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Quantitative resistance to *Botrytis cinerea* from *Solanum neorickii*

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Abstract Tomato (*Solanum lycopersicum*) is susceptible to gray mold (*Botrytis cinerea*). Quantitative resistance to *B. cinerea* was previously identified in a wild relative, *S. neorickii* G1.1601. The 122 F_3 families derived from a cross between the susceptible *S. lycopersicum* cv. Moneymaker and the partially resistant *S. neorickii* G1.1601 were tested for susceptibility to *B. cinerea* using a stem bioassay. Three putative quantitative trait loci (pQTL) were detected: pQTL3 and pQTL9 reducing lesion growth (LG) and pQTL4 reducing disease incidence (DI). For each pQTL, a putative homologous locus was identified recently in another wild tomato relative, *S. habrochaites* LYC4. pQTL3 was confirmed by assessing disease resistance in BC_3S_1 and BC_3S_2 progenies of *S. neorickii* G1.1601. pQTL4 was not statistically confirmed but the presence of the *S. neorickii* resistance allele reduced DI in all three tested populations. The reduction in LG of pQTL9 was not confirmed but rather, this locus conferred a reduced DI, similar to observations in the QTL study using *S. habrochaites.* The results are discussed in relation to other disease resistance loci identified in studies with other wild tomato relatives.

Keywords *Botrytis cinerea* · Gray mold · Quantitative trait locus (QTL) · *S. neorickii* · Marker assisted selection (MAS)

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Introduction

Botrytis cinerea [teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel] is a necrotrophic fungus with a wide host range (Jarvis 1977; Elad et al. 2004). Modern hybrid tomato (*Solanum lycopersicum*) cultivars are susceptible to *B. cinerea* although some cultivars show a certain level of quantitative resistance (ten Have et al. 2007). The presumed polygenic inheritance has limited the success of breeding for resistance to *B. cinerea*.

Quantitative resistance to *B. cinerea* has been identified in several wild relatives of *S. lycopersicum* (Urbasch 1986; Egashira et al. 2000; Nicot et al.

2002; ten Have et al. 2007). A stem bioassay suitable to quantify susceptibility of tomato to *B. cinerea* was used to screen a collection of wild tomato accessions (ten Have et al. 2007), and two parameters were calculated: the proportion of outgrowing lesions or disease incidence (DI) and lesion growth (LG) rate expressed as the increase in lesion size in mm/day. All four tested accessions of *S. habrochaites* showed quantitative resistance (ten Have et al. 2007). *S. habrochaites* LYC4 was used previously to study the genetic basis of this resistance (Finkers et al. 2007a, b) and a total of ten quantitative trait loci (QTL) were identified illustrating the genetic complexity of resistance to *B. cinerea*. Also *S. neorickii* G1.1601 showed a certain level of resistance (ten Have et al. 2007). A F_2 mapping population of *S. lycopersicum* cv. Moneymaker × *S. neorickii* G1.1601, previously developed to identify QTLs for resistance to *Oidium neolycopersici* (Bai et al. 2003), was screened for susceptibility to *B. cinerea*. Since F_2 seeds were no longer available, F_3 families were used for the analysis. Segregating BC_3S_1 families and BC_3S_2 plants were generated in order to confirm the effects identified in the F_3 analysis. We report the identification of (putative) QTLs, from *S. neorickii* G1.1601 involved in resistance to *B. cinerea*. Results of this study were compared to previously identified QTLs for resistance to *B. cinerea* in *S. habrochaites* LYC4 (Finkers et al. 2007a, b).

Materials and methods

Plant material

Three tomato accessions were used in this study: *S. lycopersicum* cv. Moneymaker (hereafter referred to as *SL*), *S. neorickii* G1.1601 (hereafter referred to as *SN*) and *S. habrochaites* LYC4 (hereafter referred to as *SH*). A cross between *SL* and *SN* was made and an F_2 population (n = 209) developed as described in detail by Bai et al. (2003). From this F_2 population, F_3 seeds of each genotype were collected, but only 122 F_2 plants produced enough F_3 seeds for further study. Marker data for 75 of the 122 F_2 plants used in this study were available.

For confirmation of the QTLs, three selected F_3 plants were backcrossed to *SL* to obtain BC_1 seeds. Two subsequent backcrosses to *SL* resulted in a BC_3 progeny of 53 plants and marker assisted selection (MAS) was used to select three plants containing either QTL. Three BC_3S_1 families (n = 86 each) were genotyped to select plants homozygous *SL* or *SN* for the putative QTLs. A selected set of BC_3S_1 genotypes homozygous *SN* for the region of interest was grown to produce BC_3S_2 seeds.

Experimental setup and stem assay

For each of the 122 F_3 families, five seedlings were grown and their susceptibility to *B. cinerea* was evaluated. For logistic reasons the disease assays were divided (at random) into 13 experiments with equal numbers of plants (50 plants/week). Eight *SL* controls were included in each experiment.

For the BC_3S_1 families, six replicates were grown by taking cuttings of each genotype including a set of *SL*, *SN*, and *SH* controls. To assess susceptibility to *B. cinerea* in the BC_3S_2 plants, two experiments were performed. In each experiment, three replicates, grown from seeds, of each genotype was tested.

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 five cm length and the top of each segment was inoculated with a droplet of 5 µl inoculum, containing $\sim 10^6$ conidia per ml. Inoculum of *B. cinerea* strain B05.10 was prepared according to Benito et al. (1998). Incubations were performed at 15°C in the dark at 100% relative humidity. 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 (DI). The LG rate was calculated as the increase in lesion size between day four and five (mm/day) for the infected stem pieces.

DNA isolation and marker analysis

Twelve plants of each F_3 family were grown and one leaf was harvested from each plant and pooled for DNA isolation in order to deduce the original F_2 genotype. The AFLP™ and CAPS analysis of the F_3, BC_3, and BC_3S_1 populations were performed as described previously (Finkers et al. 2007a, b). The following ten AFLP primer combinations were used for genotyping: P14M48, P14M49, P14M50,

P14M60, P14M61, P15M48, P18M50, P18M51, P22M50, and P22M51. AFLP primer nomenclature and adapter sequences have been described previously by Bai et al. (2003).

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 *SN* were determined using the CAPS digestion approach described by Bai et al. (2004). Markers, PCR conditions and restriction endonucleases used for genotyping are presented in Table 1.

Data analysis

Marker data were analyzed and a genetic linkage map was calculated with Joinmap® 3.0 (van Ooijen and Voorrips 2001). The susceptibility of the F_2 genotype was estimated by taking the means of the replicated disease assays of five F_3 plants. Phenotypic data of the BC_3S_1 and BC_3S_2 plants were analyzed using the general linear model (GLM) approach as implemented in SPSS 12.0 (SPSS Inc., Chicago, IL, USA). The minimal adequate models for DI and LG were determined independently in the data of the BC_3S_1 families and BC_3S_2 plants. This resulted in the

Table 1 Primer sequences, lengths of PCR products and enzymes revealing a polymorphism for CAPS/SCAR markers

Marker name	Chromosome	Primer sequence (5′-3′)	Observed PRC product length (bp)	Annealing temperature (°C)	Marker type	Enzyme	Source[a]
TG40	3	GCGAGCTCGAATTCAATTCCAAC CGGGATTTTAGTTTTTCCGATCC	450	55	CAPS	*Alu*I	PBR
TG599	3	GCATGCCTGCAGAGTGGTC ATTCGCTACCTTGAGGGCTG	350	65	CAPS	*Bsp*LI	PBR
TG549	3	ATGGAGAGAAGCTGGAACAC TTCTTAGAGCCCACCAGCAC	400	55	CAPS	*Mse*I	PBR
T1143	3	GGAGAATGGGCATCTACAAA CCTTTAGGATGGATTCCG	*SN*: 1580 + 1050/ *SL*: 1000	55	SCAR		PBR
T0707	4	TCGTGGATTATGGGCTTCTT GGTAAGGCTGCAACACATCA	560	55	CAPS	*Dde*I	PBR
TG339	4	GAAACCTTACCCCTCTA CGCTGTTTCTTGCCATTT	500	46	CAPS	*Hinf*I	PBR
TG272	4	GATTTTGCCCCCTCTACCA ACATCTTTTCCTTCCCTCTGC	352	55	CAPS	*Hinf*I	PBR
TG264	4	GGAACAGGTCAGGACAGCAT TGGCTAACTGACGAAGACGA	520	55	CAPS	*Mnl*I	PBR
T1405	4	CACCAACAACTAGCCCTTGA AAGCAATTCCTCCAGCTTCA	535	55	CAPS	*Bsa*JI	SGN
TG555	4	AATTCGGAGCTCACTGCTTC AGTACGGCATGCTTGCTATC	430	55	CAPS	*Hpy*CH4IV	PBR
TG10	9	ATGATATCCACACCCCTGGA ATGCCTCGAAATTCAAATGC	587	55	CAPS	*Hae*III	PBR
Tm2a	9	AGCGTCACTCCATACTTGGAATAA AGCGTCACTCAAAATGTACCCAAA	1600	53	CAPS	*Acc*I	Sobir et al. (2000)
TG551	9	CAACGAAAACCTTGGCACTC GAGATGAGCAGCATATGGAG	350	55	CAPS	*Hpy*CH4IV	PBR

[a] PBR: developed at Wageningen University laboratory of Plant Breeding, mainly using data from SGN. SGN: primers published in the SGN database (http://www.sgn.cornell.edu) or published previously and references are given

following models: DI = constant + genotype + block and LG = constant + genotype + block + genotype × block.

Quantitative trait locus analysis of the F_3 and BC_3S_1 populations were performed using the Kruskal–Wallis analysis as embedded in MapQTL® 5.0 (van Ooijen 2003). Data of the grouped BC_3S_2 plants were analyzed by comparing mean observations of each group to the mean observation of *SL* using a Dunnett test (Dunnett 1955). A probability of $P < 0.05$ was used to refer to a QTL as significant. Linkage maps were drawn using MapChart (Voorrips 2002). The correlation between traits was examined by interpreting Pearson correlation coefficients.

Results

Analysis of the F_3 lines

F_3 seeds of the cross between the susceptible cultivar *S. lycopersicum* cv. Moneymaker (*SL*) and *S. neorickii* G1.1601 (*SN*) were available (Bai et al. 2003) in sufficient quantity for 122 of the original 209 F_2 plants. For 75 of these 122 F_2 individuals, marker data were available; therefore we decided to (re) genotype all 122 F_3 families. Ten AFLP primer combinations resulted in a total of 234 markers: 120 *SL* specific and 114 *SN* specific. 192 AFLP markers were placed on the paternal and maternal linkage maps (data not shown).

A quantitative *B. cinerea* disease assay on stem segments (ten Have et al. 2007) yielded data on DI and LG. The frequency distributions of both traits suggested normal, quantitative trait characteristics (data not shown). The susceptible control, *SL*, showed a DI and LG comparable to previous experiments (Table 2; Finkers et al. 2007a). Kruskal–Wallis (KW) analysis identified three linkage groups putatively containing a QTL (pQTL) for decreased susceptibility to *B. cinerea*. Based on the map of Bai et al. (2003) these linkage groups could be assigned to Chromosomes 3, 4, and 9 and the pQTLs will be referred to as pQTL3, pQTL4, and pQTL9 accordingly. To integrate the maternal and paternal linkage groups of Chromosome 3, 4, and 9, thirteen co-dominant CAPS markers were developed (Table 1). Using the integrated linkage maps, the effect of each pQTL was recalculated using KW analysis (Table 3). All three pQTL regions showed a skewed segregation resulting in a deficit of plants homozygous *SL* for pQTL3 and pQTL4 and a deficit of plants homozygous *SN* for pQTL9 (Table 3). In spite of the correlation between DI and LG (Pearson: $r = 0.258$ and $P < 0.01$), all pQTLs were associated with a single trait: pQTL3 and pQTL9 conferred a reduced LG while pQTL4 conferred a reduced DI.

Confirmation of the QTLs using BC_3S_1 and BC_3S_2 plants

Fifty-three BC_3 plants were genotyped using AFLP and three BC_3 plants were selected heterozygous for the alleles of either pQTL3, pQTL4 or pQTL9 in a genetic background as similar as possible to the recurrent parent *SL* (Table 4). Only one BC_3 plant was identified containing the *SN* allele of pQTL9. However, this plant was also heterozygous for the pQTL3 and pQTL4 alleles. Three BC_3S_1 families were grown and MAS was used to obtain a more balanced test design (Table 4). In 3 of the 20 plants homozygous for the *SN* allele of pQTL9, *SN* alleles of pQTL3 and pQTL4 were absent. As a result, experiments aimed at confirming pQTL9 were only performed using BC_3S_2 lines.

While assessing susceptibility to *B. cinerea* in the BC_3S_1 families, only the first experiment showed a sufficient level of infection (mean DI of 63%;

Table 2 Mean disease incidence (DI) and lesion growth (LG) of the controls

Population	*SL*			*SN*			*SH*		
	n[a]	DI	LG	*n*	DI	LG	*n*	DI	LG
F_3	104	70 ± 7	6.9 ± 0.3						
BC_3S_1	14	70 ± 7	6.6 ± 0.3	7	71 ± 9	5.1 ± 0.3	7	45 ± 9	4.8 ± 0.4
BC_3S_2	57	55 ± 3	5.3 ± 0.2	11	36 ± 7	5.1 ± 0.4	4	16 ± 12	1.8 ± 0.9

[a] Number of plants tested

Table 3 Effect of the pQTLs identified in the F_3 population

Marker	QTL	DI (%)				LG (mm/day)			
		SL[a]	*h*	*SN*	*P*[b]	*SL*	*h*	*SN*	*P*
TG599	pQTL3-LG	51 (11)	50 (49)	45 (33)	0.410	5.6 (11)	5.4 (49)	5.0 (33)	0.063
TG339	pQTL4-DI	59 (13)	47 (31)	47 (47)	0.092	5.7 (13)	5.0 (31)	5.3 (47)	0.188
TG551	pQTL9-LG	47 (31)	50 (53)	49 (19)	0.643	5.6 (31)	5.2 (53)	5.0 (19)	0.022

Mean values for disease incidence (DI) and lesion growth (LG) are presented, along with their significances as determined using a Kruskal–Wallis test

[a] *SL* denotes homozygous for the alleles of *S. lycopersicum*, *SN* homozygous for the alleles of *S. neorickii*, and *h* describes the heterozygous class. The number of observations within each class is indicated between parentheses

[b] Significance

Table 4 Description of the three BC_3S_1 families

QTL	Trait	Additional in BC_3 plants	Segregation of the BC_3S_1 families[a]	Number of BC_3S_1 plants evaluated[a, b]
pQTL3	LG	Parts C2 and C6	21:33:16	17:17:16
pQTL4	DI	Parts C1 and C12	21:40:25	17:17:15
pQTL9	LG	pQTL3, pQTL4 and part C12	25:41:20	20:10:20

Each pQTL and the additional introgressions for each family are described. Initially, 86 BC_3S_1 plants were grown for each family. After marker assisted selection (MAS), 50 plants were selected to be evaluated for susceptibility to *B. cinerea*

[a] Segregation ratio: homozygous *SL*: heterozygous: homozygous *SN*

[b] Plants, homozygous *SN* were grown to produce BC_3S_2 seeds

observed 4 days post inoculation). Replicate experiments in subsequent weeks showed a low level of infection (mean DI < 20%) and were therefore discarded. In the first assay, the control *SL* showed the expected level of susceptibility (Table 2; Finkers et al. 2007a). However, the susceptibility of the controls *SN* and *S. habrochaites* LYC4 (*SH*) was higher than previously reported (Finkers et al. 2007a; ten Have et al. 2007) suggesting an overall high disease pressure in this experiment.

Susceptibility to *B. cinerea* in BC_3S_2 lines was assessed in two independent experiments. The mean DI of the two experiments was 29 and 62%, respectively, and yielded, on average, five independent observations for each BC_3S_2 line. The susceptible control *SL* and the partial resistant control *SH* showed the expected level of susceptibility (Table 2) but *SN* was more susceptible than previously reported (ten Have et al. 2007).

Experiments aimed at confirming pQTL3, in the BC_3S_1 population, did not lead to identification of a significant reduction of LG, yet the presence of the homozygous *SN* resistance allele resulted in a reduced LG (Table 5; $P = 0.273$). A significant reduction was observed while testing the BC_3S_2 lines (Table 6; group baa; $P < 0.001$). pQTL4, reducing DI, could not significantly be confirmed using either BC_3S_1 (Table 5; $P = 0.413$) or BC_3S_2 lines (Table 6; group aba; $P = 0.645$). However, in both BC_3S_1 population and BC_3S_2 lines, a reduction in DI was observed in the presence of the *SN* resistance allele. The LG reducing effect of pQTL9 was not confirmed in the BC_3S_2 lines (Table 6; group aab; $P = 1.000$). Instead, a 9% reduction in DI was observed for lines homozygous for the *SN* allele of pQTL9 ($P = 0.665$).

In addition, BC_3S_2 lines were tested in which a combination of loci were present (Table 6). Two plants were homozygous for the *SN* alleles of pQTL4 and pQTL9 and showed a lower DI than plants containing either *SN* alleles of pQTL4 or pQTL9. The 22% lower DI than *SL* of these plants was, however, not significant (group abb; $P = 0.056$). Plants containing a combination of the *SN* alleles of pQTL3 (higher DI and lower LG compared to *SL*) and pQTL9 (lower DI and similar LG compared to *SL*; group bab) were as susceptible as *SL*. However, the observed mean was not deviating from the mean estimated from lines containing each QTL separately.

Table 5 Effect of pQTL3 or pQTL4 in its respective BC_3S_1 family

Marker	QTL	DI (%)				LG (mm/day)			
		SL[a]	*h*	*SN*	*P*[b]	*SL*	*h*	*SN*	*P*
TG599	pQTL3-LG	69 (16)	67 (17)	72 (16)	0.734	5.0 (16)	5.2 (17)	4.3 (16)	0.273
TG339	pQTL4-DI	68 (17)	52 (17)	58 (15)	0.413	6.1 (17)	6.2 (17)	6.4 (15)	0.549

Mean values for disease incidence (DI) and lesion growth (LG) are presented

[a] *SL* denotes homozygous for the alleles of *S. lycopersicum*, *SN* homozygous for the alleles of *S. neorickii*, and h describes the heterozygous class. The number of observations within each class is indicated between parentheses

[b] Significance

Table 6 Estimated mean values for disease incidence (*DI*) and lesion growth (*LG*) of the BC_3S_2 lines

QTL genotype[a]	*n*[b]	Mean	DI (%)[c]	SE	Mean	LG (mm/day)	SE
aab	3	46	0.665	7	5.8	1.000	0.28
aba	8	48	0.645	5	4.9	0.104	0.19
abb	2	33	0.056	8	5.2	0.989	0.38
baa	13	62	1.000	4	4.5	<0.001	0.16
bab	1	61	1.000	11	5.0	0.906	0.38
SN	11	36	0.095	7	5.1	0.946	0.35
SH	4	16	0.012	12	1.8	0.001	0.91
SL	57	55		3	5.3		0.19

Means of each line/trait were compared to the mean of *S. lycopersicum* cv. Moneymaker (*SL*) using a Dunnett test

[a] aab = QTL genotype at Chromosomes 3, 4, and 9, a = Homozygous *SL*, and b = homozygous *SN*

[b] *n* = Number of lines/BC_3S_2 genotype tested

[c] Significance

Discussion

While performing the *B. cinerea* stem assays, susceptibility of the controls varied between experiments (Table 2). In experiments with a harsher infection, quantitative resistance of *SN* is less robust than the quantitative resistance of *SH*. The higher resistance of *SH* suggests the presence of a larger number of QTLs, or more effective QTLs. Stem morphology and vascular development of *SH* stems might also play a role in resistance (Coaker et al. 2002). Variation in the bioassays and environment influences the confirmation of QTLs for resistance to *B. cinerea* (Finkers et al. 2007a). External influences can be minimized by growing plants in climate rooms but this is logistically not feasible for experiments on this scale.

A correlation between DI and LG was observed in data of the F_3 families ($r = 0.258$; $P < 0.01$), but no significant correlation was observed in the *SH* F_2 population ($r = 0.173$; $P > 0.05$; Finkers et al. 2007a). These correlations are lower than the correlation observed while testing the *SH* IL population ($r = 0.65$; $P < 0.01$; Finkers et al. 2007b). The heterogeneous genetic background of F_2 and F_3 plants and/or the type of bioassay used may obscure the obvious relationship which was observed between DI and LG in the *SH* IL population (Finkers et al. 2007b).

Initially, three pQTLs were identified while analyzing the F_3 families. The resistance allele from each pQTL was derived from *SN*. The LG reducing effect of the pQTL3 resistance allele could be confirmed using BC_3S_2 lines. Because of this, pQTL3 will now be referred to as QTL3. No significant confirmation was obtained for the DI reducing effect of the pQTL4 resistance allele. Nevertheless, the presence of the pQTL4 resistance allele reduced DI in all three tested populations. For this reason, this QTL is still regarded as interesting for commercial tomato resistance

breeding. Criteria for significance were not met due to the lack of replications (BC_3S_1 and BC_3S_2) or the lack of the number of lines tested per group (BC_3S_2). The LG reducing effect of the pQTL9 resistance allele was not confirmed, but instead we observed a decreased DI. Additional experiments are required to confirm pQTL4 and pQTL9.

The position of each *SN* QTL was compared to previously mapped QTLs, conferring resistance to *B. cinerea*, from *SH* (Finkers et al. 2007a, b). Interestingly, both pQTL4 and pQTL9 from *SN* are located at positions homologous to the *SH* QTLs *Rbcq*4a and *Rbcq*9b (Fig. 1). CAPS analysis of the *SH* introgression lines (IL) containing *Rbcq*3 (Finkers et al. 2007b) does not exclude the possibility that *SN* QTL3 is at a homologous position. We postulate that the three *SN* (p)QTLs identified in this study have QTLs at homologous positions in *SH*.

Experiments aimed at confirming the LG reducing effect of pQTL9 unexpectedly resulted in the identification of a pQTL reducing DI. Previous analysis of the *SH* IL population, resulted in identification of two QTLs on Chromosome 9: *Rbcq*9a reducing LG and *Rbcq*9b reducing DI (Finkers et al. 2007b). The region homologous to *Rbcq*9a is homozygous *SL* in this set of BC_3S_2 plants and may explain why only a reduced DI was observed. Future experiments aimed at confirming the reduction in LG should focus on testing lines containing *SN* alleles in the region homologous to *Rbcq*9a.

The *SN* pQTL9 did not confer a reduction in DI in the *SL* × *SN* F_3 population (Table 3), as was also observed for the *SH Rbcq*9b in the *SL* × *SH* F_2 population (Finkers et al. 2007a). Segregation of multiple DI-reducing loci combined with the underrepresentation of *SN* alleles for Chromosome 9 might have obscured identification of this locus. Several BC_3S_2 lines contained multiple QTLs. Two plants contained both pQTL4 and pQTL9 (group abb) and the additional reduction in DI of these lines suggests a fully additive model, showing the potential of pyramiding these two pQTLs.

Figure 1 shows a comparison between (p)QTL positions identified in this study and previously mapped QTLs conferring resistance to *B. cinerea* (Finkers et al. 2007b), mapped positions of resistance genes (R-genes), R-gene analogs mapped by Zhang et al. (2002) and QTLs conferring resistance to other diseases (Tables 7, 8). The positions of

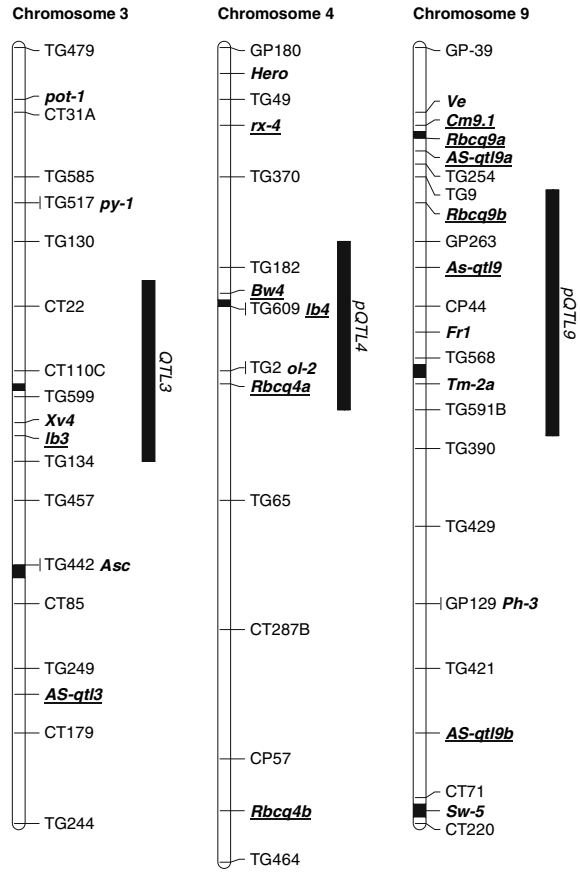

Fig. 1 Overview of resistance loci mapped on tomato Chromosomes 3, 4, and 9: markers are from the core RFLP map (Tanksley et al. 1992). *Closed blocks* within the *bars* show the approximate locations of mapped RGAs (Zhang et al. 2002). The approximate locations of monogenic resistance genes (*R genes*) and quantitative trait loci (*QTL*, *underlined*) for disease-resistance are shown. For clarity, some loci were renamed for ease of display. Explanation of abbreviations of the R genes or QTLs is presented in Tables 7 and 8, respectively. The approximate locations of (p)QTLs identified in this study are presented as *bars* on the right hand side of each chromosome

B. cinerea QTLs may be homologous with the positions of R-genes or QTLs conferring resistance to *Phytophtora infestans* and *Xanthomonas campestris* (Chromosome 3); *Ralstonia solanacearum*, *P. infestans,* and *O. neolycopersici* (Chromosome 4); *Alternaria solani*, *Fusarium oxysporum,* and TMV (Chromosome 9). It remains to be determined whether loci at homologues positions involve the same genes triggering a general defense mechanism such as papillae formation or phytoalexin production

Table 7 Qualitative resistance genes mapped on the tomato Chromosomes 3, 4, and 9

Chromosome	Locus	Pathogen	Common name	Resistance originating from[a]	Reference
3	*Asc*	*Alternaria alternata*	Black mold	*S. chilense* G1.1701, *S. chmielewskii* CPRO731089, *S. neorickii* G1.1604, *S. pennellii* LA716 and G1.1611, *L. peruvianum* G1.1860 and *S. pimpinellifolium* G1.1704	van der Biezen et al. (1995)
9	*Fr1*	*Fusarium oxysporum*	Fusarium vascular wilt	*L. peruvianum*	Vakalounakis et al. (1997)
4	*Hero*	*Globodera rostochiensis*		*S. pimpinellifolium* LA1792	Ganal et al. (1995)
4	*ol-2*	*Oidium neolycopersici*	Powdery mildew	*S. lycopersicum* var. *cerasiforme*	Ciccarese et al. (1998) and De Giovanni et al. (2004)
9	*Ph-3*	*Phytophthora infestans*	Late blight	*S. pimpinellifolium* L3708	Chunwongse et al. (2002)
3	*pot-1*	Potato virus Y	PVY	*S. habrochaites* PI247087	Legnani et al. (1995) and Parrella et al. (2002)
3	*Py-1*	*Pyrenochaeta lycopersici*	Corky root rot	*L. peruvianum*	Doganlar et al. (1998)
3	*pot-1*	Tobacco etch virus	TEV	*S. habrochaites* PI247087	Parrella et al. (2002)
9	*Tm-2a*	Tomato mosaic virus	TMV	*L. peruvianum*	Young et al. (1988)
9	*Sw-5*	Tomato spotted wilt virus	TSWV	*L. peruvianum*	Stevens et al. (1991, 1995)
9	*Ve*	*Verticillium dahliae*	Verticillium wilt	*L. peruvianum*	Zamir et al. (1993) and Diwan et al. (1999)
3	*Xv4*	*Xanthomonas campestris*	Bacterial spot	*S. pennellii* LA716	Astua-Monge et al. (2000)

[a] According to the new nomenclature *L. peruvianum* is divided 4 *Solanum* species (Peralta et al. 2005). This division is unknown for most accessions mentioned in this table

or whether the observed homologous positions are coincidental. The recessive gene *ol-2*, identified in *S. lycopersicum* var. *cerasiforme* and mapped at a position homologous to pQTL4, is involved in papillae formation (Bai et al. 2005). The observation that pQTL4 acts dominant implies that resistance to *B. cinerea* and to *O. neolycopersici* cannot be conferred by the same gene. However, it cannot be excluded that each species contains a similar ancestral gene, which has diverged into alleles conferring specificity to different pathogens. The isolation of *B. cinerea* R-genes, followed by complementation and subsequent testing of these plants for resistance to multiple pathogens, might resolve such questions.

The resistance alleles of pQTL4 and *Rbcq*4a act dominantly in reducing DI (Finkers et al. 2007a). Over-dominance of pQTL4 was observed in the BC_3S_1 population, but an independent confirmation is needed. Dominant QTLs for resistance to *B. cinerea* are advantageous in commercial F_1 hybrid cultivar development. Disease tests, using the stem assay, resulted in identification of QTLs either reducing DI or reducing LG (Finkers et al. 2007a). QTLs generally contributed to both a lower DI and LG in a greenhouse assay on mature plants (Finkers et al. 2007b). QTL3 and pQTL4 might therefore be effective in reducing both DI and LG when used in commercial breeding programs. Besides *S. habrochaites* LYC4, *S. neorickii*

Table 8 Quantitative resistance loci with at least one locus mapped on the tomato Chromosomes 3, 4 or 9

Chromosome	Locus	Pathogen	Common name	Resistance originating from	Reference
9	As-QTL9	*Alternaria solani*	Early blight	*S. arcanum* LA2157	Chaerani et al. (2006)
3 and 9	*AS-QTL3* and *AS-QTL9a&b*	*Alternaria solani*	Early blight	*S. habrochaites* PI126445	Foolad et al. (2002) and Zhang et al. (2003)
3, 4, and 9	*Rbcq3, Rbcq4a&b,* and *Rbcq9a&b*	*Botrytis cinerea*	Gray mold	*S. habrochaites* LYC4	Finkers et al. (2007a, b)
3, 4, and 9	*QTL3, pQTL4,* and *pQTL9*	*Botrytis cinerea*	Gray mold	*S. neorickii* G1.1601	This study
9	*Cm9.1*	*Clavibacter michiganensis*	Bacterial canker	*S. arcanum* LA2157	van Heusden et al. (1999)
3 and 4	*lb3* and *lb4*	*Phytophtora infestans*	Late blight	*S. habrochaites* LA2099	Brouwer et al. (2004) and Brouwer and St. Clair (2004)
4	*Bw4*	*Ralstonia solanacearum*	Bacterial wilt	*S. lycopersicum* Hawaii 7996	Thoquet et al. (1996a, b)
4	*rx-4*	*Xanthomonas campestris*	Bacterial spot	*S. lycopersicum* Hawai 7998	Yang et al. (2005)

G1.1601 is an alternative source for QTLs conferring resistance to *B. cinerea*.

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