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Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction pepper – *Phytophthora capsici* Leonian

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Abstract To study the resistance of pepper to *Phytophthora capsici*, we analyzed 94 doubled-haploid (DH) lines derived from the intraspecific F_1 hybrid obtained from a cross between Perennial, an Indian pungent resistant line, and Yolo Wonder, an American bell-pepper susceptible line, with 119 DNA markers. Four different criteria were used to evaluate the resistance, corresponding to different steps or mechanisms of the host-pathogen interaction: root-rot index, receptivity, inducibility and stability. Three distinct ANOVA models between DNA marker genotypes and the four disease criteria identified 13 genomic regions, distributed across several linkage groups or unlinked markers, affecting the resistance of pepper to *P. capsici*. Some QTLs were criterion specific, whereas others affect several criteria, so that the four resistance criteria were controlled by different combinations of QTLs. The QTLs were very different in their quantitative effect (R^2 values), including major QTLs which explained 41–55% of the phenotypic variance, intermediate QTLs with additive or/and epistatic action (17–28% of the variance explained) and minor QTLs. Favourable alleles of some minor QTLs were carried in the susceptible parent. The total phenotypic variation accounted for by QTLs reached up to 90% for receptivity, with an important part due to epistasis effects between QTLs (with or without additive effects). The relative impact of resistance QTLs in disease response is discussed.

Key words *Capsicum annuum* · Molecular markers · Polygenic disease resistance · Epistasis · Doubled-haploid lines

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Introduction

Phytophthora root rot, caused by the soilborne fungus *Phytophthora capsici* Leonian, is a major problem to the cultivation of pepper (*Capsicum annuum* L.) throughout the world (Yoon et al. 1989). *P. capsici* may attack roots at all developmental stages of the crop, causing a sudden wilt and a further collapse of the infected plants. Aerial organs may be attacked too by splashing water and soil carrying inoculum during heavy rainfalls (Black et al. 1991). Several resistant sources were found in local populations of the cultivated species *C. annuum* (Kimble and Grogan 1960; Saini and Sharma 1978; Guerrero-Moreno and Laborde 1980; Pochard et al. 1983; Matsuoka 1984). Attempts to transfer the resistance to *P. capsici* into different genetic backgrounds by backcrossing led to a rapid decrease of the resistance level certainly due to the loss of some genetic factors useful for the resistance. Conversely, the recombination of different resistant lines by recurrent selection led to transgressive genotypes, suggesting that the resistance is polygenically controlled and that the different accessions used bear different resistance genes (Palloix et al. 1990).

Despite decades of interest in resistance to *P. capsici*, little is known about the genetic, cellular and molecular mechanisms underlying the host-plant resistance. Numerous authors have described artificial inoculation methods for performing resistance tests (Smith et al. 1967; Pochard et al. 1976; Saini and Sharma 1978; Pochard and Daubèze 1980; Pochard et al. 1983; Barksdale et al. 1984; Peter et al. 1984; Reifschneider et al. 1986; Palloix et al. 1988a, Bosland and Lindsey 1991; Alcantara and Bosland 1994). These tests varied depending on the use of a qualitative or quantitative rating system and whether they involved specific isolates or a mixture of isolates. Consequently, the genetics of resistance were interpreted differently depending on the inoculation tests (environmental conditions, plant stage, isolate, disease rating system) and the resistant line studied. A number of authors agreed, however, that the resistance was controlled by several genes (Smith et al. 1967;

Guerrero-Moreno and Laborde 1980; Pochard and Daubèze 1980; Pochard et al. 1983; Kim et al. 1989; Gil Ortega et al. 1991, 1992) probably with epistatic effects (Palloix et al. 1990; Bartual et al. 1991; Reifschneider et al. 1992; Bartual et al. 1994). Several inoculation procedures and resistance criteria were shown to be necessary to evaluate the different components of this complex resistance. For most of the resistant genotypes, resistance was partial (Palloix et al. 1988 b) i.e. resistant lines reduced the extent of pathogen development within the compatible host.

The recent development of molecular markers makes it possible to investigate the inheritance of complex traits and to locate and manipulate individual genetic factors associated with these traits. Recent studies have demonstrated that molecular mapping is a powerful approach for identifying quantitative trait loci (QTLs) controlling complex resistance (for a review see Lefebvre and Chèvre 1995). Determining the mode of inheritance, the number, the chromosomal location and the effects of genetic factors involved in resistance to *P. capsici* would facilitate its incorporation into breeding lines.

In the present paper, we report the identification of QTLs controlling partial resistance to *P. capsici*, expressed under different screening conditions or different plant developmental stages, on a molecular linkage map based on a doubled-haploid (DH) population of pepper. Doubled haploid lines allow for several phenotypic evaluations and an unlimited number of markers to be continuously added to the linkage map. The number, location, individual and epistatic effects of QTLs, and the parental allelic contribution of each QTL associated with resistance are discussed.

Materials and methods

Plant and fungal material

The *C. annuum* Perennial, a small fruited pungent Indian line (supplied by J. Singh, University of Punjab, Ludhiana, India) partially resistant to *P. capsici*, and an American bell-pepper susceptible variety, Yolo Wonder, were crossed. An F_1-derived DH population was developed using in vitro androgenesis (Dumas de Vaulx 1990). Phenotypic resistance evaluations, as described below, were conducted with 94 DH lines, the two parents and the F_1. This same DH population has previously been used for the construction of a linkage map (Lefebvre et al. 1995).

A moderately aggressive isolate of *P. capsici* (S101) that reveals the resistance of Perennial was used (Clerjeau et al. 1976). The mycelium was grown on V8 juice-Agar medium (around 20°C, 12 h fluorescent light/12 h darkness). Mycelial plugs (diameter 4 mm) were taken off at the periphery of 5–7-day-old Petri-dish (diameter 10 cm) cultures.

Artificial inoculation methods and *P. capsici* resistance evaluation

Two artificial inoculation methods are currently performed to evaluate the resistance and are used in recurrent selection breeding programs (Palloix et al. 1990). They allow the measurement of four distinct quantitative resistance criteria. The same criteria were measured in the DH population to dissect the resistance to *P. capsici* into discrete genetic factors with the use of molecular markers. The resistance tests were conducted in a controlled growth chamber at 22°C with 12 h of light.

The tests on roots were conducted, as previously described by Palloix et al. (1988 a), on young plantlets (3-week-old seedlings) held in glass containers filled with a nutritive solution. For each DH line, two containers of 20 plantlets each were inoculated by dipping four mycelial plugs of *P. capsici* into the containers, arranged in two blocks. Each block had one container of each line. The containers were randomized within blocks. Root necrosis due to zoospore infection was observed 7 days after inoculation. The 20 plantlets were individually sorted by employing a semi-quantitative scale from 0 to 5 according to the extent of root necrosis. A mean necrotic root-rot index was then calculated for each line (mean of the 40 individual ratings, because of no significant block effect). Two independent trials were conducted (1991 and 1994). The mean of the two trials (80 plants) was the experimental unit for QTL analyses.

The stem inoculations were performed as described by Pochard et al. (1976). Plants at the first flowering stage were cut off below the first flower. A mycelial plug of *P. capsici* was placed on the fresh section of the main stem and wrapped with an aluminum sheet for a period of 3 days. The fungal infection provoked a necrosis of the stem that progressively killed the susceptible parent Yolo Wonder. The progression of necrosis toward the bottom of the stem was measured every 3–4 days and the speed of fungal invasion (mm/day) was calculated. Three quantitative criteria were used to evaluate the resistance, according to Pochard and Daubèze (1980) and Pochard et al. (1983). The receptivity is the initial speed of stem-necrosis progression, over the first 3 days after inoculation (mm/day). It corresponds to the ability of the genotype to offer a favourable tissue to the development of the mycelium. The inducibility is the decrease of speed necrosis between the 3rd and 10th day after inoculation (mm/day^2). It corresponds to the induction of fungistatic activity in infected stems that will progressively brake or stop the fungal progression in resistant genotypes. The stability is the mean speed of stem necrosis between the 14th and the 21st day after inoculation (mm/day). It expresses the ability of the genotype to maintain the fungistatic activity over a long time period. These three criteria correspond to distinct resistance components since different combinations were observed in different genotypes (Pochard et al. 1983; Palloix et al. 1990). For each resistance criterion, the mean of eight plants per DH line was the experimental unit for QTL analyses.

Molecular data analysis

Procedures for plant DNA isolation, RFLP and RAPD analysis were described elsewhere (Lefebvre et al. 1993, 1995). The DNA clones used as RFLP probes were supplied by S.D. Tanksley of Cornell University (Ithaca, New York, USA). The map was established with the MapMaker software (Lander et al. 1987), as described by Lefebvre et al. (1995), with a minimal Lod score of 3 and a maximum recombination rate of 0.3. Genetic distances between markers were estimated using the mapping function of Kosambi (1944).

Statistical analysis

Original data were used for analyses because data transformations did not improve normality. Differences between the resistance means of the DH lines were tested for the four criteria by using a generalized linear model (PROC GLM of Statistical Analysis System – SAS – SAS Institute Inc. 1989) to partition total variation into effects of lines and errors ($Pij=\mu+Li+Eij$ where Pij is the mean disease score of the jth repetition belonging to the ith DH line, μ is the mean of all the data, Li the DH line i effect and Eij the residual). Heritabilities (h^2) were calculated from the ANOVA with the formula $h^2=\sigma_g^2/[\sigma_g^2+(\sigma_e^2/n)]$ with σ_g^2 the genetic variance, σ_e^2 the environmental variance and n the number of independent tests (n=2 for the root-rot index, n=1 for the three stem resistance criteria). Dominance effects are non-existent in doubled-haploid lines. The Pearson coefficient was calculated to determine phenotypic correlations among trait measurements.

The effect of all the chromosomal regions marked by a marker locus was assessed by a one-way analysis of variance (ANOVA) using genotypes of the marker locus as the main effect on the phenotype (Pij=μ+Mi+Eij where Pij is the mean disease score of the jth genotype having the ith marker, μ is the mean of all the DH lines, Mi the marker i effect and Eij the residual). The PROC GLM procedure (SAS) was used to determine associations between molecular markers and the four criteria of quantitative resistance to *P. capsici*. A total of 119 analyses were performed for each resistance criterion (because of association tests between the 119 markers and the phenotype). A significance level of $P<5\cdot10^{-3}$ was employed (expecting 0.6 false-positives by chance alone). In order to decrease the error component, and consequently to detect regions with minor effects, in a second step we performed a two-way ANOVA model with a first component (Mi) corresponding to the markers linked to the major-effect genetic factors; two were detected with the one-way ANOVA (Pijk=μ+Mi+Mj+Eijk where Pijk is the mean disease score of the kth genotypes having the ith and jth markers, Mi and Mj the marker i and j effects respectively and Eijk the residual). Consequently, the second component (Mj) identified minor-effect genetic factors. A total of 234 analyses were performed for each criterion (117 remaining markers for Mj separately tested with the two markers linked to major-effect genetic factors for Mi). We kept a significance level of $P<5\cdot10^{-3}$ (expecting 1.2 false-positives). Finally, we carried out a two-way ANOVA model with an interaction component between all pairs of markers in order to detect genomic regions acting in epistasis (2nd order) (Pijk=μ+Mi+Mj+Mi*Mj+Eijk). A total of 7021 analyses were performed for each criterion [the (119*118)/2 marker combinations]. A significance level of $P<5\cdot10^{-4}$ was employed (expecting 3.5 false-positives). Homogeneity of variances for marker classes was tested using Fisher's criterion as described in Snedecor and Cochran (1957). The magnitude of the marker-associated phenotypic effect is described by the coefficient of determination (R^2), which is the fraction of the total variance accounted for by the marker genotypes.

Table 1 Upper triangle: (*1*) Pearson phenotypic correlation coefficients calculated on the means per line [[a] Probability under the hypothesis H_0 that the correlation is null; *: $P=10^{-1}$; 10^{-2}, **: $P=10^{-2};10^{-3}$, ***: $P<10^{-3}$]. Lower triangle: (*2*) genetic correlations and (*3*) environmental correlations calculated on repeats inside each experiment. Note: tests on roots and tests on stems are independent. Tests on roots were conducted on the 94 DH lines, the two parents, the F_1 hybrid and two additional lines. Tests on stems were conducted on 93 of the 94 DH lines. Genetic and environmental correlations can be calculated for a couple of variables measured on the same individuals

Item		Root-rot index (1994)	Mean root-rot index	Receptivity	Inducibility	Stability
Root-rot index (1991)	(*1*) [a]	0.81 ***	0.95 ***	0.70 ***	0.29 **	0.72 ***
Root-rot index (1994)			0.96 ***	0.76 ***	0.34 ***	0.78 ***
Mean root-rot index				0.77 ***	0.33 **	0.80 ***
Receptivity					0.21 *	0.76 ***
Inducibility	(*2*)			0.23		0.50 ***
	(*3*)			−0.35		
Stability				0.77	0.52	
				0.13	0.06	

Results

Disease resistance tests

Homogeneity between the two independent root test experiments was tested by ANOVA. Both experimental and experiment×genotype effects were significant (α=0.05) but explained only 2.5% and 7.6% of the total variance, respectively. Indeed the attack in 1991 (mean root-rot index: 3.26) was slightly more aggressive than in 1994 (mean root-rot index: 2.75). Moreover, a high correlation between the two experiments was found (r=0.81, Table 1). Thus, the mean values of the both root test experiments were considered in the further analyses.

Figure 1 shows the frequency distribution for the four resistance criteria in the DH population. They have multimodal distributions. These distributions suggested that these criteria were controlled by an oligogenic or polygenic system. A bimodal tendency suggested the effect of a major genetic factor. The four criteria were independently inherited since different combinations of values of these criteria were found in the progeny. Root-rot index, receptivity and stability were however partially correlated (Table 1). Inducibility was weakly correlated with the three other criteria. Phenotypic and genotypic correlations were similar due to low environmental correlations. Phenotypic correlations between criteria suggested that certain genetic factors may be involved in several resistance components, resulting from pleiotropic effects or closely linked genes.

The genetic variances in the DH population were highly significant for the four resistance criteria ($P<10^{-4}$), which means that the different levels of resistance found in the DH population had a genetic basis. The heritability of the genotype means was high for three of the criteria of resistance to *P. capsici* (0.83 for root-rot index, 0.78 for receptivity and stability), but not for inducibility (0.46).

The linkage map

Most markers used have been previously mapped by Lefebvre et al. (1995). This map includes 119 markers (56 RFLP, 59 RAPD, one isozyme and three phenotypic markers). A total of 110 markers were mapped on 12 linkage groups spanning a total of 954.2 cM (Kosambi) of the *C. annuum* genome. All 119 loci, including the nine remaining unlinked, were used in further QTL analyses. In comparison with the previously published map (Lefebvre et al. 1995), the designation of linkage groups was conserved, except that the two previous linkage groups, 2 and Pourpre, were now joined into a single linkage group, Pourpre, and two new linkage groups, which include new markers, were named 2 and 12. Two markers, tg312 on linkage group 5 and B04_0.45 on linkage group Pourpre, were located with the command "TRY" of MapMaker with a minimal Lod score of 2. This new map is estimated to represent almost 70% of the *C. annuum* genome, thanks to the comparison with the pepper genome length estimated by Lefebvre et al. (1995).

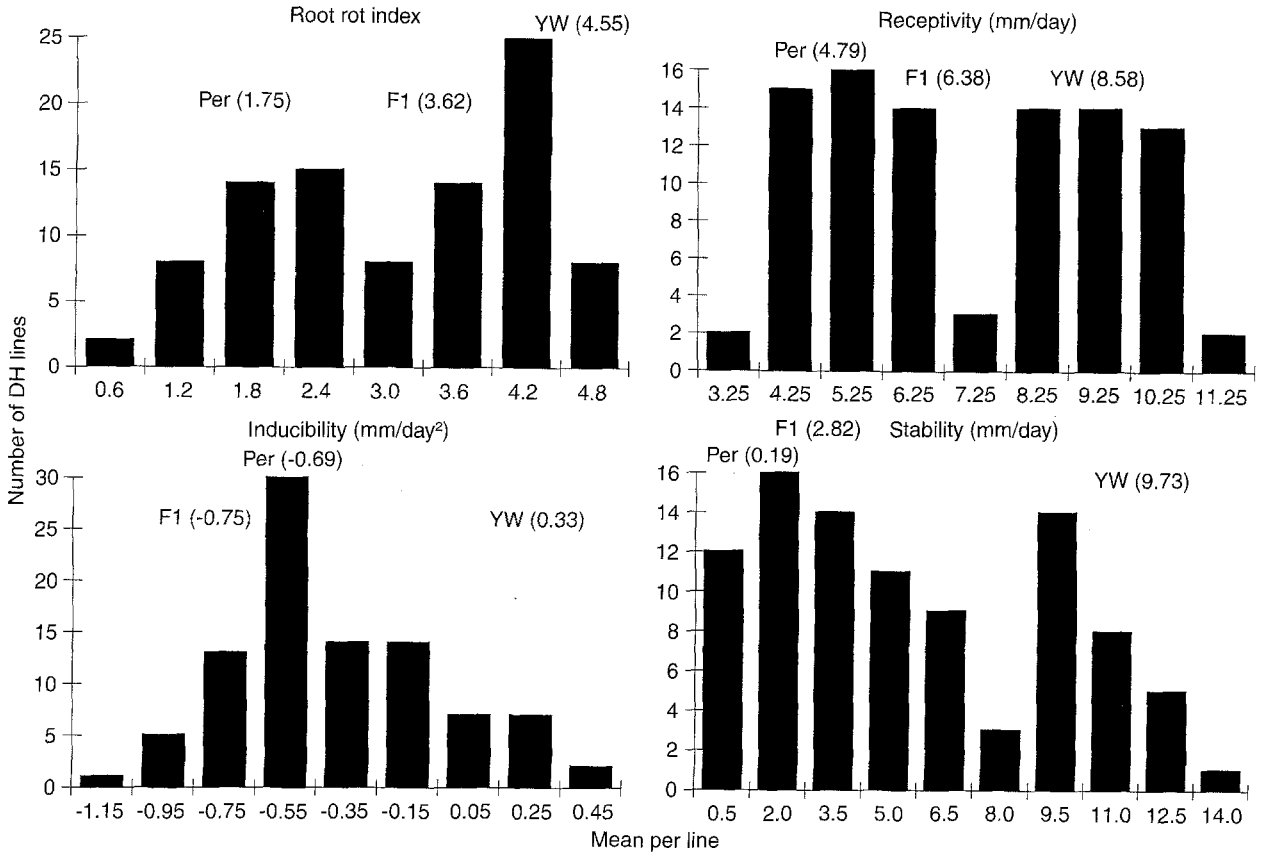


Fig. 1 Frequency distribution of root-rot index, receptivity, inducibility and stability in the DH population. Note: *Per*: Perennial, *YW*: Yolo Wonder, F_1: F_1 hybrid

Mapping of the resistance QTLs

The first ANOVA model revealed two major-effect QTLs associated with *P. capsici* resistance (Table 2). These two regions were involved in three of the four resistance criteria. The first one explained 41–55% of the total variance, depending on the criterion, the second one 19–25%. One is linked to an unmapped marker (tg483) and the other is linked to the marker B04_0.45 (linkage group Pourpre). These two QTLs were detected with a significance level lower than 10^{-5} and resistant alleles were carried in the resistant parent.

The second ANOVA model, with either tg483 or B04_0.45 as first component, detected three new QTLs with minor effects (Table 3). Two of these QTLs showed an effect on the root-rot index. They were located on the two extremities of the Brun chromosome (about 70 cM between them) and have opposite effects: one resistant allele was derived from the resistant parent whereas the other one was from the susceptible parent. The third QTL was significant only for receptivity and had a reverse effect compared to the parental behavior (i.e. the allele inherited from the susceptible parent showed higher resistance). One minor and reverse-effect QTL, located on the Brun chromosome, is linked to the locus *L* which confers resistance to tobacco mosaic virus (TMV). Yolo Wonder, susceptible to *P. capsici*, is resistant to TMV.

The third ANOVA model detected ten genomic regions with interactive effects (Table 4). These second order epistatic effects may explain up to 28% of the phenotypic var-

Table 2 Summary of the results from the first ANOVA model ($P<5\cdot10^{-3}$). Note: bold-type font indicates the more resistant class

Resistance criterion	Marker	Linkage group	P	R^2	Phenotypic mean of DH lines with allele of	
					Yolo Wonder	Perennial
Root-rot index	tg483	Unlinked	$<10^{-8}$	0.42	3.85	**2.39**
	B04_0.45	Pourpre	$1.1\cdot10^{-5}$	0.19	3.44	**2.38**
Receptivity	tg483	Unlinked	$<10^{-8}$	0.55	8.90	**5.56**
	B04_0.45	Pourpre	$1.3\cdot10^{-6}$	0.23	7.95	**5.68**
Stability	tg483	Unlinked	$<10^{-8}$	0.41	7.61	**2.96**
	B04_0.45	Pourpre	$4\cdot10^{-7}$	0.25	6.49	**2.65**

Table 3 Summary of the results from the second ANOVA model ($P<5\cdot10^{-3}$). Note: bold-type font indicates the more resistant class. In many cases, several closely linked markers were significantly associated with a trait, probably due to the presence of only one QTL localized within the cluster. Accordingly, we considered that QTLs were identified by the marker locus that displayed the highest R^2 value

Resistance criterion	Marker (Mj) (+ frist component, Mi)	Linkage group	*P*	R^2	Phenotypic mean of DH lines with allele (for Mj) of	
					Yolo Wonder	Perennial
Root-rot index	P13 0.9[a] (+ tg483)	Brun	$2.6\cdot10^{-4}$	0.56	3.40	**2.75**
	L (+ tg483)	Brun	$4.1\cdot10^{-3}$	0.56	**2.82**	3.27
Receptivity	ct259 (+ B04_0.45)	5	$1.9\cdot10^{-3}$	0.32	**6.62**	7.86

[a] tg104, E08_0.5, tg379, B11_1.0 were also detected

Table 4 Summary of the results from the third ANOVA model ($P<5\cdot10^{-4}$). Note: bold-type font indicates the more resistant classes

Resistance criterion	Markers in interaction	Linkage groups	*P*	R^2	Phenotypic mean of DH lines with alleles of			
					YY[a]	YP	PY	PP
Root-rot index	tg55B-tg83B[b]	1–8	$1.7\cdot10^{-4}$	0.17	3.28	**2.50**	**2.79**	3.89
	ct120-ct114A	Brun-unlinked	$1.8\cdot10^{-4}$	0.28	4.10	**2.85**	**2.50**	3.64
	R19_0.75-N09_0.7	2-Noir	$3.8\cdot10^{-4}$	0.19	**2.48**	3.44	3.21	**2.31**
Receptivity	ct120-ct114A[c]	Brun-unlinked	$2.1\cdot10^{-4}$	0.26	8.92	**6.91**	**6.34**	8.98
	R19_0.75-N09_0.7[d]	2-Noir	$8.7\cdot10^{-6}$	0.28	**5.40**	7.86	8.06	**5.99**
Inducibility	tg57-tg483[e]	Pourpre-unlinked	$1.6\cdot10^{-4}$	0.23	–0.16	**–0.54**	**–0.52**	–0.37
	R13_1.8-B11-1.0[f]	1-Brun	$3.7\cdot10^{-4}$	0.17	**–0.55**	–0.30	–0.24	**–0.54**
Stability	ct268-ct114A	5-unlinked	$4.7\cdot10^{-4}$	0.25	8.01	**3.27**	**4.10**	5.82
	ct268-B11_1.0[g]	5-Brun	$3.7\cdot10^{-4}$	0.17	**4.92**	6.65	7.05	**3.27**
	R19_0.75-N09_0.7	2-Noir	$1.6\cdot10^{-4}$	0.21	**3.36**	6.38	6.25	**2.73**

[a] Combination of two alleles from Yolo Wonder (YY), two alleles from Perennial (PP), the allele of Yolo Wonder for the first marker and the allele of Perennial for the second marker (YP) and conversely, the allele of Perennial for the first marker and the allele of Yolo Wonder for the second marker (PY). In many cases, other interactions of markers closely linked to the first one were significantly associated with a trait, probably due to the presence of only one QTL localized within the cluster. Accordingly, we considered that QTLs were identified by the marker loci that displayed the highest R^2 value.
[b] The interaction tg55B-B07_0.5 was also detected
[c] The interactions tg379-ct114A and B11_1.0-ct114A were also detected
[d] The interaction R19_0.75-AE04_2.3 was also detected
[e] The interaction D11_1.3-tg483 was also detected
[f] The interaction R13_1.8-tg379 was also detected
[g] The interaction ct268-P13_0.9 was also detected

iance. The interaction may occur between two alleles of the same parent but also between one allele of Perennial and one allele of Yolo Wonder. The two more-resistant classes always corresponded to symmetrical combinations of alleles from the same parent or of alleles of both parents, as constrained by the interaction contrast of 2×2 tables corresponding to the unique degree of freedom for interaction.

Complex ANOVA models were written to determine the global R^2 for each criterion, corresponding to the total part of variance explained by the set of the different QTLs revealed by the three consecutive ANOVA models (Table 5). Global R^2 values obtained with additive QTLs on one hand and epistatic QTLs on the other hand were similar and reached up to 73% and 62% of the variance, respectively. Combining both types of QTL effects in a global model explained 21–90% of the observed variance, depending on the criterion considered.

Table 5 Number of QTLs (#) and part of the total phenotypic variance explained by the set of QTLs (R^2). Note: for multiple QTL models, we included all the markers that had significant association with the trait, except for clusters of markers significantly associated with the trait and for which only the marker with the highest R^2 value was considered

Resistance criterion	Additive effects		Epistatic effects		Additive and epistatic effects	
	#	R^2	#	R^2	#	R^2
Root-rot index	4	73%	6	62%	9	84%
Receptivity	3	65%	4	48%	7	90%
Inducibility	0	0%	4	21%	4	21%
Stability	2	54%	5	50%	7	76%

Discussion

Mapping putative QTLs for *P. capsici* resistance

Quantitative resistance to *P. capsici* was studied in a doubled-haploid progeny of pepper derived from an intraspecific cross between Perennial, a resistant line, and Yolo Wonder, a susceptible line. A total of 119 markers were employed to construct an intraspecific pepper map that represents almost 70% of the genome. The search for QTLs was performed by different ANOVA models. A total of 13 different QTLs were detected confirming the polygenic nature of the resistance. QTLs were distributed across several linkage groups and unlinked markers. The four resistance criteria were controlled by different combinations of QTLs (Fig. 2). The total phenotypic variation accounted for by QTLs reached up to 90% for receptivity. One QTL linked to tg483 had a major effect on resistance. Alleles of some minor QTLs had reverse effects compared to the parental behavior. Important epistatic effects between QTLs (with or without additive effects) were detected. The different QTLs are associated with the described interaction, but a confrontation between another resistant line and fungal isolate may reveal new QTLs specific to the cross and isolate of *P. capsici* employed (Bubeck et al. 1993; Lefebvre 1993; Leonards-Schippers et al. 1994).

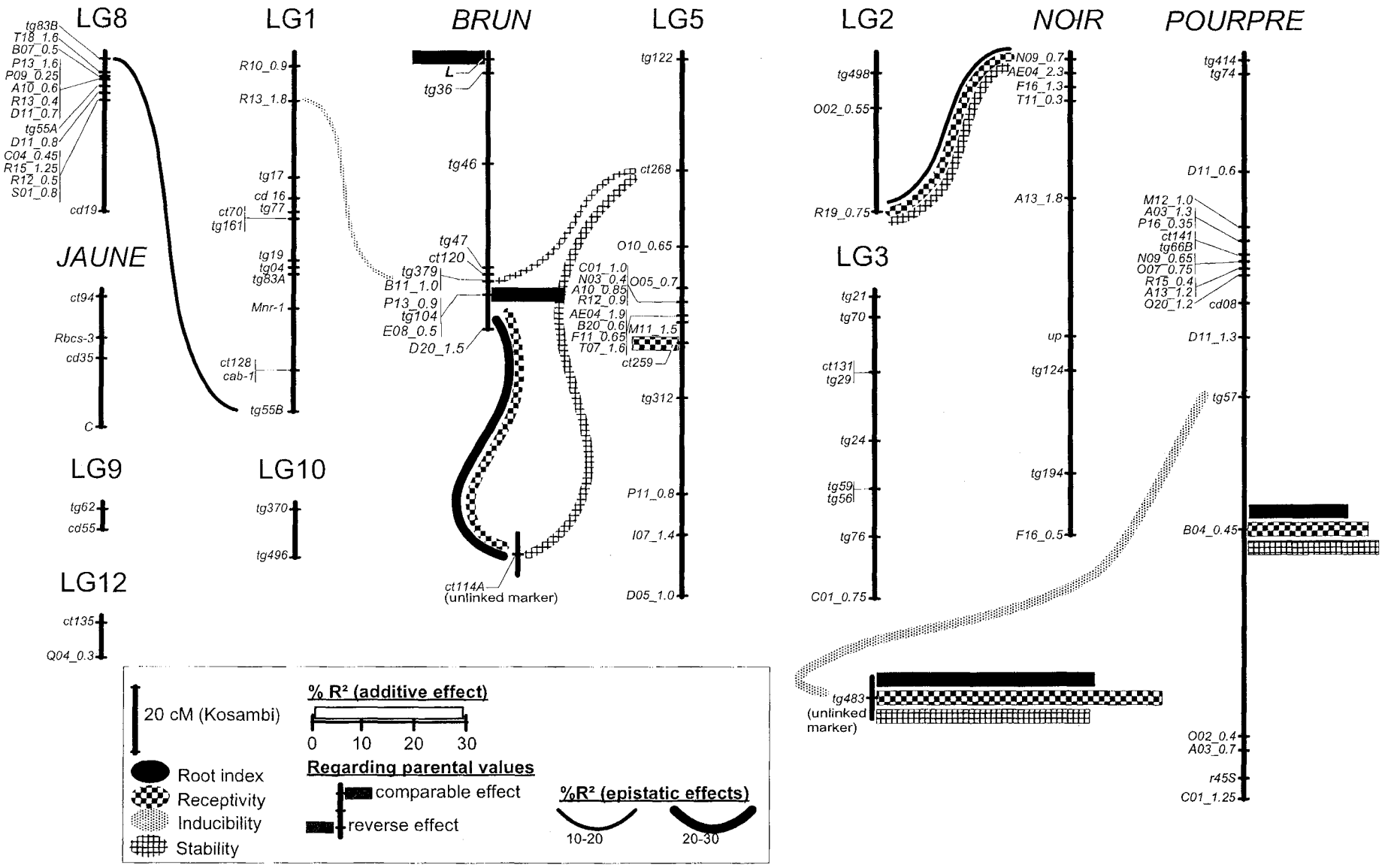
ANOVA was prefered to interval mapping (Lander and Botstein 1989) because of the non-normality of our data and the tolerance of ANOVA to non-normality when the class variances are equal, a condition that was met in our data set. Moreover, the ANOVA method allows one to look for QTLs linked to isolated markers (unsaturated map).

Differential effects of QTLs

The QTLs detected were very different in their quantitative effect (R^2 values), including one major QTL which explained 41–55% of the phenotypic variance, intermediate QTLs with additive or/and epistatic action (17–28% of the variance explained) and minor QTLs. Involvement of a major QTL in quantitative resistance to diseases has also been found by other authors, and led to the expectation that the resistance could be mapped as a qualitative character (Landry et al. 1992; Dirlewanger et al. 1994; Kreike et al. 1994; Wang et al. 1994; Ferreira et al. 1995). In our case, the major effect of the QTL linked to tg483 was probably responsible for the bimodal shape of the distributions for root-rot index, receptivity and stability; but breeding experience, as well as the other QTLs detected, clearly showed that other genetic factors are involved in resistance expression. Our results, in particular, showed the contribution of the susceptible parent to the expression of the resistance of pepper to *P. capsici*, as already suspected by Pochard and Daubèze (1980). Two additive QTLs and at least four epistatic QTLs from the susceptible parent were shown to be favourable to the resistance. Several authors pointed out the useful variability existing in susceptible lines (Bubeck et al. 1993; Figdore et al. 1993; Freymark et al. 1993, 1994; Nodari et al. 1993; Schön et al. 1993; Young et al. 1993; Danesh et al. 1994; Dirlewanger et al. 1994; Kreike et al. 1994; Leonards-Schippers et al. 1994). This confirms that defense mechanisms are also developed by the susceptible parent but their activity remains insufficient to stop fungal progression. Exploitation of such reverse-effect genetic factors is difficult since the phenotypic effects are weak. However their detection through markers should facilitate a more exhaustive and effective exploitation of quantitative variability in breeding programs.

Epistasis was shown to control a large part of the resistance of pepper to *P. capsici*. To-date, very few authors have detected significant epistasis between QTLs (see references in the following). Interactions were generally tested only between markers significantly associated with a putative QTL, or else the significance level (α value) of the interactions were not conclusive (Nienhuis et al. 1987; Nodari et al. 1993; Schön et al. 1993). Only Wang et al. (1994) has identified several digenic epistatic effects at significance levels between 10^{-2} and 10^{-4}. In our study, digenic epistasis was evident at levels ranging from $5 \cdot 10^{-4}$ to $8 \cdot 10^{-6}$. Moreover, most of the interactions were significant for several resistance criteria and/or were significant for several tightly linked markers. Involvement of additive×additive and higher-order epistasis in resistance to *P. capsici* have been reported by Bartual et al. (1991 1994) in pepper crosses including several sources of resistance. Interactions between different sources of resistance were also suggested by results from recurrent selection (Palloix et al. 1990). Additional information was obtained from the present QTL analysis concerning the relative effects of additivity and epistasis; the global-value R^2 due to epistasis (21–62% depending on resistance components) was almost similar to that due to additivity (0–73%). Moreover, some interactions were detected between QTLs without any additive effects suggesting that some genes may be effective only in the presence of other genes. Thus, the detection of epistasis between QTLs requires looking for interaction effects between all the markers. Additional new information obtained from QTLs is that favourable epistasis can occur not only between alleles from the same parent (i.e. from the resistant or susceptible parent) but also between alleles from different parents (i.e. one from each parent). This underlines the influence of the susceptible genetic background. The loss of such favourable gene combinations can be responsible for the difficul-

Fig. 2 Localization of QTLs affecting the resistance to *P. capsici* on the intraspecific map of pepper. Note: *vertical lines* indicate linkage groups (LGs). The LGs assigned to chromosomes (Lefebvre et al. 1995) are indicated by a French color name (Pochard 1970). *Symbols for loci* are shown on the left of vertical lines. *Horizontal bars* indicate factors associated with resistance criteria. *Length of bars* indicate percent of variation accounted for by marker locus. *Bars on the right* of the LG mean that a favourable allele for the resistance is carried in the resistant parent; conversely *bars on the left* mean that a favourable allele is carried in the susceptible parent. *Curved lines* indicate interactions between QTLs (epistasis). The *thickness of curved lines* is proportional to the effect of epistasis. Different symbols are used for the different resistance criteria


LG8
tg83B
T18_1.6
B07_0.5
P13_1.6
P09_0.25
A10_0.6
R13_0.4
D11_0.7
tg55A
D11_0.8
C04_0.45
R15_1.25
R12_0.5
S01_0.8
cd19
LG1
R10_0.9
R13_1.8
tg17
cd 16
ct70
tg77
tg161
tg19
tg04
tg83A
Mnr-1
ct128
cab-1
tg55B
BRUN
L
tg36
tg46
tg47
ct120
tg379
B11_1.0
P13_0.9
tg104
E08_0.5
D20_1.5
ct114A
(unlinked marker)
LG5
tg122
ct268
O10_0.65
C01_1.0
N03_0.4
A10_0.85
R12_0.9
AE04_1.9
B20_0.6
F11_0.65
T07_1.6
O05_0.7
M11_1.5
ct259
tg312
P11_0.8
I07_1.4
D05_1.0
JAUNE
ct94
Rbcs-3
cd35
C
LG9
tg62
cd55
LG10
tg370
tg496
LG12
ct135
Q04_0.3
LG2
tg498
O02_0.55
R19_0.75
N09_0.7
AE04_2.3
F16_1.3
T11_0.3
NOIR
A13_1.8
up
tg124
tg194
F16_0.5
LG3
tg21
tg70
ct131
tg29
tg24
tg59
tg56
tg76
C01_0.75
tg483
(unlinked marker)
POURPRE
tg414
tg74
D11_0.6
M12_1.0
A03_1.3
P16_0.35
ct141
tg66B
N09_0.65
O07_0.75
R15_0.4
A13_1.2
O20_1.2
cd08
D11_1.3
tg57
B04_0.45
O02_0.4
A03_0.7
r45S
C01_1.25
20 cM (Kosambi)
% R² (additive effect)
0 10 20 30
Regarding parental values
comparable effect
reverse effect
%R² (epistatic effects)
10-20
20-30
Root index
Receptivity
Inducibility
Stability

ties in introgressing the resistance into susceptible cultivars through backcrossing. Conversely, from the contribution of alleles from the susceptible parent, as well as the interactions between resistance QTLs from different origins, one might expect transgressive progenies that could be selected using the above markers.

Components of resistance

The different components of resistance, namely root-rot index, receptivity, inducibility and stability, are controlled by different sets of QTLs. We propose that QTLs, specific for one component or one inoculated organ, are named "specialist QTLs" whereas QTLs involved in several resistance criteria are named "generalist QTLs". The different criteria correspond to different steps of the host-pathogen interaction and of the resistance expression (Pochard et al. 1983). The specialist QTLs may be specifically expressed or induced during these different steps or in particular organs. They may correspond to different defense mechanisms that are also present in susceptible plants (reverse QTLs). Organ-specific defense mechanisms were reported by Molot et al. (1985) who showed that the pepper phytoalexin (capsidiol) was accumulated in the aerial parts but never in roots of plants infected by *P. capsici*. Young et al. (1993) also showed that the expression of QTLs involved in the resistance of mungbeans to powdery mildew depended on the time after infection. Conversely, the generalist QTLs which are independent of time or organ were probably the cause of the phenotypic correlations observed between the resistance criteria. These QTLs may be constitutive or expressed all along the host-pathogen interaction and might correspond to resistance genes. Besides these hypotheses, the phenotypic dissection of complex resistance allows one to detect several, and more simple, components that correspond to distinct genetic factors (QTLs). Thus it proved to be pertinent for both breeding and QTL mapping.

Some genomic regions appeared essential to the expression of resistance. The region of the Brun chromosome linked to the marker tg379 had an additive effect on the root-rot index and epistatic effects with three others regions involved in the four resistance criteria. Likewise, the epistatic effect between linkage group 2 and the Noir chromosome had a large effect on three criteria. This interaction was detected at a very low threshold of type-I error probability (10^{-5}). A QTL was also detected in the vicinity of the locus *L*, which controls the hypersensitive resistance to TMV. At this locus, both resistance factors (to TMV and *P. capsici*) were from Yolo Wonder. The same resistance or defense factor might be active against these two pathogens, or this genomic region may correspond to duplicated resistance genes as observed in lettuce (Maisonneuve et al. 1994). At present, it is not possible to conclude whether generalist QTLs correspond to pleiotropic genes or to clusters of tightly linked genes. Several authors have observed QTLs in the vicinity of loci affecting specific resistance (Freymark et al. 1993; Dirlewanger et al. 1994; Leonard-Schippers et al. 1994; Wang et al. 1994). Robertson (1989) suggested that genes for qualitative effects and genes affecting quantitative traits could be allelic.

The molecular basis for quantitative resistance to plant pathogens is poorly known. Mapping specific functional genes may provide information concerning their contribution to quantitative resistance responses. Several authors have demonstrated the co-segregation of resistance QTLs with genetic loci coding for functional genes induced in defense mechanisms (peroxidase, chalcone isomerase, chalcone synthase, phenylalanine ammonia-lyase, 4-coumarate coA ligase, Pathogenesis-Related proteins) (Giese et al. 1993; Nodari et al. 1993; Leonards-Schippers et al. 1994; Ferreira et al. 1995). Such genes provide good candidates to further explore the function of QTLs involved in polygenic resistances, and to inquire if induction of defense genes by recognition genes may be related to epistatic interactions.

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