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ORIGINAL PAPER

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Identification of QTLs for *Ralstonia solanacearum* race 3-phylotype II resistance in tomato

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Abstract Resistance against a *Ralstonia solanacearum* race 3-phylotype II strain JT516 was assessed in a $F_{2:3}$ and a population of inbred lines (RIL), both derived from a cross between *L. esculentum* cv. Hawaii 7996 (partially resistant) and *L. pimpinellifolium* WVa700 (susceptible). Resistance criteria used were the percentage of wilted plants to calculate the AUDPC value, and bacterial colonization scores in roots and stem (hypocotyl and epicotyl) assessed in two independent greenhouse experiments conducted during the cool and hot seasons in Réunion Island, France. Symptoms were more severe during the cool season trials. Heritability estimates in individual seasons ranged from 0.82 to 0.88, depending on resistance criterion. A set of 76 molecular markers was used for quantitative trait loci (QTL) mapping using the single- and composite- interval mapping methods, as well as ANOVA. Four QTLs, named *Bwr-* followed by a number indicating their map location, were identified. They explained from 3.2 to 29.8% of the phenotypic variation, depending on the resistance criterion and the season. A major QTL, *Bwr-6*, and a minor one, *Bwr-3*, were detected in each season for all resistance criteria. Both QTLs showed stronger effects in the hot season than in the cool one. Their role in resistance to *R. solanacearum* race 3-phylotype II was subsequently confirmed in the RIL population derived from the same cross. Two other QTLs, *Bwr-4* and *Bwr-8*, with intermediate and minor effects, respectively, were only detected in the hot season, demonstrating that environmental factors may strongly influence the expression of resistance against the race 3-phylotype II strain JT516. These QTLs were compared with those detected in the RIL population against race 1-phylotype I strain JT519 as well as those detected in other previous studies in the same genetic background against other race 1-phylotype I and II strains. This comparison revealed the possible occurrence of some phylotype-specific resistance QTLs in Hawaii 7996.

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Introduction

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is one of the most prevalent bacterial plant diseases, affecting over 250 different species, including economically important crops such as tomato, potato, peanut and banana (Hayward 1991, 1994; Prior et al. 1998). BW is a soil-borne pathogen that enters the plant either at sites of secondary root emergence or at root tips, and spreads rapidly throughout the vascular system (Kelman and Sequeira 1965; Schmit 1978; Vasse et al. 1995). Bacterial colonization of the stem results in browning of the xylem, foliar epinasty and lethal generalized wilting (Buddenhagen and Kelman 1964). BW is widely distributed in tropical and subtropical humid countries (Buddenhagen 1986).

Traditionally, *R. solanacearum* has been classified into five races and six biovars according to host range and biochemical properties, respectively (Buddenhagen et al. 1962; Hayward 1964, 1991, 1994; He et al. 1983). Phylogenetic analyses based on different molecular approaches including RFLP sequence analysis of the

16S–23S rRNA intergenic spacer region (ITS), polygalacturonase and endoglucanase genes, and PCR-RFLP of the *hrp* genes region, reported that *R. solanacearum* may be regarded as a species complex. This species involves four different phylotypes related to the geographical origin of the strains, namely *Asiaticum* (phylotype I), *Americanum* (phylotype II), *Africanum* (phylotype III) and *Indonesian* (phylotype IV) (Cook and Sequeira 1991, 1994; Cook et al. 1989, 1991; Fegan and Prior 2005; Fegan et al. 1998; Poussier et al. 2000a, b).

Tomato crops can be infected by highly diverse race 1 lowland tropical strains of *R. solanacearum*, which are distributed in all four phylotypes. The race 3 highland temperate strains belonging to phylotype II (race 3-phylotype II), while being primarily adapted to potato (brown rot disease), are also pathogenic to tomato in natural environments. Because of the high susceptibility of tomato germplasm to race 3 (Carmeille et al. 2006), the outbreaks reported in Europe (Janse 1996; Prior et al. 1998) and the recent fortuitous introduction of race 3 strains in the USA (Kim et al. 2003; Williamson et al. 2002), this pathogen could compromise tomato production in such temperate regions, historically unfavorable to the establishment of tropical strains.

The use of genetic resistance appears to be the cheapest, most efficient and environmentally friendly method available to control BW. However, regarding race 3-phylotype II strains, only partial resistance sources have been identified, particularly in the *L. esculentum* cv. Hawaii 7996 (Carmeille et al. 2006). To date, genetic analysis of resistance to race 3-phylotype II strains has never been reported. In contrast, resistance to race 1 strains was extensively studied and was shown to be under polygenic control (Acosta et al. 1964; Anaïs 1986; Anand et al. 1993; Monma and Sakata 1993). Quantitative trait loci (QTLs) controlling resistance to race 1 strains (phylotypes I and II) have been identified in tomato progenies derived from different resistance sources (Danesh et al. 1994; Thoquet et al. 1996a, b; Wang et al. 2000). These studies revealed both shared and strain-specific QTLs.

In the present paper, we report the use of molecular markers in $F_{2:3}$ and RIL populations from a cross between *L. esculentum* cv. Hawaii 7996 and *L. pimpinellifolium* cv. West Virginia 700 (WVa700) to identify QTLs governing partial resistance to a race 3-phylotype II strain of *R. solanacearum* using two types of resistance criteria based on symptom development and bacterial colonization. These results are compared to those obtained for BW resistance to a race 1-phylotype I strain in the RIL progeny, and to other race 1 strains in previously published papers.

Materials and methods

Plant material

A set of 189 F_3 families derived from randomly chosen F_2 plants from a cross between *L. esculentum* cv. Hawaii 7996 (partially resistant) and *L. pimpinellifolium* cv. WVa700 (susceptible) (Thoquet et al. 1996a) was kindly supplied by INRA (Toulouse, France). Tomato F_3 plants were transplanted after sowing in individual 11 cm diameter plastic pots and were grown in a loam–peat (2:1) mixture. Then, F_3 plants were maintained in a greenhouse and propagated by cuttings. Until production of lateral roots, cuttings were placed under temperature- and humidity-controlled greenhouse conditions (25°C, relative humidity of 95%). Experiments were also conducted on a set of 107 random RIL (F_8 families) derived from the cross-Hawaii 7996 × WVa700 (Thoquet et al. 1996a) and supplied by AVRDC (Taiwan). Each family consisted of randomly selected seeds sown in individual pots of sowing plates containing a loam–peat (2:1) mixture.

Bacterial strains

In the $F_{2:3}$ and the RIL experiments, the *R. solanacearum* race 3-phylotype II strain JT516 was used. JT516 was isolated from *Solanum tuberosum* (L.) in Réunion Island (Nicole 1995) and can be considered as a representative (in terms of genetic diversity) of worldwide race 3 strains (Poussier et al. 1999, 2000b). Cultures of *R. solanacearum* were routinely grown (2 days, 28°C) onto selective modified Granada and Sequeira medium plates (Granada and Sequeira 1983; Poussier et al. 1999). Bacterial suspensions were prepared in 0.01 M Tris/HCl buffer at pH 7.2 (Sigma, St. Louis, USA) and spectrophotometrically adjusted to approximately 10^8 CFU per ml ($OD_{600\ nm} = 0.125$). The suspensions were further diluted in 0.01 M Tris/HCl buffer to reach the required concentration (10^7 CFU/ml). In the RIL experiment, the race 1-phylotype I strain JT519, isolated in Réunion Island from *Pelargonium asperum* (Nicole 1995; Poussier et al. 1999, 2000b), was also used. Inoculum preparation and inoculation procedure were the same as described for the resistance assays to race 3-phylotype II strain JT516 except that 10^8 CFU/ml were obtained when $OD_{600\ nm} = 0.210$ for strain JT519.

Inoculation procedure and resistance assays

Resistance tests on the $F_{2:3}$ population were conducted in a greenhouse at CIRAD (St Pierre, 21°S, 55.3°E, Réunion Island, France) during the cool (15–25°C) and hot seasons (20–30°C), respectively. Replications per season were performed over two different years and each $F_{2:3}$ family was represented by 10 to 24 individuals per replication. Within a season, only common families between the two replicates were used in the data set, corresponding to 140 and 114 $F_{2:3}$ families for the cool and hot season, respectively. Both parental lines and the susceptible *L. esculentum* cv. Roma line (Vilmorin, France) were used as controls in each replication.

Tomato plants were inoculated at the 6–8 fully expanded leaves stage. The cuttings were uprooted and dipped for 30 min in 300 ml of inoculum. Inoculated plants were immediately transplanted into individual pots and placed in a greenhouse. Plant roots were not artificially wounded before inoculation. All plants were numbered and distributed randomly in two distinct greenhouses to minimize possible environmental effects.

Disease development was assessed twice weekly, between the 7th and 28th days after inoculation (dai) by scoring each plant as resistant (no symptom) or susceptible (at least one leaf wilted). The proportion of wilted plants was then calculated in each $F_{2:3}$ family, together with control lines. The area under the disease progression curve value (AUDPC) was further determined as AUDPC $= \sum_{i=1} [(X_i + X_{i+1})/2] \times t_i$, where x_i was the proportion of wilted plants at date i ($i = 1$ being the first notation) and t_i the number of days between scoring date i and scoring date $i+1$. The first interval of time was between 0 and 7 dai.

Bacterial colonization in plants was rated by visual evaluation on a semi-quantitative 0 to 4 scale with an increment of 0.5. This scale was developed using different serial dilutions of the bacteria (10 to 10^9 CFU/ml). Fifty microliters of each bacterial suspension were spread onto Granada and Sequeira selective medium plates using the three sectors technique and incubated at 28°C for 3–4 days. The bacterium population was assessed based on the number of sectors in which the bacteria had grown and the quantity of bacteria in each sector. According to these experiments, a 0.5 colonization score increment in our rating scale corresponded to a multiplicative factor of 10 CFU/ml, with, therefore, scores ranging from 0 to 4 ($>10^8$ CFU/ml). Bacterial colonization scores in root system, hypocotyl (lower stem, above the collar) and epicotyl (upper stem, to the apex) from each resistant plant were assessed at 28 dai, and named CSR, CSH and CSE, respectively. After rinsing roots with tap water, root, hypocotyl and epicotyl fragments were separately grounded with a pestle in 5 ml of 0.01 M Tris/HCl buffer at pH 7.2 (Sigma, St. Louis, USA). Fifty microliters of each resulting suspension were spread and bacterium population was scored after incubation at 28°C for 3–4 days. The same procedure was used for some randomly selected susceptible plants at different dai and resulted in score 4 for CSR, CSH and CSE in these susceptible plants. Consequently, a colonization score 4 was attributed to each susceptible plant in each replicate. For each $F_{2:3}$ family, an average colonization score was calculated for each type of plant fragment, i.e. root system, hypocotyl and epicotyl. The three resulting colonization scores CSR, CSH and CSE were then separately analyzed.

For the RIL experiment, two trials were conducted using strains JT516 and JT519. For each trial, two replications (in two different greenhouses) were performed during the hot season for JT519 and the cool season for JT516, corresponding to the optimum temperatures for each strain (Nicole 1995), using 10 plants per family per replication. Parental lines and cultivar 'Roma' were used as controls in each replication of this experiment. Plants were numbered and distributed randomly. Disease development was assessed twice weekly from 7 to 28 dai, and AUDPC was determined for each RIL. Only bacterium colonization scores CSR and CSH were estimated in this RIL population.

Statistical analyses

Statistical analyses of phenotypic data were performed using SAS (SAS, v8.2, SAS Institute, Cary, NC, USA). For both $F_{2:3}$ and RIL experiments, the means of each resistance criterion were estimated for each family by Proc LS Means. Normality of data and residual error distributions were estimated with the *W*-test (Shapiro and Wilk 1965) using PROC UNIVARIATE. Original data and residual errors were close to normality and then used for analyses (data transformations did not improve normality). Phenotypic variance components were estimated using analysis of variance (PROC GLM) performed on mean score per family for each criterion. Broad-sense heritability of family means was calculated at the experimental design level for each season and across seasons as $H^2 = \sigma^2_F / \left[\sigma^2_F + \left(\sigma^2_e / r\right)\right]$ and $H^2 = \frac{\sigma^2_F}{\sigma^2_F + \frac{1}{s}\sigma^2_{F\times S} + \frac{1}{rs}\sigma^2_e}$, respectively, with r, the number of replications, s, the number of seasons, σ^2_F, the genetic variance between families, $\sigma_{F\times S^2}$, the family × season variance, and σ^2_e, the error variance. Exact 90% confidence intervals of H^2 were calculated from Knapp et al. (1985). Coefficients of phenotypic correlations among the different resistance criteria based on adjusted entry means of the $F_{2:3}$ families and of the RIL were computed with PROC CORR using Pearson's product moments. These coefficients were also calculated to assess phenotypic correlations between the cool and the hot seasons for the $F_{2:3}$ experiment, and between the two strains for the RIL experiment.

Linkage map

The genetic map used for the $F_{2:3}$ experiment is based on molecular data provided by Thoquet et al. (1996a), and Wang et al. (2000). MAPMAKER v3.0b program (Lander et al. 1987) was used for map construction with a minimum LOD score of 3 and a maximum recombination fraction of 0.5. Distances between markers were calculated applying the Haldane mapping function (Haldane 1919). The map is composed of 76 markers with an average distance between markers of 12.8 cM for a total length of 950 cM, and is estimated to cover about 75% of the tomato genome.

For the RIL experiment, DNA from the parents and the 107 F_8 progeny was extracted according to the protocol described by Lefebvre et al. (1995). RFLP analysis was conducted as described in Lefebvre et al.

(1995) using RFLP probes tagging regions of QTLs located on three chromosomes using appropriate restriction enzymes (Thoquet et al. 1996a; N. Grimsley, personal communication): TG515/*Bgl*II for chromosome 3, TG178/*Eco*RI, TG118/*Eco*RV, TG153/*Bgl*II, CP18/*Eco*RV for chromosome 6 and TG564/*Bam*HI for chromosome 12.

QTL detection

The association of each marker with the resistance was assessed by ANOVA (SAS, PROC GLM) on non-averaged data to account for replication effect. Association between a marker and resistance was declared significant at $P < 0.01$ ($F_{2:3}$ experiment) or $P < 0.05$ (RIL experiment). A QTL effect was estimated by the ratio of the variance explained by the marker to the total variance (SSM/SST), from the two-way ANOVA including a replication effect without interaction, with SSM variance due to marker, and total variance SST = SSM + SSY + SSR with SSY variance due to replication and SSR residual variance. For a given resistance criterion, the proportion of the variance explained by all QTLs affecting this criterion was similarly obtained with all markers significantly associated with this resistance criterion using a multi-marker ANOVA and taking into account the replication effect.

In the $F_{2:3}$ experiment, single interval mapping (SIM) (Lander and Botstein 1989) and composite interval mapping (CIM) (Zeng 1994) methods were also used with the QTL CARTOGRAPHER software (Basten et al. 2000). CIM was performed with a window size of 10 cM, and five cofactors were identified corresponding to the markers with the highest P value (by stepwise regression using the forward/backward option). For each resistance criterion, empirical thresholds corresponding to a comparison-wise type-I error of $P < 0.05$ were computed by permutation test (1,000 permutations) for SIM and CIM. For the different resistance criteria assessed during the two seasons, similar LOD thresholds of 3.15 and 3.35 were obtained for SIM and CIM, respectively. A one-LOD support interval was constructed for each QTL, as described by Lander and Botstein (1989). Two QTLs with non-overlapping support intervals were regarded as being different. The proportion of phenotypic variance explained by an individual QTL was estimated by the partial coefficient of determination (R^2) in SIM and CIM models using averaged data. The total phenotypic variation explained by all the QTLs detected by QTL CARTOGRAPHER for a given resistance criterion was estimated using a multi-marker ANOVA (SAS, PROC GLM) with molecular markers close to the QTLs peaks using averaged data.

The dominance ratio DR = $2|d/a|$ (where a and d are the additive and dominance estimates, respectively) was estimated from the results of CIM analysis to describe the type of gene action at each QTL: additive for DR < 0.20; partially dominant for 0.21 ≤ DR < 0.80; dominant for 0.80 ≤ DR < 1.20; overdominant for DR > 1.20 (Stuber et al. 1987).

Results

Phenotypic evaluation

Resistance to race 3-phylotype II strain JT516 in the $F_{2:3}$ population

The parent Hawaii 7996 was found to be highly resistant against race 3-phylotype II strain JT516 in the greenhouse trials during both cool and hot seasons, with only 8.5 and 0% of wilted plants at 28 dai, respectively. In both susceptible genotypes WVa700 and Roma, the majority of the symptoms appeared between 6 and 10 dai for the hot season, and after 10 dai for the cool season. In both seasons, wilting progressed rapidly until 15 dai, and then evolved slowly to stabilize at 22–23 dai. Although the same bacterium inoculum's concentration was used for both seasons, symptoms were more severe in the cool season than in the hot season, as indicated by the maximum percentage of wilted plants observed for both susceptible genotypes (87.0% vs. 49.5%, and 94% vs. 46.3% for WVa700 and Roma, respectively).

For all resistance criteria, we observed a continuous frequency distribution of $F_{2:3}$ families between the parents (Fig. 1). For the AUDPC criterion during the cool season, $F_{2:3}$ families followed a near-normal distribution, whereas during the hot season, they were skewed towards resistance. In contrast, these $F_{2:3}$ families displayed asymmetric distributions skewed towards the susceptible parent for the colonization scores assessed during the cool as well as the hot season. For all resistance criteria, distributions showed that disease severity during the cool season was higher than during the hot season. This difference among the two seasons was also observed for the mean values of parents and $F_{2:3}$ families obtained for each resistance criterion (Table 1). The variance component of the genotype × season interaction was significant at $P < 0.001$ for each resistance criterion (Table 1) and, consequently, data from the two seasons were treated separately for QTL mapping. The heritability ranged from 0.82 to 0.88 depending on the criterion and the season (Table 1), indicating that most of the phenotypic variation observed resulted from genetic factors.

Resistance to race 3-phylotype II strain JT516 in the RIL population

Only one of the two replications performed on the RIL population inoculated by race 3-phylotype II strain JT516 was successful, the other one being severely damaged by mildew attack. Thus, the results from only one replication are presented (Table 2). At 28 dai,

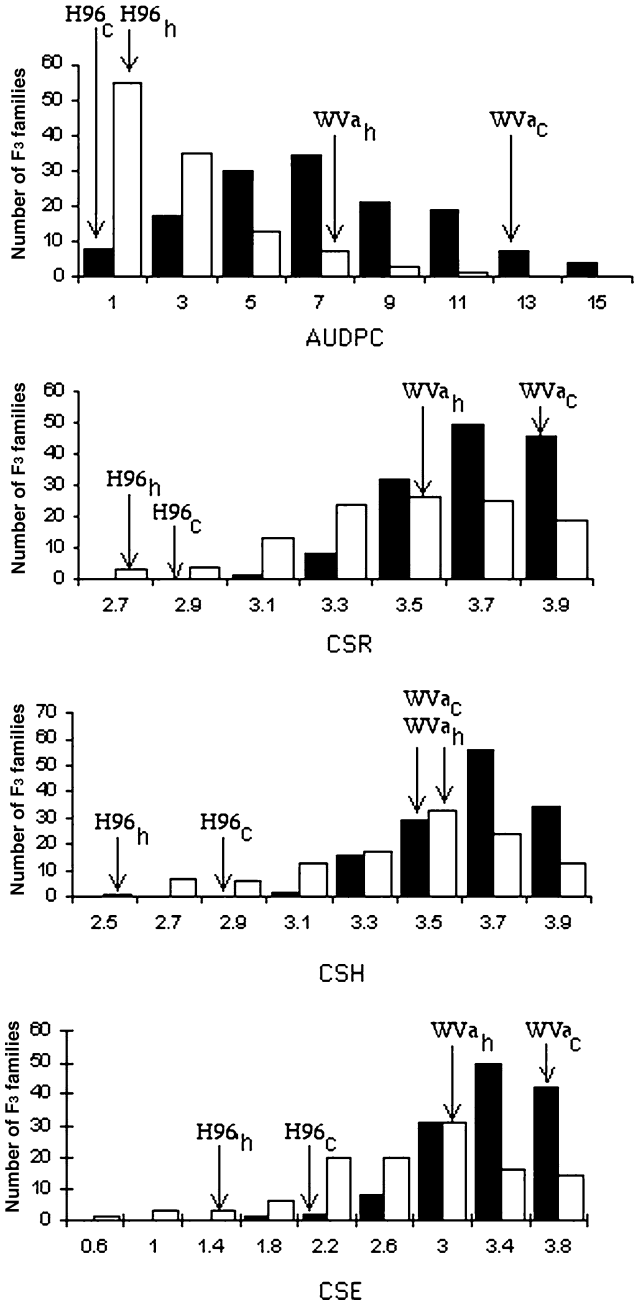

Fig. 1 Frequency distribution for the four resistance criteria against race 3-phylotype II strain JT516 measured in the cool (*solid bars*) and hot seasons (*open bars*) in the $F_{2:3}$ population. Parental mean values are indicated *H96* (Hawaii 7996) and *WVa* (WVa700), with subscripts c and h to represent cool and hot seasons, respectively

Hawaii 7996 was moderately resistant to the strain JT516 (20% of wilting), whereas WVa700 and Roma were highly susceptible (90 and 100% of wilting, respectively). A continuous distribution of RIL between the parents was observed for all resistance criteria; for AUDPC, RIL followed near-normal distribution, whereas for colonization scores CSR and CSH, they were skewed toward the susceptible parent (data not shown).

Resistance to race 1-phylotype I strain JT519 in the RIL population

After inoculation with the strain JT519, the resistant parent Hawaii 7996 did not show any symptoms of wilting (0% of wilting) whereas, at 28 dai, the susceptible lines WVa700 and Roma exhibited 80 and 95% of wilted plants, respectively. A continuous distribution of RIL between the parents was observed for all resistance criteria. For the colonization score CSR, RIL followed near-normal distribution, whereas, for AUDPC and CSH, they were skewed toward the resistant parent (data not shown). Symptoms observed in this RIL population with race 1-phylotype I strain JT519 were less severe than with race 3-phylotype II strain JT516. The heritability was high, varying from 0.89 to 0.95, depending on the criterion (Table 2).

Correlations among resistance criteria

Phenotypic correlations between the four resistance criteria AUDPC, CSR, CSH and CSE assessed during the cool and hot seasons were analyzed in the $F_{2:3}$ population inoculated with race 3-phylotype II strain JT516 (Table 3). Within a season, all criteria were significantly correlated with each other (with positive values ranging from 0.67 to 0.94, and $P < 0.0001$) suggesting several shared genetic factors. Correlation coefficients were stronger among colonization scores (0.87 to 0.94) than between each colonization score and AUDPC (0.67 to 0.74). Low correlation coefficients (0.46 to 0.56) were observed between the two seasons for the same resistance criterion, confirming differences in BW resistance expression among seasons and, therefore, confirming the need to analyse data separately from the two seasons.

In the RIL population, considering each strain, all criteria were significantly correlated with each other (with positive values ranging from 0.67 to 0.88, and $P < 0.0001$). Low correlation coefficients were observed between strains JT516 and JT519 (0.22 to 0.26) for the same resistance criterion, suggesting the existence of different genetic factors involved in the resistance to each strain.

QTL analysis of resistance to race 3-phylotype II strain JT516

Using a previously established genetic linkage map covering about 75% of the *Lycopersicon* genome (Thoquet et al. 1996a; Wang et al. 2000), four QTLs of resistance to the race 3-phylotype II strain JT516 of *R. solanacearum* were identified in the $F_{2:3}$ population (Fig. 2). They were named *Bwr-* for 'bacterial wilt resistance' followed by a number indicating their chromosomal location. The estimation of the percentage of phenotypic variation explained by these QTLs was more precise with ANOVA than with CIM or SIM, because

Table 1 Estimates of means, range of variation, variance components and heritabilities for the four criteria used to study resistance against race 3-phylotype II strain JT516 of *R. solanacearum* in a $F_{2:3}$ population derived from the cross between Hawaii 7996 and WVa700

Criterion	Season	Parents and control[a]			$F_{2:3}$ population						
		H96	WVa	Roma	Mean[b]	Range	σ_F^2[c]	$\sigma_{F\times S}^2$	σ_e^2	H^2[d]	90% CI[e]
AUDPC	Cool	0.6	13.0	15.3	7.1 (4.6)	0–19	22.47		9.79	0.82	
	Hot	0.0	6.5	6.4	2.7 (2.7)	0–13	8.55		3.20	0.84	
	Across							0.56		0.71	0.58–0.81
CSR	Cool	2.9	3.9	4.0	3.7 (0.3)	2.7–4	0.09		0.03	0.84	
	Hot	2.6	3.6	3.8	3.5 (0.4)	2.4–4	0.15		0.06	0.84	
	Across							0.01		0.60	0.41–0.73
CSH	Cool	3.0	3.9	4.0	3.7 (0.3)	2.5–4	0.14		0.05	0.84	
	Hot	2.5	3.5	3.8	3.4 (0.4)	1.4–4	0.21		0.08	0.83	
	Across							0.01		0.59	0.40–0.72
CSE	Cool	2.4	3.8	3.9	3.4 (0.7)	0.9–4	0.58		0.21	0.85	
	Hot	0.7	2.9	3.3	2.8 (0.9)	0.3–4	1.08		0.29	0.88	
	Across							0.08		0.57	0.36–0.70

[a]H96, *L. esculentum* cv. Hawaii 7996; WVa, *L. pimpinellifolium* cv. WVa700; Roma, *L. esculentum* cv. Roma
[b]Standard error is given in parenthesis
[c] σ_F^2, $\sigma_{F\times S}^2$, σ_e^2, estimates of the variances between families, of families × season interactions, and residual, respectively. All variances were significant at $P < 0.001$
[d] H^2, Broad-sense heritability
[e]90% CI, confidence interval of H^2

Table 2 Estimates of means, range of variation, variance components and heritabilities for the three resistance criteria against race 3-phylotype II strain JT516 and race 1-phylotype I strain JT519 of *R. solanacearum* in the RIL population derived from the cross between Hawaii 7996 and WVa700

Criterion	Parents and control[a]			RIL population				
	H96	WVa	Roma	Mean[b]	Range	σ_F^2[c]	σ_e^2	H^2[d]
Strain JT516 (cool season)								
AUDPC	1.6	10.4	18.8	8.6 (2.8)	3–17	–	–	–
CSR	2.7	4.0	4.0	3.8 (0.2)	3–4	–	–	–
CSH	1.6	4.0	4.0	3.6 (0.4)	2–4	–	–	–
Strain JT519 (hot season)								
AUDPC	0.0	17.1	21.0	3.3 (4.7)	0–22	34.71	4.38	0.94
CSR	2.8	3.9	4.0	3.3 (0.4)	2–4	0.25	0.06	0.89
CSH	0.7	3.5	4.0	1.6 (1.1)	0–4	2.14	0.22	0.95

[a]H96, *L. esculentum* cv. Hawaii 7996; WVa, *L. pimpinellifolium* cv. WVa700; Roma, *L. esculentum* cv. Roma
[b]Standard error is given in parenthesis
[c] σ_F^2, σ_e^2, Estimates of the variances between families and residual, respectively. All variances were significant at $P < 0.001$
[d] H^2, Broad-sense heritability

Table 3 Pearson phenotypic correlation coefficients among resistance criteria estimated in the $F_{2:3}$ population for resistance to race 3-phylotype II strain JT516: for the cool season (plain letters) and the hot season (italic letters), and for a given resistance criterion between seasons (bold letters); and in the RIL population for resistance to race 3-phylotype II strain JT516 (plain letters) and race 1-phylotype I strain JT519 (italic letters) and for a given resistance criterion between the two strains (bold letters). All phenotypic correlation coefficients were significant at the 0.0001 probability level

Criterion	$F_{2:3}$, hot and cool seasons, JT516				RIL, JT516 and JT519		
	AUDPC	CSR	CSH	CSE	AUDPC	CSR	CSH
AUDPC	**0.53**	0.74	0.71	0.72	**0.22**	0.69	0.74
CSR	*0.70*	**0.46**	0.94	0.91	*0.67*	**0.25**	0.88
CSH	*0.67*	*0.93*	**0.51**	0.94	*0.83*	*0.78*	**0.26**
CSE	*0.69*	*0.88*	*0.87*	**0.56**			

the replication effect was taken into account (Table 4). Therefore, in the study, the effects of the QTLs will be discussed based on ANOVA results using the ratio of the variance explained by the marker to the total variance. Individual QTLs explained from 3.2 to 29.8% of the phenotypic variation observed in the $F_{2:3}$ population. For all QTLs, only alleles from the resistant parent *L. esculentum* cv. Hawaii 7996 contributed to the

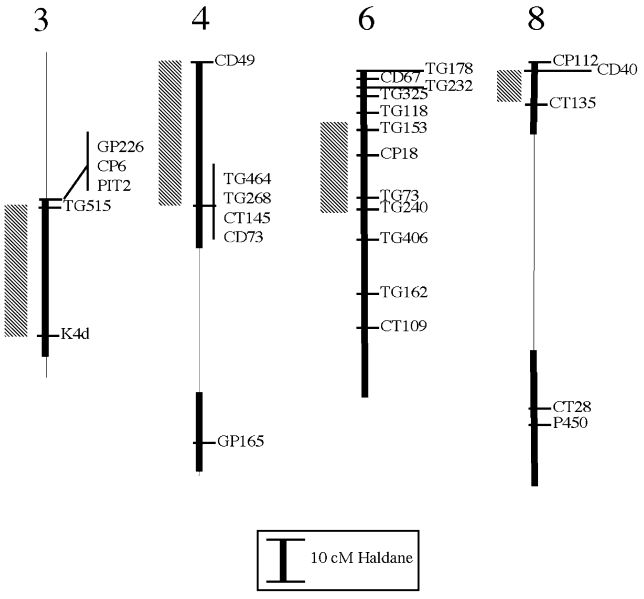

Fig. 2 Map location of the QTLs involved in resistance to *R. solanacearum* race 3- phylotype II strain JT516 in the $F_{2:3}$ population. QTLs for resistance are represented on the left of linkage groups by *hashed bars* and named *Bwr-* for bacterial wilt resistance followed by a number indicating their chromosomal location

resistance phenotype. When considering all resistance criteria together, four QTLs were detected during the hot season, while only two were detected during the cool season. Therefore, the total phenotypic variation explained by all the QTLs detected during the hot season (24.1 to 28.9%) was higher than during the cool season (4.0 to 14.2%).

The QTL *Bwr-6* located in the centromeric region of chromosome 6, in the vicinity of RFLP marker TG73, was detected by all methods, for all resistance criteria, and in both seasons. This QTL showed the strongest effect and explained up to 9.0% of the phenotypic variation observed in the cool season, and up to 29.8% in the hot season, depending on the criterion. The gene action of *Bwr-6* was partially dominant in favor of resistance for all resistance criteria in the two seasons, except for CSE in the cool season where it was additive (Table 4). The presence of *Bwr-6* for AUDPC, CSR and CSH criteria was confirmed in the RIL population tested for resistance against strain JT516 (Table 5) where it explained up to 8.1% of the variation of these traits.

A minor QTL located on chromosome 3 between the markers TG515 and K4d, and named *Bwr-3*, was significantly detected by ANOVA in the $F_{2:3}$ population, in both seasons, for all resistance criteria, except for CSE in the cool season. It was also detected by CIM and SIM for AUDPC in the hot season. This QTL was found to be overdominant and to explain 3.2 to 5.0% of the phenotypic variation in the cool season, and 5.6 to 9.3% in the hot season, depending on the criterion. The presence of *Bwr-3* for AUDPC was confirmed in the RIL population where it accounted for 13.2% of the variation of this criterion (Table 5).

One additional QTL was detected in the hot season for all resistance criteria, when using ANOVA, and only for colonization scores, when using CIM and SIM. This QTL, located on chromosome 4 in the vicinity of marker CD73, and named *Bwr-4*, explained 6.8 to 12.2% of the phenotypic variation, depending on the resistance criterion. It was additive for CSE and partially dominant for CSR and CSH (Table 4).

Finally, *Bwr-8*, an overdominant minor QTL on chromosome 8 was detected only by the CIM method for CSH criterion in the hot season, and will be thereafter referred to as putative (Table 4).

QTL analysis of resistance to race 1-phylotype I strain JT519

In order to determine whether QTL specificity can be observed or not according to the strain used, QTLs for resistance to race 3-phylotype II strain JT516 were compared to those controlling resistance to race 1-phylotype I strain JT519 using the RIL population. The search for QTLs for resistance to race 1-phylotype I strain JT519 in specific regions on chromosomes 3, 6 and 12 in our RIL population using ANOVA revealed two QTLs, *Bwr-6* and *Bwr-12*, explaining, respectively, from 11.5 to 17.2%, and from 14.2 to 22.5% of the phenotypic variation in the RIL population, depending on the resistance criterion (Table 5). These two QTLs were found to be stable across different resistance criteria (AUDPC, CSR and CSE).

Comparison of QTLs for BW resistance (race 1 and race 3 strains) in Hawaii 7996

The QTLs detected for resistance to race 3-phylotype II and/or race 1-phylotype I strains from Réunion Island matched those detected in previous studies on the same $F_{2:3}$ population for resistance to other *R. solanacearum* strains (Table 6). Knowing that QTL map position fluctuates with environmental factors as well as imprecision in quantitative evaluation of the phenotypes, we declared that QTLs co-localizing in the different studies, and differing in their estimated positions by less than 20 cM, might be considered as identical.

Of the four QTLs involved in Hawaii 7996 resistance to strain Pss4 (race 1-phylotype I; Wang et al. 2000), QTLs on chromosomes 6 and 8 co-localize with the QTLs *Bwr-6* and *Bwr-8* for resistance to the race 3-phylotype II strain JT516. However, the major-effect QTL against Pss4 was identified as being located on chromosome 12, and co-localizes with *Bwr-12* only detected against race 1-phylotype I strain JT519 in our RIL population. A total of seven genomic regions were involved in Hawaii 7996 resistance to strain GMI8217

Table 4 QTLs detected for resistance to race 3-phylotype II strain JT516 of *R. solanacearum* based on CIM, SIM and ANOVA analyses in the $F_{2:3}$ population derived from the cross between Hawaii7996 and WVa700

Criterion	QTL[a]	CIM						SIM		ANOVA		
		Mk Int[b]	LOD[c]	R^2 (%)[d]	a[e]	d[e]	$2\|d/a\|$[f]	LOD[c]	R^2(%)[d]	Mk[g]	P[k]	% [h]
Cool season												
AUDPC	*Bwr-3*		–	–	–	–	–	–	–	TG515	0.0026	5.0
	Bwr-6	TG153–CP18	6.73	19.1	−2.20	−0.83	0.75	7.03	22.6	TG 73	$< 10^{-4}$	9.0
	Total R^{2i}			20.1						% global[j]		14.2
CSR	*Bwr-3*		–	–	–	–	–	–	–	TG515	0.0064	3.5
	Bwr-6	CP18–TG73	4.76	13.0	−0.11	0.03	0.55	6.98	23.3	TG73	$< 10^{-4}$	6.9
	Total R^2 [i]			17.7						% global[j]		9.0
CSH	*Bwr-3*		–	–	–	–	–	–	–	TG515	0.0054	3.2
	Bwr-6	CP18–TG73	5.04	16.2	−0.12	0.02	0.26	5.04	16.2	TG73	0.0003	4.5
	Total R^2 [i]			15.3						% global[j]		7.4
CSE	*Bwr-6*	CP18–TG73	4.72	15.6	−0.24	0.01	0.05	4.72	15.6	TG73	0.0007	4.0
	Total R^2 [i]			14.4						% global[j]		4.0
Hot season												
AUDPC	*Bwr-3*	TG515–K4d	3.38	8.7	−0.80	−0.89	2.24	–	–	TG515	$< 10^{-4}$	9.3
	Bwr-4		–	–	–	–	–	–	–	CD73	0.0010	6.8
	Bwr-6	CP18–TG73	9.42	31.4	−2.09	−0.29	0.28	8.10	30.2	TG73	$< 10^{-4}$	20.1
	Total R^2 [i]			38.2						% global[j]		28.1
CSR	*Bwr-3*		–	–	–	–	–	–	–	TG515	0.0018	6.1
	Bwr-4	CD49–CD73	3.84	9.2	−0.13	0.04	0.70	4.06	15.3	CD73	0.0001	12.2
	Bwr-6	TG73–TG240	11.92	31.3	−0.27	0.06	0.47	12.58	43.5	TG73	$< 10^{-4}$	29.8
	Total R^2 [i]			49.2						% global[j]		28.9
CSH	*Bwr-3*		–	–	–	–	–	–	–	TG515	0.0020	5.7
	Bwr-4	CD49–CD73	4.80	13.2	−0.17	0.03	0.32	4.58	17.1	CD73	$< 10^{-4}$	11.4
	Bwr-6	CP18–TG73	13.71	34.1	−0.31	0.09	0.57	12.45	42.9	TG73	$< 10^{-4}$	27.4
	Bwr-8	CD40–CT135	3.61	6.9	−0.10	0.11	2.24	-	-			-
	Total R^2 [i]			53.5						% global[j]		27.3
CSE	*Bwr-3*		–	–	–	–	–	–	–	K4d	0.0014	5.6
	Bwr-4	CD49–CD73	3.56	11.71	−0.33	−0.02	0.11	3.62	13.7	CD73	$< 10^{-4}$	9.7
	Bwr-6	TG73–TG240	11.20	31.01	−0.63	0.10	0.32	11.95	38.6	TG73	$< 10^{-4}$	26.2
	Total R^2 [i]			46.17						% global[j]		24.1

[a]QTL, the name *Bwr-* (for BW resistance) was given. The number after *Bwr-* indicates the chromosome location
[b]Mk Int, Marker Interval from the original map reference (Thoquet et al. 1996a; Wang et al. 2000)
[c]LOD, Maximum value of the log-likelihood in the marker interval (values are superior to the LOD-thresholds: 3.15 for IM and 3.35 for CIM)
[d]R^2 (%), Partial coefficient of determination, i.e., percentage of phenotypic variation explained by the QTL calculated by QTL CARTOGRAPHER using averaged data from the two replications (years)
[e]*a* and *d* Additive and dominance 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 (Hawaii 7996)
[f]$2|d/a|$ Dominance ratio with *a* and *d* being the additive and the dominance estimates, respectively
[g]Mk, Markers linked to resistance with the strongest effect ($P < 0.01$) by ANOVA
[h]% Proportion of the total variance explained by the marker at the QTL (in %) with ANOVA and taking the replication effect into account
[i]Total R^2, sum of the effects for a resistance criterion explained by markers linked to QTL and calculated using multiway ANOVA on averaged data (in %)
[j]% global, Proportion of the total variance explained by all the markers linked to QTL affecting the respective trait (in %) with ANOVA and taking the replication into account
[k]*P* Probability

(race 1-phylotype II; Thoquet et al. 1996a, b). Four of these QTLs, on chromosomes 3, 4, 6 and 8, were mapped at the same position and with similar effect on resistance than *Bwr-3*, *Bwr-4*, *Bwr-6* and *Bwr-8*.

Discussion

L. esculentum cv. Hawaii 7996 was previously identified as the most tolerant tomato accession to race 3-phylotype II strain JT516 (Carmeille et al. 2006). QTL mapping was therefore initiated on the available $F_{2:3}$ population from the cross between Hawaii 7996 and WVa700, using a previously established genetic map (Thoquet et al. 1996a; Wang et al. 2000), and on a RIL population derived from the same genetic background, using six RFLP markers correlated with resistance to *R. solanacearum* strains (Thoquet et al. 1996a, b; Wang et al. 2000). In $F_{2:3}$ and RIL experiments, resistance of Hawaii 7996 was confirmed and inoculation conditions were successful as attested by the percentage of wilting of the susceptible controls.

Table 5 Biometrical parameters (single-factor ANOVA, $P \leq 0.05$) of QTLs confirmed against the strains JT516 (race 3-phylotype II) and JT519 (race 1-phylotype I) in the RIL population

Criterion	QTL[a]	Strain JT516, cool season			Strain JT519, hot season		
		Marker[b]	P[c]	%[d]	Marker[b]	P[c]	%[d]
AUDPC	Bwr-3	TG515	0.0007	13.2	–	–	–
	Bwr-6	TG153	0.0130	8.1	TG153	$< 10^{-4}$	11.5
	Bwr-12	–	–	–	TG564	$< 10^{-4}$	14.2
CSR	*Bwr-6*	TG153	0.0160	8.0	TG153	$< 10^{-4}$	17.2
	Bwr-12	–	–	–	TG564	$< 10^{-4}$	16.4
CSH	*Bwr-6*	TG153	0.0210	7.1	TG153	$< 10^{-4}$	13.3
	Bwr-12	–	–	–	TG564	$< 10^{-4}$	22.5

[a]QTL, the name *Bwr-* (for BW resistance) was given. The number after *Bwr-* indicates the chromosome location
[b]Marker, Markers linked to resistance ($P < 0.05$) by ANOVA.
[c]*P* Probability
[d]%, Proportion of the total variance explained by the marker at the QTL by the marker with ANOVA taking the replication effect into account (for strain JT519)

Genetic architecture of BW race 3-phylotype II resistance

Overall, the polygenic nature of BW resistance was confirmed by the detection of four QTLs (including one putative) located on four chromosomes, as expected from the continuous distribution observed for all criteria between the parents, which indicated a complex genetic basis for resistance. Moreover, the skewed distributions observed in the hot season and for colonization scores suggest the occurrence of several minor-effect resistance loci and epistasis (which was not tested).

For each resistance criterion, the comparison between broad-sense heritability and the total phenotypic variation explained by all QTLs together showed that an important part of phenotypic variation remained unexplained. This can be due to the small population size used for QTL detection, which could have prevented detection of QTLs with weak effects on the resistance, and/or to the unsaturated linkage map used in this study (75% genome coverage). Thus, other QTLs may be present in genomic regions not covered by the linkage map. Moreover, during the $F_{2:3}$ cool season experiment, the proportions of the total variance explained by markers were particularly low, in relation to (1) the lower levels of resistance observed (e.g. higher severity of symptoms) and (2) the strong replicate effect observed in this experiment.

Bwr-6, which showed the strongest effect, was detected using the different resistance criteria, whatever the season, and was stable across generations $F_{2:3}$ and RIL. Another genomic region with minor effect, *Bwr-3*, detected in both seasons with each resistance criterion, also appeared stable across generations.

The expression of resistance differed markedly between seasons with an increase towards susceptibility in the cool season, probably due to the fact that temperatures were closest to the optimal temperatures of race 3 strains, referred to as a 'temperate race' (French 1986). In the hot season, the lower severity level observed with all resistance criteria can be put in relation with the detection of two additional resistance QTLs, *Bwr-4* and the putative *Bwr-8*. Thus, these QTLs detected only during the hot season might be ineffective under cool

Table 6 Strains used in the different QTL mapping analyses for BW resistance in tomato line Hawaii 7996 using the same segregating populations from a cross Hawaii 7996 × WVa700

Strain	Race[a]	Phylotype[b]	Geographic origin[c]	QTL location[d]					Ref[e]
				3	4	6	8	12	
Pss4	1	I	Taiwan	–	–	+	+	+	Wang et al. 2000 ($F_{2:3}$)
JT519	1	I	Réunion Is.	–	nt	+	nt	+	This study (RIL)
GMI8217	1	II	Guadeloupe	+	+	+	+	–	Thoquet et al. 1996a, b ($F_{2:3}$)
JT516	3	II	Réunion Is.	+	+	+	+	–	This study ($F_{2:3}$)
JT516	3	II	Réunion Is.	+	nt	+	nt	–	This study (RIL)

[a]Races defined by Buddenhagen et al. (1962) and He et al. (1983)
[b]Phylotypes defined by Fegan and Prior (2005)
[c]Geographical origin: region where strain was isolated
[d]The number is the chromosome number of the QTLs detected in this study which are active (+), inactive (–) or not tested (nt) against the strain considered
[e]Reference indicates the authors who have performed QTL analyses. Segregating populations used are indicated in parenthesis

conditions, or bacteria might overcome resistance under optimal conditions. The lack of stability across seasons emphasizes that multi-environment tests have to be performed to accurately evaluate BW resistance in tomato.

Because resistance to race 1 strains in tomato was shown to be associated with limiting bacterial concentration rather than bacterial penetration (Grimault and Prior 1993; Grimault et al. 1994), we tested the appropriateness of using colonization scores as resistance criterion for detecting resistance QTLs. The QTLs, *Bwr-3, Bwr-4* and *Bwr-6*, involved in preventing wilting of the plants (AUDPC) were also involved in limiting bacterial colonization in the roots and stem (CSR, CSH, CSE). The putative *Bwr-8* QTL, detected only for CSH trait, could be specifically involved in limiting bacterial colonization of the stem. In the end, the use of these colonization scores was helpful to gain in accuracy for QTL detection.

QTL controlling BW resistance and phylotype-specificity

This is the first QTL study for resistance to a highland race 3-phylotype II strain of *R. solanacearum* in tomato. Previous studies involving diverse race 1 strains suggested the occurrence of strain-specific resistance QTLs in Hawaii 7996 (Thoquet et al. 1996a, b; Wang et al. 2000). All race 3 strains (including JT516) are phylogenetically closely related to a group of race 1 strains (including GMI8217) and cluster in phylotype II. This group is highly distant to the phylotype I cluster containing another group of race 1 strains (including Pss4 and JT519) (Fegan and Prior 2005). Taking into account this recent classification of *R. solanacearum* strains into phylotypes, considering QTLs detected for the same resistance trait (percentage of wilted plants and/or AUDPC) in independent studies with distinct strains (Danesh et al. 1994; Thoquet et al. 1996a, b; Wang et al. 2000) in the same genetic background (Hawaii 7996 × Wva700) and assuming that QTLs detected in the same position in these different studies are identical QTLs, we suggest that the proposed strain-specificity of some of the QTLs should rather be interpreted in terms of a phylotype-specificity. Therefore, it is possible to distinguish broad-spectrum QTLs from phylotype-specific QTLs in Hawaii 7996.

The comparison of the results from the different studies performed on QTL mapping for BW resistance in tomato emphasizes the key role of *Bwr-6*, localized on tomato chromosome 6. Actually, this QTL was detected in all studies using phylotype I strains (Pss4 and JT519) and phylotype II strains (GMI8217 and JT516) in different geographical locations, and is present in two different genetic backgrounds (Hawaii 7996 as well as L285) (Danesh et al. 1994; Thoquet et al. 1996a, b; Wang et al. 2000). Therefore, *Bwr-6* can be considered as a broad-spectrum QTL. This QTL contributes to the largest phenotypic variation in all studies, except for resistance to race 1-phylotype I strain Pss4 (Wang et al. 2000) where the major QTL was detected on chromosome 12. This QTL on chromosome 12, *Bwr-12*, was also detected as the major QTL against the race 1-phylotype I strain JT519 in our RIL experiment. QTL *Bwr-12*, which is active against the phylotype I strains Pss4 (race 1) and JT519 (race 1), but not against the phylotypes II strains JT516 (race 3) and GMI8217 (race 1), may, therefore, be considered as a phylotype I-specific QTL in Hawaii 7996. Similarly, the QTL on chromosome 3, *Bwr-3*, which was found to be active against GMI8217 and JT516 phylotype II strains, and not against Pss4 and JT519 phylotype I strains, might be considered as a phylotype II-specific QTL.

In conclusion, these preliminary observations suggest that the genetic architecture of the tomato line Hawaii 7996 resistance against *R. solanacearum* is in part related to the phylotype of the bacteria rather than to the strain or the race, and provide testable hypotheses regarding the specificity of the QTL. Environmental factors (temperature) seem to play a key role as well, as one extra QTL (*Bwr-4*) plus a putative one (*Bwr-8*) were detected during the hot season experiments. QTL *Bwr-4* could be regarded as a putative phylotype II-specific QTL, as it was active against strains GMI8217 and JT516, but not against Pss4. However, this has to be further investigated, because *Bwr-4* appears as environmentally sensible. QTL mapping studies using *R. solanacearum* strains chosen according to the new classification scheme proposed by Fegan and Prior (2005), and using different environmental conditions, will be conducted to validate these hypotheses.

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