Theor Appl Genet (2001) 103:962–971 © Springer-Verlag 2001

N. Chantret · D. Mingeot · P. Sourdille · M. Bernard
J.M. Jacquemin · G. Doussinault

A major QTL for powdery mildew resistance is stable over time and at two development stages in winter wheat

Received: 5 October 2000 / Accepted: 1 March 2001

Abstract Despite the large impact of powdery mildew in wheat cultivated areas, little has been done to study powdery mildew resistance by QTL analysis up to now. The objective of the present paper is to present how the genetic basis of powdery mildew resistance in the resistant wheat line RE714 have been studied by QTL analysis at the adult plant stage over the course of 3 years, and at the vernalized seedling plant stage, and a comparison between the results obtained. Two segregating populations (DH and $F_{2:3}$) were derived from the cross between the resistant line (RE714), and a susceptible line (Hardi); these were analysed for powdery mildew resistance at the adult plant stage in the field under natural infection conditions in 1996, 1997 and 1998. The DH population was also tested for powdery mildew resistance at the vernalized seedling stage with four different isolates of powdery mildew. At the adult plant stage, a total of three QTLs (on chromosomes 5D, 4A and 6A) and five QTLs (on chromosomes 5D, 6A, 7A and 7B) were found for the DH and $F_{2:3}$ populations, respectively. The genetic control of resistance was found to be polygenic but involved a major QTL (on chromosome 5D), which was detected each year and which explained a high proportion of the variability observed (28.1%–37.9%). At the vernalized seedling stage, two QTLs were found (on chromosomes 5D and 7B) and the QTL detected on chromosome 5D was common to the four isolates tested. The comparison between the two development stages showed that the QTL on chromosome 5D was detected in all the different environments tested and again explained a high proportion of the variability. Different molecular interpretations of this QTL have also been discussed.

Keywords *Triticum aestivum* · *Blumeria graminis* f. sp. *tritici* · QTL mapping · Disease resistance

Communicated by F. Salamini

N. Chantret (✉) · G. Doussinault
I.N.R.A. Station d'Amélioration des Plantes,
Domaine de la Motte, 35653 Le Rheu Cedex, France
e-mail: chantret@rennes.inra.fr
Fax: (33)-2-23-48-51-20

D. Mingeot · J.M. Jacquemin
Centre de Recherches agronomiques – CRA,
Départmente de Biotechnologie, 234, Chaussée de Charleroi,
5030 Gembloux, Belgium

P. Sourdille · M. Bernard
I.N.R.A. Station d'Amélioration des Plantes,
Domaine de Crouelle, 234 Avenue du Brézet,
63039 Clermont-Ferrand Cedex 2, France

Introduction

Powdery mildew of wheat, caused by the aerial fungus *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis* DC. f. sp. *tritici*), is common in all the wheat growing areas. This disease can dramatically affect the yield, especially if the climate is temperate, humid or under maritime influence (Jenkyn and Bainbridge 1978; Bennett 1984).

Two kinds of resistance were identified: a qualitative resistance and a quantitative resistance. The qualitative resistance is observed from the seedling plant stage against isolates of the pathogen and is controlled by race-specific resistance genes named '*Pm*' (Powdery mildew). More than 30 *Pm* genes have been identified so far and assigned to wheat chromosomes or linked to genetic markers: *Pm1*, *Pm2*, *Pm3*, *Pm4*, *Pm6*, *Pm12*, *Pm13*, *Pm21*, *Pm24* or *Pm25* (McIntosh et al. 1998; Shi et al. 1998; Cenci et al. 1999; Huang et al. 2000; Tao et al. 2000).

The quantitative resistance to powdery mildew is evident in cultivars possessing no identified *Pm* genes or when the natural populations of powdery mildew overcome the *Pm* genes. This resistance is generally observed at the adult plant stage. Because this resistance delays infection, development and reproduction of the pathogen, it is partial and was termed either 'adult resistance' (Bennett 1981b; Griffey 1993; Griffey and Das 1994), 'slow mildewing' (Roberts and Caldwell 1970; Shaner 1973) or 'partial resistance' (Hautea et al. 1987).

Quantitative resistance has also been identified at the seedling plant stage (Bennett 1981; Royer et al. 1984; Chantret et al. 2000).

Most of the genetic studies of quantitative resistance to powdery mildew at the adult plant stage were performed using diallel methods (Hautea et al. 1987; Griffey and Das 1994; Das and Griffey 1995) or, in one case, monosomic analysis (Chae and Fischbeck 1979). The development of molecular markers now allows for a better understanding of the genetic control of quantitative disease resistance in plants (Young 1996).

However, in bread wheat, very few publications deal with powdery mildew resistance QTLs at the adult plant stage. Keller et al. (1999) identified a total of 18 QTLs for powdery mildew resistance on a wheat x spelt population over five different environments. Among these QTLs some were stable between the different environments tested (Keller et al. 1999). In a recent study, Chantret et al. (2000) detected a powdery mildew resistance QTL on chromosome 5D with three different isolates at the seedling plant stage. William et al. (1997) detected three QTLs for resistance to leaf rust. This lack of studies in bread wheat can be mainly explained by the large amount of work required to build genetic maps for this allohexaploid species. Alternatives to overcome this drawback can be the use of bulked segregant analysis (BSA) (Michelmore et al. 1991; William et al. 1997; Chantret et al. 2000) to detect QTLs or the use of microsatellites to help increase the efficiency of mapping (Röder et al. 1995, 1998).

The winter wheat line RE714 was obtained from two interspecific crosses, and is resistant to powdery mildew at different development stages: seedling, vernalized seedling and adult plant (Robe and Doussinault 1995; Robe et al. 1996; Chantret et al. 1999). Two powdery mildew resistance genes (*MlRE* and *Pm4b*) and one QTL have been mapped at the seedling plant stage (Robe and Doussinault 1995; Chantret et al. 2000). The *MlRE* gene was described as having a residual effect on the adult resistance of RE714 (Chantret et al. 1999). The aim of the present study was to map and compare powdery mildew resistance QTLs detected at the adult plant stage and at the vernalized plant stage in the winter wheat line RE714. The test method for the vernalized seedling stage was originally devised to predict the adult plant resistance (Robe et al. 1996). During this study, the ability of this method to predict the adult plant resistance was evaluated by comparing QTL results. Another objective of the study was to use the vernalized seedling stage to characterise the QTLs in terms of race-specificity.

Materials and methods

Plant material

Two populations from the cross between RE714 and Hardi (a susceptible cultivar that does not possess any race-specific resistance genes) were used. Forty four doubled-haploid (DH) lines were produced using anther culture (Hybrinova, 32480 Pouy Roquelaure, France; Agrogène, 77550 Moissy Cramayel, France). One hundred and forty F_2 plants were issued from a single F_1 hybrid plant by self-pollination and the 140 $F_{2:3}$ families were obtained by self-pollination of each of the 140 F_2 plants. The disease resistance tests and the genotyping were done on the F_3 plants for each family to predict the phenotype and genotype of the F_2 parental plant.

Disease assessments

Adult plant tests

Field trials were conducted at Le Rheu (France) under natural disease infection over the course of 3 years for the DH lines (1996, 1997 and 1998) and 2 years for the $F_{2:3}$ population (1997 and 1998). The cultivar Barbee (highly susceptible) was used as a contaminator. According to Chantret et al. (1999), the DH lines were sown as three replicate hillplots and the $F_{2:3}$ population as two replicates in a row design with 7 to 11 F_3 plants per row. The level of disease at the adult-plant stage was scored on three different dates for each year, using a 1–9 scale based on sporulation intensity, leaf area covered by sporulating colonies, and distribution of symptoms along the plants (1: no visible symptoms – 9: heavy sporulation).

Each year, the virulence composition of the powdery mildew population was assessed by using a set of differential hosts as described by Chantret et al. (1999).

Vernalized seedling test

The vernalized seedling tests were conducted in controlled conditions as explained in Chantret et al. (1999). Four isolates of *B. graminis* f. sp. *tritici* were used for vernalized seedling tests: 96–22, 95–44, 96–47 and 93–25. The line RE714 was completely susceptible to these four isolates (scores between 8 and 9) at the seedling plant stage whereas it was resistant at the vernalized seedling plant stage (scores between 0 and 3).

Molecular marker assay

DNA extractions, digestions, Southern blotting and hybridization were performed as described by Lu et al. (1994) and by Mingeot and Jacquemin (1999). For the $F_{2:3}$ population, DNA was extracted from a freshly harvested equal amount of leaf tissues bulked from 13 F_3 plants of each $F_{2:3}$ family, 5 weeks after sowing.

The RFLP probes used were previously described as follows: FBA and FBB by Nelson et al. (1995a, b, c), CDO, BCD and WG by Heun et al. (1991), KSU by Gill et al. (1991), ABC by Kleinhofs et al. (1993), and GBX by Mingeot and Jacquemin (1999).

PCR amplifications and microsatellite detections were performed as described in Röder et al. (1995) and Tixier et al. (1997).

Statistical analysis

Data analysis

Each set of data from the DH lines (resistance assessments at three dates for 3 years and vernalized seedling scores with four isolates) was analyzed separately using a generalized linear model (PROC GLM, Statistical Analysis System: SAS 1989), according to the one-way analysis of variance model $Y_{ij}=\mu+G_i+R_j+e_{ij}$, where Y_{ij} is the score of the genotype i in the replicate j, μ is the general mean, G_i is the effect of the genotype i, R_j is the effect of replicate j and e_{ij} the residual. Predicted values were plotted against the corresponding residuals to verify their independence. A genotypic value was estimated for each DH line from the analysis of variance after having been adjusted for the replicate effect. The same method was used for the adult plant disease scores of the $F_{2:3}$ population, using plot effects instead of replicate effects. Sixteen to twenty two F_3 plants were used to estimate the genotypic value of each F_2 parental plant after adjusting for the plot effect.

At the adult plant stage, within each assessment and each population, heritabilities (h^2) were estimated from the analysis of variance by the formula: $h^2=\sigma_g^2/[\sigma_g^2+(\sigma_e^2/n)]$, where σ_g^2 is the genetic variance ($\sigma_g^2=1/n(MS_e-MS_g)$), σ_e^2 the environmental variance ($\sigma_e^2=MS_e$) and n is the number of replicates.

For each year and each population, using the genotypic values, the area under the disease progress curve (AUDPC) was calculated for individuals from both populations using the following formula: $AUDPC=\Sigma_i[(x_i+x_{i+1})/2]t_i$, where x_i is the genotypic values on date i, t_i the time in days between dates i and i+1 (Bjarko and Line 1988).

The Normality of the AUDPC distributions was assessed by using the PROC UNIVARIATE procedure (SAS). The Kendall coefficient was calculated with the PROC CORR procedure (SAS) to determine correlations from AUDPC values.

Genetic mapping

The computer software MAPMAKER (Lander et al. 1987) was used to construct both maps. The LOD score threshold used for the DH and $F_{2:3}$ populations was 2 and 3 respectively. The assignment of the linkage groups to the wheat chromosomes was done using the Chinese-Spring nullisomic-tetrasomic lines (Sears 1966) for the GBX RFLP markers, and by comparison with the reference maps for the other RFLP and microsatellite markers (Röder et al. 1998).

QTL mapping

DH population

Due to the number of unlinked markers, the association between markers and resistance variation was assessed using a one-way analysis of variance (Proc GLM, SAS 1989). The significance threshold was assigned to a value of 0.001 in order to limit false positive detection. To avoid the model being over fitted, only the two strongest QTLs were considered and associated in a two-way analysis of variance (without including the interaction between them). When more than one marker was significant in the same linkage group, the ‘Interval mapping’ method was used [computer program MAPMAKER/QTL 1.9 (Lander and Botstein 1989)] to assess the position of the QTLs. The closest marker to the peak (of the LOD curve) was chosen for the two-way analysis of variance. For each multiple-factor model, predicted values were plotted against the corresponding residuals to verify their independence. The confidence intervals were defined as the distance equivalent to a LOD decrease of 1 on each side of the position of the maximum LOD value. QTLs detected in different environments were considered to be the same when the confidence intervals overlapped.

$F_{2:3}$ population

QTL detection in the $F_{2:3}$ population was carried out with the ‘Interval mapping’ method. A LOD score of 3 was chosen as a threshold. The multiple QTL hypothesis was performed by fixing the major QTLs and re-scanning the genome. The presence of an additional QTL was declared if the total LOD score was at least three LOD score units larger than the previous fixed QTL LOD score.

For both populations, QTL effects were estimated by the percentage of phenotypic variation explained by the QTL (R^2, ratio of sum of squares for the closest marker to the LOD score peak to the total sum of squares).

When the distributions (AUDPC) were not normal, nonparametric tests were made, using the Wilcoxon method (PROC Npar1way, option Wilcoxon, SAS), to verify markers that were declared to be significant.

Results

Resistance tests

Adult plant tests

Heritabilities were calculated from each data set (three for each year and for each population) and are presented in Table 1. They were all between 0.83 and 0.96, indicating a good estimation of the genotypic value for the DH-line assay as well as for the $F_{2:3}$ population assay. Distributions of genotypic values for AUDPC for the DH lines in 1996, 1997 and 1998 and for the $F_{2:3}$ population in 1997 and 1998 are presented in Fig. 1. In Table 1 are the mean AUDPC for both populations (in 1996, 1997 and 1998) and values of the control lines. Differences between years were observed from these data, especially those from the $F_{2:3}$ population (Fig. 1) although no significant genotype x year interaction was found. The disease severity in 1997 was lower than in 1996 and much lower than in 1998, the year having the strongest disease severity. Consequently, data from different years were used separately for QTL mapping.

Correlation coefficients between the different years are presented in Table 2. They were high for both populations (0.57 to 0.78), indicating that some resistance factors might be identical from one year to another (Table 2).

The continuous distributions of genotypic values for AUDPC suggest a polygenic control of the resistance. This observation was confirmed by the normality of the distributions for AUDPC in 1997 and 1998 for the $F_{2:3}$ population according to the Shapiro-Wilk W statistic (P=0.17 and P=0.07 respectively). On the other hand,

Table 1 Means of AUDPC of the DH and the $F_{2:3}$ populations from the cross between RE714 and Hardi and of the control lines (RE714, Hardi, F_1, DH196, DH5), and ranges of the heritabilities obtained in 1996, 1997 and 1998 in field conditions, for powdery mildew resistance. Since, three assessments were done for each year, heritabilities are presented as intervals from the lower to the higher value

Population	Mean	RE714	Hardi	F_1	DH196	DH5	h^2
DH 1996	165±76	76	327	–	84	250	(0.84–0.95)
DH 1997	126±52	70	188	–	51	152	(0.83–0.90)
DH 1998	177±52	122	240	235	132	203	(0.92–0.94)
$F_{2:3}$ 1997	72±19	34	109	97	37	79	(0.89–0.92)
$F_{2:3}$ 1998	195+41	90	277	257	95	211	(0.94–0.96)

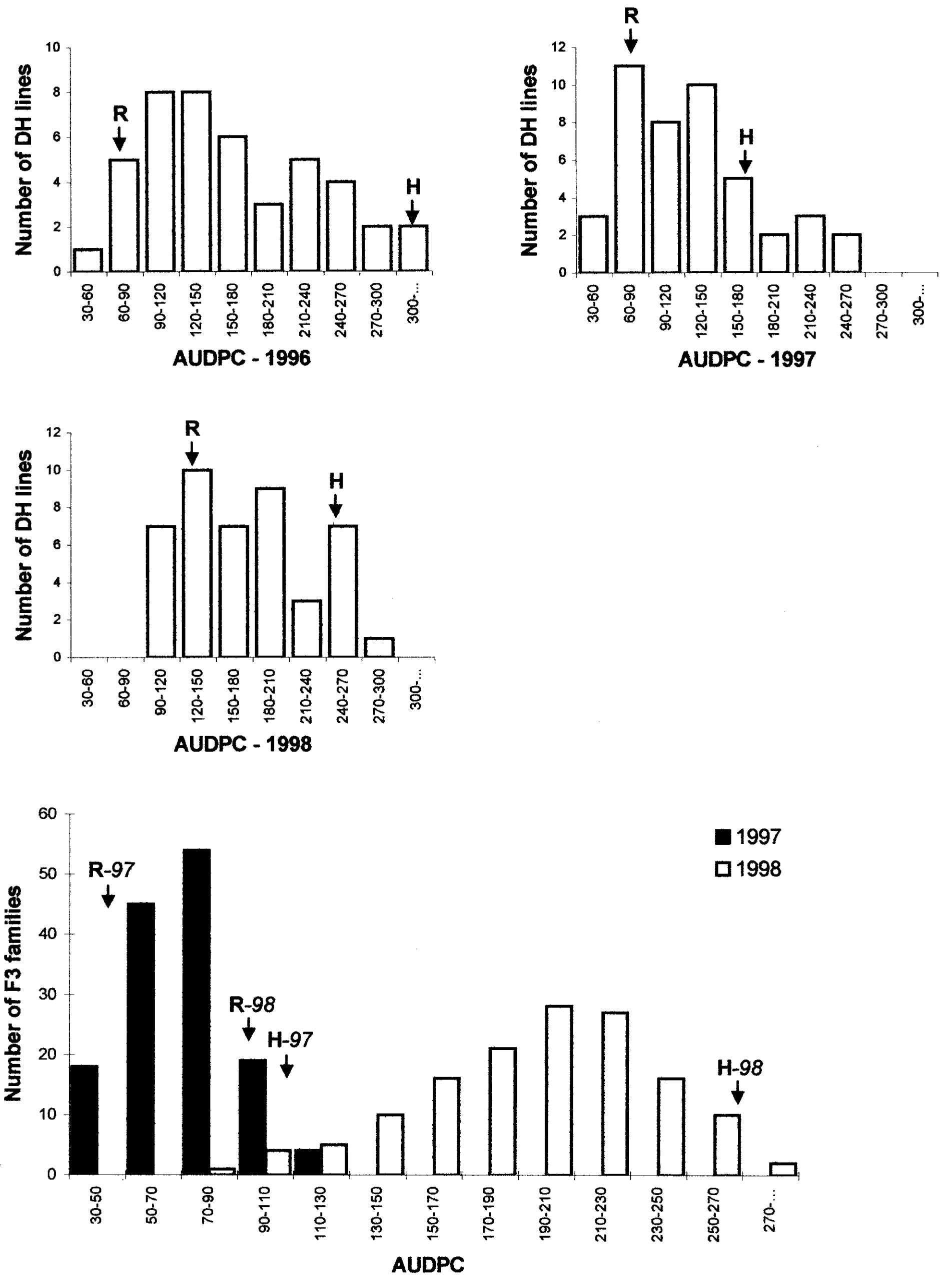

Fig. 1 Distribution of genotypic values for the area under the disease progress curve (AUDPC) at the adult plant stage for the DH population (in 1996, 1997 and 1998) and for the $F_{2:3}$ population (in 1997 and 1998) obtained from the cross between RE714 and Hardi. The scores of the parental lines are indicated by *arrows*: R=RE714, H=Hardi

Table 2 Kendall correlation coefficients among adult AUDPC for the DH population (1996, 1997 and 1998) and the $F_{2:3}$ population (1997 and 1998), and vernalized seedling scores for the DH population with the isolates 96–22, 95–44, 96–47, 93–25

Population	DH 96	DH 97	DH 98	96–22	95–44	96–47	$F_{2:3}$ 97
DH 97	0.71*						
DH 98	0.70*	0.78*					
96–22	0.33*	0.28	0.34*				
95–44	0.44*	0.46*	0.51*	0.47*			
96–47	0.45*	0.49*	0.53*	0.58*	0.60*		
93–25	0.45*	0.53*	0.54*	0.55*	0.66*	0.71*	
$F_{2:3}$ 98							0.57*

* P<0.0023 (=0.05/22)

distributions of AUDPC for the DH population were not normal (P=0.004, P=0.006 and P=0.019 for 1996, 1997 and 1998 respectively), probably because of the smaller number of individuals of the DH population (44) compared to the $F_{2:3}$ population (140).

The composition of the population of powdery mildew in terms of virulence genes was almost the same for 1996, 1997 and 1998. In particular, both *Pm4b* and *MlRE* were overcome during the 3 years.

Table 3 Powdery mildew resistance QTLs detected at adult plant stage, on the AUDPC data, for the DH and the $F_{2:3}$ populations issued from the cross between RE714 and Hardi in 1996, 1997 and 1998. Chromosome involved (Chrom.), marker closest to the peak of LOD curve (Marker), Position, Probability associated to each marker in the two-factor model for the DH population or LOD for the $F_{2:3}$ population, individual R^2 (Ind.) and total R^2 (Tot.) obtained for the complete model (percentage of variability explained); mean scores of AUDPC for individuals carrying either RE714 or Hardi allele for each marker for the DH population or additivity and dominance for the $F_{2:3}$ population

DH population

Year	Chrom.	Marker	Position	Probability	R^2		Means	
					Ind.	Tot.	RE714	Hardi
1996	5D	Xcfd26	+4 cM	0.0001	37.7		116.6	223.0
	6A	*MlRE*	–	0.0001	12.2	75.2	139.8	199.7
1997	5D	XgbxG083c	+0 cM	0.0001	33.5		95.8	164.2
	4A	XgbxG036	+0 cM	0.0359	4.9	60.5	117.0	143.1
1998	5D	XgbxG083c	+0 cM	0.0001	37.9		145.6	216.9
	4A	XgbxG036	+0 cM	0.0043	6.9	71.7	166.2	196.3

$F_{2:3}$ population

Year	Chrom.	Marker	Position	LOD	R^2		Genetic effects	
					Ind.	Tot.	Additivity	Dominance
1997	5D	Xcfd26	+2 cM	9.92	28.1		13.33	11.06
	6A	Xgwm427	+8 cM	+3.15	8.8		7.28	–1.10
	7A	Xfba069	+28 cM	+3.50	2.9	53.6	9.00	–7.50
1998	5D	Xcfd8B9	+2 cM	15.28	37.4		32.73	19.45
	6A	Xgwm427	+6 cM	3.93	13.4		18.24	–8.64
	7B	Xgwm577	+0 cM	3.70	1.7		8.55	7.59
	7A	Xgwm344	+4 cM	+5.43	6.4	67.4	14.72	–9.68

Vernalized seedling test

Segregations obtained in vernalized seedling tests with isolates 96–22, 95–44, 96–47 and 93–25 were those presented in Chantret et al. (1999). A significant isolate and an isolate×genotype effect ($P \leq 0.01$) was found. Distributions were not Gaussian according to the Shapiro-Wilk W statistic ($P<0.001$) but intermediate classes (i.e. classes 4, 5 and 6) were well represented. The transformations tested (square root, arcsin and logarithm) did not restore the normality. Resistance scores were relatively well correlated two by two (Table 2), in spite of the low difference for resistance to isolate 96–47 for which more DH lines were resistant (median=1.7 for resistance to 96–47 instead of 7.2, 7.3 and 7.7 for resistance to 96–22, 95–44 and 93–25 respectively; Chantret et al. 1999).

Mapping

DH population

The map obtained with the DH population was composed of 161 loci of which 127 were assembled into 27 linkage groups while 34 remained unlinked. The total distance covered was 993 cM, which represented about 30% of the genome size.

The two powdery mildew specific resistance genes *MlRE* and *Pm4b* were also added to this map. *Pm4b*, previously located on chromosome 2A (Zeller et al. 1993) was found at 4.8 cM from the locus Xgbx3110b. The *MlRE* gene was previously described and assigned to chromosome 6A (Chantret et al. 2000), but remained unlinked to the other markers assigned to chromosome 6A at a LOD threshold of 2.

$F_{2:3}$ population

Different linkage groups were constructed with the $F_{2:3}$ population according to (1) the results of the QTL detection on the DH population and (2) the previous results published in Chantret et al. (2000). The population was genotyped for 68 loci (36 RFLPs and 32 microsatellites), which allowed the construction of ten linkage groups, covering 510 cM. The loci for the *MlRE* gene described in Chantret et al. (2000) were not added to the map for QTL detection since they were derived from phenotypic data.

QTL mapping

Adult plant resistance of the DH population

For each year, different chromosome areas were found significant with the analysis of variance (Table 3, Fig. 2). For the 3 years, several markers on chromosomes 5D and on chromosome 4A were significant at 0.001%. In

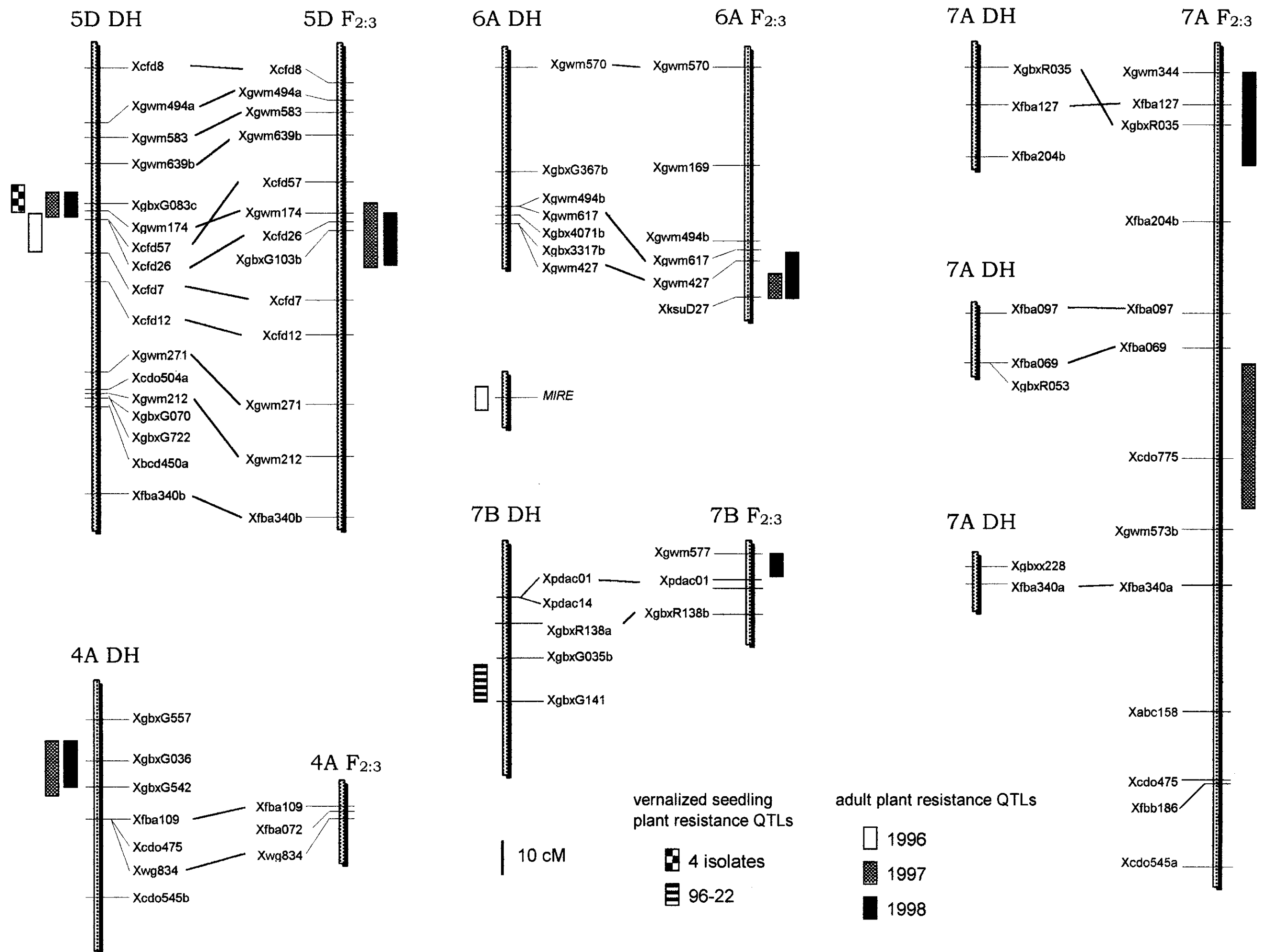

Fig. 2 Linkage groups and powdery mildew resistance QTLs detected on the DH and the $F_{2:3}$ populations derived from the cross between RE714 and Hardi. Only the linkage groups on which some QTLs have been detected are represented. The QTL length represents the confidence interval: the distance equivalent to a LOD decrease of 1 on each side of the position of the maximal LOD value

1996, an additional QTL was detected at the *MlRE* locus. Since the number of individuals in this population was very low, only the two most significant QTLs were selected for 1996, i.e. the QTL on chromosome 5D and the one at the *MlRE* locus.

The location, probabilities, individual and total R^2, and means for genotypes of the population having either the RE714 or the Hardi allele for each QTL are given in Table 3. The QTL located on chromosome 5D was detected during the 3 years of the experiment. For each year, this QTL presented the strongest effect, explaining 37.7, 33.5 and 37.9% of the genetic variation for 1996, 1997 and 1998, respectively. Another QTL was detected in 1996, at the *MlRE* locus, despite the fact that the powdery mildew population in the field overcame the gene. The QTL located on chromosome 4A explained 4.9 and 5.9% of the genetic variability in 1997 and 1998 respectively. This QTL was also detected in 1996 but was less strong than the two others presented in Table 3. For these three QTLs, the resistant alleles were provided by RE714, the resistant parent.

Adult plant resistance of the $F_{2:3}$ population

The characteristics and location of the QTLs detected in the $F_{2:3}$ population are presented in Table 3. A total of five QTLs were detected. Among these, two QTLs located on chromosomes 5D and 6A, were detected for both years 1997 and 1998. The most significant was the QTL detected on chromosome 5D, which explained 28.1 and 37.4% of the genetic variability, in 1997 and 1998 respectively. The QTL detected on chromosome 6A explained 8.8 and 13.4% of the genetic variability, in 1997 and 1998, respectively. The QTLs detected on chromosome 7A were located at different positions depending on the year of experiment (Fig. 2). The QTL detected in 1997 was at a proximal position, close to the centromere, on the long arm of chromosome 7A, while the one detected in 1998 was located in the distal part of the long arm. Overall, the confidence intervals did not overlap suggesting that two different QTLs on chromosome 7A were involved in the adult plant resistance to powdery mildew in

Table 4 Powdery mildew resistance QTLs detected at the vernalized seedling stage in the DH population from the cross between RE714 and Hardi for the four isolates. Chromosome involved (Chrom.), marker closest to the peak of LOD curve (Marker), Position, Probability associated to each marker in the two-factor model, individual R^2 (Ind.) and total R^2 (Tot.) obtained for the complete model (percentage of variability explained); mean scores of AUDPC for individuals carrying either RE714 or the Hardi allele for each marker

Isolate	Chrom.	Marker	Position	Probability	R^2		Means	
					Ind.	Tot.	RE714	Hardi
96–22	5D	Xgwm639b	+6 cM	0.0003	21.6		5.42	7.72
	7B	XgbxG035b	+12 cM	0.0061	11.3	46.3	5.74	7.40
95–44	5D	XgbxG083c	+0 cM	0.0001	56.9	56.9	5.35	8.38
96–47	5D	XgbxG083c	+0 cM	0.0001	54.9	54.9	0.95	4.30
93–25	5D	XgbxG083c	+0 cM	0.0001	61.7	61.7	5.96	8.63

1997 and in 1998. These QTLs explained a lower part of the variability for both years (2.9 and 6.4 for 1997 and 1998 respectively). An additional QTL was detected in 1998, located on chromosome 7B and explaining only 1.7% of the variability. For both years, the total genetic variability explained by the QTLs detected was high, ranging between 37 and 61%. For all these QTLs, the resistance alleles were derived from RE714, the resistant parent.

The dominance values of the QTLs detected on chromosomes 5D and 7B (in 1998 only) were between 7.59 and 19.45 (Table 3), and the resistant alleles (from RE714) were recessive to the susceptible allele. The dominance values of the other QTLs (located on chromosomes 6A and 7A for both years) were between –9.68 and –1.10 (Table 3), and the dominant alleles were from RE714, the resistant parent.

Vernalized seedling plant resistance of the DH population

The characteristics and locations of the QTLs detected at the vernalized seedling stage in the DH population are presented in Table 4. For three isolates (95–44, 96–47, 93–25), only one QTL was significant at 0.001%, located on chromosome 5D. For the isolate 96–22, two QTLs were significant, one on chromosome 5D (at the same location as for the three other isolates) and the other on chromosome 7B.

The QTL on chromosome 5D was detected for the four isolates tested and explained a large proportion of the genetic variability (between 21 and 61%). This QTL was also detected with non-parametric methods (data not shown). For 96–22, the QTL detected on chromosome 7B explained less of the variation (11.3%) and was not detected with the three other isolates. For all these QTLs, RE714 carried the resistant allele.

Discussion

In the present study, we report the detection of powdery mildew resistance QTLs at two development stages (adult and vernalized seedling) using two populations (44 DH lines and 140 $F_{2:3}$ families) derived from the same parents: RE714 and Hardi. The $F_{2:3}$ population was already used to detect a powdery mildew resistance QTL at the seedling plant stage (Chantret et al. 2000).

Powdery mildew resistance QTLs at the adult plant stage

The values of heritabilities obtained from the evaluation of the adult plant resistance to powdery mildew were high (from 0.83 to 0.85 for the DH population and from 0.89 to 0.96 for the $F_{2:3}$ population), indicating that powdery mildew resistance is highly heritable and that the disease-assessment procedure was well conducted for both experimental assays. Other authors have also found high heritabilities for powdery mildew resistance: 0.97 (Keller at al. 1999) and between 0.57 and 0.94 (Das and Griffey 1994).

The shape of the distribution, especially for the $F_{2:3}$ population (Fig. 1), suggests a polygenic control of the resistance. We detected a total of three QTLs for the DH population and five for the $F_{2:3}$ population. Assuming that the QTLs detected on chromosomes 5D and 6A are the same for the two populations, the total number of QTLs detected was six. This result confirmed that the powdery mildew resistance observed was under polygenic control. The total variability explained by the models we presented was very high for the DH population (75.2, 60.5 and 71.7% respectively for 1996, 1997 and 1998) and also high for the $F_{2:3}$ population (37.0 and 61.1 respectively for 1997 and 1998). Depending on the study, the number of powdery mildew resistance QTLs detected is extremely variable: from two in barley (Heun 1992; Backes et al. 1996) to 18 in bread wheat (Keller et al. 1999), although the total phenotypic variance explained by the models are similar: between 43.6 and 72.0% (Keller et al. 1999). The lower number of QTLs detected in the present study compared to the earlier report of Keller et al. (1999) may be explained by the partial coverage of our maps. However, since the total phenotypic variances explained by the models are similar, these opposite results are more probably the consequence of differences in the composition of the powdery mildew population. As suggested by Parlevliet and Zadoks (1977), quantitative resistance could result from

a complex of "polygene-for-polygene" interactions, and resistance to different populations of powdery mildew can involve a different number of genes according to the complexity of the populations.

For each year of experimentation, the QTL detected on chromosome 5D was the strongest, explaining between 28.1 and 37.9% of the variability, and most likely was responsible for the high correlations between the different years of experimentation for both populations (Table 2). This QTL was also detected in 1996 and 1998, with the DH population and in two different environments in Belgium (Mingeot, D. communication personal). Finding one or two major QTLs is common for many disease resistance QTL analyses (Chen et al. 1994; Pecchioni et al. 1996; Caranta et al. 1997; Rector et al. 1998; Geffroy et al. 2000). Under the hypothesis that the genetic factors involved at this QTL are the same for the different environments, the stability observed suggests that the genes involved were fundamental for the plant to reach a good level of powdery mildew resistance independently of the natural population of the pathogen. This hypothesis could also be expressed in terms of race non-specificity: the QTL detected on chromosome 5D may be stable because it could be of a race non-specific nature. Börner et al. (2000) strongly suggested that a major gene involved in the genetic control of resistance to stripe rust in barley was a race non-specific factor because it was efficient with a large number of different strains of the pathogen. In the present study, the genetic variability of the pathogen did not change significantly between the different years so it is also possible that the QTL detected on chromosome 5D was involved in a specific interaction with the same part of the powdery mildew population each year.

The QTL detected close to the *MlRE* gene was described as the residual effect of this gene (Chantret et al. 1999). Using the $F_{2:3}$ population, the residual effect of *MlRE* corresponded to the QTL detected on the distal part of chromosome 6A since *MlRE* was located with this population, and at the same position (Chantret et al. 2000). With the $F_{2:3}$ population, only the data from molecular markers were considered to avoid any bias when constructing a multifactor model (Chantret et al. 2000). Considering both populations, the residual effect of the *MlRE* gene was stable over the 3 years even if, using the DH population, it seemed to be 'hidden' by the QTLs detected on chromosomes 5D and 4A. By contrast, the size of the $F_{2:3}$ population allowed this QTL to be considered in a multifactor model that showed the persistence of the residual effect of the *MlRE* gene.

Two other QTLs were detected on chromosome 7A with the $F_{2:3}$ population, and may correspond to two different factors, each specific to 1 year of the experiment. Other authors have found some year-specific QTLs (Pilet et al. 1998). The QTL detected on chromosome 4A with the DH population was not detected using the $F_{2:3}$ population (LOD<1) maybe because the coverage of the $F_{2:3}$ population map was not efficient enough. We also detected a QTL on chromosome 7B using the $F_{2:3}$ population that was not present on the DH population, most likely because the DH population was not large enough to allow a statistically significant detection of this minor QTL.

Powdery mildew resistance QTLs at the vernalized seedling plant stage

With the four isolates tested, we found two QTLs involved in resistance at the vernalized seedling plant stage. Either the resistance at the vernalized seedling stage was controlled by only a limited number of genes or all the QTLs involved could not be detected. For each of the four isolates tested, a QTL was detected on chromosome 5D, in the same chromosomal area (between markers Xgwm639b and Xgwm174; Fig. 2). This QTL explained a large part of the variation of the trait (21.6 to 61.7%), and is responsible for the high correlations found between the resistances to the four isolates. This suggests that either a unique gene, or a cluster of genes, is involved in resistance to the four isolates. Others authors have also reported some QTLs involved in the resistance to different strains of the same pathogen. Krieke et al. (1994) found one major locus for the resistance to two different pathotypes of *Globodera pallida* in potato; Caranta et al. (1997) also discussed the possibility that a common resistance factor of pepper to different strains of potyviruses could be procured by one gene with a pleiotropic effect or by several linked genes. An additional QTL, located on chromosome 7B, was also detected only with the isolate 96–22 at the threshold level chosen. This QTL might also exist with the clone 95–44 but did not reach significance (P=0.0095 and 0.0036 at loci XgbxG035b and XgbxG141 respectively). It was not detected with the two other clones. This QTL explained more than 10% of the variability and was clearly race-specific since it was not detected with all the isolates. Other studies have demonstrated that quantitative resistance can be controlled by resistance factors that are race-specific (Geffroy et al. 2000).

Comparison between development stages

The comparison between the different development stages yielded the following results: the detection of a major QTL located on the long arm of chromosome 5D in all the different resistance test conditions. This QTL was stable at the adult plant stage (for 3 years and at three locations) and was also detected at the vernalized seedling stage with the four isolates tested. This QTL may correspond to one gene efficient in all these different environments or to several linked resistance genes. Under the hypothesis that the same gene is activated we can make some observations; first, this strong common factor, which is the major QTL for both developmental stages, showed that the vernalized seedling stage might be predictive of the adult plant resistance since it allowed the detection of the main adult resistance factor. The second observation concerned the possi-

bility that this QTL could be a non-specific resistance factor. As previously discussed, it can be hypothesised that we found an adult resistance component, which acted as a non-specific factor. Recently, Börner et al. (2000) described an adult plant resistance to a genetically diverse natural population of stripe rust controlled by a non-specific factor. In the present study, the factor involved was not a major gene but a QTL explaining only a part of the variability, which can be also non-specific. In this case, this factor becomes particularly interesting for understanding which kind of resistance mechanism could be associated with its expression.

We cannot exclude the possibility that we observed the effect of several clustered genes. Under this hypothesis, such a cluster of genes could be of interest since each of the involved genes could have a partial but strong effect, even when several isolates of the pathogen were used.

This resistance QTL was also detected at the seedling plant stage with three different isolates (Chantret et al 2000). If the same resistance factor was involved, it would mean that this adult plant resistance factor is expressed at the seedling plant stage, with different isolates, but with a lower effect. Considering the three development stages, this QTL seemed to be constantly involved through the development of the plant.

This new genetic factor could be used in marker-assisted selection because it was stable over 3 years and two locations, and may be race non-specific, which is an indication of possible durability. Also, this QTL was strongly involved in the resistance and explains a high proportion of the variability. Moreover, this QTL was mapped with microsatellites, which are PCR markers; hence, it is easy to use in a breeding program.

The other resistance factors detected at the adult plant stage were not found at the vernalized seedling plant stage (except maybe the one on chromosome 7B). Although this could be due to the poor coverage of the map in the DH population, it seemed that the vernalized seedling plant stage could not be used as a perfect predictor. This development stage was a more-powerful test method, which, in addition to adult and seedling plant stages, gave an idea of the resistance expression during the complete development of the plant.

Acknowledgments The authors thank Marie-Thérèse Pavoine for technical assistance, Hélène Muranty for helping in the QTL analysis and C. Quandalle for donating a portion of the doubled-haploid lines.

References

Backes G, Schwarz G, Wenzel G, Jahoor A (1996) Comparison between QTL analysis of powdery mildew resistance in barley based on detached primary leaves and on field data. Plant Breed 115:419–421

Bennett FGA (1981) The expression of resistance to powdery mildew infection in winter wheat cultivars. I. Seedling resistance. Ann Appl Biol 98:295–303.

Bennett FGA (1984) Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathol 33:279–300

Bjarko ME, Line RF (1988) Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. Phytopathology 78:457–461

Börner A, Röder MS, Unger O, Meinel A (2000) The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. Theor Appl Genet 100:1095–1099

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

Cenci A, D'Ovidio R, Tanzarella OA, Ceoloni C, Porceddu E (1999) Identification of molecular markers linked to *Pm13*, an *Aegilops longissima* gene conferring resistance to powdery mildew in wheat. Theor Appl Genet 98:448–454

Chae YA, Fischbeck GW (1979) Genetic analysis of powdery mildew resistance in wheat cultivar 'Diplomat'. Z Pflanzenzüchtg 83:272–280

Chantret N, Pavoine MT, Doussinault G (1999) The race-specific resistance gene to powdery mildew, *MlRE*, has a residual effect on adult plant resistance of winter wheat line RE714. Phytopathology 89:533–539

Chantret N, Sourdille P, Röder M, Tavaud M, Bernard M, Doussinault G (2000) Location, mapping of the powdery mildew resistance gene *MlRE* and detection of a resistance QTL by bulked segregant analysis (BSA) with microsatellites in wheat. Theor Appl Genet 100:1217–1224

Chen FQ, Prehn D, Hayes PM, Mulrooney D, Corey A, Vivar H (1994) Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). Theor Appl Genet 88:215–219

Das MK, Griffey CA (1994) Heritability, number of genes governing adult-plant resistance to powdery mildew in Houser and Redcoat winter wheats. Phytopathology 84:406–409

Das MK, Griffey CA (1995) Gene action for adult plant resistance to powdery mildew in wheat. Genome 38:277–282

Geffroy V, Sevignac M, De Oliveira JCF, Fouilloux G, Skroch P, Thoquet P, Gepts P, Langin T, Dron M (2000) Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. Mol Plant-Microbe Interact 13:287–296

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34: 362–374

Griffey CA (1993) Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat. Plant Dis 77:618–622

Griffey CA, Das MK (1994) Inheritance of adult-plant resistance to powdery mildew in Knox 62 and Massey Winter Wheats. Crop Sci 34:641–646

Hautea RA, Coffman WR, Sorrells ME, Bergstrom GC (1987) Inheritance of partial resistance to powdery mildew in spring wheat. Theor Appl Genet 73:609–615

Heun M (1992) Mapping quantitative powdery mildew resistance of barley using a restriction fragment length polymorphism map. Genome 35:1019–1025

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrels ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000) Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor Appl Genet 101:407–414

Jenkyn JF, Bainbridge A (1978). Biology, pathology of cereal powdery mildews. In: Spencer DM (ed) The powdery mildews. Academic Press, London pp 284–321

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat×spelt population. Theor Appl Genet 98:903–912

Kleinhofs A, Kilian A, Saghai MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, et al. (1993) A molecular, isozyme, morphological map of barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kreike CM, de Koning JRA, Vinke JH, van Ooijen JW, Stiekema WJ (1994) Quantitatively inherited resistance to *Globodera pallida* is dominated by one major locus in *Solanum spegazzinii*. Theor Appl Genet 88:764–769

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

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental, natural populations. Genomics 1:174–181

Lu YH, Merlino M, Isaac PG, Staccy J, Bernard M, Leroy P (1994) A comparative analysis between [^{32}P], digoxigenin-labelled single-copy probes for RFLP detection in wheat. Agronomie 14:33–39

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. Proc 9th Int Wheat Genet Symposium 2–7 August, University of Saskatchewan, Saskatoon, Canada, pp 123–127

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci 88:9828–9832

Mingeot D, Jacquemin JM (1999) Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98:1132–1137

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995a) Molecular mapping of wheat: major genes, rearrangements in homoeologous groups 4, 5, 7. Genetics 141:721–731

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995b) Molecular mapping of wheat. Homoeologous group 2. Genome 38:516–524

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995c) Molecular mapping of wheat. Homoeologous group 3. Genome 38:525–533

Parlevliet JE, Zadoks JC (1977) The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica 26:5–21

Pecchioni N, Faccioli P, Toubia-Rahme H, Valè G, Terzi V (1996) Quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) is dominated by one major locus. Theor Appl Genet 93:97–101

Pilet ML, Delourme R, Foisset N, Renard M (1998) Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent *Leptosphaeria maculand* (Desm.) Ces. et de Not., in Winter rapeseed (*Brassica napus* L.). Theor Appl Genet 96:23–30

Rector BG, All JN, Parrott WA, Boerma HR (1998) Identification of molecular markers linked to quantitative trait loci for soybean resistance to corn earworm. Theor Appl Genet 96:786–790

Robe P, Doussinault G (1995) Genetic analysis of powdery-mildew resistance of a winter-wheat line, RE714, and identification of a new specific-resistance gene. Plant Breed 114:387–391

Robe P, Pavoine MT, Doussinault G (1996) Early assessment of adult plant reaction of wheat (*Triticum aestivum* L.) to powdery mildew (*Erysiphe graminis* f. sp. *tritici*) at the five-leaf seedling stage. Agronomie 16:441–451

Roberts JJ, Caldwell RM (1970) General resistance (slow mildewing) to *Erysiphe graminis* f. sp. *tritici* in Knox wheat. Phytopathology 60:1310

Röder MS, Plaschke J, König SU, Börner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of the wheat genome. Genetics 149:2007–2023

Royer MH, Nelson RR, MacKenzie DR, Diehle DA (1984) Partial resistance of near-isogenic wheat lines compatible with *Erysiphe graminis* f. sp. tritici. Phytopathology 74:1001–1006

SAS Institute (1989) SAS/STAT user's guide, version 6, 4th edn. SAS Institute, Cary, North Carolina

Sears ER (1966) Nullisomic tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulation and plant genetics. Oliver, Boyd, London, pp 29–45

Shaner G (1973) Evaluation of slow-mildewing resistance of Knox wheat in the field. Phytopathology 63:867–872

Shi AN, Leath S, Murphy JP (1998) A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology 88:144–147

Tao W, Liu D, Liu J, Feng Y, Chen P (2000) Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis. Theor Appl Genet 100:564–568

Tixier MH, Sourdille P, Röder M, Leroy P, Bernard M (1997) Detection of wheat microsatellites using a non-radioactive silver-nitrate staining method. J Genet Breed 51:175–177

William HM, Hoisington D, Singh RP, Gonzalez-de-Leon D (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40:253–260

Young ND (1996) QTL mapping, quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501

Zeller FJ, Lutz J, Reimlein EL, Limpert E, Koenig J (1993) Identification of powdery mildew resistance gene in common wheat (*Triticum aestivum* L.). II. French cultivars. Agronomie 13: 201–207