line adapted to the tropics, almost spineless, with dark purple fruits of intermediate shape was created at INRA (Guadeloupe). It recombines BW resistance factors from MM127, a Turkish *S. melongena* line, and MM134, a *S. aethiopicum* Aculeatum Group accession, (Ano et al. 1990, 1991). The susceptible parent MM738 is one of the parents of the F_2 mapping population that was used to establish the reference map of eggplant (Doganlar et al. 2002; Wu et al. 2009), and is a European commercial-type line, almost spineless and hairless, with globose dark purple fruits. MM738 (P1), used as female parent, was crossed with AG91-25 (P2) to generate F_1, and then F_2, BC_1P1, BC_1P2 generations. A population of 178 F_6 recombinant inbred lines (RILs) was obtained by single seed descent without selection from 178 F_2 plants produced by the selfing of one single F_1 plant.

Bacterial strains

The four *Ralstonia solanacearum* strains used in this study, CMR134, PSS366, GMI1000, and PSS4, belong to phylotype I (Table 1). They were chosen according to their degree of aggressiveness on the RILs parents as previously reported (Lebeau et al. 2011). All are highly aggressive on the susceptible parent MM738, but display different levels of aggressiveness against the resistant parent AG91-25.

Experimental design

The RILs, parents, and derived generations were planted at the experimental station of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in Saint-Pierre, Réunion island (lowland tropical environment, 140 m elevation, 21°S, 55.3°E). Trials with RILs, parents, as well as F_1, F_2, and the first backcross generations (BC_1) on the two parents were conducted in two greenhouses, one per replication, with a complete randomized design, each plant constituting one experimental unit. In each replication (greenhouse), five plants were grown for each RIL family, 20 plants for the F_1, 100 plants for the F_2 and BC_1 generations, and 125 plants for the two parents. Single trials were carried out at different seasons with strain CMR134 (July–August 2008, cool, wet season, 16 ± 2/26 ± 7 °C and 90 ± 4/71 ± 15 % relative humidity (RH), night/day respectively), strain PSS366 (September–November 2008, cool, dry season, 19 ± 2/31 ± 7 °C and 84 ± 6/56 ± 16 % RH), and strain GMI1000 (March–May 2009, hot, wet season, 22 ± 3/32 ± 7 °C and 90 ± 7/68 ± 18 % RH). Two successive trials were carried out with strain PSS4 during the cool, dry season (September–October 2009, 19 ± 3/32 ± 7 °C and 81 ± 6/52 ± 16 % RH) and during the hot, wet season (April–May 2010, 21 ± 2/30 ± 6 °C and 89 ± 4/63 ± 17 % RH).

Inoculation and disease assessment

Ralstonia solanacearum strains were grown at 30 °C on Kelman's triphenyltetrazolium chloride (TZC) solid medium complemented with 0.5 g yeast extract (Kelman 1954). Inoculum consisted of a suspension of bacterial cells harvested from 48-h-old cultures by flooding plates with 10 mL of Tris buffer (Trizma 0.01 M pH 7.1; Sigma, St. Louis, USA). The concentration of each bacterial suspension was determined by measuring the optical density (600 nm) and adjusted to 10^8 CFU mL^{-1} (colony-forming unit). The inoculum was prepared for each replication as follows: 2 L of inoculum (10^8 CFU mL^{-1}) was diluted to 1 % (v/v), so that each individual plant was infected with an average of 100–150 mL at 10^6 CFU mL^{-1}. The plants were inoculated at the 4–5 fully expanded leaf stage with the bacterial suspension delivered through the drip irrigation system. The soil substrate was drenched with inoculum right after the plants' roots were wounded with a knife.

Disease development was assessed twice a week for 6–7 weeks according to a disease scale, 0: asymptomatic plant, 1: one wilting leaf, 2: less than 50 % wilted leaves, 3: more than 50 % wilted leaves, and 4: completely wilted leaves (dead plant). At each scoring date the disease score

Table 1 Characteristics of *Ralstonia solanacearum* strains used in the study

Strain	Alternative name	Host of isolation	Geographical origin	Phylotype–sequevar
PSS366	RUN155	*Solanum lycopersicum*	Taiwan	I-15
CMR134	RUN215, CFBP7058	*Solanum scabrum*	Cameroon	I-13
GMI1000	RUN54, JS753	*Solanum lycopersicum*	French Guyana	I-18
PSS4	RUN157, CIP410	*Solanum lycopersicum*	Taiwan	I-15

PSS AVRDC collection, Shanhua, Taiwan; *CMR* Cameroon strain; *RUN* collection at CIRAD-INRA Réunion; *CFBP* Collection Française des Bactéries Phytopathogènes, Angers, France

was calculated for each line, parent, and generation as the mean rating of all plants from each replication. The proportion of wilted plants (W) was calculated for each line, parent, control and generation after scoring each plant as resistant (no symptom) or susceptible (at least one leaf wilted). The area under the disease progression curve (AUDPC) (Jeger and Viljanen-Robinson 2001) was further determined as $\sum_{i=1}^{n-1} \frac{X_i+X_{i+1}}{2} \times (t_{i+1} - t_i) \times \frac{1}{t_n - t_1}$, where X_i is the mean wilting symptoms rating (disease score) at the ith date ($i = 1$ corresponds to the inoculation day), t_i was the time at the ith observation, and n the total number of observations. At the end of the assay, a 0.5-cm-long stem section was sampled at the base of each plant and transferred to 5 mL of Tris buffer. Stem sections were stored for 1–2 h at room temperature to allow bacteria to stream out of the xylem vessels. An aliquot of 50 µL from each sample was streaked onto modified Granada and Sequeira selective medium plates (Granada and Sequeira 1983; Poussier et al. 1999) and incubated at 28 °C for 3–4 days. Stem sections from which characteristic *R. solanacearum* colonies were isolated, were scored as positive for the presence of bacteria. From these results, a colonization index (CI) was calculated as $N_{WP} + (N_S \times R_S)$, where N_{WP} is the percentage of wilted plants; N_S, the percentage of symptomless plants; and R_S, the percentage of symptomless plants colonized by the bacteria (Grimault and Prior 1994; Prior et al. 1996).

Greenhouse data analysis

Analyses of variance were conducted for each trial, each trait, and also across trials (seasons) for strain PSS4, using the Proc GLM procedure of SAS (SAS Institute 1999). An additional analysis was conducted for strain PSS4 using Proc MIX procedure with the family factor considered to be random, and replication and season to be fixed effects. For PSS4, the best linear unbiased predictors (BLUPs) (Henderson 1975) were calculated by adding the general mean of the trial to the solution of the random family effect. Broad sense heritability was calculated for each trial and across trials for PSS4 from the variance components, according to Hallauer and Miranda's formula (1981). The exact 90 % confidence interval of h^2 was calculated from Knapp et al. (1985).

DNA extraction

A bulk of young leaves was collected from ten plants per RIL at the seedling stage. Genomic DNA was extracted using a modified CTAB procedure (Doyle and Doyle 1990). DNA quality was assessed by 1 % (*w/v*) agarose gel electrophoresis in comparison with a standard series of lambda DNAs. DNA concentration was evaluated by the Thermo Scientific NanoDrop® 8000 spectrophotometer (NanoDrop products, Wilmington, USA).

Marker analysis

DNA-AFLP analysis was performed as described by Vos et al. (1995). Genomic DNA was digested with two restriction enzymes, *EcoR*I and *Mse*I, and then ligated to adapters (AFLP® Core Reagent kit, Invitrogen, Carlsbad, California, USA). The pre-amplification reaction was performed with a pair of primers based on the adapter sequences, each having one selective nucleotide (*Eco*-A and *Mse*-C). The selective amplification of preamplified fragments was performed with eight *EcoR*I primers (Applied Biosystem) and eight *Mse*I primers (GeneCust, Dudelange, Luxembourg), each with three selective nucleotides. The sequences of the 3′ selective nucleotides were: *EcoR*I plus AAC, AAG, ACA, ACC, ACG, ACT, AGC, or AGG; and *Mse*I plus CAA, CAC, CAG, CAT, CTA, CTC, CTG, or CTT. The 5′ end of *EcoR*I primers was labeled with fluorescent dye. The PCR fragments were separated by capillary electrophoresis using an Applied Biosystems 3130*xl* Genetic Analyzer. The AFLP data were analyzed using GelCompar II® software, version 4.6 (Applied Maths, Saint-Martens-Latem, Belgium). Only polymorphic AFLP bands ranging from 50 to 500 bp were scored (1 if present, 0 if absent) and analyzed. The 64 primer combinations yielded a total of 163 markers.

A panel of 835 microsatellite (SSR) primers designed from eggplant (Nunome et al. 2003a, 2009; Stagel et al. 2008) as well as tomato sequences (SGN) was screened for polymorphism on the mapping parents. A total of 34 SSRs showing polymorphism between the two parents were used to genotype the RIL population. PCR and amplified product visualization were performed as described by Dintinger et al. (2005).

In addition, sequence-related amplified polymorphism (SRAP) screening was carried out on the parents (Budak et al. 2004) for a total of 238 primer combinations. The PCR amplification conditions were as described by Li and Quiros (2001). In addition, 66 10-mer or 12-mer RAPD primers were screened on the parents. PCR amplification was carried out using a thermal cycler (9700, Applied Biosystems) with the following cycling parameters: 1 cycle at 94 °C for 3 min; 35 cycles at 94 °C for 1 min, 40 °C for 45 s, 72 °C for 2 min, and final extension at 72 °C for 10 min. Amplification products were separated by electrophoresis on 4.5 % agarose gels, stained with ethidium bromide, and photographed under UV light.

Linkage map analysis

The segregation of each locus was checked for deviation from the Mendelian ratio expected for a RIL population (1:1) by standard χ^2 tests. Linkage analysis was performed using the MAPMAKER/EXP ver. 3.0b program (Lander et al. 1987). All pairs of linked markers were first identified using the 'group' command LOD $\geq$3, $r = 0.3$. The 'order' command was used to establish the framework order of markers within linkage groups (LGs) and the 'ripple' command was used to verify the order. The groups where the 'order' command failed to find a starting order were analyzed using the 'compare' command. Markers were retained within the framework map only if the LOD value for 'ripple' command was $\geq$3. All remaining markers were assigned to intervals within the framework using the 'try' command. After determination of the most-likely marker order, recombination frequencies between loci were converted into map distances in cM applying the Haldane function (Haldane 1919).

QTL analysis

Because data of the experiments with CMR134, PSS366 and GMI1000 resulted in non-normal distributions of RILs, genetic factor analysis was carried out on all data with R/qtl package (Broman et al. 2003) of R software (R Development Core Team 2011) which performs two models of non-normal phenotypes analysis (scanone function): (1) binary traits interval mapping (model = "binary") using maximum likelihood analysis calculated from mixtures of Bernoulli distributions and (2) nonparametric interval mapping (model = "np") using the extension of the Kruskal–Wallis test. For binary traits interval mapping, a LOD score was determined. For nonparametric interval mapping, the statistic following a χ^2 distribution under the null hypothesis of no linkage was converted to the LOD scale. Although the resulting statistic was not a true LOD score, i.e., a $\log_{10}$ likelihood ratio, that value was considered as close to that from standard interval mapping. Depending on the strain and resistance trait, a 1 % LOD score threshold was performed by a permutation test (1,000 permutations) via the n.perm argument to scanone function to test the significance of each putative genetic factor. The interval estimate of genetic factors location was calculated by lodint function, which computes the position interval corresponding to LOD values higher than LOD-max-1; the expandtomarkers argument allows definition of the nearest flanking markers of the interval's higher and lower limits.

For PSS4 trials, in addition to nonparametric interval mapping (np), QTL analysis was carried out using PLABQTL software (Utz and Melchinger 1996), which performs single interval mapping (SIM) using multiple regression of phenotypic data on marker genotypic data as described by Haley and Knott (1992), and composite interval mapping (CIM) using a set of markers as cofactors for the background control (Jiang and Zeng 1995; Zeng 1994). The multiple regression approach used by PLABQTL has the advantage of robustness when the distribution of the residuals is not Gaussian. Both permutation tests (Doerge and Churchill 1996) and Bonferonni χ^2 approximation (Zeng 1994) indicated that the empirical average LOD score threshold across different traits, was equal to 4.0. Hence, each putative QTL displaying a LOD $\geq$4.0 is considered significant. QTL position was estimated at the point where the LOD score reached its maximum in the region under consideration. A one-LOD graph was constructed for each QTL as described by Lander and Botstein (1989). The proportion of phenotypic variance explained by a given QTL was determined by the square of the partial correlation coefficient (R^2). Estimate of the additive effect of each QTL was obtained including all putative QTL detected for the respective trait. The proportion of phenotypic variance explained by all QTL was determined by the adjusted coefficient of determination (R^2_{adj}) obtained by fitting the model of multiple regression on the additive effects of all putative QTLs.

Analysis of the association of markers with bacterial wilt resistance in segregating RILs population

For CMR134, PSS366 and GMI1000, the effect of genotype at markers linked to QTL of resistance was analyzed using a generalized linear model (glm) with a binomial distribution and a logistic (logit) link function considering the resistance indices *W*, CI, and SCO_{AUDPC}, using combined data from experiments with the three strains. The resistance index SCO_{AUDPC} was transformed into a binomial variable by noting "0" when SCO_{AUDPC} was equal to zero and "1" when SCO_{AUDPC} was higher than zero. For PSS4, the effect of genotype at markers linked to QTL of resistance also was analyzed using glm with binomial distribution and a *logit* link function for resistance indices *W* and CI, although SCO_{AUDPC} was analyzed using a classical linear model (*lm*) with normal distribution. A χ^2 test was performed to assess the significance of differences between the two parental genotypic classes at the most closely linked marker of QTL. For flanking markers of QTL, mean of the different parental and recombined genotypic classes was compared using Tukey's test.

Results

Disease traits analysis

The susceptible parent MM738 had a mean percentage of wilted plants (*W*) ranging from 85 to 96 % and a mean

colonization index (CI) ranging from 85 to 98 %, depending on the strain inoculated (Table 2). On the other hand, the parent AG91-25 was scored as highly resistant to strains CMR134, PSS366 and GMI1000, with almost no plants showing symptoms and very few plants colonized by the bacteria. All the generations (F_1, F_2, BC_1s) derived from the MM738 × AG91-25 cross were also found to be almost totally resistant to these three strains, with no or very few plants showing symptoms or asymptomatic colonization when inoculated by these strains. The RILs population was highly resistant with a mean *W* value close to 5 % and a mean CI value varying from 6 to 10 %, depending on the strain. On the other hand, the resistance of AG91-25 was overcome by the aggressive strain PSS4 after 5 weeks, with mean *W* values of 50 and 81 % and mean CI values of 64 and 84 % observed in seasons 1 (cool) and 2 (hot), respectively. Although the same inoculum concentration was used for both seasons, the

Table 2 Estimates of mean of parents and MM738 × AG91-25 progenies, variance components and heritabilities for the maximum wilting percentage (*W*) and colonization index (CI) and AUDPC of the score (SCO_{AUDPC}) used to study resistance against four phylotype I strains (CMR134, PSS366, GMI1000 and PSS4) of *R. solanacearum*

	Mean[a]							Variances[b]				
	MM738	AG91-25	F_1	F_2	BC_1P1	BC_1P2	F_6	$\hat{\sigma}^2_F$	$\hat{\sigma}^2_{F\times S}$	$\hat{\sigma}^2_e$	$\hat{h}^{2c}$	$Ci^d_{P=90\ \%}$
CMR134												
W (%)	86.5 (9.2)	0.2 (0.2)	0.0 (0.0)	0.5 (0.5)	2.6 (0.5)	0.0 (0.0)	4.2 (0.9)	–	–	–	–	–
CI (%)	90.6 (7.6)	1.2 (0.8)	2.5 (2.5)	2.5 (0.5)	3.6 (0.5)	1.0 (1.0)	6.0 (1.0)	–	–	–	–	–
SCO_{AUDPC}	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	–	–	–	–	–
PSS366												
W (%)	90.4 (4.4)	0.0 (0.0)	0.0 (0.0)	2.0 (1.0)	1.0 (1.0)	3.6 (3.6)	5.3 (1.1)	–	–	–	–	–
CI (%)	93.1 (3.5)	5.5 (3.0)	0.0 (0.0)	6.0 (2.0)	3.5 (1.5)	6.7 (3.6)	10.3 (1.3)	–	–	–	–	–
SCO_{AUDPC}	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	–	–	–	–	–
GMI1000												
W (%)	84.6 (6.2)	0.0 (0.0)	5.0 (0.0)	0.5 (0.5)	0.6 (0.6)	1.0 (1.0)	4.3 (0.8)	–	–	–	–	–
CI (%)	86.5 (5.1)	0.2 (0.2)	7.5 (2.5)	4.0 (1.0)	2.2 (1.1)	3.1 (2.1)	8.3 (1.0)	–	–	–	–	–
SCO_{AUDPC}	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	–	–	–	–	–
Grouping[e]												
W (%)	87.2 (3.8)	0.1 (0.1)	1.7 (1.1)	1.0 (0.4)	1.4 (0.5)	1.5 (1.2)	4.6 (0.5)	–	–	–	–	–
CI (%)	90.1 (3.2)	2.3 (1.1)	3.3 (1.7)	4.2 (0.9)	3.1 (0.6)	3.6 (1.5)	8.2 (0.6)	–	–	–	–	–
SCO_{AUDPC}	2.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	–	–	–	–	–
PSS4												
S1												
W (%)	94.6 (2.1)	49.6 (6.8)	50.0 (15.0)	61.3 (2.5)	75.4 (0.4)	57.0 (0.0)	80.7 (1.1)	92.6***	–	291.0	0.39	–
CI (%)	98.3 (1.3)	64.3 (5.8)	65.0 (30.0)	72.8 (1.3)	84.9 (2.1)	77.5 (6.5)	87.9 (0.9)	NS	–	224.7	–	–
SCO_{AUDPC}	2.6 (0.1)	0.7 (0.2)	1.1 (0.2)	1.4 (0.1)	1.8 (0.0)	1.2 (0.0)	2.0 (0.0)	0.14***	–	0.25	0.53	
S2												
W (%)	96.0 (2.4)	81.2 (11.6)	94.7 (0.0)	88.2 (7.8)	97.4 (0.6)	91.4 (3.6)	90.1 (0.9)	NS	–	214.4	–	–
CI (%)	97.2 (2.0)	84.4 (9.2)	94.7 (0.0)	90.3 (5.7)	97.4 (0.6)	97.0 (2.0)	92.4 (0.8)	NS	–	185.5	–	–
SCO^f_{AUDPC}	3.0 (0.2)	2.1 (0.5)	2.8 (0.2)	2.5 (0.4)	2.9 (0.2)	2.4 (0.2)	2.6 (0.0)	0.04*	–	0.23	0.28	
Comb.												
W (%)	95.3 (1.6)	65.4 (6.8)	72.4 (14.3)	74.7 (8.5)	86.4 (6.4)	74.2 (10.1)	85.4 (0.7)	NS	NS	323.9	–	–
CI (%)	97.7 (1.0)	74.3 (8.0)	79.9 (15.0)	81.5 (5.6)	91.2 (3.7)	86.7 (6.0)	90.1 (0.6)	NS	NS	244.4	–	–
SCO_{AUDPC}	2.8 (0.2)	1.4 (0.5)	2.0 (0.5)	1.9 (0.4)	2.3 (0.3)	1.8 (0.4)	2.3 (0.0)	0.07*	NS	0.41	0.50	0.37–0.59

NS non-significant, *S1* season 1, *S2* season 2, *Comb.* combined season

Significant at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, respectively

[a] Standard error is given in parenthesis

[b] $\hat{\sigma}^2_F$, $\hat{\sigma}^2_e$, and $\hat{\sigma}^2_{F\times S}$ are the respective estimates of the variances between families, of families × season interaction, and residual, respectively

[c] $\hat{h}^2$, broad-sense heritability

[d] $Ci_{P=90\ \%}$, 90 % confidence interval of $\hat{h}^2$

[e] Group combining data from strains CMR134, PSS366 and GMI1000

[f] Analysis of variance using mix procedure for the variable SCO_{AUDPC} at season 2

inoculation pressure obtained with this strain was slightly higher in the second test (hot season) than in the first one (cool season). The generations derived from the MM738 × AG91-25 cross, were all highly susceptible to PSS4, especially in season 2, with mean *W* values higher than 88 %, and mean CI values higher than 90 %.

The distributions of RILs frequencies were compared between strains, using the Tukey's all pair comparison and the χ^2 tests. These tests indicated that the segregation of RILs according to the *W* or CI values was not affected by strain, greenhouse, and strain–greenhouse interaction factors (data not shown). Thus, the experimental data with strains CMR134, PSS366 and GMI1000 were combined in a single dataset, considering each strain as a replication.

The data obtained from the experiments with strains that were controlled by AG91-25 resistance (CMR134, PSS366, and GMI1000) resulted in non-normal distributions of phenotypes (Fig. 1). Thus, nonparametric analysis was preferred to the classical QTLs analysis based on the analysis of variance. The analysis of variance of data from the experiments with strain PSS4, using the GLM procedure, revealed that the genotypic variance component was not significant for *W* and CI, except for *W* in season 1. Among the traits showing a significant genotypic variance, the highest value of heritability was found for the AUDPC calculated on score data (SCO_{AUDPC}) in season 1 as well as across the two seasons. This trait was found to be significantly correlated with *W* and CI for season 1 and across seasons. In season 2, the genotypic variance was non-significant for all traits analyzed with GLM procedure. A significant genotypic variance was found only for the SCO_{AUDPC} when analyzed with MIX procedure. In the analysis across the two seasons using the GLM and MIX procedures, a highly significant ($P < 0.001$) effect of the season was found for SCO_{AUDPC}, although the genotype × environment interaction ($\hat{\sigma}^2_{F\times S}$) was not significant. Low values of heritability for this latter trait were in agreement with the hypothesis that the most important part of phenotypic variance was due to environment effects.

Segregation analysis and genetic linkage map

A total of 1,323 primer combinations of different molecular markers were screened on the parental lines, with a very low level of polymorphism found for all types of markers, except AFLP which yielded zero to eight informative bands per primer combination. In this way, 179 polymorphic markers were obtained from 64 *EcoRI*/*Mse*I AFLP primer combinations, and 163 of these markers could be genotyped on the RILs population of 178 individuals. From the 459 eggplant SSRs screened, 32 (7.0 %) were polymorphic and could be genotyped, while only two out of 376 (0.5 %) screened tomato SSRs were polymorphic. From the 358 SRAP, RGA, and SRAP/RGA combinations screened, only two polymorphic markers (0.5 %) could be genotyped (Supplementary Table 2). From the 66 RAPD primers screened, only one marker (1.5 %) showed clear polymorphism and could be genotyped. In this way, we identified a total of 200 polymorphic molecular markers. In addition to these molecular markers, one morphologic marker (presence/absence of hairs on plant stalks) was also used. A genetic map was built (Fig. 2) that consisted of 119 markers positioned on 18 linkage groups (LGs). All loci were significantly linked (LOD threshold $\geq$3) to one of these 18 LGs with a maximum distance of 25.5 cM, corresponding to a stringent recombination frequency (*r*) of 0.2, before applying the Haldane mapping function. Markers not included on the map were extremely distorted, not linked to any linkage group or tightly clustered with other markers. The map spanned a total of 884 cM with an average distance of 8.8 cM between markers. Twenty out of the 119 mapped loci (16.8 %) showed highly significant ($P < 0.001$) distortion from the expected Mendelian 1:1 segregation ratio for parental alleles. The proportion of susceptible parent MM738 genome among the 178 F_6 individuals ranged from 15.7 to 70.5 %, with a mean of 45.1 ± 9.5 %, which did not differ significantly from the proportion expected under Mendelian segregation, 50 %.

QTL analysis

QTL analysis was performed on SCO_{AUDPC} traits using genotypic data from the 119 marker loci and the phenotypic data obtained on 176 RILs (phenotypic data not available for two RILs of a total of 178 tested) from the single experiments with the four *R. solanacearum* strains (phylotype I) and from the mean of the combined (CMR134-PSS366-GMI1000) strains data (Table 3). For CMR134, PSS366, GMI1000, and the combined data of these strains, SCO_{AUDPC} was found to be significantly correlated with *W* and CI (data not shown). QTL analysis was also performed with quantitative data from experiments against strain PSS4, using BLUP values obtained from the disease score traits as selected in individual trials and across seasons (Table 4). For strains CMR134, PSS366, GMI1000, and the combined data, both nonparametric and binary methods resulted in the detection of a major locus on LG2 in the interval between AFLP markers CRO432b and CSI447b. In addition, a minor QTL was detected on linkage group 3. The LOD-peak of the major QTL, named *ERs1*, was detected at the same position for the three strains, close to marker COX067a (Figs. 2, 3). No QTL was detected at this locus for strain PSS4, suggesting

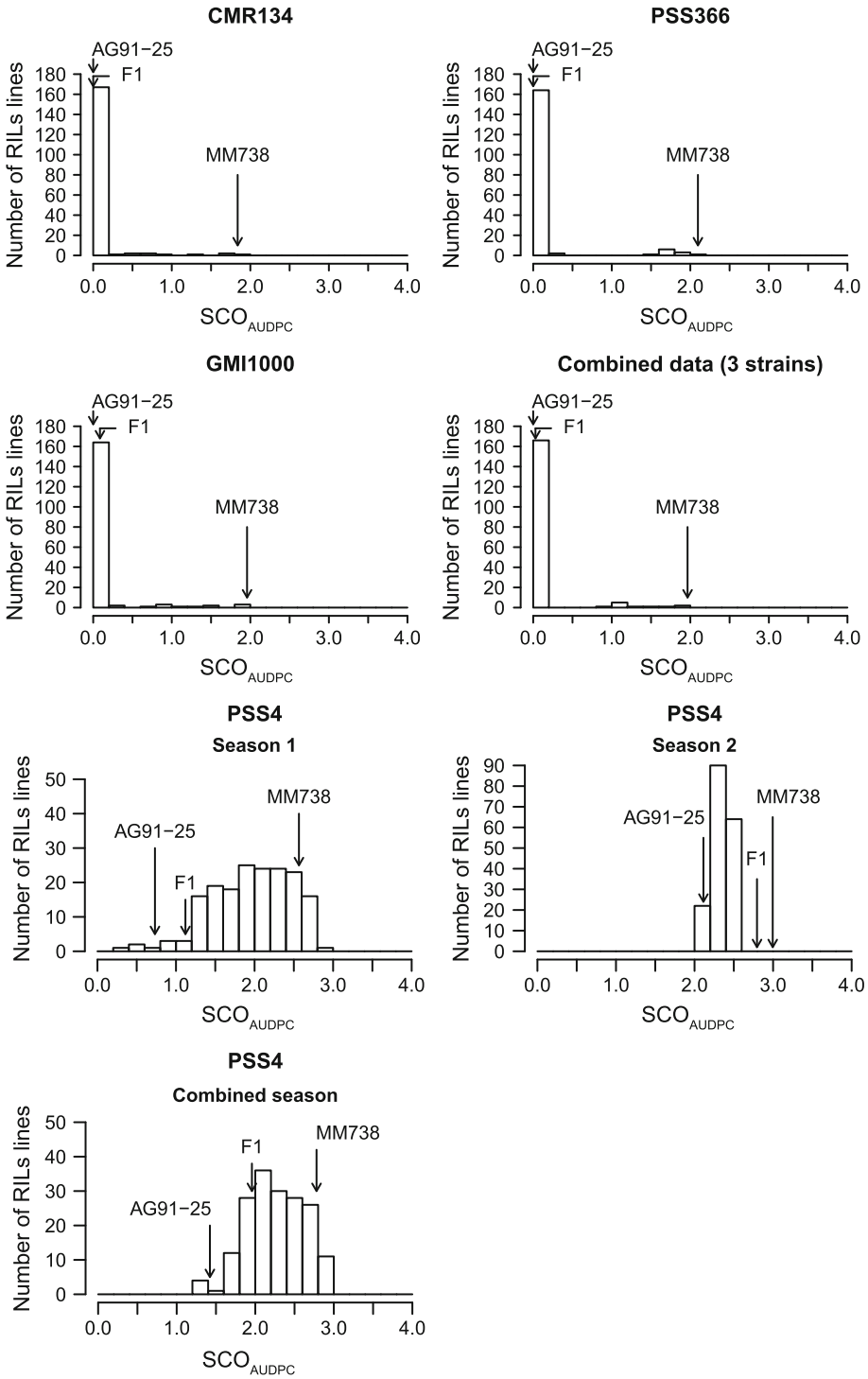

Fig. 1 Frequency distribution of RIL F_6 population for AUDPC calculated with the mean wilting symptoms rating (disease score) SCO_{AUDPC}, when tested against phylotype I strains CMR134, GMI1000, PSS366, PSS4 (season 1, 2, and combined season 1&2), and the combined data for the three strains CMR134, PSS366, and GMI1000. The *arrows* indicate the mean of the parents (MM738 and AG91-25) and of their F_1

that *ERs1* is completely overcome by this aggressive strain (Tables 3, 4). Nevertheless, one to three minor QTLs, depending on the method of QTLs analysis, were found to be associated with partial resistance observed at an intermediate stage of disease progress with PSS4. These QTLs, which were located on LG2 and LG13, explained 19–36 % of the phenotypic variation for the variable disease score.

Association of markers with bacterial wilt resistance in segregating RILs population

The AFLP marker COX067a found on LG2, closely linked to the resistance locus *ERs1*, was extremely distorted in favor of resistant parent AG91-25. At COX067a, the genotype of susceptible parent MM738 was observed only in 13 RILs, representing 7.4 % of total lines, whereas the

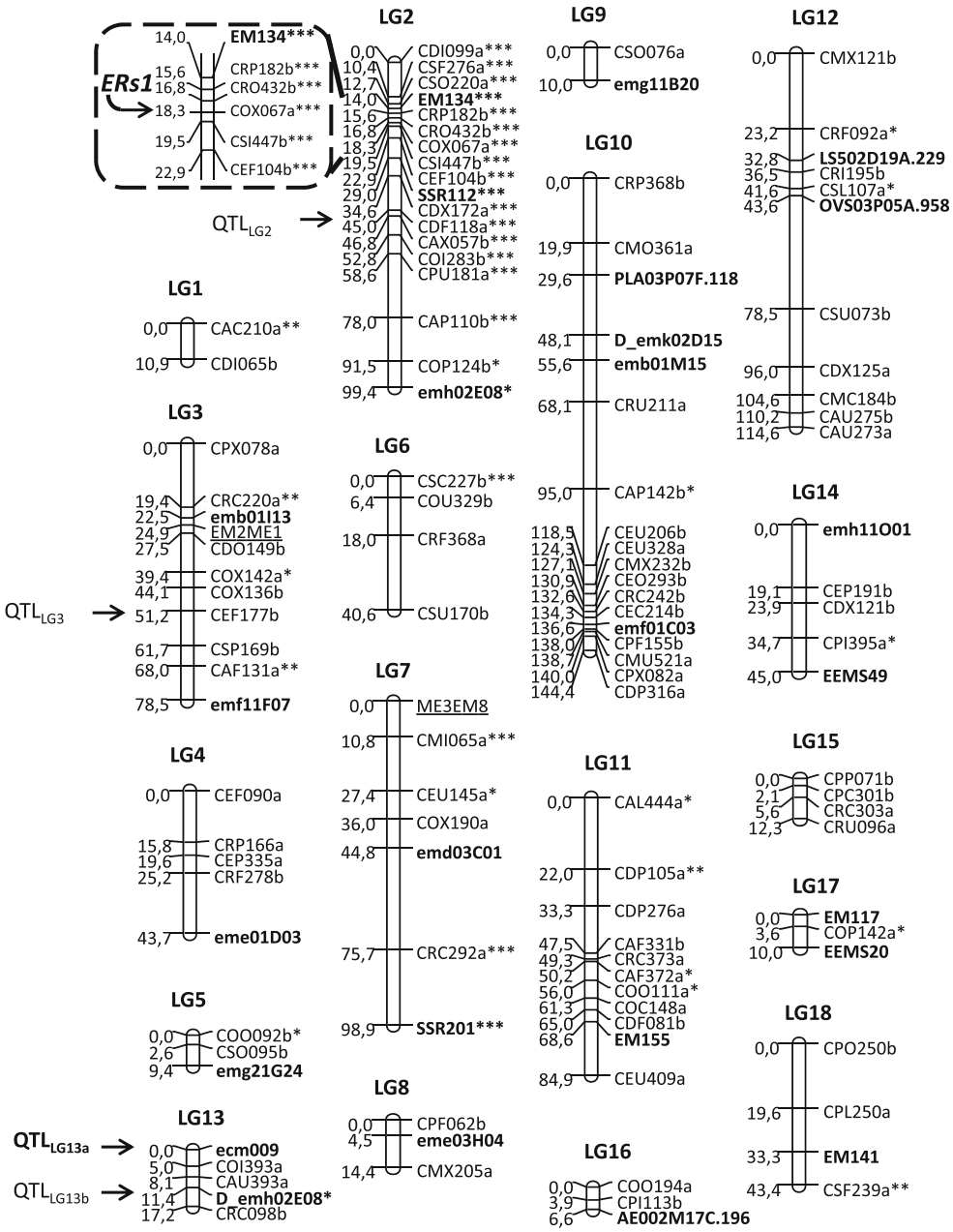

Fig. 2 Linkage map for 119 markers AFLP, SSR and SRAP, based on 178 F_6 families derived from the cross MM738 × AG91-25 and position of quantitative trait loci (QTL) for resistance to *R. solanacearum* phylotype I strains CMR134, PSS366, GMI1000 and PSS4. The 26 markers indicated in *bold* are SSRs. The two *underlined* markers are SRAPs. The *inset* corresponds to an enlarged fragment of the LG2 with *ERs1* positioned at the level of the closest marker. The *asterisk* indicates the degree of distortion of each marker from Mendelian segregation ratios significant at $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

genotype of resistant parent AG91-25 was observed in 157 RILs, six RILs having missing data at this marker. The difference between the two parental genotypic classes at COX067a for *W*, CI and SCO_{AUDPC} mean was highly significant (Table 5), showing the strong association of this locus with resistance. Among lines carrying the susceptible parent genotype at this marker, only two could be considered as resistant, the other ones being susceptible (Table 6). Among the 11 susceptible RILs, eight were of parental susceptible genotype and three of recombined genotype between CRO432b and COX067a, or between COX067a and CSI447b. It is noteworthy that the two resistant RILs with parental susceptible genotype at marker COX067a presented recombinant genotypes at both intervals. All the RILs carrying AG91-25 allele at this marker could be considered as resistant, with 50 lines presenting no wilting as well as no colonization for any strain, 85 lines presenting no wilting and CI on 3.3–10.7 % of plants, and 22 lines presenting wilting on 3.3–6.7 % of plants and CI on 3.3–20.0 % of plants (data not shown). When we also included the two flanking markers CRO432b and CSI447b within the confidence interval carrying *ERs1*, all these RILs were of parental genotype at the three markers except two RILs that were recombinant.

At the SSR marker ecm009 found on LG13 closely linked to the QTL specifically detected against PSS4 strain,

Table 3 Genetic factors detected for resistance to *R. solanacearum* strains (phylotype I) CMR134, PSS366, GMI1000 and PSS4 based on nonparametric and binary methods in the RILs F_6 population derived from the cross between MM738 and AG91-25

Strain	Trait	LG	Position (cM)[a]	Closest marker	Marker interval	Nonparametric		Binary	
						$LOD^{b}_{threshold}$	LOD[c]	$LOD_{threshold}$	LOD[d]
CMR134	SCO_{AUDPC}	2	18 (17–20)	COX067a	CRO432b-CSI447b	3.3	22.8	3.7	12.1
		3	51 (49–54)	CEF177b	COX136b-CSP169b		7.1		4.3
PSS366	SCO_{AUDPC}	2	18 (17–19)	COX067a	CRO432b-CSI447b	3.7	26.7	3.7	14.1
		3	51 (49–54)	CEF177b	COX136b-CSP169b		7.7		4.7
GMI1000	SCO_{AUDPC}	2	18 (17–20)	COX067a	CRO432b-CSI447b	3.6	21.1	3.6	12.0
		3	51 (48–55)	CEF177b	COX136b-CSP169b		4.5		–
Grouping[e]	SCO_{AUDPC}	2	18 (17–20)	COX067a	CRO432b-CSI447b	3.5	15.0	3.4	9.2
		3	51 (49–55)	CEF177b	COX136b-CSP169b		4.4		–
PSS4									
S1	SCO_{AUDPC}	13	0 (0–3)	ecm009	ecm009-COI393a	3.5	12.8	–	–
		2	47 (46–48)	CAX057b	CDF118a-COI283b		8.8		–
S2	SCO^{f}_{AUDPC}	13	0 (0–3)	ecm009	ecm009-COI393a	3.3	11.2	–	–
		2	47 (46–48)	CAX057b	CDF118a-COI283b		8.4		–
Comb.	SCO_{AUDPC}	13	0 (0–3)	ecm009	ecm009-COI393a	3.3	12.2	–	–
		2	47 (46–48)	CAX057b	CDF118a-COI283b		8.3		–

LG linkage group, *S1* season 1, *S2* season 2, *Comb.* combined season

[a] Position of the QTL on the LG and confidence interval given in parenthesis

[b] $LOD_{threshold}$, is calculated by a permutation test (1,000 permutations) for each analysis method, for each trial and combination of trials

[c] LOD, value resulting from nonparametric statistic by nonparametric model

[d] LOD, maximum value of the log-likelihood I the marker interval for binary model

[e] Combined data from strains CMR134, PSS366 and GMI1000

[f] QTL analysis on BLUPs values for the variable SCO_{AUDPC} at season 2

the differences between the two parental genotypic classes for *W*, CI, and SCO_{AUDPC} were significant, although it was very slim (Table 5). When considering the two flanking markers ecm009 and COI393a, the two parental genotype classes also were found significantly different, although recombined genotype classes were not different from parental ones.

Discussion

Accurate phenotypic evaluation is a prerequisite for QTL mapping. Under natural conditions, *R. solanacearum* is not uniformly spread across a field or a greenhouse. Therefore, artificial inoculation is essential for obtaining a reliable plant response. Moreover, this is the only way to test resistance against identified strains and to investigate the specific versus generalist response of the detected genes/QTL. Inoculation of root-wounded individual plants 10 days after transplanting using the drip irrigation system was shown to be effective and reliable. Nevertheless, some heterogeneity of plant growth observed in different RILs when inoculated may increase the microenvironment component of variance and, therefore, reduce the precision of resistance tests.

Solanum melongena AG91-25 was previously identified as one of the resistant accessions exhibiting the most variable response to different strains representing phylotype I (Lebeau et al. 2011). Phylotype I is the most geographically widespread clade and the most important one in terms of global economic impact as well as genetic and phenotypic diversity (Hayward 1991, 1994). Gene/QTL mapping was therefore carried out using a panel of bacterial strains representing different levels of virulence within this phylotype to inoculate a RILs-F_6 population derived from the intraspecific cross MM738 × AG91-25. In this way, it was possible to evaluate the effectiveness and stability of the resistance factors involved, and to assess whether an aggressive strain may break these factors. A major advantage of using a population of nearly homozygous RILs is that this population also could be used for repeated tests against different strains representing the other three main phylotypes, in different environments. Consequently, it will be possible to further investigate the specificity of this resistance versus *R. solanacearum* genetic diversity, and to estimate resistance durability throughout large

Table 4 QTLs detected for resistance to *R. solanacearum* strain PSS4 (phylotype I) based on CIM and SIM analyses in the RILs F_6 population derived from the cross between MM738 and AG91-25

Strain	Trait	LG	QTL		CIM			SIM		
			Position (cM)[a]	Marker interval	LOD[b]	R^{2c}	a^d	LOD	R^2	a
PSS4										
Season 1	SCO_{AUDPC}	13	0 (0–2)	ecm009-COI393a	16.2	37.5	−0.29**[g]	15.2	35.6	−0.25**
		13	12 (8–16)	D_emh02E08-CR098b	–	–	–	10.2	23.3	NS
		2	48 (46–50)	CAX057b-COI283b	–	–	–	8.2	19.3	NS
	Total R^{2e}					31.5			32.2	
Season 2	SCO^f_{AUDPC}	13	0 (0–2)	ecm009-COI393a	5.1	13.8	−0.06**	14.2	33.7	−0.04**
		13	12 (10–16)	D_emh02E08-CR098b	–	–	–	10.2	23.5	NS
		2	48 (46–50)	CAX057b-COI283b	–	–	–	8.6	20.2	NS
	Total R^2					28.1			29.7	
Combined season	SCO_{AUDPC}	13	0 (0–2)	ecm009-COI393a	16.3	37.6	−0.21**	15.5	36.2	−0.16**
		13	14 (8–16)	D_emh02E08-CR098b	–	–	–	10.6	24.3	NS
		2	48 (46–50)	CAX057b-COI283b	–	–	–	8.5	20.0	NS
	Total R^2					30.2			31.6	

LG linkage group

[a] Position of the QTL on the LG and confidence interval given in parenthesis

[b] LOD, maximum value of the log-likelihood I the marker interval (values are superior to the LOD-thresholds: 4 for SIM and CIM)

[c] R^2, partial coefficient of determination, i.e., percentage of phenotypic variation explained by the QTL calculated by PLABQTL on averaged data, adjusted for cofactors in case of CIM

[d] a, additive estimates. The sign of a indicates the origin of the allele contributing to the resistance: here all of these alleles come from the resistant parent (AG91-25)

[e] Total R^2, total adjusted coefficient of determination, i.e., the percentage of phenotypic variance explained by all QTL, calculated by PLABQTL on averaged data, fitting the model of multiple regression on the additive effects of all putative QTL affecting the respective trait

[f] QTL analysis on BLUPs values for the variable SCO_{AUDPC} at season 2

[g] Significance at *0.05 and **0.01 probability level; *NS* not significant

cropping areas. In spite of their contrasting response to BW infection, the two parents displayed a very low level of polymorphism for DNA markers. A total of 179 AFLP polymorphic bands were detected from 64 primer combinations, but only 32 polymorphic markers were obtained from the survey of 459 eggplant SSR, and very few polymorphisms were revealed by RAPD, SRAP, RGA, and SRAP/RGA techniques. These results confirm the low frequency of DNA polymorphism in the eggplant genome observed in other parentage (Nunome et al. 2001). In the end, only 91 AFLP, 26 SSR and 2 SRAP markers were genotyped to construct a genetic linkage map of *S. melongena*. The observed number of LGs does not correspond to the expected number of 12 LGs for a comprehensive linkage map of eggplant ($2n = 24$). In our study, the high rate of distorted markers may have resulted in an overestimation of the recombination frequency between these markers, and thus, may have contributed to the formation of several small LGs, as described by Lyttle (1991). These small LGs may also be due to incomplete coverage of the genome. Most of the distorted markers were mapped on LG2, suggesting the presence of a specific region of the genome where structural differences or loci may affect recombination. This region could correspond to a part of the *S. aethiopicum* Aculeatum group genome introgressed into AG91-25. Despite a relatively low number of markers, the genetic length of the present map is comparable to the estimate provided by Nunome et al. (2001) for an intraspecific F_2 population.

We demonstrated that AG91-25 resistance to *R. solanacearum* phylotype I strains is conferred by a major dominant gene, named *ERs1*. Genetic factor analysis with both nonparametric and binary methods indicated the presence of a locus with at least one major dominant gene positioned on LG2, in a region with markers highly skewed in favor of the resistant parent AG91-25. This locus was accurately positioned in a confidence interval of 2.5 cM and flanked by two closely linked markers at a distance of less than 2 cM. *ERs1* localized at the 18 cM position of LG2 and was associated with the marker COX067a. It is assumed that this locus will be very efficient for controlling BW resistance, strongly reducing both colonization and

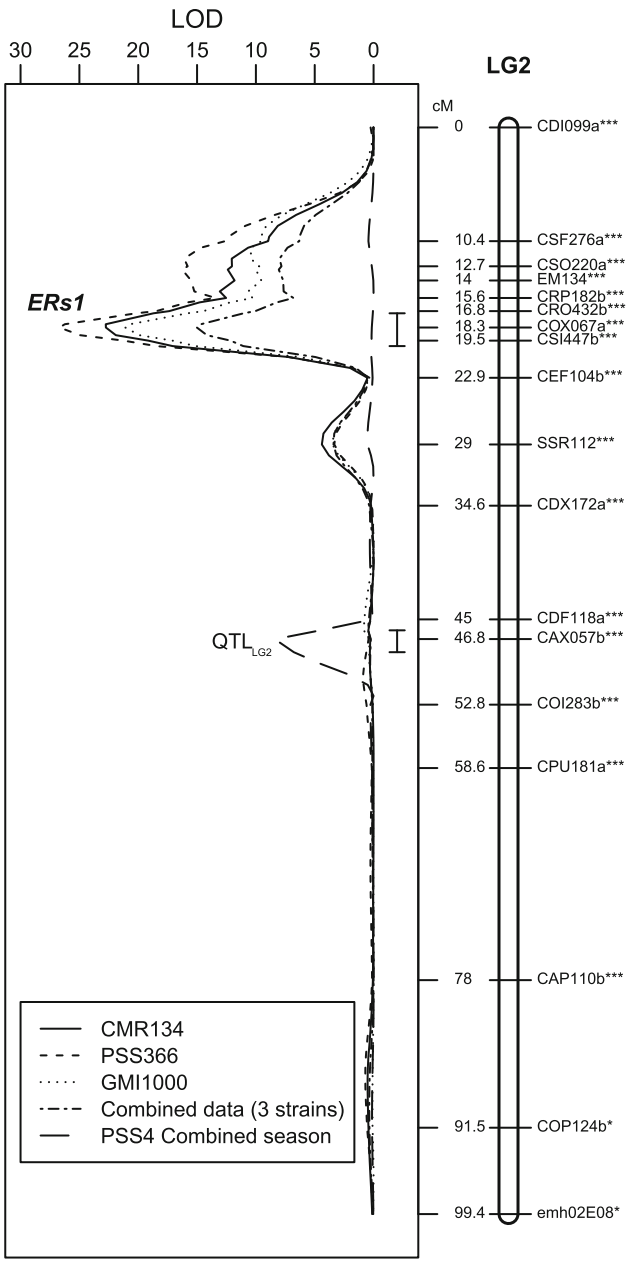

Fig. 3 LOD plot for the SCO_{AUDPC} QTL detection on LG2 for resistance to *R. solanacearum* strains CMR134, PSS366, GMI1000 and PSS4 based on nonparametric method in the RILs F_6 population derived from the cross between MM738 and AG91-25. The *lines* drawn to the side of the ERs1 and QTL_{LG2} position represent the confidence interval. The *asterisk* indicates the degree of distortion of each marker from Mendelian segregation ratios at the *0.05, **0.01, and ***0.001 probability levels

wilting of plants when inoculated by strains displaying a virulence profile similar to CMR134, PSS366 and GMI1000. Since all the RILs carrying the AG91-25 allele at COX067a were resistant while the ones carrying the MM738 parent allele were susceptible, except two with recombined genotypes at flanking markers, the presence of *ERs1* may be considered as a necessary condition for controlling CMR134, PSS366 and GMI1000 strains. The putative minor QTL positioned in LG3 did not modify the action of this major gene and, consequently, might be disregarded in breeding. Nevertheless, the detection of only one major gene is not consistent with the results obtained from Mendelian analyses on F_2 and BC_1s that suggest rather an oligogenic than a monogenic inheritance of resistance, the number of major QTL depending on both the trait and the strain (Supplementary Table 1). Discrepancies between Mendelian and QTL analyses may be caused by a strong segregation distortion of alleles in favor of the resistant parent in the region of the major gene. This distortion may be due to the introgression of a large portion of wild *S. aethiopicum* genome in this region of AG91-25, resulting in a proportion of resistant plants much higher than expected for one major dominant gene hypothesis. Moreover, we cannot disprove the hypothesis that other major genes or QTLs exist. It is possible that such loci were not detected because of incomplete map coverage and/or lack of precision in QTL detection. Actually, the appearance of the LOD curve in *ERs1* region could suggest the possible presence at this locus of two QTLs that are not clearly distinguishable by our analysis.

We also demonstrated that this major resistance gene was strain-specific, and was overcome by strain PSS4. The only reliable QTL for this strain was detected on LG13 (QTL_{LG13a}) and was associated with the capacity to delay disease progress. Thus, AG91-25 resistance failed to stop infection by PSS4 in our experiments because this aggressive strain broke down the resistance contributed by the major gene detected on LG2. Although the difference between allelic classes at the ecm009 marker for *W*, CI and SCO_{AUDPC} values was low, it was statistically significant and consistent with the detection of resistance QTL located close to ecm009. We also observed lines appearing more resistant than the resistant parent AG91-25, although we cannot prove if these are transgressive or just artifacts (Fig. 1).

The manifestation of eggplant's resistance to BW was reported to be similar to the one described in tomato, i.e., limitation of bacteria spread within the xylem vessels, such that the more resistant a plant is, the less colonized its stem tissues will be (Grimault et al. 1994; Grimault and Prior 1994). Vasse et al. (2005) observed that resistance was initiated at a very early stage, activating mechanisms that prevent the multiplication and spread of the bacteria in association with accumulation of phenolic compounds in the roots. The fact that only 23–49 % of the colonized RILs developed wilting symptoms (Supplementary Table 1), suggests the existence of a second resistance mechanism acting at a later stage and dependent on the first mechanism. This hypothesis is consistent with the observed pleiotropic effects of *ERs1* on traits CI and *W*.

Table 5 Mean and standard deviation values of *R. solanacearum* (phylotype I) strains CMR134, PSS366, GMI1000 and PSS4 resistance indices (*W*, CI and SCO_{AUDPC}) in RILs F_6 population derived from the cross between MM738 and AG91-25 of each marker genotype

LG	Marker	Genotype[a]	RILs number	*W* (%)[b]	CI (%)	SCO_{AUDPC}
2	COX067a	A	13	56.3 (3.8)a[c]	63.3 (4.2)a	1.1 (0.1)a
		B	157	0.5 (0.1)b	3.9 (0.3)b	0.0 (0.0)b
13	ecm009	A	76	91.0 (0.8)a	93.9 (0.7)a	2.3 (0.0)a
		B	83	80.0 (1.2)b	86.6 (1.0)b	1.9 (0.0)b
	Flanking markers	AA	70	90.7 (0.9)a	93.8 (0.7)a	2.3 (0.0)a
	ecm009-COI393a	AB	6	95.0 (1.8)a	95.0 (1.8)a	2.5 (0.1)a
		BA	7	78.2 (4.3)b	84.6 (3.3)b	1.9 (0.1)b
		BB	69	80.2 (1.3)b	86.8 (1.0)b	1.9 (0.0)b

LG linkage group

[a] For the COX067a and ecm009 markers, respectively, the closest marker of *ERs1* and QTL_{LG2}, the parental genotypes were MM738 (A) and AG91-25 (B); for the flanking markers (ecm009-COI393a) of QTL_{LG2}, the parental genotypes were MM738 (AA) and AG91-25 (BB), and the recombined genotypes were AB and BA

[b] The mean and standard deviation of the wilting percentage (*W*), the colonization index (CI), and the AUDPC calculated with the disease score (SCO_{AUDPC}) were calculated from combined three strains (CMR134-PSS366-GMI1000) data for *ERs1* on LG2, and from combined season PSS4 data for QTL_{LG2} on LG13. For all variables, the statistical analysis was performed using GLM model with binomial distribution and logit-link function, except for SCO_{AUDPC} in the case of PSS4 where it was performed using a linear model with normal distribution

[c] For the COX067a and ecm009 markers, the χ^2 test indicated that parental genotypic classes A and B were significantly different ($P < 0.001$); for the flanking markers (ecm009-COI393a) of QTL_{LG2}, the Tukey's all-pair comparisons indicated the difference between the genotypic classes AA, AB, BA and BB

Table 6 Mean and standard deviation values of resistance indices (*W*, CI and SCO_{AUDPC}) for *R. solanacearum* phylotype I strains CMR134, PSS366, GMI1000 (combined data) in 13 RILs F_6 derived from the cross between MM738 and AG91-25 carrying the MM738 allelic form (A) at marker COX067a closely linked to *ERs1*

RILs	CRO432b[a]	COX067a	CSI447b	*W* %[b]	CI %	SCO_{AUDPC}
G074	A	A	A	63.3 (9.5)	73.3 (9.9)	1.1 (0.2)
G110	A	A	A	50.0 (13.4)	63.3 (12.0)	1.0 (0.2)
G136	A	A	A	72.5 (8.3)	80.0 (10.3)	1.6 (0.2)
G140	A	A	A	60.8 (14.2)	74.2 (16.4)	1.2 (0.2)
G145	A	A	B	83.3 (6.1)	90.0 (6.8)	1.8 (0.2)
G148	B	A	B	6.7 (4.2)	6.7 (4.2)	0.1 (0.1)
G170	B	A	B	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
G224	A	A	A	83.3 (6.1)	93.3 (4.2)	1.8 (0.2)
G239	A	A	B	56.7 (10.9)	63.3 (12.0)	1.0 (0.2)
G281	A	A	A	49.2 (14.0)	53.3 (16.1)	1.1 (0.2)
G293	A	A	A	61.7 (9.8)	68.3 (11.7)	1.2 (0.2)
G341	A	A	A	54.2 (11.9)	60.8 (11.0)	1.1 (0.2)
G360	B	A	A	90.0 (4.5)	96.7 (3.3)	1.7 (0.2)

[a] For the COX067a marker, the closest marker of *ERs1*, and flanking markers, the parental genotypes were MM738 (A) and AG91-25 (B)

[b] The mean and standard deviation of the wilting percentage (*W*), the colonization index (CI), and the AUDPC calculated with the disease score (SCO_{AUDPC}) were calculated from combined three strains (CMR134-PSS366-GMI1000) data

The identification of this strain-specific major BW-resistance gene constitutes a major finding and an important step toward untangling the complex genetics of *R. solanacearum*–plant interactions. Research efforts are now needed to investigate the stability and specificity of this gene toward other phylotypes of *R. solanacearum*. More information on this question is crucial for practical control of the disease in production areas where bacterial populations can be mixed. The strain-specificity of the major BW resistance gene from eggplant AG91-25 led us to hypothesize that our set of strains may carry different virulence/avirulence gene repertoires. The hypothesis of a

gene for gene interaction must be confirmed by testing AG91-25 resistance against genome-sequenced strains belonging to phylotype I and other phylotypes. The recent classification scheme of *Ralstonia solanacearum* diversity into several phylotypes (Fegan and Prior 2005), as well as the characterization of a core collection of tomato, eggplant, and pepper accessions representative of the principal sources of resistance (Lebeau et al. 2011), provides the necessary foundation for further investigation of the stability of resistance when confronted by the genetic diversity of *Ralstonia solanacearum* and for exploration of the molecular bases of resistance and its breakdown by certain virulent strains.

In conclusion, the BW resistance in eggplant accession AG91-25 is conferred by a combination of (1) at least one major dominant gene, named *ERs1*, which was demonstrated to be efficient and stable against three strains of phylotype I, and (2) a disease progress-delaying QTL specifically acting against the virulent strain PSS4 that totally broke down *ERs1*. Our study is the first one that mapped resistance genes in eggplant against well-characterized *R. solanacearum* strains. *ERs1* may be involved in a gene for gene interaction with bacterial effectors, as it was not functional against the virulent strain PSS4 which completely overcame resistance. The action of *ERs1* seems to be minimally influenced by environmental factors (temperature and humidity) and mostly dependent on the inoculum strain. These properties suggest promising prospects for breeders. In a breeding program for commercial F_1 hybrids of eggplant, the allele from the resistance source AG91-25 can be introgressed into breeding material using the molecular markers mapped less than 1 cM from the resistance gene. The conversion of these AFLP markers into breeder-friendly sequence characterized amplified region (SCAR) markers will provide a very convenient tool allowing routine marker-assisted selection for BW resistance in diverse genetic backgrounds. A codominant SCAR marker, if made available, will permit differentiation of homozygous susceptible and heterozygous resistant plants in BC_1 or F_2 populations. This would allow elimination of individuals that do not carry the favorable allele at a very early stage of selection by screening their genomes. Such selection could therefore avoid time-consuming and costly large-scale artificial inoculations, and also compensate for the fact that BW is quarantined at most locations and cannot be inoculated in classical resistance tests. Since one of the linked markers was found to be almost colocalized with the gene, this suggests that virtually the probability of recombination that may occur between the marker and the gene is low. Therefore, just one phenotypic test for resistance will be necessary at the end of the backcrossing process, to ascertain that the resistance allele has indeed been inherited with the marker allele tagging the *ERs1* locus. In addition the linked markers will provide a starting point for exploring the diversity of resistance in eggplant germplasm as well as for exploring the synteny of *R. solanacearum* resistance genes in solanaceous crops. It would also be interesting to map BW resistance genes from other sources of eggplant germplasm and investigate whether they are allelic to *ERs1*. Because of the lack of anchor markers on our intraspecific map, we cannot yet identify potential syntenic QTLs for BW resistance in the tomato or pepper genomes. However, we can use the sequences of the flanking markers CRO432b and COX067a, or derived-SCAR markers as probes for assigning the position of our BW resistance gene to a particular region of the eggplant reference map and to the corresponding colinear genomic regions in tomato and pepper.

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