Theor Appl Genet (1998) 97: 398–406

M. L. Pilet · R. Delourme · N. Foisset · M. Renard

Identification of QTL involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg resistance (*Leptosphaeria maculans*) in winter rapeseed (*Brassica napus* L.)

Received: 21 November 1997 / Accepted: 3 March 1998

Abstract Quantitative trait loci (QTL), involved in the polygenic field resistance of rapeseed (*Brassica napus* L.) to light leaf spot disease, were mapped using 288 DNA markers on 152 doubled-haploid (DH) lines derived from the cross 'Darmor-*bzh*' × 'Yudal'. Over two years (1995 and 1996), the DH population was evaluated for light leaf spot resistance on leaves (L) and stems (S), and for blackleg disease resistance in same field trials. For the L resistance criterion, a total of five and seven QTL were detected in 1995 and in 1996 respectively, accounting for 53% and 57% of the genotypic variation. For the S criterion, three and five QTL were identified in 1995 and in 1996 respectively, explaining 29% and 43% of the genotypic variation. The locations of the QTL detected were quite consistent over the two years (4- and 2-year common QTL for L and S, respectively). Three genomic regions, located on the DY5, DY10 and DY11 groups, were common to the resistance on leaves and stems. In comparison with the QTL for blackleg resistance described by Pilet et al. (1998), two regions on the DY6 and DY10 groups, were associated with the two disease resistances. These 'multiple disease resistance' ('MDR') QTL may correspond to genes involved in common resistance mechanisms towards the two pathogens or else to clusters of resistance genes.

Key words *Pyrenopeziza brassicae* · *Leptosphaeria maculans* · *Brassica napus* L. · QTL mapping · Colocalizations

Communicated by F. Salamini

M. L. Pilet (✉) · R. Delourme · N. Foisset[1] · M. Renard
I.N.R.A., Station d'Amélioration des Plantes, BP 29,
35653 Le Rheu Cedex, France
Fax: (33).02.99.28.51.20
E-mail: pilet@rennes.inra.fr

Present address:
[1] DNA LandMarks Inc., P.O. Box 6, St-Jean-sur-Richelieu, J3B6Z1, Québec, Canada

Introduction

Light leaf spot, caused by *Pyrenopeziza brassicae* Sutton and Rawlinson (anamorph *Cylindrosporium concentricum* Grev.), is considered to be the most damaging disease of winter rapeseed (*Brassica napus* L.) in the UK (Yarman and Giltrap 1989; Hardwick et al. 1991). It is capable of producing up to a 50% reduction in yield (Rawlinson et al., 1978). This disease has become increasingly problematic in France since its appearance in 1978 (Brun et al. 1979; Penaud and Regnault 1983) and in Germany since the end of the eighties (Amelung and Daebeler 1991). Symptoms are expressed from the two-leaf stage to the harvest, and affects all organs of the plant (leaves, petioles, stems, pods). The severity of epidemics increases with temperature (Figueroa et al., 1995 a) and leaf wetness (Figueroa et al., 1995 b). Many other *Brassica* crops are affected by light leaf spot including vegetable brassicas, especially cauliflower, broccoli, brussels sprouts, turnip, etc. (Maddock and Ingram 1981).

Disease control by cultural practice involves destruction of debris and crop rotation. Chemical control can be achieved by the application in the autumn (Figueroa et al. 1994; Sutherland et al. 1995) of fungicides such as carbendazim and prochloraz. However, the extensive use of these fungicides imposes a selection pressure on the pathogen population which might cause resistant strains to appear. Moreover, the occurrence of the sexual stage in the UK (Lacey et al. 1987), Ireland and New Zealand (Cheah et al. 1980) provides the opportunity for enhanced genetic variability of the pathogen.

The use of resistant cultivars remains the least expensive, and environmentally most sustainable, method of control. Among *Brassica* species, a wide variation in disease resistance has been reported (Rawlinson et al. 1978; Walker et al. 1995). Several differential interactions between cultivars of *B. napus* and isolates of *P. brassicae* have been described (Maddock et al. 1981), but did not allow the separation of physiological races

of the pathogen. The exploitation of this variability has led to the creation of partially resistant double-low varieties. No study has been published concerning the genetics of light leaf spot resistance in rapeseed. In *Brassica oleracea*, Simons and Skidmore (1988) identified specific resistances which were expressed as dominant characters. Molecular tools have been used to study the mechanisms of *P. brassicae* pathogenicity and sexual morphogenesis (Ashby 1997). But, until now, the identification of molecular markers linked to light leaf spot resistance in *Brassicas* has not been reported in the literature.

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not. [anamorph *Phoma lingam* (Tode : Fr.) Desm.], is the most economically important disease of *Brassica* species in Australia, Europe and North America. The interaction between oilseed *Brassica* spp. and *L. maculans* has been widely studied. Many aspects of this interaction were reviewed by Rimmer and Van den Berg (1992). Mapping of blackleg resistance genes has been initiated by several research teams (for a review see Delourme et al. 1995).

At I.N.R.A., the search for molecular markers linked to blackleg resistance has been recently developed because of the inadequacy of chemicals to reduce the disease and the quantitative nature of the resistance. In a previous study, we have identified QTL (quantitative trait loci) contributing to field resistance to *L. maculans* at the adult plant stage (Pilet et al. 1998).

In the present paper, we first report on the identification, characterization and stability of QTL associated with resistance to light leaf spot. Then, we compare the localization and additive effects of the QTL detected for light leaf spot and blackleg resistance.

Materials and methods

Plant material

The segregating population used is a doubled-haploid (DH) progeny of 171 DH lines derived from the F_1 'Darmor-*bzh*' × 'Yudal'. The spring inbred line 'Yudal' is very susceptible to light leaf spot and blackleg. The dwarf line 'Darmor-*bzh*' originated from the winter single-low (low erucic acid) variety 'Jet Neuf', which is very susceptible to light leaf spot (Jeffery et al. 1989). The extension of light leaf spot in Europe was partially related to the wide culture of 'Jet Neuf', which is highly resistant to *L. maculans*. Since then, 'Jet Neuf' has been re-converted in the double-low variety 'Darmor' (1983), which is resistant to blackleg and less susceptible to light leaf spot than 'Jet Neuf' (Brun et al. 1989).

Genetic map

The segregating DH population developed from the cross 'Darmor-*bzh*' × 'Yudal', and the genetic map elaborated from 152 DH lines, were both described previously in Foisset et al. (1996). New markers have been added and the map used in the present study comprises 288 markers (predominantly RAPD and RFLP) on 19 linkage groups and covers 1954 cM.

Field assessment

The 152 DH lines were tested for their resistance to *P. brassicae* and *L. maculans* in the same field trial at Le Rheu, France, in 1995 and in 1996. The field experiment was conducted in a randomized incomplete block design with three replicates and six blocks per replicate. Infected rapeseed stubble collected from the previous-year trial was scattered through the field at the three-leaf stage. Each replicate was composed of four-row plots (2.5 m^2) of parental, DH, and control lines. The controls were three winter-type *B. napus* cultivars showing different levels of light leaf spot resistance ['Falcon' (resistant), 'Eurol' (moderately resistant), 'Shogun' (susceptible)] and of blackleg resistance ['Falcon' (partially resistant), 'Eurol' (moderately resistant), 'Shogun' (susceptible)].

Notations of light leaf spot severity were carried out on two organs at two development stages : on leaves (L) at the stem extension stage and on stems (S) when the pods were formed. All the DH lines were assessed on the same dates. A visual qualitative disease score was attributed for each plot using a 1–11 scale, based on the percentage of plants infected and the intensity of disease: 1 = healthy appearance of the plot, 3 = a few attacked and weakly infected plants, 5 = 25–50% of weakly infected plants, 7 = 50–75% of moderately infected plants or a few plants strongly attacked at the sites of infection, 9 = 75–90% of severely attacked plants, 11 = 100% of very severely attacked plants.

Blackleg resistance was evaluated as described by Pilet et al. (1998), using two criteria:

- the percentage of dead plants (P) by counting the total number of plants per line at the stem extension stage and before maturity;
- the mean disease index (I) on 30 plants scored using the following scale: 0 = no disease, 1 = epidermis necrotic spot, 3 = superficial and one-side necrosis, 5 = superficial complete girdling or one-side deep necrosis, 7 = deep necrosis and complete girdling, plant yellow, 9 = broken crown, dead plant.

Statistical analysis and QTL mapping

Data from 1995 and 1996 for L and S notations were analysed with the same procedure as for the blackleg data in Pilet et al. (1998). A generalized linear model (PROC GLM of the Statistical Analysis System, SAS Institute Inc. 1989) was used for statistical analysis of data from 1995, 1996 and genotype × year interaction. The model was: $P_{ijk} = \mu + L_i + R_j + B_{k/j} + e_{ijk}$ where P_{ijk} is the mean disease score of the ith DH line located in the kth block of the j_{th} replicate, μ the mean of all the data, L_i the DH line i effect, R_j the replicate j effect, $B_{k/j}$ the block k effect in the j_{th} replicate, and e_{ijk} the residual. Normality of residuals was assessed by using the PROC UNIVARIATE procedure. L and S genotypic values of each DH line were estimated from ANOVA after freeing replicate, block and residual components. For each resistance measurement, the estimated genotypic value per DH line was the experimental unit for correlations and QTL analysis. The Kendall coefficient was calculated with the PROC CORR procedure (SAS) to determine correlations from genotypic values. Within each year, strict heritability (h^2) was also estimated from ANOVA by the formula: $h^2 = \sigma^2_g/[\sigma^2_g + (\sigma^2_e/n)]$, with $\sigma 2_g$ being the genetic variance [$\hat{\sigma}^2_g = 1/n$ (MSe-MSg)], σ^2_e the environmental variance [$\hat{\sigma}^2_e$ = MSe], and n the number of replicates per line.

QTL were mapped by 'Interval mapping' for L and S criteria using the computer program MAPMAKER/QTL 1.9 (Lincoln et al., 1992). Putative QTL for L and S were identified in 1995 and in 1996 using a LOD (log of odds likelihood ratio that a QTL is present vs absent) threshold of 2.8, corresponding approximately to an overall α-type-I error of 5% (Lander and Botstein 1989). Then, the multiple-QTL hypothesis was tested by fixing the first QTL detected and re-scanning the genome. A new QTL was identified if the total LOD with the new QTL was more than 2.0 LOD of the total LOD of the

fixed QTL. Due to non-normality of some genotypic distributions, QTL mapping was also performed by a non-parametric method, using the option 'NP Scan' of Mapmaker/QTL (Kruglyak and Lander 1995) with a Z_w threshold of 3.6, which corresponds to a LOD threshold of 2.8. Markers associated with light leaf spot resistance detected by 'Interval mapping' were checked by multiple regression of the trait on the markers with a significance level of $P < 5.10^{-2}$. The normality of the residual variation after QTL detection was also verified for each resistance trait.

Results

Light leaf spot resistance data

Data from 1995 and 1996 were analysed separately because of significant genotype × year interactions ($P = 0.0001$ for L and S). Heritabilities calculated within each year were very high ($h^2 = 0.89$ and 0.83 in 1995, $h^2 = 0.89$ and 0.87 in 1996, for L and S respectively). The analysis of variance indicated highly significant effects of genotype and replicate ($P = 0.0001$). The residual variation was distributed normally in accordance with the Shapiro-Wilk W statistic ($P = 0.81$ and 0.05 in 1995, $P = 0.12$ and 0.94 in 1996, for L and S respectively).

The continuous genotypic distributions (Fig. 1) suggest a polygenic control of the resistance at the two developmental stages studied. In accordance with the Shapiro-Wilk W statistic, these distributions were Gaussian in 1996 for S ($P = 0.30$) but not in 1995 for L and S ($P = 0.0001$) nor in 1996 for L ($P = 0.003$). The transformations tested (arc sine, square root) did not restore the normality. The severity of light leaf spot on leaves and stems was higher in 1995 than in 1996, as indicated by the mean scores of the DH population and by the genotypic values of the controls (Table 1). The control 'Darmor-*bzh*' evaluated in the two years is a sister line of the parental line used to generate the DH population. This control is more susceptible than the parent, which explains the high scores observed. In 1995, the mean values of the variety 'Darmor' in French official registration trials were 4.0 and 1.9 for the resistance on leaves and on stems, respectively.

Correlations between L and S criteria are higher in 1995 than in 1996 (Table 2). The inter-year correlations are more important for L than for S (Table 2). However, they are all significant at the α-risk of 0.001, corresponding to the global risk (0.05) divided by the number of comparisons. Between blackleg resistance traits (I and P) and light leaf spot resistance traits (L and S), correlations are weaker and half of them are not significant. In 1996, I and S were negatively correlated.

Mapping QTL associated with light leaf spot resistance and comparison with QTL involved in blackleg resistance

The characteristics and localization of the QTL detected for light leaf spot resistance are presented in

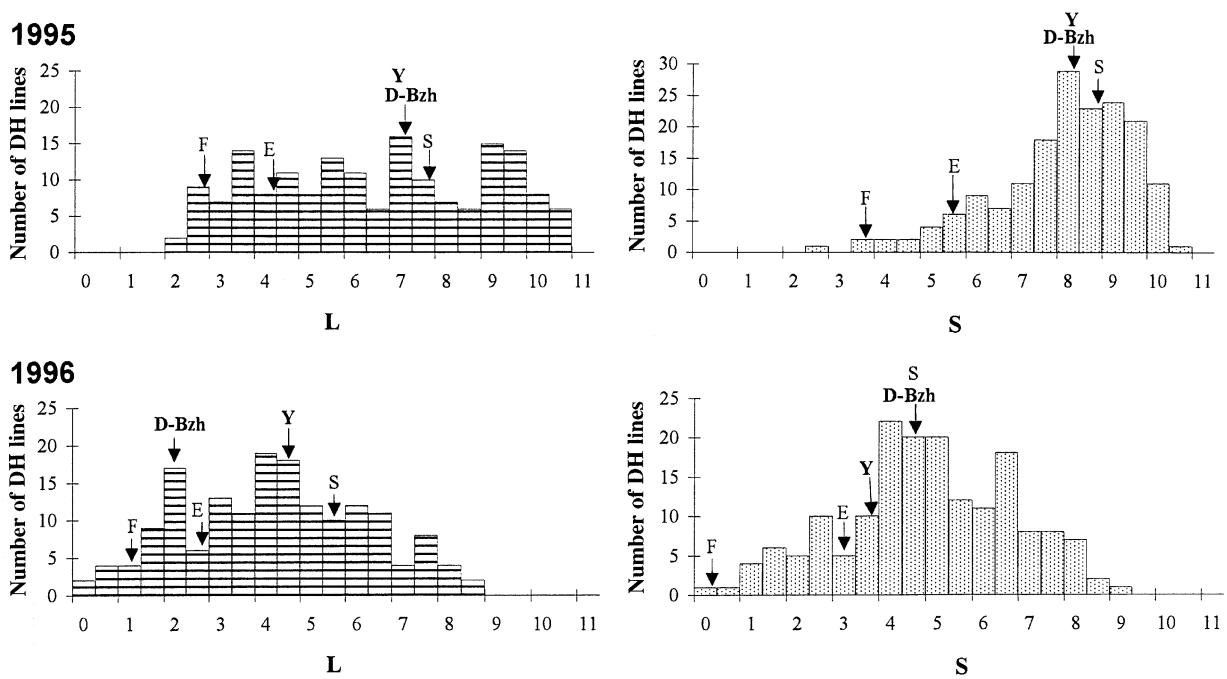


Fig. 1 Distributions of genotypic values estimated in 1995 and 1996 for light leaf spot resistance on leaves (*L*) and and on stems (*S*) in the DH population 'Darmor-*bzh*' × Yudal. Control lines: D-*bzh* ('Darmor-*bzh*'), Y ('Yudal'), F ('Falcon'), E ('Eurol'), S ('Shogun')

Table 1 Genotypic scores of the DH population 'Darmor-*bzh*' × 'Yudal' and controls ('Darmor-*bzh*', 'Yudal', 'Falcon', 'Eurol', 'Shogun') estimated in 1995 and 1996 for light leaf spot resistance on leaves (L) and stems (S)

Item	1995		1996	
	L	S	L	S
DH population	6.3 ± 2.4	7.6 ± 1.5	4.0 ± 2.0	4.6 ± .1.9
'Darmor-*bzh*'	7.1	8.4	2.4	4.7
'Yudal'	7.0	8.3	4.6	3.8
'Falcon'	2.6	3.4	1.2	0.4
'Eurol'	4.3	5.6	2.9	3.5
'Shogun'	7.4	8.8	5.7	4.7

Table 3 and Fig. 2. All the QTL identified by the non-parametric method were also detected using the usual parametric approach. Only the results obtained from the parametric method are presented in this paper.

From scoring light leaf spot resistance on leaves (L), five and seven QTL, explaining 53% and 57% of the genotypic variation, were identified in 1995 and 1996, respectively. Four QTL, located on DY5, DY6, DY10 and DY12 were detected in both years. On the DY12 group, the QTL has a negative effect, indicating that the allele of 'Darmor-*bzh*' at this locus contributed to an increase in susceptibility. On the DY11 group, the confidence intervals of the QTL detected for the two years overlap. Two other QTL were specifically revealed in 1996 on the DY4 and DY1 groups.

From scoring on stems (S), three and five QTL were associated with light leaf spot resistance in 1995 and in 1996, respectively. They explained a smaller portion of the genotypic variation than all the QTL detected for L in each of the two years (29% in 1995 and 43% in 1996). Two QTL detected in 1995 on the DY5 and DY10 groups were also revealed in 1996. On the DY11 group, the confidence intervals of the two QTL are situated end to end, each of them being specific to one year. Two other QTL were specifically expressed in 1996 with negative effects; the first of these is linked to the dwarf gene '*bzh*' on the DY6 group and has the strongest weight (− 1.38), contributing individually to 17% of the genotypic variation; the second one, located on the DY3 group, has the weakest effect (− 0.81).

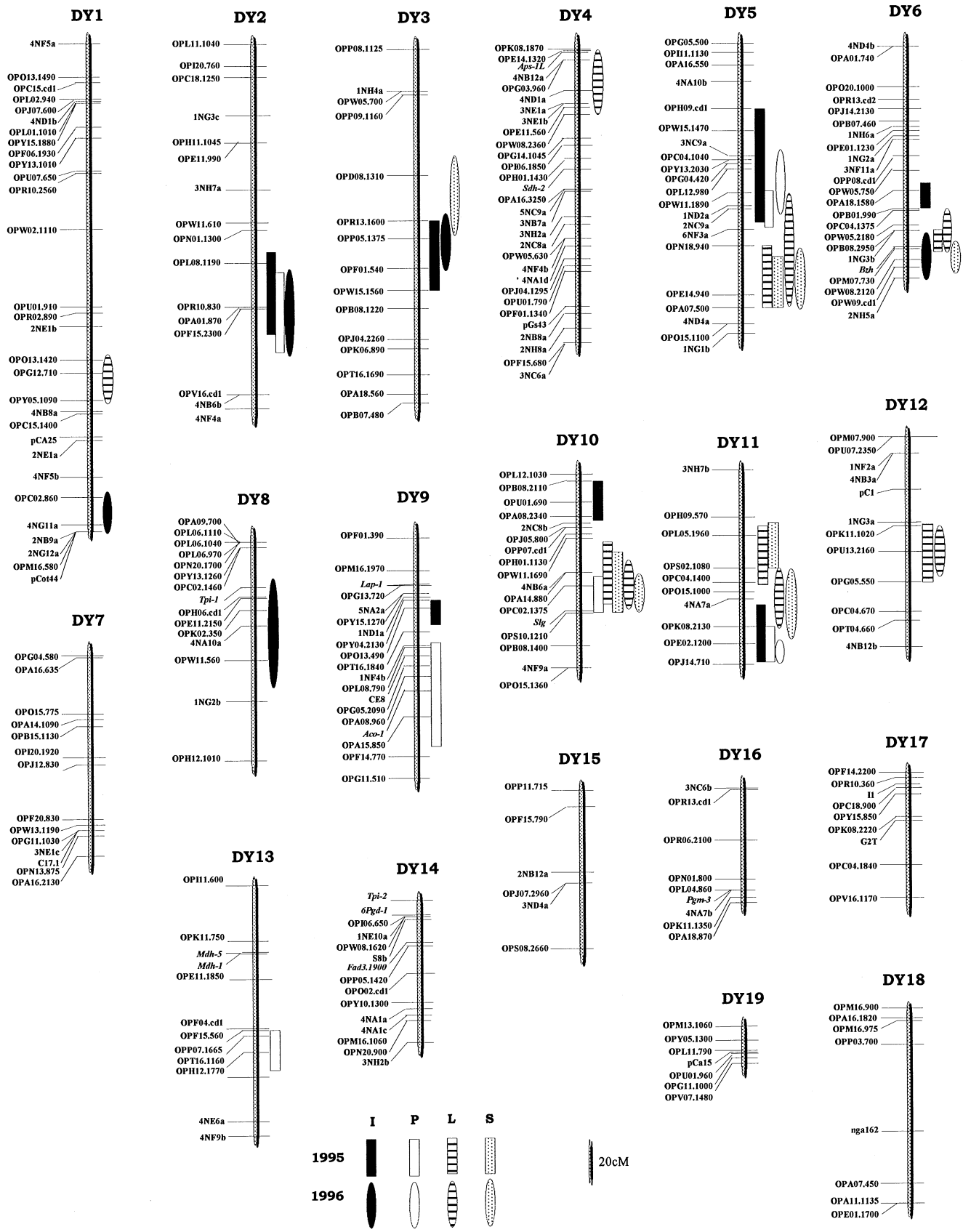
Table 2 Kendall genotypic correlation coefficients among light leaf spot and blackleg resistance criteria assessed in 1995 and 1996. I = blackleg resistance index; P = proportion of lost plants supposed to be due to backleg; L = light leaf spot resistance on leaves; S = light leaf spot resistance on stems

	L95	S95	L96	S96	I95	P95	I96	P96
L95								
S95	0.58							
L96	0.58	0.44						
S96	0.31	0.43	0.30					
I95	*0.26*	*0.17*	*0.23*	*0.03**				
P95	*0.3*	*0.27*	*0.27*	*0.21*	0.42			
I96	*0.13**	*0.04**	*0.14**	*− 0.15**	0.38	0.23		
P96	*0.16**	*0.21*	*0.15**	*0.16**	0.15*	0.26	0.16*	

*: $P > 0{,}001$

Table 3 QTL for light leaf spot resistance on leaves (L) and on stems (S) detected by 'Interval mapping' in the DH 'Darmor-*bzh*' × Yudal' population: position, LOD score, individual effect and contribution to the genotypic variation. The QTL position from the first marker of the interval is expressed in centimorgans. When a QTL was detected with the multiple-QTL model, the increase of total LOD with the new QTL is indicated with the sign ' + '. 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. R^2 is the percentage of genotypic variation explained by all the QTL detected. Note: the bold-type font indicates the common QTL between the two years for each resistance criterion

1995						1996					
Trait	Position	Linkage group	LOD	Weight	R^2 (%)	Trait	Position	Linkage group	LOD	Weight	R^2 (%)
L	**OPN18.940 + 20**	**DY5**	**5.6**	**2.13**		L	4NA7a + 2	DY11	4.2	1.40	
	OPA14.880 + 4	**DY10**	**4.3**	**1.96**			**1NG3b + 0**	**DY6**	**3.9**	**1.35**	
	OPL05.1960 + 6	DY11	3.0	1.23			OPG12.710 + 0	DY1	3.4	1.10	
	OPB08.2950 + 0	**DY6**	**+ 4.0**	**1.50**			**OPC02.1375 + 0**	**DY10**	**+ 5.0**	**1.32**	
	OPK11.1020+12	**DY12**	**+ 2.7**	**− 1.07**	53.5		**OPN18.940 + 18**	**DY5**	**+ 3.2**	**1.21**	
							OPU13.2160 + 0	**DY12**	**+ 3.5**	**− 0.99**	
							OPG03.960 + 6	DY4	+ 2.1	− 0.79	57.3
S	**OPN18.940 + 22**	**DY5**	**+ 4.1**	**1.04**		**S**	OPM07.730 + 0	DY6	5.7	− 1.38	
	OPL05.1960 + 4	DY11	+ 2.7	0.97			**OPC02.1375 + 0**	**DY10**	**+ 3.7**	**0.99**	
	OPA14.880 + 6	**DY10**	**+ 2.1**	**0.74**	28.8		4NA7a + 6	DY11	+ 2.4	0.92	
							OPN18.940 + 18	**DY5**	**+ 2.2**	**1.00**	
							OPD08.1375 + 22	DY3	+ 2.1	− 0.81	42.7


DY1
4NF5a
OPO13.1490
OPC15.cd1
OPL02.940
OPJ07.600
4ND1b
OPL01.1010
OPY15.1880
OPF06.1930
OPY13.1010
OPU07.650
OPR10.2560
OPW02.1110
OPU01.910
OPR02.890
2NE1b
OPO13.1420
OPG12.710
OPY05.1090
4NB8a
OPC15.1400
pCA25
2NE1a
4NF5b
OPC02.860
4NG11a
2NB9a
2NG12a
OPM16.580
pCot44
DY2
OPL11.1040
OPI20.760
OPC18.1250
1NG3c
OPH11.1045
OPE11.990
3NH7a
OPW11.610
OPN01.1300
OPL08.1190
OPR10.830
OPA01.870
OPF15.2300
OPV16.cd1
4NB6b
4NF4a
DY3
OPP08.1125
1NH4a
OPW05.700
OPP09.1160
OPD08.1310
OPR13.1600
OPP05.1375
OPF01.540
OPW15.1560
OPB08.1220
OPJ04.2260
OPK06.890
OPT16.1690
OPA18.560
OPB07.480
DY4
OPK08.1870
OPE14.1320
Aps-1L
4NB12a
OPG03.960
4ND1a
3NE1a
3NE1b
OPE11.560
OPW08.2360
OPG14.1045
OPI06.1850
OPH01.1430
Sdh-2
OPA16.3250
5NC9a
3NB7a
3NH2a
2NC8a
OPW05.630
4NF4b
4NA1d
OPJ04.1295
OPU01.790
OPF01.1340
pGs43
2NB8a
2NH8a
OPF15.680
3NC6a
DY5
OPG05.500
OPI11.1130
OPA16.550
4NA10b
OPH09.cd1
OPW15.1470
3NC9a
OPC04.1040
OPY13.2030
OPG04.420
OPL12.980
OPW11.1890
1ND2a
2NC9a
6NF3a
OPN18.940
OPE14.940
OPA07.500
4ND4a
OPO15.1100
1NG1b
DY6
4ND4b
OPA01.740
OPO20.1000
OPR13.cd2
OPJ14.2130
OPB07.460
1NH6a
OPE01.1230
1NG2a
3NF11a
OPP08.cd1
OPW05.750
OPA18.1580
OPB01.990
OPC04.1375
OPW05.2180
OPB08.2950
1NG3b
Bzh
OPM07.730
OPW08.2120
OPW09.cd1
2NH5a
DY7
OPG04.580
OPA16.635
OPO15.775
OPA14.1090
OPB15.1130
OPI20.1920
OPJ12.830
OPF20.830
OPW13.1190
OPG11.1030
3NE1c
C17.1
OPN13.875
OPA16.2130
DY8
OPA09.700
OPL06.1110
OPL06.1040
OPL06.970
OPN20.1700
OPY13.1260
OPC02.1460
Tpi-1
OPH06.cd1
OPE11.2150
OPK02.350
4NA10a
OPW11.560
1NG2b
OPH12.1010
DY9
OPF01.390
OPM16.1970
Lap-1
OPG13.720
5NA2a
OPY15.1270
1ND1a
OPY04.2130
OPO13.490
OPT16.1840
1NF4b
OPL08.790
CE8
OPG05.2090
OPA08.960
Aco-1
OPA15.850
OPF14.770
OPG11.510
DY10
OPL12.1030
OPB08.2110
OPU01.690
OPA08.2340
2NC8b
OPJ05.800
OPP07.cd1
OPH01.1130
OPW11.1690
4NB6a
OPA14.880
OPC02.1375
Slg
OPS10.1210
OPB08.1400
4NF9a
OPO15.1360
DY11
3NH7b
OPH09.570
OPL05.1960
OPS02.1080
OPC04.1400
OPO15.1000
4NA7a
OPK08.2130
OPE02.1200
OPJ14.710
DY12
OPM07.900
OPU07.2350
1NF2a
4NB3a
pC1
1NG3a
OPK11.1020
OPU13.2160
OPG05.550
OPC04.670
OPT04.660
4NB12b
DY13
OPI11.600
OPK11.750
Mdh-5
Mdh-1
OPE11.1850
OPF04.cd1
OPF15.560
OPP07.1665
OPT16.1160
OPH12.1770
4NE6a
4NF9b
DY14
Tpi-2
6Pgd-1
OPI06.650
1NE10a
OPW08.1620
S8b
Fad3.1900
OPP05.1420
OPO02.cd1
OPY10.1300
4NA1a
4NA1c
OPM16.1060
OPN20.900
3NH2b
DY15
OPP11.715
OPF15.790
2NB12a
OPJ07.2960
3ND4a
OPS08.2660
DY16
3NC6b
OPR13.cd1
OPR06.2100
OPN01.800
OPL04.860
Pgm-3
4NA7b
OPK11.1350
OPA18.870
DY17
OPF14.2200
OPR10.360
I1
OPC18.900
OPY15.850
OPK08.2220
G2T
OPC04.1840
OPV16.1170
DY18
OPM16.900
OPA16.1820
OPM16.975
OPP03.700
nga162
OPA07.450
OPA11.1135
OPE01.1700
DY19
OPM13.1060
OPY05.1300
OPL11.790
pCa15
OPU01.960
OPG11.1000
OPV07.1480
I
P
L
S
1995
1996
20cM

Comparing the genomic regions identified for L and S, two QTL located on the DY5 and DY10 groups were associated with light leaf spot resistance both on leaves and on stems in 1995 and in 1996. On the DY11 group, two overlapping year-specific regions are common to both L and S. Close to the dwarf gene on the DY6 group, two regions specific to L and S were detected with opposite effects. Another region specific to L was identified on the DY12 group for the two years.

Five linkage groups include QTL for blackleg and light leaf spot resistance. On the DY6 and DY10 groups, some QTL of the two disease resistances coincide. On the DY3, DY5 and DY11 groups, the confidence intervals of some QTL for resistance to the two pathogens overlap (Fig. 2).

Discussion

Light leaf spot resistance assessment

In this paper, we report for the first time on the genetic analysis of field resistance to light leaf spot in *B. napus*. The nature of isolates could not be established, however, since the characterization of groups within the pathogen has not yet been done. From infected crop debris, ascospores of the sexual stage *P. brassicae* can be disseminated by wind for considerable distances (McCartney et al. 1986; McCartney and Lacey 1991), whereas conidia of its asexual stage are spread by rain splash for short distances (Rawlinson et al. 1978; Fatemi and Fitt 1983). In France, the sexual stage has not been shown to occur naturally on oilseed rape; only the conidial cycle may operate, explaining the frequent observation of localized contamination. In our field trials, homogeneity of contamination is expected since rapeseed stubble infected by the two pathogens was uniformly scattered to increase inoculum pressure.

Heritabilities of resistance on leaves and on stems were high within each year. This suggests that the genotypic values were well estimated from the phenotypic values despite the lack of knowledge about pathogen variability and resistance assessment by visual semi-quantitative notations.

Mapping QTL for light leaf spot resistance

We have identified genomic regions contributing to light leaf spot field resistance in the DH population from the cross between moderately resistant 'Darmor-*bzh*' and susceptible 'Yudal'. Although the difference in resistance levels between 'Darmor-*bzh*' and 'Yudal' is less important for light leaf spot than for blackleg, large variability was observed in the progeny for the resistance to light leaf spot.

From two-years data (1995 and 1996), we detected a total of ten genomic regions associated with resistance on leaves and on stems, distributed on 8 of the 19 linkage groups of the genetic map. The high number of QTL detected, as well as continuous genotypic distributions, confirm the polygenic nature of this field resistance.

The stability of QTL between 1995 and 1996 is moderate, since four of the eight QTL detected for L and two of the six QTL detected for S were common to both years.

Three QTL, located on the DY5, DY10 and DY11 linkage groups, were common to resistance on both L and S : two of them were revealed for the two years (on DY5 and DY10); the other one, on DY11, consists of two regions, each specifically expressed in one year. In each of these regions, the alleles of 'Darmor-*bzh*' had strong effects on resistance. Several studies have revealed QTL, which were common to several steps of plant pathogen interaction (Freymark et al. 1993; Danesh et al. 1994; Ferreira et al. 1995; Lefebvre and Palloix 1996). In the current study, the common-organ regions were probably the cause of the correlations observed between L and S criteria. They may correspond to genes expressed, independently of time or organ, at several developmental stages or during all the host-pathogen interaction.

We also identified four other genomic regions with negative weights (on DY3, DY4, DY6 and DY12), suggesting that alleles of 'Darmor-*bzh*' contributed to increased susceptibility. This is confirmed by the observation of an important number of DH lines more susceptible than 'Yudal'.

Two overlapping regions with opposite effects were identified around the '*bzh*' gene: the first one corresponds to resistance at the leaf stage (both years) and the second one to resistance at the stem level. The dwarf trait could have interacted with light leaf spot resistance. The large morphological differences between dwarf and normal plants could have substantially influenced levels of resistance and/or notations.

Earliness also influenced the assessment of resistance since all the DH lines segregating for this trait were scored on the same dates. Three QTL for earliness are co-linear with QTL for resistance to *P. brassicae* on the DY6, DY11 and DY12 groups, among which one QTL is close to the '*bzh*' gene (unpublished data).

Comparison of QTL for light leaf spot and blackleg resistance

A total of 10 and 13 QTL were revealed over two years from light leaf spot (L and S criteria) and blackleg

Fig. 2 Linkage map locations of QTL contributing to field resistance to blackleg (I = index, P = proportion of lost plants) and light leaf spot (L = on leaves, S = on stems), identified in the cross 'Darmor-*bzh*' × 'Yudal'. The QTL lengths are the confidence intervals where the likelihood of the presence of a QTL is within tenfold (1 LOD) of its maximal value. The names of loci with biased segregation are preceded by an *asterisk* and the letter *D* (for 'Darmor-*bzh*') or *Y* (for 'Yudal') according to the favored parental line (Foisset et al. 1996).

resistance (I and P criteria) evaluation, respectively. A large number of QTL were detected for each disease resistance, even though the variation of scores between 'Darmor-*bzh*' and 'Yudal' is higher for blackleg resistance than for light leaf spot resistance. The number of QTL detected with negative effects is higher for light leaf spot resistance (four QTL) than for blackleg resistance (two QTL). This is not surprising since 'Darmor' was selected for blackleg resistance.

The level of genotypic variation explained by all the QTL detected for one resistance criterion never exceeded 57%. This may result from the size of our mapping population, which did not permit the identification of QTL with very small effects for the two polygenic resistances studied.

Two regions, located on DY6 and DY10, were considered to be involved in the resistance to the two fungi in 1995 or in 1996. They do not coincide with the blackleg resistance QTL with major effects. Common QTL for different pathogen resistance genes have been widely reported in the literature (Lefebvre and Chèvre 1995; Jung et al. 1996; Young 1996). In the interaction *B. oleracea*/*Xanthomonas campestris*, Camargo et al. (1995) identified two regions associated with young plant resistance, which contained markers common to a region of *B. napus* associated with cotyledon and stem resistance to *L. maculans*. Some studies tried to identify positive correlations between resistances to different pathogens with the goal of applying selection for multiple disease resistance ('MDR') (Hill and Leath 1975; Nyhus et al. 1989). Mitchell-olds et al. (1995) reported a positive genetic correlation in levels of resistance to *Peronospora parasitica* and *L. maculans* in *Brassica rapa*.

In our study, the 'MDR' QTL may include clusters of genes involved in each of the two resistances. The existence of such clusters have been demonstrated in several plant/pathogen systems (Martin et al. 1993; Witsenboer et al. 1995; Concibidio et al. 1996; Saghai Maroof et al. 1996). The dissection of the 'MDR' QTL detected would then be of great interest to associate mechanisms of resistance with each locus. This approach would require tests controlling the nature of isolate(s) and the homogeneity of contamination, as well as elaborating a fine scale of notations.

Otherwise, the 'MDR' QTL may correspond to genes involved in common mechanisms of resistance induced by *L. maculans* and *P. brassicae*, independently or in interaction. In 1995 and 1996, the evaluation of resistance to *P. brassicae* and *L. maculans* was performed in the same trial. In 1995, the mean levels of susceptibility in the DH population were higher than in 1996 for the two diseases, suggesting that the inoculum pressure of the two pathogens was higher in 1995 than in 1996. In fact, the development of the two fungi are influenced by identical climatic conditions (high moisture, mean temperature). However, we cannot exclude the hypothesis that possible negative or positive interactions between the two pathogens could enhance or decrease the levels of plant resistance to *P. brassicae* and/or *L. maculans*. The different development pathways of the two fungi in the plant could suggest the absence of a direct interaction between these pathogens. *L. maculans* infects oilseed rape by an intercellular systemic pathway, colonizing intercellular spaces and spreading down the petiole and the stem in xylem vessels (Hammond et al. 1985). The infection with conidia from the asexual stage of *P. brassicae* remains superficial: they penetrate through the cuticle, produce hyphae which spread between the cuticle and epidermal cells and differentiate conidiophores in acervula (Rawlinson et al. 1978; Martin 1990). However, Chamberlain et al. (1995) reported that culture extracts of *P. brassicae* stimulated sexual and/or asexual reproduction of about 30 other species of fungi. Awasthi et al. (1997) studied the effect of *Albugo candida* on resistance to *Peronospora parasitica*, and vice versa in rapeseed-mustard. Since we cannot exclude the hypothesis of an interaction between *L. maculans* and *P. brassicae*, trials where the two pathogens are inoculated simultaneously is of great interest : it may reveal further 'MDR' QTL which may not be detected when inoculating with only one pathogen. It is all the more interesting since, in field conditions of cultivation, rapeseed can be infected by several pathogens at the same time. To validate the hypothesis that the 'MDR' regions are QTL resulting from the interaction between the two pathogens, field experiments could be performed stopping the development of *P. brassicae* by using fungicides.

These results provide DNA markers linked to disease resistance which could be used to assist the selection of rapeseed resistant to light leaf spot and blackleg. However, further mapping studies need to be conducted in other locations and in crosses between relevant breeding materials in order to validate the usefulness of the identified DNA markers.

Acknowledgments We are very grateful to H. Brun and X. Tanguy for their assistance in the resistance tests, to B. Landry and M. Manzanares for their critical review of the manuscript and to the breeding experimental team of the farm of I.N.R.A. for their help in performing the disease trials. This research was supported by a grant from M.E.S.R., and by CETIOM and PROMOSOL.

References

Amelung D, Daebeler F (1991) Occurrence of the fertile apothecia and the epidemiology of *Pyrenopeziza brassicae* Sutton & Rawlinson (Anamorph: *Cylindrosporium concentricum*) in the German Democratic Republic. IOBC/WPRS Bull no. 14 : 147–150

Ashby AM (1997) A molecular view through the looking glass: the *Pyrenopeziza brassicae Brassica* interaction. Adv Bot Res Inc Adv Plant Pathol 24 : 31–70

Awasthi R, Nashaat N, Heran A, Kolte S, Singh U (1997) The effect of *Albugo candida* on the resistance to *Peronospora parasitica*, and vice versa, in rapeseed-mustard. ISHS Symposium on brassicas, 10th Crucifer Genetic Workshop, p 49

Brun H, Renard M, Jouan B, Tanguy X, Lamarque C (1979) Observations préliminaires sur quelques maladies du colza en France: *Sclerotinia sclerotiorum*, *Cylindrosporium concentricum*, *Ramularia armoraciae*. Sci Agron Rennes, pp 7–17

Brun H, Renard M, Tribodet M, Plessis J, Tanguy X (1989) Apport de la lutte génétique contre les maladies du colza. Phytoma 404 : 36–40

Camargo LEA, Williams PH, Osborn TC (1995) Mapping of quantitative trait loci controlling resistance of *Brassica oleracea* to *Xanthomonas campestris* pv. *campestris* in the field and greenhouse. Phytopathol 85 : 1296–1300

Chamberlain M, Ertz S, Ingram DS (1995) Effects of extracts of *Pyrenopeziza brassicae* on the reproductive development of other species of fungi. Mycologia 87 : 846–856

Cheah LH, Hartill WFT, Corbin JB (1980) First report of the natural occurrence of *Pyrenopeziza brassicae* Sutton et Rawlinson, the apothecial state of *Cylindrosporium concentricum* Greville, in brassica crops in New Zealand. NZ J Bot 18 : 197–202

Concibidio VC, Young ND, Lange DA, Denny RL, Danesh D, Orf JH (1996) Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode. Theor Appl Genet 93 : 234–241

Danesh D, Aarons S, McGill GE, Young ND (1994) Genetic dissection of oligogenic resistance to bacterial wilt in tomato. Mol Plant-Microbe interact 7 : 464–471

Delourme R, Chèvre AM, Eber F, Foisset N, Pilet ML, Barret P, Brun H, Tanguy X, Renard M (1995) Mapping of blackleg resistance genes. Blackleg Newslett 5 : 4–6

Fatemi F, Fitt BDL (1983) Dispersal of *Pseudocercosporella herpotrichoides* and *Pyrenopeziza brassicae* spores in splash droplets. Plant Pathol 32 : 401–404

Ferreira ME, Rimmer SR, Williams PH, Osborn TC (1995) Mapping loci controlling *Brassica napus* resistance to *Leptosphaeria maculans* under different screening conditions. Phytopathology 85 : 213–217

Figueroa L, Shaw MW, Fitt BDL, Mccartney HA, Welham SJ (1994) Effects of previous cropping and fungicide timing on the development of light leaf spot (*Pyrenopeziza brassicae*), seed yield and quality of winter oilseed rape (*Brassica napus*). Ann Appl Biol 124 : 221–239

Figueora L, Fitt BDL, Shaw MW, McCartney HA, Welham SJ (1995 a) Effects of temperature on the development of light leaf spot (*Pyrenopeziza brassicae*) on oilseed rape (*Brassica napus*). Plant Pathol 44 : 51–62

Figueroa L, Fitt BDL, Welham SJ, Shaw MW, McCartney HA (1995 b) Early development of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in relation to temperature and leaf wetness. Plant Pathol 44 : 641–654

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

Hammond KE, Lewis BG, Musa TM (1985) A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. Plant Pathol 34 : 557–565

Hardwick NV, Fitt BDL, Wale SJ, Sweet JB (1991) Oilseed rape diseases. Home Grown Cereals Authority Research Review OS4

Hill AL, Leath KT (1975) Genotypic and phenotypic correlations for reaction to five foliar pathogens in alfalfa. Theor Appl Genet 45 : 254–258

Jeffery DC, Jones DG, Jenkins PD (1989) Effects of early infections of light leaf spot (*Pyrenopeziza brassicae*) on oilseed rape (*Brassica napus*). Aspects Appl Biol 23 : 409–415

Jung GW, Coyne DP, Skroch PW, Nienhuis J, Arnaudsantana E, Bokosi J, Ariyarathne HM, Steadman JR, Beaver JS, Kaeppler SM (1996) Molecular markers associated with plant architecture and resistance to common blight, web blight and rust in common beans. J Am Soc Hort Sci 121 : 794–803

Kruglyak L, Lander ES (1995) A nonparametric approch for mapping quantitative trait loci. Genetics 139 : 1421–1428

Lacey ME, Rawlinson CJ, Mccartney HA (1987) First record of the natural occurrence in England of the teleomorph of *Pyrenopeziza brassicae* on oilseed rape. Trans Br Mycol Soc 89 : 135–140

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

Lefebvre V, Chèvre AM (1995) Tools for marking plant disease and pest resistance genes: a review. Agronomie 15 : 3–19

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 Technical Report 2nd edition

Maddock SE, Ingram DS (1981) Studies of survival and longevity of the light leaf spot pathogen of brassicas, *Pyrenopeziza brassicae*. Trans Br Mycol Soc 77 : 153–159

Maddock SE, Ingram DS, Gilligan CA, (1981) Resistance of cultivated brassicas to *Pyrenopeziza brassicae*. Trans Br Mycol Soc 76 : 371–382

Martin D (1990) Cylindrosporiose du colza – Cycle de développement et épidémiologie, observation des premières phases de la contamination par les conidies. La Défense des Végétaux 262 : 16–21

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spirey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring resistance in tomato. Science 262 : 1432–1435

McCartney HA, Lacey ME (1991) Spread of light leaf spot (*Pyrenopeziza brassicae*) in oilseed rape crops in the United Kingdom. In: McGregor DI (ed) Proc 8th Int Rapeseed Congr, Saskatoon, Canada, pp 454–459

McCartney HA, Lacey ME, Rawlinson CJ (1986) Dispersal of *Pyrenopeziza brassicae* spores from an oilseed rape crop. Agric Sci 107 : 299–305

Mitchell-olds T, Vaughn James R, Palmer MJ, Williams PH (1995) Genetics of *Brassica rapa* (syn. campestris). 2. Multiple disease resistance to three fungal pathogens: *Peronospora parasitica*, *Albugo candida* and *Leptosphaeria maculans*. Heredity 75 : 362–369

Nyhus KA, Russel WA, Guthrie WD, Martinson CA (1989) Reaction of two maize synthetics to anthracnose stalk rot and northern corn leaf blight following recurrent selection for resistance to Diplodia stalk rot and European corn borer. Phytopathology 79 : 166–169

Penaud A, Regnault Y (1983) Revue bibliographique sur une nouvelle maladie du colza, *Cylindrosporium concentricum* (Grev.). Informations interne CETIOM No. 81

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

Rawlinson CJ, Sutton BC, Muthyalu G (1978) Taxonomy and biology of *Pyrenopeziza brassicae* sp. nov. (*Cylindrosporium concentricum*), a pathogen of winter oilseed rape (*Brassica napus* ssp. *oleifera*). Trans Br Mycol Soc 71 : 425–439

Rimmer SR, Van Den Berg CGJ (1992) Resistance of oilseed *Brassica spp.* to blackleg caused by *Leptosphaeria maculans*. Can J Plant Pathol 14 : 56–66

Saghai Maroof MA, Yue YG, Xiang ZX, Stromberg EL, Rufener GK (1996) Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. Theor Appl Genet 93 : 539–546

Simons AJ, Skidmore DI (1988) Race-specific resistance to light leaf spot in *Brassica oleracea*. Trans Br Mycol Soc 90 : 431–435

Sutherland KG, Wale SJ, Sansford C (1995) Effect of different epidemics of *Pyrenopeziza brassicae* on yield koss in winter oilseed rape. Proc 9th Int Rapeseed Congr, Cambridge, UK, pp 1004–1006

Walker KC, Thomas JE, Kightley SP (1995) The effects of cultivar resistance and fungicides on the yield of oilseed rape infected with light leaf spot (*Pyrenopeziza brassicae*). Proc 9th Int Rapeseed Congr, Cambridge, UK, pp 998–1000

Witsenboer H, Kesseli RV, Fortin MG, Stanghellini M, Michemore RW (1995) Sources and genetic structure of a cluster of genes for resistance to three pathogens in lettuce. Theor Appl Genet 91 : 178–188

Yarham DJ, Giltrap NJ (1989) Crop diseases in a changing agriculture. Arable crops – a review. Plant Pathol 38 : 459–477

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