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

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*<sub>1</sub>-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*<sub>1*. This same DH population was used 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.

### 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 4 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 parental genotypes (*P*<sub>i</sub> and *E*<sub>j</sub>) and the mean disease score of the *j*th repetition belonging to the *i*th DH line, *μ* is the mean of all the data, *L* the DH line i effect and *E*<sub>ij</sub> the residual). Heritabilities (*h*<sup>2</sup>) were calculated from the ANOVA with the formula

\[\text{h}^2 = \frac{\sigma^2_g}{\sigma^2_g + (\sigma^2_n/\text{df})}\]

with *σ*<sub>g</sub> the genetic variance, *σ*<sub>n</sub> the environmental variance and *df* 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.

**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 the *P. capsici* inoculum 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*<sup>2</sup>).* This 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.