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ORIGINAL PAPER

Three QTLs for *Botrytis cinerea* resistance in tomato

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Abstract Tomato (*Solanum lycopersicum*) is susceptible to grey mold (*Botrytis cinerea*). Partial resistance to this fungus was identified in accessions of wild relatives of tomato such as *S. habrochaites* LYC4. In order to identify loci involved in quantitative resistance (QTLs) to *B. cinerea*, a population of 174 F_2 plants was made originating from a cross between *S. lycopersicum* cv. Moneymaker and *S. habrochaites* LYC4. The population was genotyped and tested for susceptibility to grey mold using a stem bioassay. *Rbcq*1, a QTL reducing lesion growth (LG) and *Rbcq*2, a QTL reducing disease incidence (DI) were identified. *Rbcq*1 is located on Chromosome 1 and explained 12% of the total phenotypic variation while *Rbcq*2 is located on Chromosome 2 and explained 15% of the total phenotypic variation. Both QTL effects were confirmed by assessing disease resistance in two BC_2S_1 progenies segregating for either of the two QTLs. One additional QTL, *Rbcq*4 on Chromosome 4 reducing DI, was identified in one of the BC_2S_1 progenies. F_2 individuals, homozygous for the *Rbcq*2 and *Rbcq*4 alleles of *S. habrochaites* showed a reduction of DI by 48%. QTLs from *S. habrochaites* LYC4 offer good perspectives for breeding *B. cinerea* resistant tomato cultivars.

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Introduction

Grey mold: *Botrytis cinerea* Pers.:Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel) is a necrotrophic pathogenic fungus with a wide host range (Elad et al. 2004; Jarvis 1977). Modern hybrid tomato (*Solanum lycopersicum*) cultivars are susceptible to *B. cinerea* although some cultivars show some quantitative resistance. During cultivation in heated greenhouses, leaves and fruit are rarely infected by *B. cinerea* but infections on stems are common (Shtienberg et al. 1998). Stem rot usually develops after pruning of lower leaves and side shoots (Verhoeff 1968) and results in lower yield or premature plant death.

Disease control in heated greenhouses currently relies on spraying systemic fungicides and/or the application of biocontrol agents. These control strategies may, however, often be ineffective as *B. cinerea* has becomes resistant against several of these agents (Elad et al. 2004). Transgenic plants displaying increased

resistance to *B. cinerea* under laboratory conditions have been described (Elad et al. 2004) but their performance under commercial culturing conditions has rarely been tested. Implementation of transgenic *B. cinerea*-resistant crops in practice is unlikely to occur in the near future because of legal restriction and lack of consumer acceptance. Development of non-transgenic cultivars by introgression of resistance genes from wild relatives into modern elite lines may provide effective disease control.

Using a segregating population of a cross between a susceptible and a (partial) resistant parent combined with molecular marker analysis, genetic linkage maps can be constructed and quantitative trait loci (QTL) can be identified. Such a strategy has been successfully applied for identifying quantitative resistance against several fungal and bacterial diseases in tomato (Bai et al. 2003; Foolad et al. 2002; van Heusden et al. 1999). Once identified, desirable QTLs can be introgressed rapidly into elite cultivars by marker-assisted selection (MAS) (Tanksley et al. 1998). Quantitative resistance to *B. cinerea* has been identified in wild relatives of *S. lycopersicum* (Egashira et al. 2000; Nicot et al. 2002; Urbasch 1986). Although the resistance levels reported in these plants are high, the presumed polygenic inheritance and the lack of a standardized bioassay for quantitatively assessing resistance have limited the success of breeding for resistance. We used a stem bioassay suitable to quantify *B. cinerea* infection (ten Have et al. 2007) to assess susceptibility to *B. cinerea* in an interspecific segregating F_2 population, obtained after crossing the susceptible cultivar *S. lycopersicum* cv. Moneymaker with the *B. cinerea* resistant accession *S. habrochaites* LYC4 (ten Have et al. 2007; Urbasch 1986). The population was phenotyped using stem bioassays and genotyped using AFLP fingerprinting and PCR based co-dominant CAPS and SCAR markers. Two QTLs were detected in a F_2 population screen, while a third locus was identified in experiments aimed at confirmation of the two initial QTLs. This is the first report of a successful QTL study for resistance against *B. cinerea* in crop plants.

Materials and methods

Plant material

An interspecific cross between *S. lycopersicum* cv. Moneymaker (hereafter referred to as *SL*) and *S. habrochaites* LYC 4 (hereafter referred to as *SH*) was made to produce F_1 seeds. Seeds of *SL* were in stock of Wageningen University and seeds of *SH* were obtained from the Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany. F_2 seeds, derived from a single F_1 plant, were sown to obtain an F_2 population of 174 individuals. The same F_1 was backcrossed to *SL* to obtain BC_1 seeds. 14 BC_1 plants were subsequently backcrossed to *SL* to obtain a BC_2 population of 59 individuals. Using marker-assisted selection, BC_2 genotypes were selected containing one of the two identified QTLs. These BC_2 plants were self pollinated to produce BC_2S_1 seeds. Two BC_2S_1 populations were grown: one population consisting of 60 BC_2S_1 individuals that segregated for the QTL for disease incidence and a second population consisting of 47 BC_2S_1 individuals that segregated for the QTL for lesion growth.

Stem assay and experimental design

Inoculum of *B. cinerea* strain B05.10 was prepared according to Benito et al. (1998). The stem assay was performed according to ten Have et al. (2007). In short, stems of 6–8 week-old plants were cut into six pieces of 5 cm length and inoculated with 5 µl inoculum, containing 10^6 conidia ml^{-1}, applied to the top of each stem segment. Incubations were performed in plastic boxes (30 × 45 cm) at 15°C in the dark at 100% relative humidity. In each box 22 genotypes were tested, including two *SL* controls. The infection progress was measured at day four and five after inoculation using a Vernier caliper. For each genotype, the percentage of successfully infected stem pieces was calculated (disease incidence, DI). The lesion growth rate (LG) was calculated as the increase in lesion size between day 4 and 5 (mm/day) only for the infected stem pieces.

To assess susceptibility in F_2 plants, cuttings were made for each F_2 genotype and tested independently in eight destructive disease assays. For each experiment, the cuttings and *SL* controls were grown and tested using a completely randomized design. Nine boxes were required for each replicate experiment.

To confirm the identified QTLs, two BC_2S_1 families were grown. For each BC_2S_1 genotype, cuttings were grown and tested using a completely randomized design. Two independent experiments were performed in which susceptibility of each BC_2S_1 genotype was tested twice resulting in a total of four replicate observations. In each experiment, both *SL* and *SH* controls were included.

DNA isolation and marker analysis

Genomic DNA was isolated from two young (rolled up) leaves using a CTAB based protocol according to Steward and Via (1993), adjusted for high throughput

DNA isolation using 1-ml micronic tubes (Micronic BV, Lelystad, The Netherlands). Leaves were ground using a Retsch 300 MM shaker at maximum speed (Retsch BV, Ochten, The Netherlands). The AFLP analysis of the F_2, BC_2 and BC_2S_1 populations was done according to Vos et al. (1995). AFLP fragments were resolved on a LI-COR 4200 DNA sequencer, essentially following the method of Myburg et al. (2001). The selective *Pst*I primer was labeled with an IRD700 or IRD 800 fluorescent label. AFLP gel images were scored using the AFLP-Quantar Pro software package (Keygene, Wageningen, The Netherlands). Primer and adapter sequences had been described by Bai et al. (2003). The following ten primer combinations were used for genotyping: P14M48, P14M49, P14M50, P14M60, P14M61, P15M48, P18M50, P18M51, P22M50 and P22M51.

Sets of CAPS and SCAR primers were obtained from the "solanaceae genomics website" (http://www.sgn.cornell.edu) or designed on sequences of genomic or cDNA clones available from the same source. Polymorphisms between *SL* and *SH* were determined using the CAPS digestion approach described by Bai et al. (2004). Marker sequences, PCR conditions and specific restriction endonucleases used for genotyping are presented online in an electronic supplement (Table S1). CAPS markers were generally separated using a 2.5% agarose gel.

Data analysis

Marker data were analyzed and a genetic linkage map was calculated with Joinmap 3.0 (van Ooijen and Voorrips 2001). Skweness of a region was determined using a Chi-square test. If all markers in a certain region were skewed at a probability of $P < 0.005$, this region was considered skewed. Phenotypic data were analyzed using the general linear model (GLM) approach as implemented in the software package SPSS 12.0 (SPSS Inc., Chicago, USA). LG data were square root transformed to obtain a normal distribution. Mean estimates for each F_2 genotype were calculated using the following models: DI = constant + genotype + experiment and LG = constant + genotype + experiment + genotype × experiment. While analyzing BC_2S_1 data, information about experimental unit structures (box) were added and resulted in the following models to estimate mean values for each genotype: DI = constant + genotype + box + experiment and LG = constant + genotype + box + experiment + genotype × experiment. QTLs were calculated using the multiple QTL mapping procedure (MQM; Jansen and Stam 1994; F_2 dataset) or Kruskal–Wallis test (BC_2S_1 dataset) as embedded in MapQTL (van Ooijen 2003). Using the permutation test of MapQTL, a genome wide significance threshold ($P < 0.05$) was calculated for each trait. Linkage maps and QTL plots were drawn using the MapChart software (Voorrips 2002). The correlation between traits was considered by interpreting Pearson correlation coefficients. Epistasis was analyzed using a two-way ANOVA and the gene action of each locus was tested using orthogonal contrast mean separation statistics (Cockerham and Zeng 1996; Table 4). Homogeneity of variance was tested using a Levene test and depending on the result, the contrast table was interpreted from the table, which does assume equal variances or from the table, which does not assume equal variances.

Results

Molecular marker analysis and genetic linkage map

An F_2 population ($n = 174$) was grown of the cross between the susceptible cultivar *S. lycopersicum* cv. Moneymaker (hereafter referred to as *SL*) and the partially resistant accession *S. habrochaites* LYC4 (hereafter referred to as *SH*; ten Have et al. 2007; Urbasch 1986). This population was used to develop a genetic linkage map. Ten AFLP primer combinations resulted in 218 AFLP markers. Initially, all markers were scored dominantly, but 69 markers (32%) could be readily scored co-dominantly using Quantar Pro software. To improve the linkage map, 51 co-dominant PCR based CAPS and SCAR markers were added (Table S1), mainly selected based on their positions in the *S. lycopersicum* LA925 × *S. pennellii* LA716 reference map (Tanksley et al. 1992). After identification of QTLs an additional set of CAPS or SCAR markers was developed that flank each of the QTLs.

CAPS markers known to be close to telomeres were often outside the region covered with AFLP markers. A representative example provided by the linkage map of Chromosome 2 (Fig. 1), where the region below P22M50-93h could only be included after addition of CAPS markers TG537, TG91 and TG620. The resulting genetic linkage map consisted of 12 linkage groups, covering a total genetic length of 1087 cM. Ten linkage groups could be assigned to chromosomes using the CAPS and SCAR markers. The last two linkage groups were assigned using common *SL* markers, which have been mapped in previous crosses using the same *SL* cultivar (Bai et al. 2003; Haanstra et al. 1999).

Skewed segregation of markers was observed in regions of Chromosome 1, 5, 7 and 9. Skewness of most regions was in favor of *SH* alleles, with the exception of

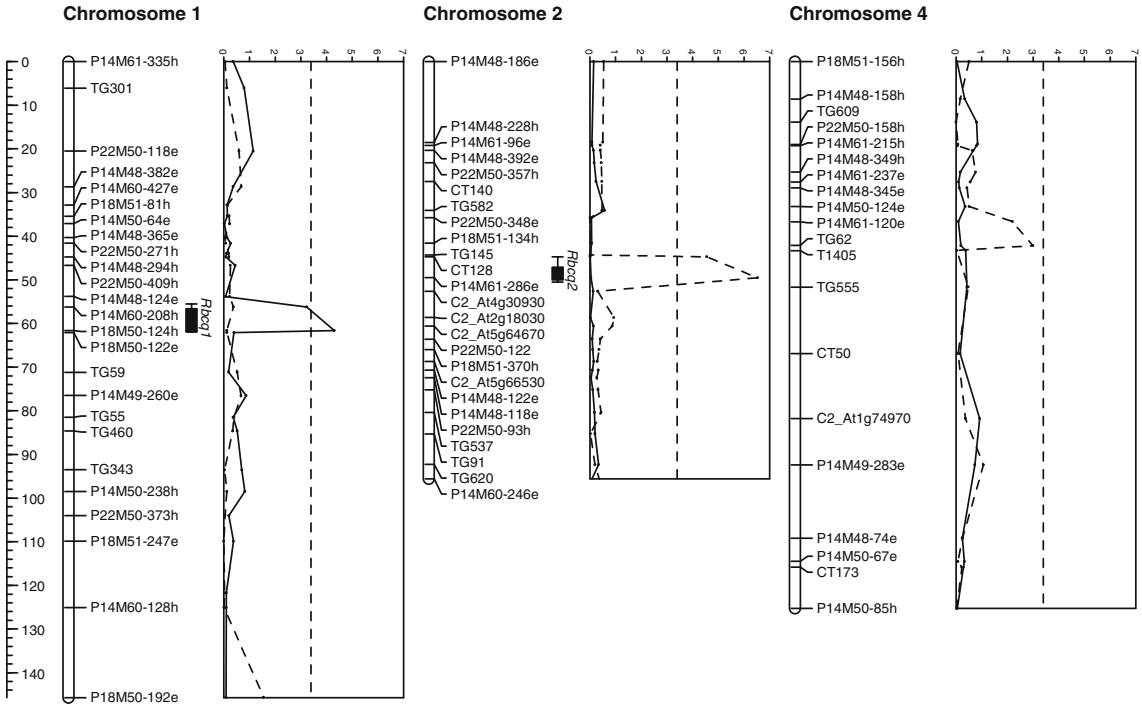

Fig. 1 The positions of QTLs for resistance to *B. cinerea* are shown on linkage maps of Chromosome 1, 2 and 4. Map positions are given in cM. QTL *Rbcq*1 on Chromosome 1 reduced lesion growth (*solid line*) while QTL *Rbcq*2 on Chromosome 2 reduced disease incidence (*dashed line*). The epistatic QTL *Rbcq*4, linked to TG62 on Chromosome 4, did not exceed the significance threshold in MQM mapping. *Bars* indicate the QTL intervals for which the *inner bar* shows a one-LOD support interval and the outer bar shows a two-LOD support interval. The LOD threshold value of 3.5 is shown as a *dashed line*

a region on Chromosome 1 where the occurrence of marker, TG224, which is linked to the *S* locus (Bernacchi and Tanksley 1997), was skewed towards *SL* while the more distal region was skewed in favor of *SH* alleles.

Botrytis cinerea resistance in the F_2 population

To assess the quantitative level of susceptibility to *B. cinerea* for each F_2 genotype, eight independent disease assays were performed resulting in phenotypic data for 172 of the 174 F_2 individuals. Two traits were evaluated for each individual genotype: disease incidence (DI), expressed as the proportion of inoculated stem pieces that were successfully infected and lesion growth (LG), expressed as the mean growth (mm/day) of *B. cinerea* lesions of infected stem pieces.

Between experiments, the mean DI varied between 13 and 52%, while the mean LG varied from 6.2 to 8.4 mm/day (Table 1). Experiments with a low mean DI will bias the analysis of our data since relatively many genotypes will have a DI of 0%. In experiments 3 and 4, 61 and 49% of the genotypes, respectively showed a DI of 0%. The data were analyzed using data of all experiments and excluding experiments 3 and 4.

Table 1 Mean disease incidence (DI) and mean lesion growth (LG) given as an estimation for the disease progress in each experiment

Experiment	DI (%)	n^a	LG (mm/day)	n^b
1	32	170	6.2	115
2	34	153	7.7	114
3	13	148	8.2	53
4	18	109	6.5	57
5	47	53	7.2	44
6	36	135	7.6	109
7	52	152	8.4	129
8	21	142	6.5	80
Estimated mean[c]	37	172	7.4	172

[a] Number of individuals that were tested in this experiment

[b] Number of individuals that had at least one outgrowing lesion

[c] Estimated from data of experiments 1–2 and 5–8. Data from experiments 3 to 4 were excluded for reasons explained in the text

Including or excluding data from experiments 3 and 4 only influenced the significance of the QTLs, described in the next section, but did not lead to a different interpretation of the data.

The mean DI of the susceptible control *SL* was 60% and the mean LG was 9.1 mm/day. Within the F_2 population the mean DI of individual genotypes varied

between 5 and 86% and the mean LG varied between 2.6 and 12.3 mm/day. No correlation was observed between both traits ($r = 0.173$, $P > 0.05$).

We obtained an estimate of DI and LG for each genotype based on six determinations. The continuous distribution of DI and LG suggests that resistance to *B. cinerea* is quantitatively inherited and likely to be controlled by more than one gene.

QTL mapping in the F_2 population

Using a multiple QTL mapping approach (MQM), two QTLs were identified, for which the LOD score exceeded the genome-wide confidence threshold of 3.5 ($P < 0.05$). The first QTL, located on Chromosome 1, reduced LG by 1.7 mm/day (LOD score of 4.3) and it explained 12% of the total phenotypic variation (Table 2, Fig. 1). This QTL was named *Rbcq*1 (Resistance to *B. cinerea* QTL on Chromosome 1). The second QTL, located on Chromosome 2, reduced DI by 22% (LOD score of 6.5) and it explained 15% of the total phenotypic variation. This QTL will be referred to as *Rbcq*2. In addition, the analyses suggested the presence of a third putative QTL, *Rbcq*4, on Chromosome 4 (LOD score 3.0) just below the genome-wide significance threshold. This QTL was confirmed in a later stage of this study and was included as a co-factor in the MQM analysis. Alleles from *Rbcq*1 and *Rbcq*2 show additive effects and the increased resistance originated, as expected, from the *SH* alleles. QTL analyses were also done on data of independent experiments. Both QTLs *Rbcq*1 and *Rbcq*2 could be detected in data of experiments 1 and 2, separately, but the LOD peak did not reach the significance threshold of 3.5.

Confirmation of QTLs

To confirm the QTLs *Rbcq*1 and *Rbcq*2, two BC_2 genotypes were selected which were heterozygous for *Rbcq*1 or *Rbcq*2. After selfing, one BC_2S_1 progeny segregated for *Rbcq*1 (BC_2S_1a, $n = 47$) and the other segregated for *Rbcq*2 (BC_2S_1b, $n = 60$). Both segregating populations were screened for susceptibility to *B. cinerea* in four replications. AFLP, CAPS and SCAR markers in the regions covering *Rbcq*1 and *Rbcq*2 were determined. Analyses of the two BC_2S_1 populations identified a significant variance between the ten boxes ($P < 0.001$ for LG and $P = 0.007$ for DI), therefore box was included in the GLM model. The mean DI during these tests was 58%, which is higher than compared to the DI of 37% observed during analysis of the F_2 population. This can be explained by assuming that additional resistance factors originating from *SH*, which were present in the F_2 population, are lacking in the BC_2S_1 genotypes. The mean DI of *SL* in these experiments was 68% while the mean LG was 6.1 mm/day. The resistant parent *SH* showed a mean DI of 37% and a mean LG of 2.2 mm/day. In population BC_2S_1a, homozygous presence of the resistance allele of *Rbcq*1 reduced LG by 0.92 mm/day (Table 2), which explains 24% of the parental variation. In the second population, BC_2S_1b, homozygous presence of the resistance allele of *Rbcq*2 reduced DI by 10%, which explained 32% of the parental variation. According to a Kruskal–Wallis test, reduction of both LG and DI were not significantly different at the $P < 0.05$ level. Nevertheless, the observed effect and magnitude of each effect confirmed the identified QTLs (Table 2).

Table 2 Summary of QTLs identified for DI and LG in multiple populations

Locus	Population	Trait	*n*	*SL*[a]	*n*[b]	H	*n*	*SH*	*n*	Significance	% explained
*Rbcq*1	F_2	LG[c]	174	8.26	(13)	7.51	(73)	6.56	(76)	LOD 4.3	12
	BC_2S_1a	LG	47	5.63	(6)	4.94	(12)	4.71	(29)	NS[d]	
	BC_2S_1b	LG	60							Not present	
*Rbcq*2	F_2	DI[e]	174	54	(25)	39	(81)	32	(50)	LOD 6.5	15
	BC_2S_1a	DI	47							Not present	
	BC_2S_1b	DI	60	61	(12)	62	(25)	51	(14)	$P < 0.1$	
*Rbcq*4	F_2	DI	174	49	(43)	39	(84)	37	(35)	LOD 3.0	7
	BC_2S_1a	DI	47	66	(7)	71	(30)	50	(9)	$P < 0.01$	
	BC_2S_1b	DI	60	61	(20)	56	(34)	63	(5)	NS	

[a] *SL* denotes homozygous for the *S. lycopersicum* allele, *SH* homozygous for the *S. habrochaites* LYC4 allele and H is used to describe heterozygous classes

[b] Number of observations for the linked co-dominant marker

[c] Expressed in mm/day

[d] Not significant

[e] Expressed in % infected pieces

Identification of an additional locus for disease incidence

To examine whether additional loci could be identified that contribute to resistance to *B. cinerea*, each BC_2S_1 genotype was AFLP fingerprinted with the same 10 AFLP primer combinations as used for fingerprinting the F_2 population. A Kruskal–Wallis test showed a significant effect ($P < 0.01$) on Chromosome 4 reducing DI in the population BC_2S_1a. Interestingly, this locus, *Rbcq*4, was already identified as a putative QTL in the F_2 population. *Rbcq*4 also segregated in the population BC_2S_1b but a contribution to a lower DI could not be observed in this population. Additional co-dominant CAPS and SCAR markers were determined in this region to allow a better calculation of the size of the effect of this locus (Table 2).

In order to determine if the combined effect of *Rbcq*2 and *Rbcq*4 would give a further decrease in susceptibility, mean values for the nine possible genotypic classes in both the F_2 and BC_2S_1b populations were calculated (Table 3) using the nearest co-dominant CAPS markers. No effect of *Rbcq*4 was detected in the population BC_2S_1b even though an effect of *Rbcq*2 and *Rbcq*4 was seen in the F_2 population. When both resistance alleles of *Rbcq*2 and *Rbcq*4 are absent, a mean DI of 68% was observed. The homozygous presence of either *Rbcq*2 or *Rbcq*4 resistance alleles reduced DI to 39 or 29% respectively while the homozygous combination of both *Rbcq*2 and *Rbcq*4 resistance alleles reduced DI to 20%. These results show that resistance alleles of both *Rbcq*2 and *Rbcq*4 reduce DI and that the effect of *Rbcq*2 is larger than the effect of *Rbcq*4. The combination of both *Rbcq*2 and *Rbcq*4 resistance alleles shows a less-than-additive epistatic interaction. The F_2 data showed that the gene action of *Rbcq*4 is partially dominant. Orthogonal contrast analysis testing for additive-by-additive, additive-by-dominant, dominant-by-additive and dominant-by-dominant epistatic interactions between *Rbcq*2 and *Rbcq*4 detected a significant additive (*Rbcq*2) by dominant (*Rbcq*4) epistatic interaction ($P = 0.047$; Table 4).

Discussion

An F_2 population was created of the cross between *S. lycopersicum* × *S. habrochaites* LYC4 and a genetic

Table 3 Mean values for disease incidence of the populations segregating for both *Rbcq*2 and *Rbcq*4

QTL on Chromosome 4 (Rbcq*4)*							
		F_2			BC_2S_1		
		SL[a]	H	*SH*	*SL*	H	*SH*
QTL on Chromosome 2 (*Rbcq*2)	*SL*[a]	68% (3)[b]	42% (9)	39% (4)	67% (4)	60% (13)	56% (2)
	H	48% (16)	34% (28)	37% (6)	65% (9)	57% (15)	75% (2)
	SH	29% (8)	34% (15)	20% (12)	53% (7)	49% (6)	51% (1)

[a] *SL* denotes homozygous for the alleles of *S. lycopersicum*, *SH* homozygous for the alleles of *S. habrochaites* LYC4 and H is used to describe heterozygous classes

[b] Number of individuals within each genotypic class

Table 4 Orthogonal contrast codes used to determine gene action and type of epistasis of the DI QTLs *Rbcq*2 and *Rbcq*4 in the F_2 dataset

Contrast[a]	$SH_2SH_4^b$	SH_2H_4	SH_2SL_4	H_2SH_4	H_2H_4	H_2SL_4	SL_2SH_4	SL_2H_4	SL_2SL_4	*P*-value[c]
A *Rbcq*2	−1	−1	−1	0	0	0	1	1	1	0.003
D *Rbcq*2	1	1	1	−2	−2	−2	1	1	1	0.803
A *Rbcq*4	−1	0	1	−1	0	1	−1	0	1	0.015
D *Rbcq*4	1	−2	1	1	−2	1	1	−2	1	0.337
$A_2 \times A_4$	1	0	−1	0	0	0	−1	0	1	0.193
$A_2 \times D_4$	−1	2	−1	0	0	0	1	−2	1	0.047
$D_2 \times A_4$	−1	0	1	2	0	−2	−1	0	1	0.541
$D_2 \times D_4$	1	−2	1	−2	4	−2	1	−2	1	0.318

[a] A indicates additive gene action; D indicates dominant gene action; subscripts denote the QTL *Rbcq*2 (2) and *Rbcq*4 (4)

[b] *SL* denotes homozygous for the alleles of *S. lycopersicum*, *SH* homozygous for the alleles of *S. habrochaites* LYC4 and H is used to describe heterozygous classes; subscripts denote the QTL *Rbcq*2 (2) and *Rbcq*4 (4)

[c] Data, excluding experiment 3 and 4, was used for orthogonal contrast analysis and test significances are reported for the test which does not assume equal variances

linkage map was constructed spanning 1,078 cM of the genome. This length is shorter than the 1,200–1,400 cM reported for other interspecific *S. lycopersicum* maps (Foolad et al. 2002; Haanstra et al. 1999; Tanksley et al. 1992). Although *Pst*I/*Mse*I derived AFLP markers have a better distribution over the genome compared to *Eco*RI/*Mse*I AFLP makers (Haanstra et al. 1999), the use of additional CAPS markers revealed that not all telomeric regions were covered by AFLP-markers. Nonetheless the map covers at least 85% of the genome. Skewness of loci was detected on several chromosomes and was often in favor of the presence of *SH* alleles. The most extreme skewness was observed on Chromosome 1 in the proximity of a region where a locus for *S*-RNAse based unilateral incompatibility has been described (Bernacchi and Tanksley 1997) and it was skewed towards homozygous *SL*. Another region on Chromosome 1 was skewed towards *SH*; QTLs have been described in this region affecting hybrid incompatibility in *S. habrochaites* LA1777 (Moyle and Graham 2005). Skewness has been described in multiple studies and is generally believed to be related to the presence of genes involved in reproductive behavior such as pollen, gamete and (or) zygote viability (Fulton et al. 1997; Monforte and Tanksley 2000; Paran et al. 1995). Skewness may result in failure to detect QTLs or misinterpretation of the effect of a QTL.

Quantitative resistance to *B. cinerea* was observed in the F_2 population between *SL* × *SH* and two loci were detected: *Rbcq*1 and *Rbcq*2. Both QTLs were validated in BC_2S_1 populations each segregating for *Rbcq*1 or *Rbcq*2 separately. In both populations, the expected decrease in resistance caused by *Rbcq*1 and *Rbcq*2 were observed (Table 2). The criteria for significance were not met due to the small population sizes ($n = 60$ and 47) and skewness of segregation. Identification of the third locus *Rbcq*4 in the BC_2S_1 population aimed at confirming *Rbcq*1 suggested that certain QTLs were not detected in the original F_2 mapping population. The experimental variation in the bioassay as well as epistatic interactions, such as shown between *Rbcq*2 and *Rbcq*4 and genotype × environment (G × E) interactions allow only the identification of major QTLs. Transgressive segregation for DI and LG, beyond the level of the susceptible control *SL*, was observed in the F_2 population showing the presence of at least one unidentified QTL contributing to resistance in the susceptible parent.

The bioassay for measuring susceptibility of tomato to *B. cinerea* (ten Have et al. 2007) has proven to be a valuable tool although the test remains difficult to standardize. While the bioassay itself is performed under standardized lab conditions, the environment in which the plants are grown can vary (e.g. day length, hours of sun and temperature), affecting their physiological condition and presumably their susceptibility in a quantitative manner. Data analysis of the single F_2 experiments never revealed the presence of a significant QTL, mainly due to the limited levels of observation (only six stem pieces were inoculated per genotype per experiment). The identification of significant QTLs in all cases required combining data of multiple independent experiments.

The mechanisms underlying the increased resistance conferred by the QTLs remain to be resolved. *B. cinerea* is a necrotroph that relies primarily on three principles for subduing its host plants, i.e. the ability to kill host cells, the ability to decompose plant tissue and the ability to counteract plant defense responses (reviewed by van Kan 2006). In order to kill host cells, the fungus is able to produce multiple phytotoxic metabolites, such as botrydial and/or botcinolide (Reino et al. 2004), which may be important for virulence of certain *B. cinerea* strains (Siewers et al. 2005). Moreover, *B. cinerea* contains at least three genes encoding phytotoxic proteins (Chagué et al. 2006; Staats et al. 2006), which are expressed during infection (Chagué et al. 2006; Schouten and van Kan Unpublished). It could be envisaged that QTLs that reduce *B. cinerea* disease incidence confer a reduced sensitivity or detoxify either of these phytotoxic factors, but this remains to be determined. The second important virulence trait for the pathogen is its ability to decompose plant tissue by a spectrum of plant cell wall-degrading enzymes (Kars and van Kan 2004; ten Have et al. 2002). QTLs that reduce *B. cinerea* lesion growth rate might be related to slower plant tissue decomposition, possibly caused by altered cell wall composition or architecture. Thirdly, the pathogen must be able to counteract the action of antifungal plant metabolites. *S. lycopersicum* produces one major phytoanticipin named α-tomatin and a spectrum of sesquiterpene phytoalexins including rishitin (Grayer and Kokubun 2001; Hammerschmidt 1999; Kuć 1995). Enzymatic detoxification of α-tomatin was shown to be an important virulence trait (Quidde et al. 1998). The QTLs identified in this study might be involved in conferring higher levels of antifungal plant metabolites, or in the production of slightly different metabolites, which cannot efficiently be counteracted by *B. cinerea*. Future studies should identify the gene(s) and unravel the mechanisms that underlie the enhanced resistance levels conferred by *Rbcq*1, *Rbcq*2 and *Rbcq*4.

Regardless of the mechanisms involved, the QTLs identified in this study provide an excellent source of resistance that can be introgressed into commercial

cultivars. Even though *Rbcq*1, *Rbcq*2 and *Rbcq*4 do not confer full resistance, it should be considered that the disease pressure in the bioassay was extremely favorable for the pathogen. Disease pressure in commercial greenhouses is lower and environmental conditions are less favorable for pathogen invasion. Thus the partial resistance conferred by the identified QTLs may in practice be sufficient to efficiently protect tomato against *B. cinerea*. Although *Rbcq*4 is not the QTL showing the largest reduction, its partial dominance, as observed in the F_2 dataset, makes it an interesting candidate for breeding resistance into F_1 hybrids, since introgression in only one parent is needed. We realize that our F_2 population is relatively small and that due to skewness in segregation, we have under-representation of several classes and therefore a balanced design will be needed to detect QTLs for resistance to *B. cinerea* to its full extent (de Vicente and Tanksley 1993). For identifying additional QTLs we have pursued to develop a complete introgression line (IL) population, offering multiple advantages. Firstly, the phenotypic variation between an IL and the control cultivar is directly associated with the introgressed segment. Secondly, each IL is homozygous allowing multiple testing. Thirdly, negative epistatic effects caused by other regions of the wild genome that may obscure a QTL are less likely to occur (Zamir 2001), allowing a more reliable assessment of quantitative disease resistance to *B. cinerea*. A complete IL population of *S. habrochaites* LYC4 in the genetic background of *SL* is under construction.

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