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ORIGINAL PAPER

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# Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*

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**Abstract** Clubroot, caused by *Plasmodiophora brassicae,* is one of the most damaging diseases of vegetable *Brassica* crops in the world. In this study, genetic control and mapping of loci implied in quantitative resistance against five isolates of *P. brassicae* were studied in the $F_1$ and $F_{2/3}$ progenies of the cross C10 (resistant kale)× HDEM (susceptible broccoli). A genetic map was constructed using RFLP, random and specific PCR-based markers. The 199 loci were assembled into nine linkage groups covering 1,226.3 cM. The $F_3$ families were assessed for resistance under controlled conditions with four single-spore isolates and one field isolate. A total of nine genomic regions were detected for clubroot resistance. Depending on the isolate, two to five QTLs were identified. The total phenotypic variation accounted for by QTLs ranged from 70% to 88% depending on the isolate. One of the QTLs (*Pb-Bo1*) was detected in all isolates and explained 20.7–80.7% of the phenotypic variation. *Pb-Bo1* had a major effect on three isolates but this effect was weaker for the last two. Five QTLs with minor effect were identified in only one isolate. To construct clubroot resistant varieties, the existence of both broad-spectrum and isolate-specific QTLs should be taken into account for the choice of genomic regions to use in a marker-assisted selection strategy.

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## Introduction

Clubroot, caused by the obligate biotrophic soil-borne pathogen *Plasmodiophora brassicae*, is one of the most damaging diseases of vegetable *Brassica* crops in the world. Infected plants show symptoms leading to gall formation on the root system. These clubs inhibit nutrient and water transport, stunt the plant growth and increase the susceptibility to wilting. In areas where *Brassica* vegetables are cultivated intensively, cultural practices or chemicals treatments either have not been successful in keeping these crops healthy, or are too expensive. Therefore, the development of resistant cultivars is the most efficient way to control clubroot for all *Brassica* crops.

Resistance to clubroot has been reported in different cruciferous species including the three most commonly cultivated: *Brassica napus*, *B. rapa* and *B. oleracea* (Crute et al. 1980). Although several sources of resistance have been identified in *B. oleracea* (reviewed by Crute et al. 1980; Crisp et al. 1989; Monteiro and Williams 1989; Dias et al. 1993; Manzanares-Dauleux et al. 2000b), few breeding programs for resistance have been successful. Clubroot resistance in *B. oleracea* is frequently incomplete, rarely expressed at a high level and is largely carried by types unsuitable for production purposes (Crute et al. 1983). Precise genetic control of resistance to clubroot in the various crops of *B. oleracea* is still unclear and information is relatively limited. Authors usually conclude that resistance is quantitative under polygenic control and involves recessive (Crute et al. 1983; Voorrips and Visser 1993) or dominant (Laurens and Thomas 1993; Grandclément et al. 1996) alleles. However, quantitative trait loci (QTL) analysis in *B. oleracea* has revealed a limited number of genetic factors implied in resistance. In field evaluations under natural infection, Moriguchi et al. (1999) detected only one QTL controlling clubroot resistance. Under controlled conditions using field isolates as inocula, one or two QTLs were identified (Landry et al. 1992; Figdore et al. 1993; Voorrips et al. 1997).

The complex nature of the interaction between *B. oleracea* resistance genes and *P. brassicae* populations is also a limitation on the effective deployment of resistance. In this pathosystem, plant-pathogen interaction has classically been considered as non-differential, but isolate-specific resistance responses have also been described (Crute 1986; Manzanares et al. 1996). Indeed, the effectiveness of the resistance genes present in *B. oleracea* has been rarely tested against a large range of pathotypes of the pathogen and in all these previous QTL investigations the question of the race-specificity of genetic factors implied in resistance was not addressed. Moreover, the interpretation of the results was often limited by non-homogeneous reactions owing to the use of field isolates of the pathogen (i.e., *P. brassicae* isolated from a single root gall, consisting of a mix of genotypes) in the resistance tests, making it difficult to define the race-specificity of the resistance genes. It has been recognized, however, that significant differences in pathogenicity exist both among field populations of *P. brassicae* and within field isolates (Buczacki et al. 1975; Crute et al. 1980; Jones et al. 1982; Schoeller and Grunewaldt 1986; Voorrips 1995; Somé et al. 1996; Kuginuki et al. 1999; Manzanares-Dauleux et al. 2001). Interaction between isolates can hide virulence factors (Jones et al. 1982), and thus a strong host resistance system against a specific pathogenic factor in the pathogen may mask the expression of other major or minor resistance genes.

The use of homogeneous *P. brassicae* isolates (single spore isolates (SSI) developed from one resting spore) can simplify and facilitate the detection of the genetic factors implied in clubroot resistance and the study of their specificity. Information on the number, effects, chromosomal location and specificity of loci controlling resistance to clubroot in *B. oleracea* would help to design breeding strategies and would greatly facilitate the development of resistant cultivars through the use of marker-assisted selection. The present study reports on the genetic analysis of resistance to clubroot from C10, an inbred line selected from a French kale landrace showing a high level of resistance to several pathotypes of *P. brassicae* (Manzanares-Dauleux et al. 2000b). The location, effects and specificity of loci involved in the control of five different *P. brassicae* isolates (four SSI and one field isolate) were compared in a $F_{2/3}$ population from the cross between the resistant line C10 and a highly susceptible broccoli (HDEM). Of the nine QTLs identified, one was common to all the isolates and the others were specific to one, two or three isolates. Depending on the isolate, the magnitude of the effect of both the common and the isolate-specific QTLs was variable.

## Materials and methods

### Plant material and pathogen isolates

The C10 line, selected from a French kale landrace (*B. oleracea* var. *acephala*), shows a high level of resistance to different isolates of *P. brassicae* (Manzanares-Dauleux et al. 2000b). HDEM is a susceptible doubled haploid (DH) broccoli (*B. oleracea* var. *italica*) from the *B. oleracea* collection of INRA. C10 was crossed as the female parent to the broccoli HDEM. A single $F_1$ hybrid plant was bud self-pollinated to obtain the $F_2$ progeny. $F_2$ plants were bud self-pollinated to generate $F_3$ families that were used to assess disease resistance and to build the genetic map.

Five different isolates of *P. brassicae* were used for the tests under controlled conditions: three SSI (Ms6, K92-16 and Pb137-522), one "selection" isolate (eH) and one field isolate (K92). Biological and molecular characterization of the isolates Ms6, K92-16, Pb137-522 and K92 was done previously (Manzanares-Dauleux et al. 2001). The "selection" isolate eH (i.e., an isolate displaying a molecular and phytopathological pattern similar to a single spore isolate), kindly provided by J. Siemens (University of Berlin), was described by Fähling et al. (2003). Isolates were characterized for pathogenicity on three differential *B. napus* cultivars according to the classification proposed by Somé et al. (1996). These isolates belong to the pathotypes P1 (Ms6 and eH), P2 (K92), P4 (K92-16) and P7 (Pb137-522).

### Resistance tests

Assessment of resistance was carried out in glasshouses as previously described (Manzanares-Dauleux et al. 2000b). Resistance tests on the $F_3$ families were conducted in a randomized block design with two blocks and two replicates per block. Two hundred and sixty-six $F_3$ families were tested with isolate Ms6, 157 with isolate K92, 150 with isolates K92-16 and Pb137-522, and 122 with isolate eH. For isolates K92, K92-16, Pb137-522 and eH, 20 plants per $F_3$ family were tested; for isolate Ms6, 40 plants per $F_3$ family were tested. Parental lines, $F_1$ progeny and a susceptible control (*B. rapa* spp. *pekinensis* cv. Granaat) were included in all replicates. The plants were scored for disease infection 6–8 weeks after inoculation and records were made using the scale described by Manzanares-Dauleux et al. (2000a). A disease index (DI) was calculated by summation of the coefficients (0, 25, 50, 75, 100) affecting each plant class frequency (Buczacki et al. 1975) of each genotype or $F_3$ family.

### DNA extraction and molecular data

Genomic DNA was extracted from a pool of 20–40 young plants of each $F_3$ family in order to reconstitute the genotype of the corresponding $F_2$ plant. A modified CTAB (cetyl-trimethyl-ammonium-bromide) protocol was used (Doyle and Doyle 1990).

RAPD markers, obtained from 54 RAPD primers from Operon Technologies Inc., were named using the kit letter in lower case and the primer number followed by the estimated molecular weight of the fragment. PCR amplifications were conducted in a 12.5 $\mu$l reaction mix containing 1.9 $\mu$M of $MgCl_2$ (Eurobio), 400 $\mu$M of dNTPs, 0.25 U of *Taq* polymerase (Eurobio), 0.2 $\mu$M of primer and 25 ng of DNA. PCR amplifications involved 30 s at 94°C, 45 cycles of 30 s at 94°C, 1 min at 35°C and 2 min 30 s at 72°C, followed by 5 min at 72°C. Amplified products were separated on a 1.8% agarose gel, stained with ethidium bromide and visualized with an UV transilluminator. Three RAPD-like markers (RA12-75, WE22 and WE49) (Kuginuki et al. 1997) and two corresponding STS (sequence tagged sites) derived markers (W22Bcrs1-2 and RA12PCR) (Kikuchi et al. 1999) linked to a clubroot resistance gene in *B. rapa* were used; primer sequences and PCR conditions are described by the authors.

Fifty-two RFLP probes which provided loci spaced on a previous *B. oleracea* map (Le Corre et al. 1994) were used. These

probes correspond to anonymous clones from different *Brassica* gDNA or cDNA libraries (Le Corre et al. 1994). Probe preparation and Southern blot analysis were performed as described in Hoisington et al. (1992). Plant DNA was digested either by *Hind*III or by *Eco*RV depending on the probe used. RFLP markers in the genetic map were designated with the prefix PBB and T followed by a number.

Twenty-two amplified consensus genetic markers (ACGM) developed from sequences of known function genes of *Arabidopsis thaliana* by Brunel et al. (1999) and Fourmann et al. (2002) were used. Markers were named by the *Arabidopsis* gene name in capitals. Another marker developed in our laboratory from the *A. thaliana* O-methyltransferase (*OMT1*) gene (accession number U70424) was mapped using the primers fwd: 5′-GGC GGA GAC ACA ATT AAC TC-3′ and rev: 5′-TGA ATA TGG CAT CAC CTT TAG GGA C-3′, and an annealing temperature of 53°C. Ten resistance gene analogues developed in *B. napus* by Fourmann et al. (2001) (named RGA followed by a number) and in *B. oleracea* by Vincente and King (2001) (BoRGLIV and BoRGLV) were amplified following conditions described by the authors. Six EST (expressed sequence tag) markers, named At followed by a letter in upper case and a number, were developed using primers designed on *A. thaliana* ESTs and kindly provided by A. Barakat and M. Delseny (Univ. Perpignan, France). PCR were conducted in a 25 $\mu$l reaction mix containing 2.5 mM of $MgCl_2$, 200 $\mu$M of dNTPs, 1 U of *Taq* polymerase, 1 $\mu$M of each primer and 50 ng of DNA. Amplifications were performed at 94°C for 2 min, 35 cycles of 94°C for 30 s, 53°C for 30 s and 72°C for 30 s, then finished by 72°C for 10 min.

### Map construction

Mapping population consisted in 157 to 266 $F_3$ families. For each segregating marker, a chi-squared analysis was performed to test for deviation from the expected segregation. Marker order was performed with MapMaker/Exp 3.1 (Lincoln et al. 1992) with the "Compare" command. Linkage groups (LG) were established with a minimum LOD threshold of 4.0. Genetic distances between markers were estimated with Kosambi and Haldane functions.

### Data analysis

Data from each isolate were analyzed using a generalized linear model (PROC GLM of Statistical Analysis System, SAS, 1989). DI genotypic values of each $F_3$ family were assessed from ANOVA after freeing block and residual components; the estimated genotypic value was the experimental unit for QTL analysis. Heritability was estimated from ANOVA with the formula $h^2=\sigma^2_g/[\sigma^2_g+(\sigma^2_e/n)]$ with $\sigma^2_g$ the genetic variance; $\sigma^2_e$ the environmental variance and $n$ the number of replicates per $F_3$ family. The Pearson coefficient was calculated to determine phenotypic correlation between DI traits measured with the different isolates.

QTL detection was performed using interval mapping (IM) and composite interval mapping (CIM) with the QTL Cartographer software package (Basten et al. 1997). Ten markers, selected by a forward-backward stepwise regression analysis, were used as cofactors in the CIM procedure, with a 10-cM window size. CIM was performed at a LOD threshold of 2.5. Markers associated to clubroot resistance with both IM and CIM methods were checked by one-way ANOVA with a significant level threshold $P\leq0.05$.

## Results

### Linkage map

Ninety-three RAPD polymorphic markers were amplified with the 54 primers. An average of 1.7 markers was obtained per RAPD primer. Fifty-eight polymorphic RFLP markers between parental lines were detected with 52 probes, with six probes each detecting two loci localized in different linkage groups. The 48 remaining markers were all RADP-like and the PCR-based specific markers.

One hundred and ninety-nine markers were scored for the $F_3$ families among which 191 were scored for 157 $F_3$ families and 8 for 266 families. Sixty-one markers were co-dominant. One hundred and ninety-four markers were assembled into nine LGs (Fig. 1) spanning a map distance of 1,374.2 centiMorgans (cM) Haldane (1,226.3 cM Kosambi), which represented one marker every 7.1 cM. Five markers were not linked to any linkage group and four markers formed an additional small group (not included in Fig. 1). Length (with Haldane function) of each LG varied from 84.4 cM (LG9) to 265.2 cM (LG1) with a distance between two markers varying from 4.6 cM (LG9) to 9.3 cM (LG4).

Thirty-five markers (17.6%) exhibited significant skewing ($P<0.05$) from the expected Mendelian ratios of 3:1 or 1:2:1. Most of the skewed loci were mapped on groups LG2, LG6 and LG8 (Fig. 1). On LG6, nine of the ten distorted markers showed an excess of C10 alleles while all the distorted loci on LG8 (eight markers) and LG2 (five markers) segregated with an excess of HDEM alleles.

### Resistance analyses

Figure 2 shows the frequency distribution for the DI criteria in the $F_{2/3}$ population tested with the five isolates. DI values of the susceptible control (*B. rapa* spp. *pekinensis* cv. Granaat) were 100 in all experiments. Block effect was significant ($P<0.01$) in tests with isolates K92-16 and K92. Whatever the isolate, the parental line C10 showed a high level of resistance (DI from 0 to 16.7), while the DH line HDEM was highly susceptible (DI=100 for all isolates). The disease reaction rating of the $F_1$ plants was variable depending on the isolate used. With the isolate Ms6, the mean disease severity of the $F_1$ progeny was slightly higher than the mid-parent value (59 versus 50), suggesting the likelihood of additivity for the resistance. For the other four isolates, the $F_1$ plants exhibited DI values similar to that of the resistant parent C10. This indicated that the resistance carried by C10 to isolates K92, K92-16, Pb137-522 and eH was dominant.

Analysis of the resistance against isolates Ms6 and eH in the $F_{2/3}$ population showed a continuous pattern of distribution, suggesting that C10 resistance was controlled by a polygenic system (Fig. 2). The DI distributions for isolates Pb137-522, K92-16 and K92 showed also a continuous, but near asymmetric, bimodal distribution (Fig. 2). The involvement of a major genetic factor for clubroot resistance would explain such behavior. The genetic variation for the DI trait was highly heritable in the $F_{2/3}$ population. Heritabilities were $h^2=0.90$ for the Ms6 and K92-16 isolates, $h^2=0.89$ for the Pb137-522

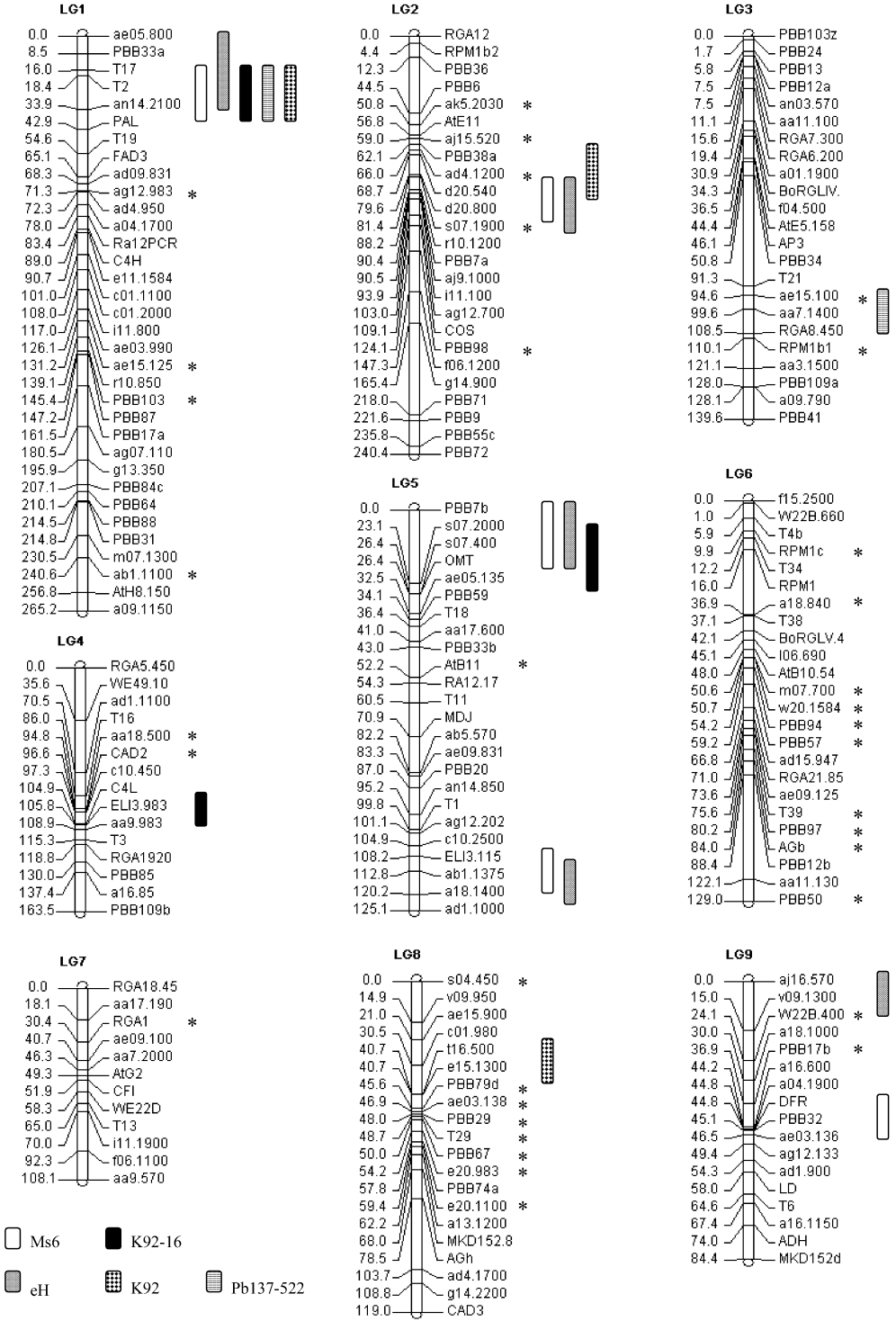


**Fig. 1** QTL localization on the genetic map of *Brassica oleracea* obtained from the C10×HDEM cross. Linkage groups are numbered LG1 to LG9. *Boxes* indicate QTL positions detected with the five isolates with one-LOD likelihood intervals. Different symbols are used for the five isolates analyzed. * indicates loci with segregation distortion

isolate, $h^2$=0.88 for the K92 isolate and $h^2$=0.86 for the eH isolate. There was a strong correlation between measures of resistance to the five isolates (from $r$=0.912 to $r$=0.427; $P$<0.001; Table 1). The lowest correlation was found between the isolate eH and the other isolates, while the highest correlation was obtained between isolates K92 and K92-16.

## QTL mapping for clubroot resistance

Composite interval mapping analysis revealed nine putative QTLs, localized on seven LGs, involved in clubroot resistance against the five isolates (Table 2, Fig. 1). All the putative QTLs, named *Pb-Bo1* to *Pb-Bo9* according to their location on the linkage groups, were confirmed by interval mapping and by one-way analysis of variance

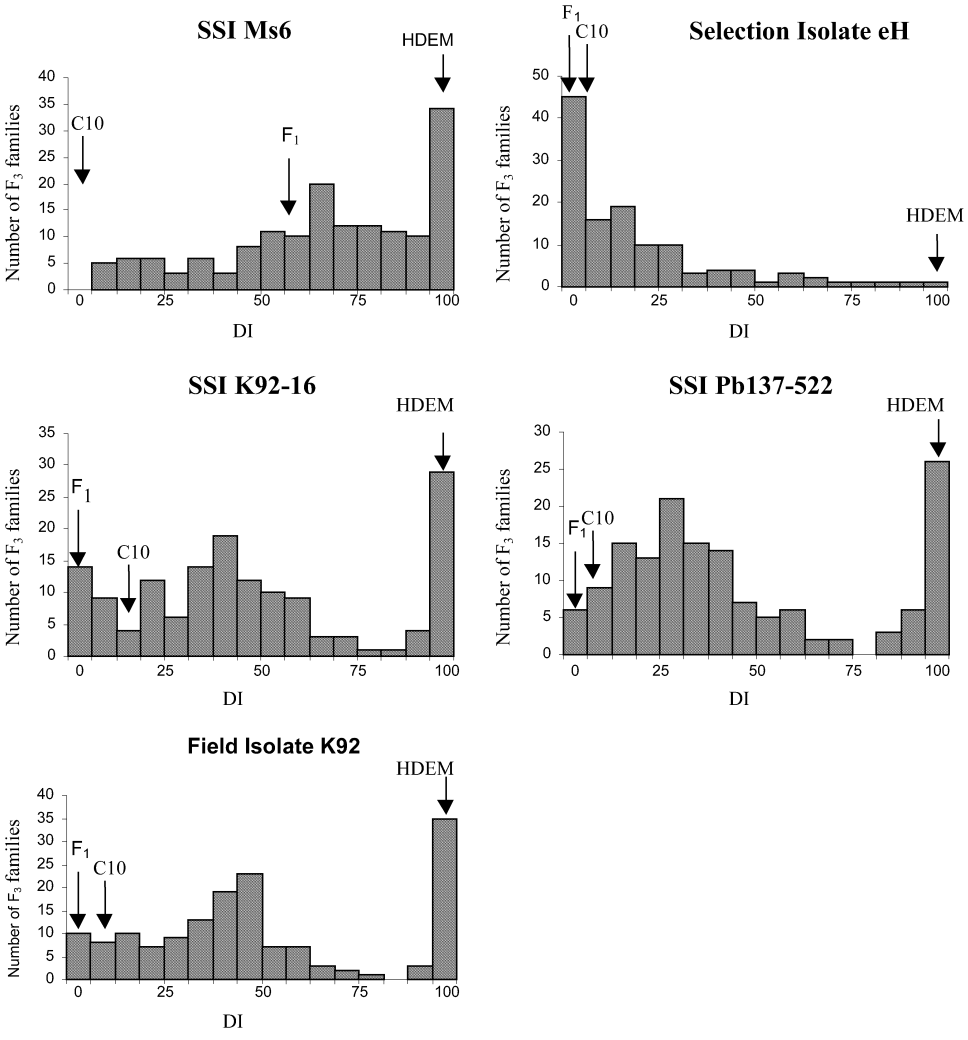


**Fig. 2** Genotypic distributions estimated for the Disease Index (DI) criteria for resistance to five isolates of *P. brassicae* in the $F_{2/3}$ population C10×HDEM. The *arrows* indicate the position of the DI mean values of the parents and the $F_1$ progeny

**Table 1** Phenotypic correlation coefficients among measures of clubroot resistance (DI) to five isolates of *Plasmodiophora brassicae* in 157 $F_3$ families derived from the cross between the C10 line and the HDEM doubled haploid line

| | K92 | Pb137-522 | K92-16 | eH |
|---|---|---|---|---|
| Ms6 | 0.792** | 0.696** | 0.804** | 0.581** |
| K92 | – | 0.897** | 0.912** | 0.485** |
| Pb137-522 | – | – | 0.884** | 0.427** |
| K92-16 | – | – | – | 0.486** |

**$P<0.001$

($P \leq 0.05$). Because the three methods provided similar results, the data shown correspond to results obtained with CIM method, which provides a more accurate estimation of the location of the putative QTLs. Depending on the isolate, between two and five QTLs were identified. With the isolates Ms6 and eH, five QTLs were detected, explaining in a multiple QTL model 78% and 70% of the total phenotypic variation, respectively. Three QTLs were identified with the isolates K92 and K92-16; when combined in a multilocus model, the percentage of phenotypic variation explained was 88% and 83%, respectively. From the data set of the isolate Pb137-522, two QTLs were detected, explaining 81% of the phenotypic variation (Table 2). One QTL located on LG1 was common to the five isolates tested. This locus, designated *Pb-Bo1,* was detected with a high LOD score and explained the largest fraction of the phenotypic variance for all of the isolates (34–80.7%), except for isolate eH (20.7%). From the data sets of the isolates Ms6, K92, K92-16 and Pb137-522, the peak of *Pb-Bo1* was located at the same position (near the RFLP marker T2). The QTL localized on LG2 (*Pb-Bo2*) detected in the isolates eH, Ms6 and K92 explained a large part of the phenotypic variation in eH (21%). Eight QTLs were identified in one, two or three isolates. Five minor-effect QTLs were each detected in only one isolate: *Pb-Bo3*, *Pb-Bo4*, *Pb-Bo8*, *Pb-Bo9a* and *Pb-Bo9b* were identified in isolates Pb137-522, K92-16, K92, eH and Ms6, respectively. Two QTLs located on the two extremities of LG5 (*Pb-Bo5a* and *Pb-Bo5b*) were identified in three and two isolates, respectively. Four of the five QTLs detected in the two isolates belonging to pathotype P1 (Ms6 and eH) were common (*Pb-Bo1, Pb-Bo2, Pb-Bo5a, Pb-Bo5b*); however the effect of these QTLs was variable depending on the isolate

**Table 2** Summary of QTLs detected for clubroot resistance against five different isolates of *P. brassicae* using a composite interval mapping method. The markers given are the left flanking marker of the test positions. The estimated position of each QTL using the CIM method along the linkage group is also shown. The phenotypic variation explained by each QTL, and the total phenotypic variation explained by all detected QTLs (using a multiple QTL model, ANOVA) is given. Parent represents the **p**arental allele which increased the resistance level for each QTL

| Isolate | QTL | Marker | Position | LOD | $R^2$ | $R^2t$ | Parent |
|---|---|---|---|---|---|---|---|
| Ms6 | *Pb-Bo1* | T2 | 20.41 | 27.5 | 34 | 78.3 | C10 |
| | *Pb-Bo2* | s07.1900 | 85.41 | 8.4 | 6.8 | – | C10 |
| | *Pb-Bo5a* | PBB7b | 0.01 | 6.2 | 4.3 | – | C10 |
| | *Pb-Bo5b* | ab1.1350 | 116.81 | 3.8 | 3 | – | C10 |
| | *Pb-Bo9b* | a04.1900 | 44.78 | 5.2 | 3.4 | – | – |
| K92 | *Pb-Bo1* | T2 | 20.41 | 64.3 | 77.4 | 87.8 | C10 |
| | *Pb-Bo2* | PBB38a | 66.01 | 5.6 | 2.5 | – | C10 |
| | *Pb-Bo8* | c01.980 | 36.49 | 4.1 | 2.1 | – | HDEM |
| Pb137-522 | *Pb-Bo1* | T2 | 20.41 | 59.5 | 80.7 | 80.7 | C10 |
| | *Pb-Bo3* | aa7.1400 | 144.46 | 2.6 | 4.7 | – | C10 |
| K92-16 | *Pb-Bo1* | T2 | 20.41 | 53.3 | 68 | 82.8 | C10 |
| | *Pb-Bo4* | aa9.983 | 112.89 | 3.9 | 2.8 | – | C10 |
| | *Pb-Bo5a* | s07.2000 | 29.27 | 4.4 | 3.3 | – | C10 |
| eH | *Pb-Bo1* | ae05.800 | 0.01 | 6.4 | 20.7 | 69.7 | C10 |
| | *Pb-Bo2* | r10.1200 | 88.21 | 13 | 21 | – | C10 |
| | *Pb-Bo5a* | PBB7b | 0.01 | 3 | 4.2 | – | C10 |
| | *Pb-Bo5b* | a18.1400 | 118.19 | 4.3 | 6.1 | – | C10 |
| | *Pb-Bo9a* | aj16.570 | 10.01 | 3 | 5.5 | – | C10 |

(Table 2). For all the putative QTLs detected, except for *Pb-Bo8*, the alleles of the resistant parent C10 were associated with resistance.

In order to understand the functions of the genes underlying the QTLs, several genes that have been described in resistance mechanisms in other plant-pathogen models were mapped. Three putative genes, *PAL* (ACGM marker), *OMT1* and *RGA8*, were located in the confidence interval of *Pb-Bo1*, *Pb-Bo5a* and *Pb-Bo3*, respectively. In one-way ANOVA, *PAL* and *OMT1* ex-plained 39% and 6% of the phenotypic variation, respectively. *RGA8* was not significantly linked to resistance with this method. Additionally, five markers linked to clubroot resistance in *B. rapa* developed by Kuginuki et al. (1997) and Kikuchi et al. (1999) were mapped. In the *B. oleracea* model described here, they were not linked to clubroot resistance.

## Discussion

The results of this study indicated that both broad-spectrum and isolate-specific QTLs are involved in the control of clubroot resistance in *B. oleracea.* Of the nine genomic regions identified, one was common to all isolates and the others were specific to one, two or three isolates. Moreover, the magnitude of the QTLs effect was variable depending on the isolates.

### *B. oleracea* map

The kale line C10 and the DH broccoli HDEM were selected for these genetic studies because they show a considerable difference in clubroot resistance and a high level of polymorphism at both the phenotypic and molecular levels. The genetic map obtained was composed of nine linkage groups (coinciding with the haploid chromosome number in *B. oleracea*) spanning a map distance of 1,226.3 cM (Kosambi). The length of the present genetic map was higher than most of the *B. oleracea* maps already published: 1,112 cM in Landry et al. (1992) or 823.6 cM in Morigushi et al. (1999), but smaller than the map described in Cheung et al. (1997) covering 1,606 cM, suggesting however a good coverage of the genome.

Seventeen per cent of the mapped loci showed a distorted segregation. These loci mostly clustered on LG2, LG6 and LG8. Biased segregation, ranging from 12% to 65%, has been reported in several *B. oleracea* mapping studies with most of the loci showing segregation ratio distortions clustered in a few linkage groups (Kianian and Quiros 1992; Landry et al. 1992; Cheung et al. 1997; Voorrips et al. 1997). These authors explained these results by either the presence of lethal alleles in the neighborhood of these markers or a quantitative selection advantage for one or more alleles. Kianian and Quiros (1992) observed that the distortion of segregation ratios increased also with the level of divergence of the parents. In the present study, the probably high divergence between the two parents could explain the biased segregation observed.

### QTL detection

By combining the results of the five isolates, nine genomic regions containing putative QTLs associated with clubroot resistance were identified. Depending on the isolate, between two and five QTLs could be detected. Moreover, the near asymmetric bimodal distribution observed with isolates Pb137-522, K92 and K92-16 and the results of the QTL analysis suggested the presence of a major genetic factor controlling clubroot resistance. Previous published studies reported the involvement of one major factor in the control of clubroot resistance in *B. oleracea*. Landry et al. (1992) mapped two QTLs (CR2a and CR2b), of which CR2a had a major effect on resistance (50% of the phenotypic variation). Because the parentage of the resistant cabbage line used by authors included a resistant *B. napus* (cv. Wilhelmsburger), the

authors concluded that the major QTL CR2a probably originates from this species (Chiang et al. 1980). Voorrips et al. (1997) found at least two QTLs for clubroot resistance in a *B. oleracea* DH population, explaining 68% of the phenotypic variation. One of these QTLs was considered as a major gene since it was responsible for half of the total additive resistance effect. It is not possible to conclude if these major factors correspond to one of the QTLs detected in this work because no markers in common with previously published *B. oleracea* maps were used. Moreover, comparison of the effects of clubroot resistance genes among the published studies is difficult, owing to the use of different resistant sources, screening methods and pathogen isolates. In the previous published studies, genetics of the resistance were analyzed using field isolates consisting of a mix of genotypes. In these isolates, mixed genetic races of the pathogen could be responsible for the unexplained remaining variance and could hide QTLs with weaker effects. In the present study, the advantage of using SSI in resistance tests is the absence of interaction between different pathogen genotypes, thus allowing a better differentiation of the resistance genes.

## Isolate-specificity of detected QTLs

The use of five different *P. brassicae* isolates allowed us to dissect and to analyze more accurately the specificity of the quantitative resistance traits studied. Of the nine QTLs detected, one QTL (*Pb-Bo1*) was common to all the isolates tested. This locus acted as a major resistance gene against Pb137-522, K92 and K92-16 isolates, but had a weaker effect against isolates belonging to the pathotype P1 (Ms6 and eH). Manzanares-Dauleux et al. (2000a) identified a dominant major gene for resistance in the *B. napus* line Darmor-*bzh*, against isolate Pb137-522 of *P. brassicae*. The same locus was implied in partial resistance against SSI K92-16 but showed a weaker effect. As in *B. napus*, it can be hypothesized that a same genomic region can act as a QTL or as a major gene according to the isolate. Other examples showing that qualitative and quantitative resistance may share a common genetic basis have been reported for other fungus and plant virus interactions (Freymark et al. 1993; Wang et al. 1994; Caranta et al. 1997). In rice, the well-characterized *Xa4* locus acted either as a major gene against avirulent pathogen races or as a QTL against virulent races (Li et al. 1999). These authors suggested that the resistance genes, once having been overcome by a pathogen strain, may retain a residual effect, and that quantitative resistance may be an accumulation of residual effects of defeated genes with qualitative effects. Five other QTLs were each detected in only one isolate. These five QTLs (*Pb-Bo3*, *Pb-Bo4*, *Pb-Bo8*, *Pb-Bo9a* and *Pb-Bo9b*) had a minor effect for isolates Pb137-522, K92-16, K92, eH and Ms6, respectively, but showed no significant effect on resistance to the other isolates. In most of the *B. oleracea* studies, the plant-*P. brassicae* interaction has been identified as non-differential (non-specific). Crute et al. (1983) pointed out that one possible explanation was that resistance was controlled by several isolate-specific genes of quantitative effect. Similar arguments have been applied by Parlevliet and Zadoks (1977) to suggest that resistance is likely to be isolate-specific and under gene-for-gene control. Isolate-specific QTLs have been described in other plant-pathogen models, and have been implicated in both partial resistance (Qi et al. 1999; Arru et al. 2003) and overall resistance (Chen et al. 2003), leading to the hypothesis of specific interaction between regions of the genome and specific determinants of the pathogen isolates. Several hypotheses have been addressed for the meaning of effect and specificity variation of QTLs detected with different isolates: (1) a QTL could be a cluster of genes either belonging or not to the same multigene family; on the potato map, the GP21-GP179 region is known to contain a cluster of resistance genes specific to different plant pathogens and to different races of a same pathogen (Rouppe van der Voort et al. 1998), (2) a single gene at the QTL could carry alleles that differ for their quantitative effect, (3) a single gene could also operate in different signaling pathways leading to the resistance. The resistance response implicates a great number of genes that could be interconnected and be required in different signaling pathways (for a review, Feys and Parker 2000). Thus, QTLs detected with different races of a pathogen or with different pathogens could overlap (Bert et al. 2002; Backes et al. 2003). Nevertheless, due to the possible biased confidence intervals, it is difficult to conclude whether putative QTLs in the same genomic region involved just one genetic factor or were the result of linkage between of several of them. Advanced intercross line populations should be analyzed and more markers placed in order to break down the linkage. Another solution would be to analyze the functions underlying the QTLs. In this study, a candidate gene approach has been initiated to characterize the QTLs involved in clubroot resistance. Two co-localizations between putative known function genes (*PAL* and *OMT1*) and QTLs (*Pb-Bo1* and *Pb-Bo5a*) have been found. PAL (phenylalanine ammonia lyase, EC 4.3.1.5) catalyzes the conversion of L-phenylalanine to trans-cinnamic acid in the first step of the phenylpropanoid pathway leading to the synthesis of defense-related compounds (lignin, phytoalexins or coumarins). OMT (O-methyltransferase, EC 2.1.1.68) catalyzes several methylation reactions in the lignin biosynthesis pathway. In many plant-pathogen interactions, a transient increase in PAL activity follows inoculation or elicitor treatment (Smith-Becker et al. 1998; Klarzynski et al. 2000; Takahashi et al. 2002; Way et al. 2002). Work is in progress in our laboratory to map other known function genes and to validate the colocalizations observed.

The QTLs we detected explained from 69.7% to 87.8% of the total phenotypic variation in response to infection with the different *P. brassicae* isolates. Heritability estimates of the DI varied from 0.86 to 0.90. The unexplained variation could be due to either non-detected

QTLs because of their weak effects or incomplete map coverage. A part of the variability can also be explained by epistatic interactions. The importance of epistatic interactions in the expression of clubroot resistance has already been observed by Manzanares-Dauleux et al. (2000a) in *B. napus*. In this species, significant epistatic interactions were found between loci with and without main effects, and between alleles either from the resistant parent or from each parent. Studies on other plant-pathogen interactions showed that digenic interaction may play a role in unexplained variation for resistance to pathogens (e.g. Lefebvre and Palloix 1996).

We have shown that the relative effectiveness of the clubroot resistance genes in *B. oleracea* can be different depending on the isolate. Moreover, QTLs may be isolate-specific and non-specific and the same QTL may have major or minor effects depending on the isolate. These results must be taken into account for the choice of the QTLs to use in a marker-assisted selection process. Therefore, studies of the stability of QTLs in different genetic backgrounds and environments would help the choice of genomic regions to incorporate in susceptible cultivars in a breeding program aimed at constructing clubroot-resistant varieties.

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