Theor Appl Genet (2000) 101:885–891

M.J. Manzanares-Dauleux · R. Delourme · F. Baron
G. Thomas

Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*

Received: 10 November 1999 / Accepted:18 February 2000

Abstract Clubroot, caused by *Plasmodiophora brassicae*, is a damaging disease of *Brassica napus*. Genetic control and mapping of loci involved in high and partial quantitative resistance expressed against two single spore isolates (Pb137–522 and K92–16) were studied in the F_1 and DH progenies of the cross Darmor-*bzh* (resistant) x Yudal (susceptible). The high level of resistance expressed by Darmor-*bzh* to isolate Pb137–522 was found to be mainly due to a major gene, which we have named *Pb-Bn1*, located on linkage group (LG) DY4. Partial quantitative resistance showed by Darmor-*bzh* to the K92–16 isolate arose from the association of at least two additive QTLs detected on LGs DY4 and DY15; the QTL on DY4, explaining 19% of the variance, was mapped at the same position as the major gene *Pb-Bn1*. Epistatic interactions between nine regions with or without additive effects were detected. The total phenotypic variation accounted for by additive and epistatic QTLs ranged from 62% to 81% depending on the isolate. For one isolate, the relative effect due to additivity was similar to that due to epistasis.

Key words *Brassica napus* · Epistasis · Molecular markers · *Plasmodiophora brassicae* · Quantitative trait loci (QTL)

Introduction

The clubroot disease, caused by the obligate biotroph *Plasmodiophora brassicae* Woron., is one of the economically most important and damaging diseases of *Brassica* crops in the world. The pathogen causes swelling of parts of the roots and sometimes of the stem into typical clubs. These clubs inhibit nutrient and water transports, stunt the growth of the plant and increase susceptibility to wilting. In areas of intensive *Brassica* cultivation (specially the three more important species, *B. oleracea*, *B. napus* and *B. rapa*), clubroot is an increasing problem. Under favorable conditions, rapeseed and swede (*Brassica napus* var. *oleifera* and *B. napus* var. *rapifera*) are severely attacked, resulting in serious losses in yield and in root crops of reduced quality (Engqvist 1994; Wallenhammar 1998). As cultural practices or chemical treatments are not effective or too expensive, the development of resistant cultivars is considered an essential step to control this disease for all *Brassica* species.

Different sources of resistance to clubroot have been found in *B. oleracea*, *B. rapa* and *B. napus* (reviewed in Crute et al. 1983); in the last species, resistant forms are relatively common in cultivars of swede and fodder rape. Breeding programs to increase clubroot resistance in *B. napus* have been conducted using both intra- and interspecific sources of resistance (Gowers 1982; Lammerink and Hart 1985; Diederichsen and Sacristán 1996; Bradshaw et al. 1997). At the intraspecific level, genetic analyses have been carried out for the five *B. napus* hosts of the European Clubroot Differential set (Buczacki et al. 1975) (ECD 06 to ECD 10) as well as on several genotypes of the Wye swede, the Ditmar swede and the York swede groups (Crute et al. 1980, 1983; Gustafsson and Fält 1986). The results of these different investigations indicate that the interaction between *B. napus* and *P. brassicae* is mainly differential, the resistance in *B. napus* being race-specific. Disagreements exist with respect to the number of genes involved in the resistance. Models have been proposed based on three, four or five differential factors inherited as single independent genes, resistance being dominant to susceptibility (Brokenshire 1982; Crute et al. 1983; Gustafsson and Fält 1986).

However, information on the genetic basis of the resistance in *Brassica* remains relatively limited, especially in the subspecies *B. napus oleifera*, and a comparison of published studies is difficult. The contradictory con-

Communicated by F. Salamini

M.J. Manzanares-Dauleux (✉) · R. Delourme · F. Baron
G. Thomas
INRA Station d'Amélioration des Plantes, BP 35327,
35653 Le Rheu Cedex, France
e-mail: manzanar@rennes.inra.fr
Fax: +33-2-23-48-51-20

clusions of different authors may be explained by the heterogeneity and the diversity of the plant materials, the methodological approaches, and the environmental conditions. Furthermore, the interpretation of results is often limited by non-homogeneous reactions owing to the use of field isolates of the pathogen (heterogeneous resting spore populations) in the tests, making it difficult to define the race-specificity of the resistance genes.

The use of homozygous lines and homogeneous pathogen isolates (single spore-derived isolates, SSI) can simplify and facilitate the understanding of the genetic mechanisms involved in this host-pathogen interaction. Moreover, information on the number, effects and chromosomal location of loci controlling resistance to clubroot in the different *Brassica* species would greatly facilitate the development of resistant cultivars and would help in the design of breeding strategies through the use of marker-assisted selection. The mapping of clubroot resistance genes in *B. oleracea* has been initiated by several research teams (Landry et al. 1992; Figdore et al. 1993; Grandclement and Thomas 1996; Voorrips et al. 1997; Manzanares et al. 1998). More recently, molecular markers linked to major resistance loci in *B. rapa* have been identified (Kuginuki et al. 1997; Matsumoto et al. 1998). However, markers linked to genetic factors controlling resistance have not been reported in *B. napus*, the amphidiploid species carrying the A and C genomes of *B. rapa* and *B. oleracea*.

The study reported here was undertaken to gain insight into the genetic basis of clubroot resistance in *B. napus* and to identify and locate loci controlling clubroot resistance that are expressed against different isolates of the pathogen. Our long-term perspective, is to compare the location, the level and the specificity of resistance conferred by the resistance genes identified in *B. napus* with those of *B. oleracea*. Here we report the detection and mapping in a doubled haploid (DH) progeny of genomic regions with major and minor effects on clubroot resistance in *B. napus*.

Materials and methods

Plant material and genetic map

The segregating population used was a DH progeny derived from the cross Darmor-*bzh* x Yudal. The parental genotype Yudal is a spring inbred line selected from a Korean population. Darmor-*bzh* is a winter dwarf line, nearly isogenic to Darmor. Manzanares-Dauleux et al. (2000) showed that Darmor-*bzh* is highly or partially resistant to clubroot, depending on the isolate. The segregating DH progeny and the genetic map established from this population have been described previously (Foisset et al. 1996). Due to the additional mapped markers, the map used in this study covers 2000 cM and comprises 388 markers [predominantly restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs)] assembled into 20 linkage groups.

Pathogen isolates

Two single spore isolates of *Plasmodiophora brassicae* were used in the resistance tests. The SSIs K92–16 and Pb137–522 were derived from two field populations isolated from cauliflower clubs as described previously (Somé et al. 1996). Isolates were maintained on the susceptible Chinese cabbage (*Brassica rapa* spp. *pekinensis* cv. Granaat); clubs were harvested, washed and stored at –20°C. Both isolates were characterized for pathogenicity on three differential *B. napus* cultivars as described previously (Somé et al. 1996). The isolates belong to pathotypes 4 (K92–16) and 7 (Pb137–522).

Resistance tests

Resistance tests were done in the glasshouse. Inoculum was prepared from slowly thawed-out clubs. The clubs were then ground in distilled water and filtered through three sieves (500-µ, 250-µ and 100-µ pore diameter). The final resting spore concentration was adjusted with distilled water to 10^7 spores/ml using a Malassez cell. Resistance tests on the DH progeny from the cross Darmor-*bzh* x Yudal were conducted with 110 DH lines (20 plants per line) in a randomized block design with 2 two replicates. Both parental lines, F_1 progeny and the susceptible control (Chinese cabbage) were included in all replicates. Seeds of each genotype were germinated directly in 12-cm-diameter plastic pots containing a steam-sterilized mixture of soil, sand and peat (1:1:1: v:v, pH 6.0). Inoculation was performed by applying 1 ml of the spore suspension to the bottom of the stem base of each seedling at the 6- to 8-day stage. The plants were grown during the spring and summer months in the glasshouse under a maximum temperature of 22°C. The soil was kept moist throughout the test. Plants were evaluated for clubroot 6–8 weeks after inoculation. Plant roots were thoroughly washed, and records were made using the Buczacki et al. scale (1975) with one supplementary quotation (2+): 0 – no visible swelling; 1 – very slight swelling usually confined to lateral roots; 2 – moderate swelling on lateral roots and taproot; 2+ – severe clubs on all roots, but some roots remain; 3 – no root left, only one big gall. 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) for each replicate of each genotype or DH line. In the qualitative analysis of resistance, DH lines with DI indices less or equal to 25% (classes 0 and 1) were rated as "resistant", and plants with indices greater than 25% (classes 2–3) were rated as "susceptible."

Statistical analysis, quantitative trait loci (QTLs) detection and mapping

Chi-square tests were used to assess the observed proportions of resistant and susceptible progeny with the expected ratio. Data from each isolate were analyzed using a generalized linear model (PROC GLM of Statistical Analysis System, SAS, 1989). DI genotypic values of each DH line were assessed from ANOVA after freeing block and residual components; the estimated genotypic value per DH line was the experimental unit for QTL analysis. Raw data were used for analysis as data transformations did not improve normality. Heritability (h^2) was estimated from ANOVA with the formula $h^2=\sigma^2_g/[\sigma^2_g+(\sigma^2_e/n)]$ with σ^2_g the genetic variance; σ^2_e, the environmental variance and n the number of replicates per line.

QTLs were mapped from 110 DH lines by interval mapping using the computer program MAPMAKER/QTL 1.1 (Lincoln et al. 1992). Putative QTLs were identified for the two isolates using a LOD (Log of Odds likelihood ratio that a QTL is present vs. absent) threshold of 2.8, corresponding approximately to an overall 5% α-type-I error risk (Lander and Bostein 1989). The multiple QTL hypothesis was performed by fixing the first detected QTL and re-scanning the genome. The presence of an additional QTL was assumed if its total LOD was more than 2.0 LOD of the previous fixed QTL LOD. Due to non-normality of the genotypic distributions, QTL mapping was also performed by a non-parametric method, using the option NP scan of MAPMAKER/QTL, with a Z_w threshold of 3.6, which corresponds to a LOD threshold of 2.8. Markers associated to clubroot resistance detected by both mapping methods were then checked by one-way ANOVA (signifi-

cance level threshold $P \leq 0.05$) and by the Kruskal and Wallis nonparametric test. Digenic epistatic interactions were checked by a two-way ANOVA model with an interaction component (SAS/IML, SAS 1989) using 146 markers scattered through the genome, including the QTL-linked markers (significance level threshold $P \leq 5 \cdot 10^{-4}$, expecting 5.2 false positives).

Results

Resistance tests

Figure 1 shows the frequency distribution for the DI criteria in the DH population tested with the two isolates. Phenotypic scores of the susceptible control were 100 in all experiments. The block effect was significant (P=0.001) in tests with the K92–16 isolate.

In the evaluation of resistance to isolate Pb137–522, Darmor-*bzh* exhibited low DI (11.0), whereas Yudal showed high DI (95.0). The disease reaction rating of the F_1 plants was 15, slightly more susceptible than the resistant parent. This indicated that the resistance carried by Darmor-*bzh* to isolate Pb137–522 was dominant. Three distinct phenotypic classes were observed in the DH progeny: 49 lines exhibited low DIs (classes 0–1), 2 lines exhibited intermediate DIs (class 2) and 59 lines showed high DIs (classes 2+ and 3). If the progeny were grouped into two phenotypic classes (DI<25 and DI>25), the observed segregation (49:61) fitted a 1:1 ratio (Chi^2=1.31, P=0.252), suggesting the involvement of one resistance gene.

In tests with the isolate K92–16, Darmor-*bzh* and Yudal exhibited a disease reaction of 50 and 100, respectively. The mean disease severity of the F_1 population was lower than the mid-parent value (66 vs 75), indicating the likelihood of incomplete dominance for partial resistance. Analysis of the resistance in the DH progeny showed a continuous pattern of distribution, suggesting that partial resistance was controlled by an oligogenic or polygenic system (Fig. 1).

Heritabilities were high for the two isolates in the DH population (h^2=0.98 and h^2=0.79 for the isolates Pb137–522 and K92–16 respectively).

Mapping genes associated to clubroot resistance

Mapping the resistance locus as a qualitative trait

The first approach used was to map the resistance of Darmor-*bzh* to isolate Pb137–522 as a Mendelian trait since DH lines could be categorized as ”resistant” or ”susceptible” as described above. The two lines showing intermediate responses were not included in the analysis. The location of the qualitative resistance gene on the DY map was analyzed with MAPMAKER/EXP version 3.0. The resistance locus mapped 5.3 cM proximal to the RAPD marker OPG03.960 and 3.2 cM distal to the RAPD marker OPV09.2100 on the linkage group (LG) DY4 (Fig. 2). We designated this locus *Pb-Bn1*, since its relation to previously identified genes in *B. napus* can not be established. This qualitative approach was not possible for isolate K92–16 because of the continuous distribution of the DI scores.

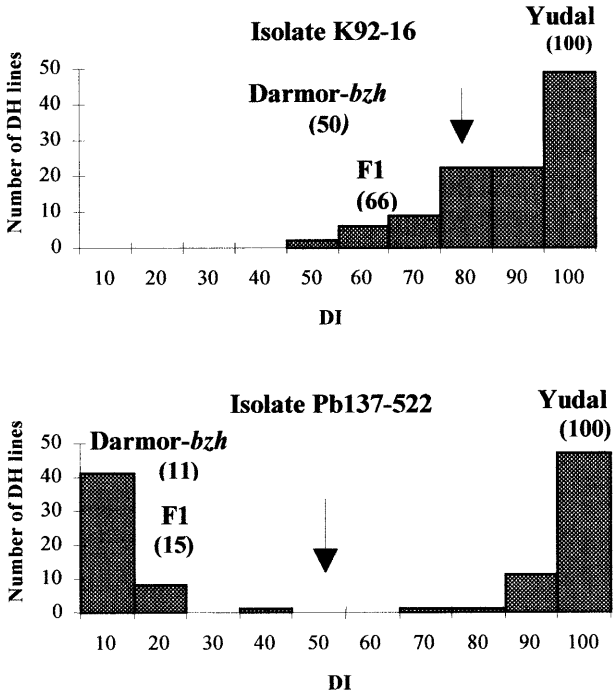

Fig. 1 Genotypic distributions estimated for DI criteria for resistance to two isolates of *P. brassicae* in the DH population Darmor-*bzh* x Yudal. *In parenthesis*, DI values for parents and F_1 generation. The *arrow* indicates the position of the mean of the DH progeny

Mapping the resistance loci as a quantitative trait

With the interval mapping analysis, two genomic regions were identified as contributing to resistance to isolate Pb137–522: one near the distal region of LG DY4, with the peak of this QTL appearing at the same relative position as the qualitative resistance gene *Pb-Bn1*, and the second on the LG DY2 (Fig. 2). The marker intervals representing the peaks of the QTLs and the LOD scores are summarized in Table 1. In a multiple QTL model, these two loci accounted for 98.3% of the phenotypic variation. The combined effects of two QTLs slightly exceeded the effect that could be explained by the QTL on DY4 alone.

In the tests with isolate K92–16, the frequency distribution of DI in the DH population was skewed towards susceptibility (Fig. 1). Two genomic regions, on LGs DY4 and DY15, were detected (Table 1, Fig. 2). The two QTLs accounted for 35.1% of the phenotypic variance under the multiple QTL model. The QTL revealed on DY4 group mapped at the same position as the QTL detected for the isolate Pb137–522 (Table 1, Fig. 2). When *Pb-Bn1* was included in the DY framework, the peak of the QTL on DY4 was exactly located at this locus. Interval mapping with the major fixed QTLs failed to reveal other QTLs.

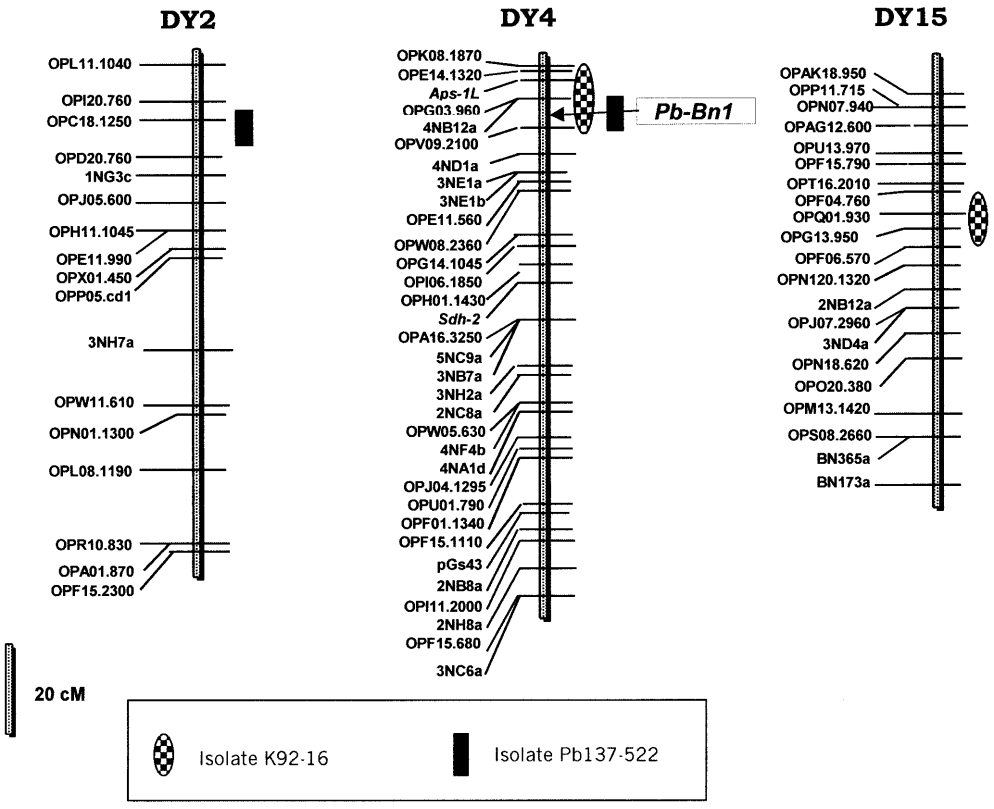

Fig. 2 Linkage map locations of the putative additive QTLs contributing to clubroot resistance identified in the cross Darmor-*bzh* x Yudal, assessed by the disease index (DI). The QTL length is the confidence interval in which the likelihood of the presence of a QTL is within tenfold (1 LOD) of its maximal value. Different symbols are used for the two isolates analyzed

Table 1 Clubroot resistance QTLs detected by interval mapping in the DH Darmor-*bzh* x Yudal population for the DI resistance criteria with two *P. brassicae* isolates: position, LOD score, individual effect and contribution to the resistance variation

Isolate	Linkage group	Marker interval	Length (cM)	Position (cM)	LOD	Weight[a]	R^2p (%)[b]	R^2t (%)[c]
Pb137–522	DY4	OPG03.960-PV09.2100	8.3	5.3	91.0	90.9	98.1	
	DY2	OPC18.1250-OPD20.760	11.8	0.0	3.85	35.4	15.0	
					91.3			98.3
K92–16	DY4	OPG03.960-OPV09.2100	8.3	2.3	4.8	12.3	19.4	
	DY15	OPQ01.930-OPG13.950	3.7	0.0	3.4	10.4	13.4	
					9.8			35.1

[a] The weight of each QTL represents the substitution effect of the two Darmor-*bzh* alleles by two Yudal alleles; i.e. the allelic contribution of each parent to the resistance

[b] R^2_p is the percentage of variation explained by each QTL

[c] R^2_t is the percentage of variation explained by all the additive QTLs detected for one isolate. The same QTLs were detected by non-parametric mapping

All of the QTLs identified by interval-mapping were also detected using the non-parametric approach and confirmed in ANOVA and in non-parametric tests (results not shown). No additional QTLs were found with the non-parametric approach. For all of the additive QTLs, the alleles of Darmor-*bzh* were associated with resistance.

Epistatic interactions

A total of 10,585 digenic interactions between 146 markers, including the QTL-linked markers, were analyzed by a two-way ANOVA. For isolate Pb137–522, 6 significant digenic interactions were detected. In the evaluation of resistance to the K92–16 isolate, 10 genomic regions with interactive effects were found, explaining independently up to 20% of the phenotypic variance (Table 2). Some of the interactions implicated the additive QTL-linked markers; the other interactions were found between regions that independently had no significant effect on resistance (Table 2). Significant marker loci and interactions were combined in complex ANOVA models to determine the global R^2 corresponding to the total part of variance explained by the set of different QTLs detected (additive and epistatic QTLs). For isolate K92–16, the phenotypic variance explained by only the additive

Table 2 Significant interactions between genetic markers as determined using two-way ANOVA for the isolates Pb137–522 and K92–16 ($P<0.0005$)

Isolate	Markers in interaction[a]	Linkage group	P	R^2	Phenotypic DI means of DH lines with alleles[b]			
					DD	DY	YD	YY
Pb137–522	OPL11.1040-**OPG03.960**	DY2-DY4	$4.8\ 10^{-5}$	3.6	*4.5*	95.7	34.0	88.0
	(OPC18.1250-OPG03.960)	(DY2-DY4)	$4.7\ 10^{-4}$	2.6	*3.8*	94.6	31.5	90.2
	(OPA09.1000-OPG11.1030)	(DY3-DY7)	$3.8\ 10^{-4}$	11.7	67.2	44.8	*28.0*	70.9
	OPA09.1000-OPN13.875	DY3-DY7	$1.3\ 10^{-4}$	13.2	70.8	43.8	*28.0*	71.2
	OPW11.560-OPY10.1300	DY8-DY14	$2.3\ 10^{-4}$	11.8	*27.9*	82.7	55.2	45.0
	OPM07.900-p11.715	DY12-DY15	$3.7\ 10^{-4}$	11.6	84.8	44.1	*33.9*	57.8
K92–16	OPY15.1880–OPA07.500	DY1a-DY5	$2.5\ 10^{-4}$	12.6	88.1	81.3	*76.1*	89.6
	(OPL02.940–OPA07.500)	(DY1a-DY5)	$3.7\ 10^{-4}$	11.1	87.1	81.7	*75.9*	89.7
	OPA01.870–OPA16.3250	DY2-DY4	$9.0\ 10^{-5}$	14.7	87.9	*78.7*	*78.2*	91.3
	OPG03.960–OPJ07.2960	DY4-DY15	$1.2\ 10^{-4}$	9.4	*61.6*	82.6	88.5	90.8
	(**OPG03.960**–OPF15.790)	(DY4-DY15)	$5.0\ 10^{-4}$	8.2	*66.7*	84.6	88.4	90.0
	OPA07.500–OPM16.1970	DY5-DY9	$8.8\ 10^{-5}$	13.7	90.8	*76.7*	82.3	89.8
	OPJ07.2960–OPM16.975	DY15-DY18	$4.6\ 10^{-7}$	20.0	89.0	*66.3*	84.6	88.7
	(OPJ07.2960–OPP03.700)	(DY15-DY18)	$2.1\ 10^{-4}$	11.7	86.8	*69.7*	85.2	88.6
	(**OPQ01.930**–OPM16.975)	(DY15-DY18)	$1.4\ 10^{-4}$	10.7	84.5	*69.2*	86.6	90.0
	(OPN18.620-OPM16.975)	(DY15-DY18)	$1.9\ 10^{-4}$	11.7	88.7	*69.7*	85.1	87.1

[a] In parenthesis, other interactions of markers closely linked to the first one that were significantly associated with resistance. We considered QTLs to be identified by the marker loci that displayed the highest R^2 value. Note: bold-type font indicates the markers of the additive QTLs

[b] Combination of two alleles of Darmor-*bzh* (DD), two alleles from Yudal (YY), the allele of Darmor-*bzh* for the first marker and the allele of Yudal for the second marker (DY) and conversely (YD). Note: italics indicates the most resistant class

QTLs was 33.9%; in the complex model including the additive and the epistatic QTLs, the global R^2 was 62.5%. For isolate Pb137–522, the percentages of phenotypic variance were 76.6% and 81.4%, in the additive and in the complex models, respectively.

Discussion

The availability of homogeneous plant material (DH lines) and of a well-characterized molecular map of the Darmor-*bzh* x Yudal DH population, together with the use of homogeneous isolates of the pathogen (single spore isolates), made it possible for the first time to identify and map Mendelian and QTLs conferring clubroot resistance in rapeseed. The results of this study indicate that in the *B. napus-P. brassicae* model analyzed, the resistance of a host genotype is expressed at high or intermediate levels and is under monogenic or polygenic control according to the isolates of the pathogen. The finding of a quantitative partial resistance response controlled by a polygenic system does not appear to fit easily into any of the schemes suggested by Crute et al. (1983) or Gustafsson and Fält (1986) for qualitative resistance in *B. napus*. The discrepancy with these previous studies may come from a different interpretation of the data (quantitative versus qualitative approach) and/or from the pathogen material used in tests (SSI versus field isolates). Categorizing plants into resistant or susceptible classes can hide intermediate levels of resistance expression that occur generally in segregating populations although not frequently in the parents. Variation for pathogenicity within field isolates of *P. brassicae* has been clearly demonstrated by several authors (Jones et al. 1982; Schoeller and Grunewaldt 1986). Interaction between isolates can hide virulence factors (Jones et al. 1982), and thus a strong host resistance system against a specific pathogenicity factor in the pathogen may mask the expression of partial resistance.

We have identified and located a major dominant gene controlling resistance to isolate Pb137–522 on LG DY4. This gene, designated *Pb-Bn1*, may be identical to one of the genes previously identified in the *B. napus* ECD hosts (Gustafsson and Fält 1986); however, it is not possible to confirm this on the basis of available published data. This locus was also identified by interval mapping using a quantitative evaluation of the response reactions to both isolates, and it mapped at the same position. For isolate Pb137–522, it had a much greater R^2 value than for isolate K92–16. Due to the possible biased confidence intervals, it is difficult to conclude if the putative QTL detected for K92–16 is due to *Pb-Bn1* itself or to closely linked genetic factors. If our results can be explained by the action of only one gene, whose effect may differ depending on the pathogen isolate, our results could be the first to demonstrate that a major gene conditioning a high level of resistance may have a weaker effect that contributes, in association with other QTLs, to the expression of a partial resistance to other isolates. Examples of qualitative and quantitative resistance possibly sharing a common genetic basis have been reported for other fungus and virus plant interactions (Freymark et al. 1993; Wang et al. 1994; Caranta and Palloix 1996; Caranta et al. 1997).

The high level of resistance expressed by Darmor-*bzh* to isolate Pb137–522 arose from the association of the

major-gene *Pb-Bn1* and one minor-effect QTL. The partial level of resistance shown by Darmor-*bzh* to isolate K92–16 arose from the association of at least two QTLs that seemed to act with additive effect, explaining the independent portion of the variation in response to clubroot infection. However, in both cases, epistasis was found to have a significant effect on resistance. To date, no epistatic QTLs involved in clubroot resistance have been reported, and few studies are available for other plant-pathogen models (Wang et al. 1994; Lefebvre and Palloix 1996; Lübberstedt et al. 1998; Pressoir et al. 1998). The total phenotypic variation accounted for by additive and epistatic QTLs ranged from 62% to 81.4%, depending on the isolate; for K92–16, the relative effect due to epistasis was almost similar to that due to additivity. The unexplained part of the genetic variation in tests with the isolate K92–16 may result from the incomplete coverage of the linkage map and/or from the moderate size of our sample (110 lines), which did not allow us to detect very low effect QTLs or to reliably identify digenic interactions; these have to be more precisely evaluated in a larger progeny. The finding that epistatic interactions were detected between loci with and without significant main effects and that favorable epistasis occurred between alleles from the same parent (Darmor-*bzh*) but also between alleles from each parent (Darmor-*bzh* x Yudal) has important implications in breeding, making the selection procedure complex. Therefore, the application of molecular markers linked to the resistance alleles would greatly simplify the breeding programs.

Clubroot-resistant cultivar development in *B. napus* has concentrated on introgressing the most effective genes from *B. rapa*, especially from ECD line 04 (Johnston 1974; Gowers 1982; Lammerink and Hart 1985) and on combining specific resistance coming from *B. rapa* and non-specific resistance from *B. oleracea* in resynthesized *B. napus* (Diederichsen and Sacristán 1996; Bradshaw et al. 1997). In *Brassicas*, one of the main problems in breeding resistant cultivars is the large variation for pathogenicity in *P. brassicae*. In agreeing with previous reports (Brokenshire 1982; Crute et al. 1983; Toxopeus et al. 1986; Bradshaw et al. 1997), our results also indicate that the relative effectiveness of the resistance genes may vary when the challenging population of the pathogen is changed. In these conditions, a broad spectrum of durable resistance may require the incorporation of several resistance genes in cultivars intended for use in a large area. Therefore, in order to define the best genetic combinations (management of resistance genes), it is necessary to know in more detail the resistance genes already present in *B. napus*, to analyze the allele relations between loci associated with partial and complete resistance, between QTLs and major gene loci, and to identify markers more closely linked to resistance genes. In addition, knowledge of the relationship between resistance factors involved in clubroot resistance in the different *Brassica* species can help gain an understanding of the genetic effects of the QTLs and major genes in different genetic backgrounds. The molecular markers associated to regions controlling resistance detected in this study might be an useful tool for (1) finding other clubroot resistance genes in the *Brassicas,* (2) the accumulation of these genes and (3) comparing the genetic factors controlling clubroot resistance in *B. oleracea* or in *B. rapa*, the two progenitors of *B. napus*. Moreover, the availability of single spore isolates, which allow differentiation of the resistance genes, and of markers closely linked to clubroot resistance loci can help to elucidate the mechanisms of this host-pathogen interaction.

Acknowledgements We thank Dr. A. Charcosset for providing the SAS/IML program for analyzing epistatic interactions. We acknowledge P. Glory and R. Horvais for technical assistance and Dr. M. Renard for his critical review of the manuscript.

References

Bradshaw JE, Gemmell DJ, Wilson RN (1997) Transfer of resistance to clubroot (*Plasmodiophora brassicae*) to swedes (*Brassica napus* L. var. *napobrassica* Peterm) from *B. rapa*. Ann Appl Biol 130:337–348

Brokenshire T (1982) The reaction of forage *Brassicas* to nine populations of *Plasmodiophora brassicae* Woron from S.E. Scotland. Ann Appl Biol [Suppl] 100:92–93

Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA (1975) Study of physiological specialization in *Plasmodiophora brassicae*: proposals for attempted rationalization through an international approach. Trans Br Mycol Soc 65:295–303

Caranta C, Palloix A (1996) Both common and specific genetic factors are involved in polygenic resistance of pepper to several potyviruses. Theor Appl Genet 92:15–20

Caranta C, Lefebvre V, Palloix A (1997) Polygenic resistance of pepper to potyviruses consists of a combination of isolate-specific and broad-spectrum quantitative trait loci. Mol Plant-Microbe Interact 10:872–878

Crute IR, Gray AR, Crisp P, Buczacki ST (1980) Variation in *Plasmodiophora brassicae* and resistance to clubroot disease in *Brassicas* and allied crops. A critical review. Plant Breed Abstr 50:91–104

Crute IR, Phelps K, Barnes A, Buczacki ST, Crisp P (1983) The relationship between genotypes of three *Brassica* species and collections of *Plasmodiophora brassicae*. Plant Pathol 32: 405–420

Diederichsen E, Sacristán MD (1996) Disease response of resynthesized *Brassica napus* L. lines carrying different combinations of resistance to *Plasmodiophora brassicae* Wor. Plant Breed 115:5–10

Engqvist LG (1994) Distribution of clubroot in Sweden and the effect of infection on oil content of oilseed rape. Sveriges Utssaedesfoeren Tidskr 2:82–86

Figdore SS, Ferreira HD, Slocum HK, Williams PH (1993) Association of RFLP markers with trait loci affecting clubroot resistance and morphological characters in *Brassica oleracea* L. Euphytica 69:33–44

Foisset N, Delourme R, Barret P, Hubert N, Landry BS, Renard M (1996) Molecular mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled haploid progeny. Theor Appl Genet 93:1017–1025

Freymark PJ, Lee M, Woodman WL, Martinson CA (1993) Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). Theor Appl Genet 87:537–544

Gowers S (1982) The transfer of characters from *Brassica campestris* L. to *Brassica napus* L.: production of clubroot-resistant oil-seed rape (*B. napus* spp. *oleifera*). Euphytica 31:971–976

Grandclement C, Thomas G (1996) Detection and analysis of QTLs based on RAPD markers for polygenic resistance to *Plasmodiophora brassicae* Woron in *Brassica oleracea* L. Theor Appl Genet 93:86–90

Gustafsson M, Fält AS (1986) Genetic studies on resistance to clubroot in *Brassica napus*. Ann Appl Biol 108:409–415

Johnston TD (1974) Transfer of disease resistance from *Brassica campestris* L. to rape *(B. napus* L.). Euphytica 23:681–683

Jones DR, Ingram DS, Dixon GR (1982) Characterization of isolates derived from single resting spores of *Plasmodiophora brassicae* and studies of their interaction. Plant Pathol 31: 239–246

Kuginuki Y, Ajisaka H, Yui M, Yoshikawa H, Hida K, Hirai M (1997) RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. Euphytica 98:149–154

Lammerink J, Hart RW (1985) 'Tina' a new swede cultivar with resistance to dry rot and clubroot. N Z J Exp Agric 13:417–420

Lander ES, Bostein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199

Landry B, Hubert N, Crete R, Chang MS, Lincoln SE, Etoh T (1992) A genetic map for *Brassica oleracea* based on RFLP markers detected with expressed sequences and mapping of resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin). Genome 35:409–420

Lefebvre V, Palloix A (1996) Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction pepper-*Phytophtora capsici* Leonian. Theor Appl Genet 93:503–511

Lincoln SE, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute, Cambridge, Mass

Lübberstedt T, Klein D, Melchinger AE (1998) Comparative QTL mapping of resistance to *Ustilago maydis* across four populations of European flint-maize. Theor Appl Genet 97:1321–1330

Manzanares MJ, Baron F, Fourmann M, Brunel D, Thomas G (1998) Genetic and molecular characterization of a quantitative source of resistance to *Plasmodiophora brassicae* using single spore derived isolates. In: Brassica Genetic Technologies for the Future. 11th Int Crucifer Genet Workshop. Montreal, Canada. pp 46

Manzanares-Dauleux MJ, Delourme R, Glory P, Thomas G (2000) Isolate-specific resistance to clubroot in *Brassica napus* is expressed at high or partial level. Cruciferae Newsl 22:57–58

Matsumoto E, Yasui C, Ohi M, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (*Brassica rapa* spp. *pekinensis*). Euphytica 104:79–86

Pressoir G, Albar L, Ahmadi N, Rimbault I, Lorieux M, Fargette D, Ghesquière A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. II. Evidence of a complementary epistasis between two QTLs. Theor Appl Genet 97:1155–1161

SAS Institute (1989) SAS/STAT users guide, version 6, 4th edn. SAS Institute, Cary, N.C.

Schoeller M, Grunewaldt J (1986) Production and characterization of single spore derived lines of *Plasmodiophora brassicae* Woron. Cruciferae Newsl 11:110–111

Somé A, Manzanares MJ, Laurens F, Baron F, Thomas G, Rouxel F (1996) Variation for virulence on *Brassica napus* L. among *Plasmodiophora brassicae* collections from France and derived single-spore isolates. Plant Pathol 45:432–439

Toxopeus H, Dixon GR, Mattusch P (1986) Physiological specialization in *P. brassicae*: an analysis by international experimentation. Trans Br Mycol Soc 87:279–287

Voorrips RE, Jongerius MC, Kanne HJ (1997) Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor Appl Genet 94:75–82

Wallenhammar AC (1998) Observations on yield loss from *Plasmodiophora brassicae* infections in spring oilseed rape. Z Pflanzenkr Pflanzenschutz (J Plant Dis Protec) 105:1–7

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421–1434