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ORIGINAL PAPER

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QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*

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Abstract Anthracnose fruit rot is an economically important disease that affects pepper production in Indonesia. Strong resistance to two causal pathogens, *Colletotrichum gloeosporioides* and *C. capsici*, was found in an accession of *Capsicum chinense*. The inheritance of this resistance was studied in an F_2 population derived from a cross of this accession with an Indonesian hot pepper variety (*Capsicum annuum)* using a quantitative trait locus (QTL) mapping approach. In laboratory tests where ripe fruits were artificially inoculated with either *C. gloeosporioides* or *C. capsici*, three resistance-related traits were scored: the infection frequency, the true lesion diameter (averaged over all lesions that actually developed), and the overall lesion diameter (averaged over all inoculation points, including those that did not develop lesions). One main QTL was identified with highly significant and large effects on all three traits after inoculation with *C. gloeosporioides* and on true lesion diameter after inoculation with *C. capsici*. Three other QTL with smaller effects were found for overall lesion diameter and true lesion diameter after inoculation with *C. gloeosporioides*, two of which also had an effect on infection frequency. Interestingly, the resistant parent carried a susceptible allele for a QTL for all three traits that was closely linked to the main QTL. The results with *C. capsici* were based on less observations and therefore less informative. Although the main QTL was shown to have an effect on true lesion diameter after inoculation with *C. capsici*, no significant QTL were identified for overall lesion diameter or infection frequency.

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Introduction

Anthracnose fruit rot is a serious disease affecting the production of peppers in tropical and subtropical regions. It is caused by several species of the fungal genus *Colletotrichum*, of which *C. gloeosporioides* and *C. capsici* occur widely. Although these fungi can also cause lesions on the leaves and stems, the most economically important damage results from the fruit rot symptoms. Symptoms on the fruit first appear as sunken, water-soaked lesions. Later acervuli appear, and often secondary rot is caused by other fungi and bacteria. Disease control involves the frequent applications of fungicides, with negative effects on farmer income and health, particularly in developing countries. Even when these measures are applied, pre- and post-harvest anthracnose fruit rot can cause severe losses (Hartman and Wang 1992).

Our study was aimed at anthracnose resistance effective in Indonesia, where hot pepper is one of the main vegetables both in production value and in the consumer diet, and where anthracnose is a particularly severe problem. In Indonesia, as elsewhere, anthracnose is caused mainly by *C. gloeosporioides* and *C. capsici*. It can result in losses of up to 60% of the marketable yield through pre- and post-harvest fruit rot (Duriat et al. 1991). Post-harvest rot is of particular importance, as the transport from farm to market usually takes several days and the temperature during transport is generally high. Also, post-harvest losses limit the potential of hot pepper as a high-value export crop.

We have developed a laboratory test method for harvested, ripe fruits and found a high correlation between the results of this method and disease incidence in field tests in Java, Indonesia. Using this test method we identified a promising source of resistance in *Capsicum chinense*, which might be of value in breeding programs.

Since the resistance of this source appeared to be inherited quantitatively, the current study was performed to establish the number, locations and effects of QTL with an effect on resistance. Although several other studies have been published on the inheritance of anthracnose resistance in *Capsicum* (Cheema et al. 1984; Park et al. 1990 (cited in Hartman and Wang 1992); Ahmed et al. 1991; Qing-Lin et al. 2002), this is to our knowledge the first report of a quantitative trait locus (QTL) study of resistance to this disease.

Materials and methods

Plant material

The two parents of the mapping population were the susceptible Indonesian hot pepper (*Capsicum annuum*) var. *Jatilaba* (East-West Indonesia, Purwakarta, Indonesia), and the resistant *C. chinense* accession PRI95030 (Plant Research International, Wageningen, The Netherlands). F_1 seed of the cross between these two parents, with *Jatilaba* as the female, was obtained easily. Individual plants of the F_1 were selfed, but all showed a high degree of sterility under Dutch glasshouse conditions. The least sterile F_1 plant was cloned, and from 20 cuttings of this plant about 750 F_2 seeds were obtained. The individual parental plants were selfed to obtain two lines for use as controls.

From about 375 seeds an F_2 population of 346 plants was raised. These were grown together with parental inbreds (three plants/line) and F_1 (four cuttings) plants as controls in 1999 in a glasshouse of Plant Research International, Wageningen, The Netherlands. No chemical pest or disease control treatments were used; pests were controlled biologically with predator organisms. Of these 346 plants, many produced only very few fruits. Early in the season 145 F_2 plants were identified that were expected to yield sufficient fruits for testing. These 145 plants were used as the mapping population, although some of them ultimately did not yield enough fruits for reliable scoring of resistance (Table 1).

Pathogen

Two Indonesian *Colletotrichum* isolates were used: one of *C. gloeosporioides* and one of *C. capsici*. Both isolates were isolated from pepper fruits from the lowland production area at Brebes (Java, Indonesia) and were obtained from the Research Institute of Vegetables (Lembang, Indonesia). The appearance of the conidia matched the description of these species by Sutton (1980). The isolates were maintained on potato dextrose agar. Three weeks before inoculum preparation they were subcultured on potato dextrose agar and incubated in the dark at 22°C. Inoculum was prepared by pouring sterile tap water on the cultures and gently scraping the spores with a glass rod. The suspension was diluted to a density of 10^5 spores per milliliter.

Resistance tests

Methodology

Resistance tests were performed on fruits harvested from glasshouse-grown plants. The fruits were harvested when they had just matured, as determined from their colour (bright red for *Jatilaba*, dark brown for PRI95030). Inoculation of fruits was carried out by dipping a sterile wooden toothpick in the inoculum and inserting it into the fruit wall, without penetrating the central fruit cavity. Depending on the size of the fruit, one, two or three inoculations were made in a line from the basal to the apical end of the fruit with at least a 3-cm separation to avoid the merging of lesions. The fruits were placed on wet tissue paper in closed plastic boxes with the inoculations facing upwards, and incubated 7 days in the dark at 28°C. Finally the diameter of the lesions was measured along the length of the fruit; if no lesion

Table 1 Number of observations and percentage explained variance for each resistance-related trait

Trait	Number of F_2 plants	Observations per plant [a]		F_2 variance explained [b](%)
		Mean	Range	
Colletotrichum gloeosporioides				
Overall lesion diameter	134	38	6–124	40
Infection frequency	134	38	6–134	30
True lesion diameter	126	28	6–129	35
Colletotrichum capsici				
Overall lesion diameter	107	26	6–60	–[c]
Infection frequency	107	26	6–60	–
True lesion diameter	104	24	6–59	23

[a]Plants with fewer than six observations were excluded from analysis. One highly fertile F_2 plant was included in the analysis, but not in the means and ranges shown here: it yielded about 2.5-fold the number of observations of the maximum of the ranges shown
[b]The percentage of phenotypic F_2 variance explained by all QTL identified for each trait
[c]–, No QTL identified

developed, a diameter of zero was recorded. Lesions showing bacterial rot were not measured.

Phenotyping

At weekly intervals, all fruits in the correct stage of development were harvested from each plant of the F_2 population and the controls. Inoculum was freshly prepared at each harvest from 2- to 4-week-old fungal cultures. The first ten harvests were inoculated with the *C. gloeosporioides* isolate; the last three harvests with the *C. capsici* isolate. Although about 20% of the plants produced too few fruits in those last three harvests to allow analysis, most plants actually produced more fruits per harvest in this final period (Table 1). Since the fertility of the F_2 plants varied, different number of fruits were harvested from each plant, and not all plants yielded fruits in every harvest.

Statistical analysis

The tests with *C. gloeosporioides* and with *C. capsici* were analysed separately. Three measures for susceptibility were used: the overall lesion diameter (averaged over all inoculations, including those that did not develop lesions); the true lesion diameter (averaged over all lesions that actually developed); the infection frequency (the fraction of inoculations resulting in a lesion).

For statistical analysis, each inoculation point was treated as an elementary observation and each weekly harvest was treated as a block. Because of the widely varying numbers of observations per plant per harvest, ANOVA analysis was not straightforward. Therefore the REML (residual maximum likelihood; GENSTAT 6 for Windows (Lawes Agricultural Trust 2002; Fixed model: Genotype; Random model: Harvest) procedure was used to calculate plant means and the residual variance of plant means for overall and true lesion diameter and infection probability.

Molecular markers and linkage map

Genomic DNA was isolated from leaf material of 145 F_2 plants, parents and F_1 plants as previously described (Van der Beek et al. 1992). The principle of amplified fragment length polymorphism (AFLP) as described by Vos et al. (1995) was applied. Polymorphic markers were detected using combinations of *Eco*RI and *Mse*I or *Pst*I and *Mse*I primers with either two (*Pst*I) or three (*Pst*I, *Eco*RI and *Mse*I) selective nucleotides. The pre-amplification primers (5′–3′) were: E00: GAC TGC GTA CCA ATT C; P00: GAC TGC GTA CAT GCA G; M02: GAT GAG TCC TGA GTA AC. The amplification primers were: E37: E00-ACG; P11, P00-AA; P14: P00-AT; P17, P00-AC; P37: P00-AAC; M47: M02-AA; M48: M02-AC; M49: M02-AG; M50: M02-AT; M51: M02-CA; M54: M02-CT; M58: M02-GT; M61: M02-TG. Fifteen primer combinations were used: E37 M51, P11 M47, P11 M48, P11 M49, P11 M50, P11 M51, P11 M54, P11 M61, P14 M48, P14 M49, P14 M50, P14 M58, P14 M61, P17 M48 and P37 M49. The *Pst*I and *Eco*RI primers were labelled with a 6-FAM or Joe fluorescent label (Eurogentec), and the AFLP fragments were resolved on a ABI 377 sequencer with a ROX GS-500 internal size standard (Applied Biosystems, Foster City, Calif.). AFLP data were scored using the GENOTYPER 2.5 programme (Applied Biosystems). AFLP fragments were scored co-dominantly when there was a distinct difference between homozygous and heterozygous peak intensities after normalisation based on a nearby reference peak.

Five primer pairs, kindly provided by Dr. I. Nagy, were used to amplify microsatellite markers (forward + reverse primer, both 5′–3′): CA-MS6: CAG AGC ACT TGA CAT GCC TT + GAT CTT TAT AGT AGC TCA TCA ATA; CA-MS12: TCA AGA ACT TGT ATT TCC TTC CC + CTT ACC TTG GTA CCC CCA CC; CA-MS22: GAT CAC ACC ATC TCT ACT AAC AGT TT + TGC ATT GCA TAT GCA TCT TTC; CA-MS23: CAC AAG TGT TGT TTC ACC TCT TTT C + GAC TCA CAT AGC CCG AAG AAA AT; and CA-MS25: TTT CCT TCA TAT CAA GCC ATA CAA + TTT TTG GTG ATG AAT TCT TTT. The PCR mix (20 µl) contained 20 ng template DNA, 2 m*M* $MgCl_2$, 1 µ*M* of each primer (Eurogentec), 0.1 m*M* of each dntp (Invitrogen, Carlsbad, Calif.), 0.5 U *Taq* DNA polymerase (Promega, Madison, Wis.). The PCR programme for all primer combinations consisted of a 3-min initial denaturation at 94°C followed by 35 PCR cycles of 30 s at 94°C, 30 s 50°C, 60 s 72°C, and a final 10-min extension at 72°C on a PTC-200 PCR machine (MJ Research, Waltham, Mass.). Each forward primer was labelled with a FITC fluorescent label, and the microsatellite patterns were analysed on an ABI 377 sequencer (Applied Biosystems).

Nucleotide binding site (NBS) profiling was carried out according to Van der Linden et al. (2004) using one NBS-region selective primer (Seq1) developed by J. Mes (5′–3′: GGT GGG GTT GGG AAG ACA AC). NBS profiling is a PCR-based method for targeting the NBS-LRR (leucine-rich repeat) class of disease resistance genes and analogues (RGAs) which produces polymorphic markers in these genes (Van der Linden et al. 2004).

A linkage map was calculated from the marker data using the software package JOINMAP 3.0 (Van Ooijen and Voorrips 2001). Altogether 266 markers were mapped: 80 co-dominant, 96 dominant for the *C. annuum* allele and 90 dominant for the *C. chinense* allele. The 266 markers consisted of 249 AFLPs (obtained with 15 primer pairs), 11 simple sequence repeats (SSRs; five primer pairs) and six RGAs (one primer pair). For the construction of the linkage map, the LOD grouping of the JOINMAP 3.0 package was used as a basis to group the markers into linkage groups. Groups of a more or less constant composition over a range of LOD values were used as a

starting point. Where such groups split up into subgroups (generally at gaps in the map) the consistency of the whole group was checked: if the order of the pairwise recombination frequencies across the gap was consistent with the order of the markers in the subgroups, the group was kept together, otherwise it was split into subgroups. Further, markers added in the "third round" (at a goodness-of-fit jump value above 5.0) were only accepted if they did not significantly change the rest of the linkage group map.

QTL mapping

For the QTL analysis, plant means based on fewer than six observations were discarded. Potential QTL for each trait were identified using the MAPQTL 4.0 package (Van Ooijen et al. 2002). Kruskal-Wallis and interval mapping analyses were initially performed to find regions with potential QTL effects. Co-dominantly scored markers in those regions were then used in various combinations as co-factors in multiple QTL models (MQM analysis, also performed with MAPQTL). Log of odds (LOD) thresholds for genome-wide $P<0.05$ were empirically determined for each trait using the PERMUTATION test of MAPQTL with 1,000 iterations.

The next step was using the GENSTAT 6.0 statistical package (Lawes Agricultural Trust 2002) for generalised linear regression of the trait on the selected markers. In this regression analysis, the marker scores (homozygous for either parent or heterozygous) were treated as different levels of the explaining factor, without imposing relative values. That is, the effects of each genotypic class were estimated separately. Regression analysis was first performed allowing all possible between-marker interactions; if interactions and/or main effects were found to be insignificant, a new regression was performed omitting those. The estimated additive and dominance effects of each marker were tested separately for significance, using Student's *t*-test (two-sided). The QTL graphs were prepared with MAPCHART 2.1 (Voorrips 2002).

Results

Resistance tests

The laboratory test method was validated using 27 accessions with varying resistance levels in three field tests in Java, Indonesia (unpublished). The field tests were artificially inoculated with an isolate of *C. capsici*. Due to natural infection both *C. capsici* and *C. gloeosporioides* were observed in the fields. The percentage of infected fruits in these tests showed correlations between 0.54 ($P=0.007$) and 0.59 ($P<0.001$) with the lesion diameter observed in a laboratory test inoculated with *C. capsici*.

The means from the REML analyses of the F_2 population and controls are listed in Table 2. In the tests with *C. gloeosporioides*, the F_1 and F_2 means were more resistant than the mid-parent values. When tested with *C. capsici*, the F_1 and F_2 means were near to the mid-parent value for overall and true lesion diameter but about equal to the susceptible parent value with respect to infection frequency.

Larger lesions and higher infection frequencies were observed in the tests with *C. capsici* than in those with *C. gloeosporioides*. In particular, the infection frequencies were so high that about two-thirds of the F_2 population showed almost 100% infection (Fig. 1).

Linkage map

A linkage map was calculated that consisted of 26 linkage groups, each with at least four markers and lengths of 14–71 cM, and six additional small groups with two or three markers each. The 26 larger groups were labelled A–Z in order of decreasing length. Their total map length was 997 cM; with the 63 cM in the six small groups, the total map length was 1,060 cM. The linkage map is available upon request from the corresponding author.

Significantly skewed segregation ratios ($P<0.01$) were observed in 12% of the loci; in all cases but one an excess of *C. chinense* alleles was found. Three complete linkage groups (L, M and Z) and the lower end of group B were skewed, accounting for 71% of the skewed loci; the remaining skewed loci were scattered over the map.

Table 2 Values of resistance-related traits for parents, F_1 and F_2 plants after inoculation with *Colletotrichum gloeosporioides* or *C. capsici*

	Overall lesion diameter (mm)	Infection frequency (%)	True lesion diameter (mm)
Colletotrichum gloeosporioides			
C. annuum	23.0±2.5[a]	100±1	22.1±2.5
C. chinense	3.1±1.1	31±10	7.2±0.3
F_1	9.4[b]	58[b]	13.2[b]
Mean F_2	8.4±4.1	55±21	12.0±2.9
Colletotrichum capsici			
C. annuum	22.2±2.6	94±1	22.8±2.6
C. chinense	3.5±0.3	61±5	6.3±1.3
F_1	14.2[b]	95[b]	14.9[b]
Mean F_2	14.3±4.4	90±12	15.4±3.6

[a]Mean ± standard deviation
[b]All four F_1 plants were scored under the same number; therefore no between-plant standard deviation could be calculated

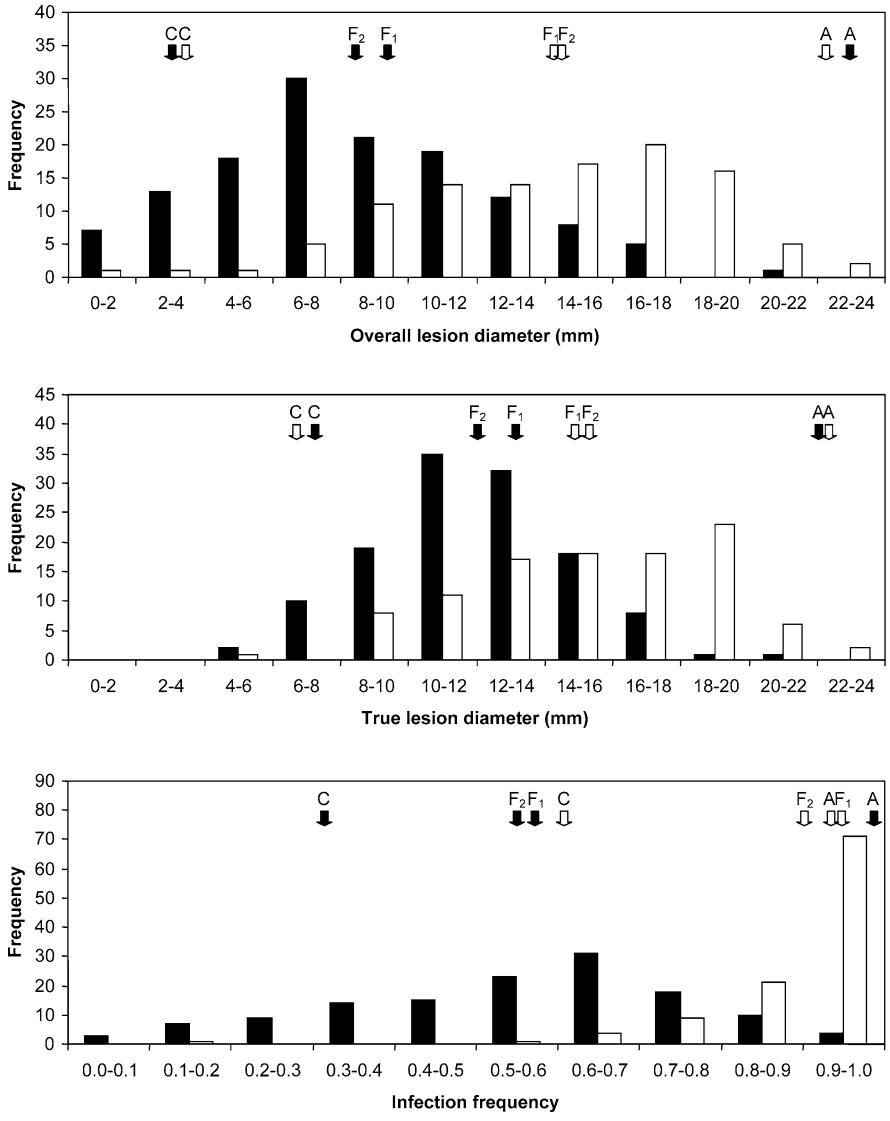

Fig. 1 Frequency distribution of overall lesion diameter, true lesion diameter and infection frequency in the F_2 population following inoculation with *Colletotrichum gloeosporioides* (*solid bars*) or *C. capsici* (*empty bars*). *Arrows* indicate the approximate means of the *Capsicum annuum* (*A*) and *C. chinense* (*C*) parents, F_1 and F_2-populations

QTL mapping

Tests with C. gloeosporioides

For overall lesion diameter three markers were found with significant QTL effects through MQM analysis: B1, B2 and H1 (Table 3, Fig. 2). The LOD score of a fourth marker (D1) did not reach the LOD threshold value of 3.78 (genome-wide, $P<0.05$), but the inclusion of this marker as co-factor considerably raised the LOD values of the other three co-factor markers. As marker D1 was a significant factor for true lesion diameter (see below) and its additive effect was significant in regression analysis, it was also included among the potential QTL for overall lesion diameter. Together, these four markers explained 39.7% of the variance of the F_2 plant means (Table 1). Remarkably, the resistant allele of QTL B2 was inherited from the susceptible parent.

For infection frequency (Table 3, Fig. 2), the LOD score of marker B1 again was highly significant. The LOD of the B2 and H1 markers were below the threshold value. However, both markers used as co-factors increased the significance of B1 and each other. As they also contributed to overall lesion size and showed large and significant additive and/or dominant effects in generalised linear regression, all three markers were included. The D1 marker, which has effects on overall lesion size and true lesion size, does not have a significant effect on infection frequency, nor does inclusion of D1 as co-factor strengthen the effects of the other markers. With the three selected markers 29.6% of the phenotypic F_2 variance could be accounted for (Table 1).

The true lesion size analysis (Table 3, Fig. 2) showed clearly significant LOD values for markers B1 and D1. The LOD values of B2 and H1 in MQM analysis were below the threshold value. Again, because these markers strengthened the effects of D1 and B1, because their effects in generalised linear regression analysis were significant and because they showed significant LOD values for overall lesion size they were included in the analysis. With the four selected markers 35.0% of the phenotypic F_2 variance was explained (Table 1).

Table 3 QTL effects for resistance-related traits after inoculation with *C. gloeosporioides* or *C. capsici*

Marker[a]	LOD[b]	Additive effect[c]	Dominance effect[c]	Parental difference explained (%)
C. gloeosporioides, overall lesion diameter				
B1	9.29	5.7***	−1.5	57.3
B2	4.67	−3.8***	1.3	38.6[d]
H1	3.74	1.5**	−1.7*	14.8
D1	2.71	1.4**	1.2	13.9
C. gloeosporioides, infection frequency				
B1	7.47	26.5***	0.7	77.1
B2	3.33	−17.8***	−1.3	51.8[d]
H1	2.92	7.0**	9.3*	20.5
C. gloeosporioides, true lesion diameter				
B1	4.92	3.1***	−1.0	42.3
B2	2.72	−2.2**	0.8	30.2[d]
H1	3.03	1.0**	−0.9*	13.4
D1	4.59	1.4***	0.9	19.2
C. capsici, true lesion diameter				
B1	3.89	1.34**	1.14*	16.3
G1	2.84	−0.63	2.22***	7.6[d]

*P<0.05; **P<0.01; ***P<0.001

[a]Co-dominantly scored markers, used as co-factors in MQM analyses. [*B1* and *B2* E37 M51_184 and P11 M49_355 on linkage group B, *D1* P11 M48_217 on linkage group D, *G1* P14 M58_199 on linkage group G *H1* P11 M48_139 on linkage group H (Fig. 2)]

[b]Log of Odds value from MQM analysis; LOD thresholds for genome-wide P<0.05: 3.78, 4.08 and 3.54 for overall lesion diameter, infection frequency and true lesion diameter (*C. gloeosporioides*), respectively, and 3.91 for true lesion diameter (*C. capsici*)

[c]The additive and dominance effects are expressed in millimeters for overall and true lesion diameter and in percentage for infection frequency

[d]The additive effects for B2 and G1 are negative, i.e. the resistant allele is inherited from the susceptible parent. The "explained" parts of the parental difference are therefore in the opposite direction

For none of these three traits significant between-locus interactions were found. Regression analysis without interaction effects showed significant main effects for each identified QTL (Table 3). In all cases the additive effect was significant, but only at marker H1 a small but significant dominance effect was found, where resistance was partially dominant.

For all three traits observed after inoculation with *C. gloeosporioides*, the two QTL with the largest effects were B1 and B2, which are closely linked on linkage group B. In each case the susceptible allele at B2 was inherited from the resistant *C. chinense* parent, while for B1, as for all other QTL, this parent contributed the resistant allele.

Tests with C. capsici

For overall lesion size and infection frequency, no markers were found with LOD values above the threshold values (3.80 and 6.91, respectively, for a genome-wide confidence level of 0.05). For overall lesion size an elevated but non-significant LOD score (3.12) was observed at marker B1 when marker G1 was used as co-factor in MQM analysis.

For true lesion diameter, the LOD score of marker B1 was equal to the threshold value when G1 was used as cofactor (Table 3, Fig. 2). Together, both markers explained 22.8% of the phenotypic F_2 variance (Table 1). No significant interaction effect between these two markers was found. The estimated additive effect of marker B1 was much smaller than that estimated for true lesion size after inoculation with *C. gloeosporioides*, although the parental difference was comparable with both pathogens. Marker G1 showed only a small and non-significant additive effect but a significant dominance effect for susceptibility.

Discussion

Linkage map

The number of linkage groups in our map, including groups of at least four markers, is 26 while the haploid number of *Capsicum* chromosomes is 12. This large number of linkage groups is probably due to the rather stringent criteria for linkage of subgroups across gaps, as also reported by Lefebvre et al. (2002). Also, the total length of our linkage map was only 1,060 cM, which is less than that of most other recently published intraspecific and interspecific *Capsicum* maps: 1,246 cM (Livingstone et al. 1999), 1,740 cM (Ben Chaim et al. 2001), 1,320 cM (Kang et al. 2001), 1,513, 1,668 and 685 cM (Lefebvre et al. 2002), and also less than the 1,832 cM of the integrated map of pepper (Paran et al. 2004). This, in combination with the large number of linkage groups indicates a still incomplete coverage of the genome.

QTL mapping

True lesion diameter and infection frequency can be interpreted as components of the more complex trait overall lesion diameter. These component traits were correlated with each other: R=0.70 in experiments with *C. gloeosporioides* and R=0.60 with *C. capsici*. In the case of *C. gloeosporioides*, where QTL were found for both component traits, three QTL (B1, B2 and H1) affected both traits, which explains the correlation. Formally we cannot exclude the possibility that different genes within the QTL regions are affecting true lesion diameter and infection frequency, but this seems very unlikely for traits that are so closely related. A fourth QTL (D1) had an effect on true and overall lesion diameter but not on infection frequency. This shows that different components of the same trait may be controlled by different genes. Separate analysis of components may therefore yield new QTL, which is illustrated by the fact that after inoculation with *C. capsici* no QTL was found for overall lesion

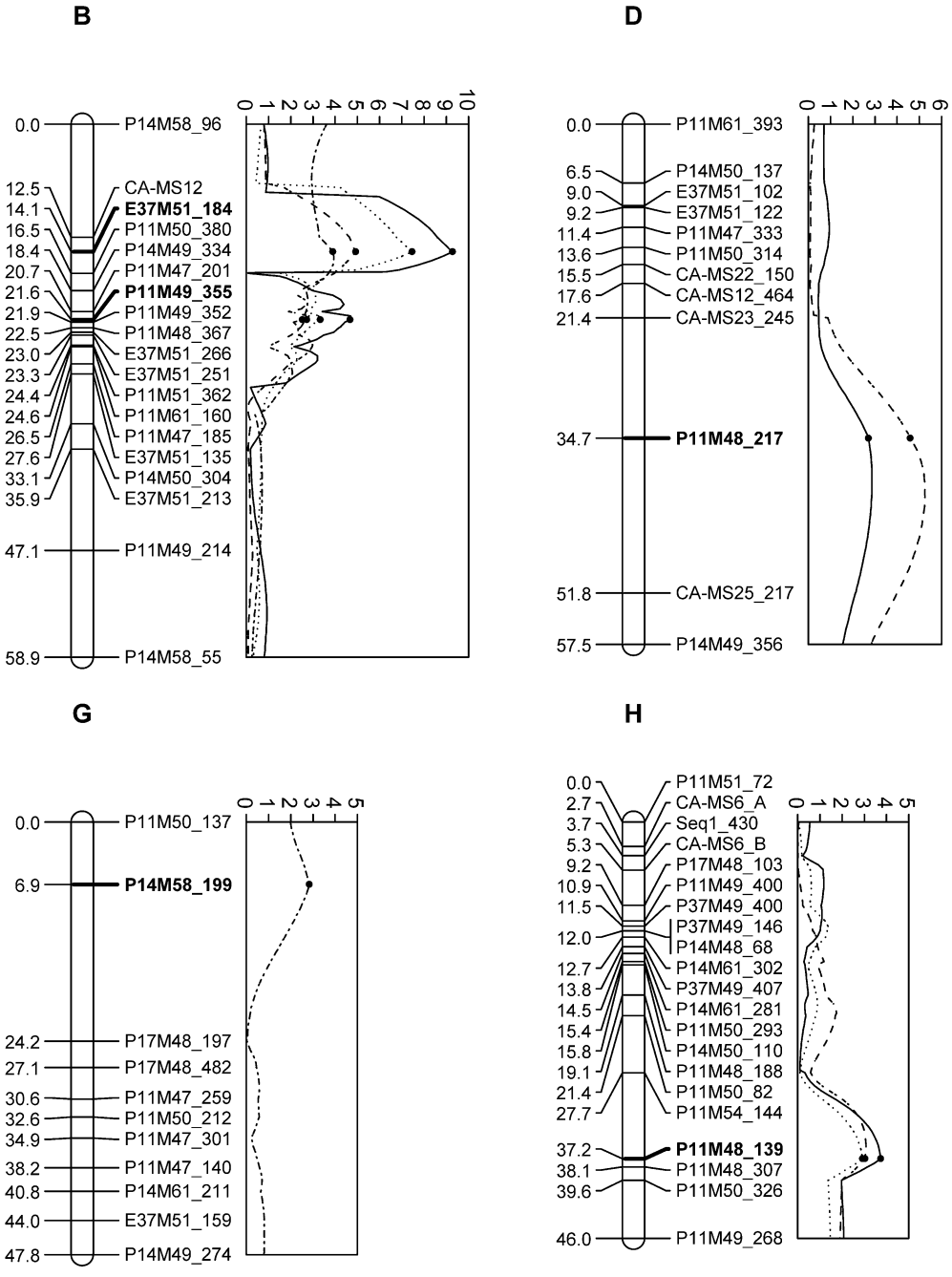

Fig. 2 LOD profiles for resistance QTLs on linkage groups B, D, G and H. Markers in *bold* were used as co-factors in MQM analyses. *Solid*, *dashed* and *dotted lines* overall and true lesion diameter and infection frequency, respectively, after inoculation with *C. gloeosporioides*, *dash-dotted lines* true lesion diameter after inoculation with *C. capsici*

diameter but that significant QTL were found for the component trait true lesion diameter.

Only three tests were performed with *C. capsici*, in contrast with ten tests with *C. gloeosporioides*. Consequently, fewer F_2 plants yielded the minimum required number of observations, and also the average number of observations per plant was lower than in the *C. gloeosporioides* tests (Table 1). This is probably a cause of the lower LOD scores for overall and true lesion size with *C. capsici* inoculation. The *C. capsici* data for infection probability were more problematic. Infection levels were so high that 66% of the population showed almost 100% infection (Fig. 1). Consequently, much of the genotypic variation for this trait was obscured.

Some important conclusions can be derived from the QTL mapping results. First, there appears to be one main QTL (B1) present on linkage group B, that is involved in resistance against both *C. gloeosporioides* and *C. capsici*. This QTL is the most important genetic factor in all the resistance-related traits studied. Also, no significant interactions occurred between different QTL. This observation implies that plant breeders will benefit from the introgression of this one QTL from the *C. chinense* parent into their material, even when neglecting the QTL on other linkage groups.

Secondly, this main QTL is closely linked to another QTL (B2, genetic distance approximately 7.5 cM), which also has an important effect on resistance against *C.*

gloeosporioides. However, the *C. chinense* parent harbours the susceptible allele at this locus. This suggests that an even higher level of resistance than that present in the *C. chinense* parent might be obtained in a recombinant homozygous genotype. Pre-breeding is needed to obtain the resistance alleles of both QTL in linkage instead of repulsion phase. This aim is easily achieved: among our 145 F_2 plants, 12 plants were homozygous resistant for one of these markers and heterozygous for the other. The average overall lesion diameter of these 12 plants was 4.4 mm, which is almost as good as that of the *C. chinense* parent (3.1 mm). Moreover, five of these 12 plants showed a smaller overall lesion diameter than that of the *C. chinense* parent, although the differences were not significant. Similarly, the average infection frequency of these 12 plants was 30.9%, which is about equal to that of the resistant parent (31.2%), and six of these 12 plants showed a lower infection frequency, which in three cases was significant ($P<0.05$).

Two field tests with F_3 lines in Indonesia were attempted to verify the results of the laboratory tests. However, both tests were lost as a result of infection from other diseases, particularly late blight (*Phytophthora* spp.) to which the *C. chinense* parent and most F_3 lines proved to be highly susceptible.

Our results indicate that between-locus interactions are mostly absent. A substantial part of the different resistance-related traits is controlled by one QTL with mostly additive effects. The additive effects of the other loci are also generally larger than the dominance effects (Table 3). Therefore, the different resistance-related traits are inherited in an intermediary or partly dominant manner. This is also indicated by the fact that the F_1 means (except for infection frequency after inoculation with *C. capsici*) are intermediate between the parents (Table 2). These conclusions deviate from those in earlier studies, which were based on intraspecific *C. annuum* crosses and did not use a QTL approach. Cheema et al. (1984) found that resistance to *C. capsici* was inherited recessively, with significant epistatic interactions. Park et al. (1990, cited in Hartman and Wang 1992) found that resistance to *C. dematium* was partly dominant. They also found significant specific combining ability effects, which would indicate that non-additive gene effects are important. Ahmed et al. (1991) reported polygenic, mostly additive inheritance of resistance to *C. capsici*. Qing-Lin et al. (2002) found evidence for a monogenic dominant inheritance of resistance to *C. capsici*.

In contrast to these earlier studies on the inheritance of resistance our results were obtained in an interspecific cross between *C. annuum* and *C. chinense*. Further, we obtained linkage information and estimates of specific QTL effects. Therefore our study offers a new opportunity for resistance breeding against anthracnose fruit rot.

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