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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.)

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Abstract Blackleg, caused by *Leptosphaeria maculans*, is one of the most important diseases of *Brassica napus*. Genomic regions controlling blackleg resistance at the adult plant stage were detected using 152 doubled-haploid (DH) lines derived from the F_1 'Darmor-*bzh*' × 'Yudal'. The rapeseed genetic map used includes 288 DNA markers on 19 linkage groups. Blackleg resistance of each DH line was evaluated in field tests in 1995 and 1996 by measuring the mean disease index (I) and the percentage of lost plants (P). From notations recovered in 1995, ten quantitative trait loci (QTL) were detected: seven QTL for I and six QTL for P, explaining 57% and 41% of the genotypic variation, respectively. Three of them were common to I and P. From data recovered in 1996, seven QTL were identified: five QTL for I and two different QTL for P, accounting for 50% and 23% of the genotypic variation, respectively. One I QTL, located close to a dwarf gene (*bzh*), was detected with a very strong effect, masking more QTL detection. It was not revealed at the same position and with the same effect in 1995. Four major genomic regions were revealed from 1995 and from 1996 with the same parental contribution. One of them, located on the DY2 group, has a resistance allele from the susceptible parent. Five- and two-year-specific QTL were detected in 1995 and 1996, respectively.

Key words *Leptosphaeria maculans* · *Brassica napus* L. · Quantitative resistance · QTL mapping · Doubled-haploid progeny

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Introduction

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not. [anamorph *Phoma lingam* (Tode:Fr.) Desm.] is the most serious disease of rapeseed (*Brassica napus* L.) and many other cruciferous species in most of the rapeseed producing areas in the world except China (Gugel and Petrie 1992). In Europe, the severity of blackleg was reported to be significant since 1966 (Lacoste 1969; Humpherson-Jones 1983). While the introduction of the resistant Winter cultivar 'Jet Neuf' in 1977 has effectively controlled the disease in Western and Eastern Europe (Gladders and Musa 1979), the development of high-yielding but moderately resistant varieties in the 1980's and the beginning of the 1990s, has contributed to an increase its severity (Gladders 1995). Because the disease cannot be efficiently controlled chemicaly, increasing blackleg resistance has become a major objective of rapeseed breeding programs. Different sources of resistance to blackleg have been characterized at various development stages (Rimmer and Van Der Berg 1992). At the interspecific level, total seedling and adult resistances of mustards (*B. nigra* L., *B. juncea* L.) have been identified and transferred into *B. napus* (Roy 1984; Struss et al. 1992; Chèvre et al. 1996; Pang and Halloran 1996a). At the intraspecific level, most of the specific seedling resistances reported were monogenic or oligogenic (Delwiche 1980; Sawatsky 1989; Stringam et al. 1992). On the basis of plant material analyzed and inoculum composition, some authors proposed a monogenic control of adult resistance in the field (Dion et al. 1995; Stringam et al. 1994), whereas others demonstrated a polygenic control (Cargeeg and Thurling 1979; Sippell et al. 1991; Fereirra et al. 1995; Pang and Halloran 1996b, c). Although many authors observed a significant correlation between seedling and adult tests (Newman and Bailey 1987; McNabb et al. 1993; Bansal et al. 1994), recent studies

suggest that seedling and adult plant resistance could be under different genetic controls (Fereirra et al. 1995; Pang and Halloran 1996c; Ballinger and Salisbury 1996).

The complexity of non-specific adult blackleg resistance, the wide pathogen variability (Williams 1992) and the constraints of field testing do not facilitate breeding for blackleg resistance through conventional methods. The development of molecular markers and the construction of genetic maps have enabled quantitative trait loci (QTL) such as quantitative resistance loci (QRL) to be localized and characterized (Young 1996). Many examples of quantitative resistance mapping have already been described in many species (for reviews, Young 1996; Lefebvre and Chèvre 1995).

In our study, we used a doubled haploid (DH) population derived from the F_1 'Darmor-*bzh*' × 'Yudal', from which a *B. napus* map was elaborated by Foisset et al. (1996). The resistant parent, 'Darmor-*bzh*', is a dwarf line, nearly isogenic (B_3F_3) to 'Darmor' (Foisset et al. 1995). 'Darmor' is derived from the Winter cultivar 'Jet Neuf' through back-crosses. The adult plant resistance of 'Jet Neuf' (Renard and Brun 1979), which is partial and non-specific, has been durable despite the wide use of this cultivar in western and eastern Europe for 10 years (Humpherson-Jones 1983; Hammond and Lewis 1987). 'Yudal' is a Spring inbred line (F_9) very susceptible to blackleg and was selected from a Korean population by pedigree breeding.

In the present paper, we report on the identification of QTL associated to blackleg resistance that were expressed in this DH population during two successive years. We assessed the number, the location, the individual effect and the parental allelic contribution of each QTL detected. We discuss the method used for the resistance assessment, the number of QTL detected and the environmental stability of the marker-QTL associations between the 2 years.

Materials and methods

Plant materials and genetic map

The segregating DH population, derived from the cross 'Darmor-*bzh*' × 'Yudal', and the genetic map established from this population were previously described by Foisset et al. (1996). Due to additional mapped markers, the map used in this study covers 1954 cM and comprises 288 markers [predominantly restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD)] assembled into 19 linkage groups.

Field experiment

The field experiment was conducted with 171 DH lines in a randomized incomplete block design with three replicates and six blocks per replicate, at Le Rheu, France, in 1995 and 1996. In each replicate, four-row plots (2.5 m^2) of parental, DH and control lines were sown. The controls were three Winter type *B. napus* cultivars that show different levels of resistance to *L. maculans* ('Falcon': partially resistant; 'Eurol': moderately resistant; 'Shogun': susceptible). Infected rapeseed stubble collected from the previous year trial was scattered through the field at the three-leaf stage to increase inoculum pressure. Blackleg severity was assessed in two ways:

- In each plot of the trial, the total number of plants was counted twice–first, at the stem extension stage, and second, before maturity–to estimate the proportion of dead plants (P) mostly as a result of blackleg.
- When the plants were just beginning to ripen, 30 plants per plot were uprooted and scored using the following scale based on the extent of external and internal necrosis at the crown: 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. A mean blackleg index (I) in each replicate was calculated for each plot from the formula:

$$I = 1/N \sum_i in_i$$

with N the total number of scored plant, i the disease score and n_i the number of plant with the score i.

Since earliness was a segregating trait in the DH population, scoring of the DH lines was done at a specific developmental stage.

Statistical analysis and QTL mapping

The same data analysis procedure has been realized for each of the two resistance criteria I and P.

Data from 1995 and 1996 as well as genotype × year interaction were analyzed using a generalized linear model (PROC GLM of Statistical Analysis System, SAS, 1989). For each year, this one-way analysis of variance (ANOVA) partitioned total variation into effects of lines, replicates, blocks and errors ($P_{ijk} = \mu + L_i + R_j + B_{k/j} + e_{ijk}$ where P_{ijk} is the mean disease score of the i^{th} DH line located in the k^{th} block of the j^{th} replicate, μ is the mean of all the data, L_i is the DH line i effect, R_j is the replicate j effect, $B_{k/j}$ is the block k effect in the j^{th} replicate and e_{ijk} is the residual). Normality of each residual distribution was assessed by using the PROC UNIVARIATE procedure. I and P genotypic values of each DH line were assessed 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 QTL analysis. The Pearson coefficient was calculated with the PROC CORR procedure (SAS) to determine correlations among criteria between or within the two years from estimated genotypic values. Heritability (h^2) was also estimated from ANOVA with the formula: $h^2 = \sigma_g^2/[\sigma_g^2 + (\sigma_e^2/n)]$ with σ_g^2 the genetic variance $[\hat{\sigma}_g^2 = 1/n(MSe - MSg)]$; σ_e^2, the environmental variance $[\hat{\sigma}_e^2 = MSe]$; and n the number of replicates per line.

QTL were mapped from 152 DH lines by 'interval mapping' using the computer program MAPMAKER/QTL 1.1 (Lincoln et al. 1992). Putative additive quantitative trait loci for I and P 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 risk of 5% (Lander and Bostein 1989). Then, 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. Markers associated to blackleg resistance detected by 'interval mapping' were then checked by multiple regression of the trait on the markers at a significance level threshold of $P < 5.10^{-3}$. Residual normality after QTL detection was also verified for each resistance trait.

Results

Blackleg resistance data

Within years heritabilities were high and similar over the 2 years for each resistance criteria (for I and P respectively $h^2 = 0.89, 0.69$ in 1995 and $h^2 = 0.88, 0.61$ in 1996). These indicate that a good estimation of the genotypic values was obtained in the experiments within each year. Due to highly significant genotype × year interactions ($P > F = 0.0001$; 0.0012 for I and P, respectively), data from 1995 and 1996 were analyzed separately. Genotype and replicate effects were highly significant for the two criteria in 1995 and in 1996 ($P > 0.01$). The disease index data (I) had a normal residual distribution as described by the Shapiro-Wilk W statistic (for I, $P = 0.99$ in 1995, $P = 0.71$ in 1996). For the proportion of lost plants (P), a square root transformation was necessary to achieve residual normality ($P = 0.98$, 0.97 in 1995 and 1996, respectively).

Figure 1 shows the genotypic frequency distributions for the two resistance criteria in the DH population. Genotypic scores of the controls were higher in 1995 than in 1996 for I ('Darmor-*bzh*': I = 3.3 and 2.0; 'Falcon': I = 4.6 and 3.9; 'Eurol': I = 6.2 and 5.2; 'Shogun': I = 7.9 and 6.9, respectively, in 1995 and 1996) and for $\sqrt{P}$ except for 'Eurol' ('Darmor-*bzh*': $\sqrt{P} = 0.29$ and 0.13; 'Falcon': $\sqrt{P} = 0.06$ and 0.05; 'Eurol': $\sqrt{P} = 0.15$ and 0.24; 'Shogun': $\sqrt{P} = 0.64$ and 0.49, respectively, in 1995 and 1996), as genotypic mean scores of the 171 DH lines (I = 6.07 ± 1.00 and 4.45 ± 1.02, $\sqrt{P} = 0.34 \pm 0.18$ and 0.27 ± 0.15, respectively, in 1995 and 1996). The I index genotypic values of the DH lines were normally distributed according to the Shapiro-Wilk statistic in 1995 ($P = 0.43$) and in 1996 ($P = 0.95$). For $\sqrt{P}$ genotypic distributions, normality hypothesis was accepted by the skewness (S) and kurtosis (K) coefficients but not by the Shapiro-Wilk statistic.

Fig. 1 Frequency genotypic distributions estimated in 1995 and 1996 for blackleg resistance index (*I*) and square root of lost plants ($\sqrt{P}$) in the DH population 'Darmor-*bzh*' × 'Yudal'. Control lines: *D-Bzh* 'Darmor-*bzh*', *Y* 'Yudal', *F* 'Falcon', *E* 'Eurol', S 'Shogun'. 'Yudal' could not be correctly assessed in 1995.

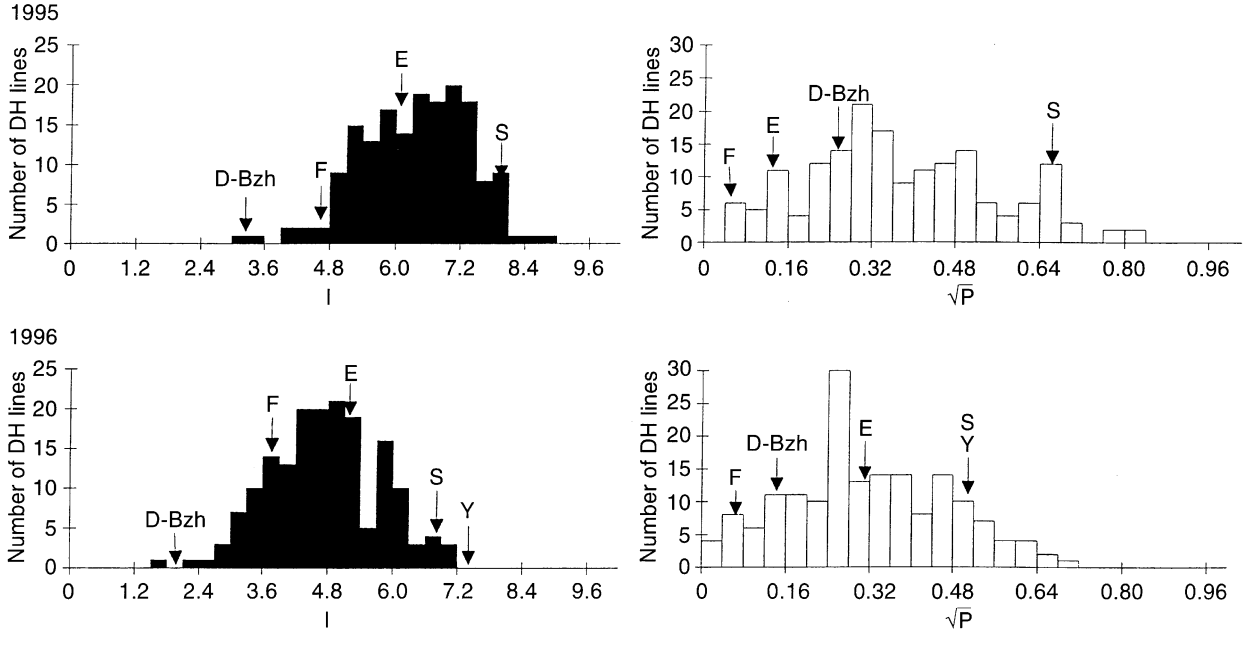


Table 1 Pearson genotypic correlation coefficients among blackleg resistance criteria (I and $\sqrt{P}$) assessed in 1995 and 1996

	I95	P95	I96	P96
I95				
P95	0.59			
I96	0.60	0.37		
P96	0.28	0.42	0.28	

($P < 0.01$)

Correlations among resistance variables are given between and within the 2 years in Table 1. All these correlations were significant ($P < 0.01$).

Mapping QTL associated to blackleg resistance

Results from 'interval mapping' are summarized in Table 2 and in Fig. 2. The QTL that were revealed in the 2 years are distributed over 10 linkage groups.

From the index data (I), 7 genomic regions were detected in 1995 and 5 in 1996, collectively accounting for 57% and 51% of the genotypic variation, respectively. Two QTL, revealed on the DY2 and DY3 groups to have important additive effects, were common to the 2 years. The one located on the DY2 group has a negative individual effect on the blackleg resistance index.

Table 2 Blackleg resistance QTL detected by 'interval mapping' in the DH 'Darmor-*bzh*' × 'Yudal' population for the two resistance criteria (I and P): their position, LOD score, individual effect and contribution to the resistance 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 in total LOD with the new QTL is indicated with the sign ' + '. The weight of each QTL represents the substitution effect of the 2 'Darmor-*bzh*' alleles by 2 'Yudal' alleles, i.e. the allelic contribution of each parent to the resistance. For P, the weight data are expressed in percentage of losses. R^2 is the percentage of genotypic variation explained by all the QTL detected. *Note*: bold-type font indicates the common QTL between the 2 years for each resistance criterion

1995						1996					
Trait	Position	Linkage group	LOD	Weight	R^2 (%)	Trait	Position	Linkage group	LOD	Weight	R^2 (%)
I	**OPL08.1190+14**	**DY2**	**3.1**	**−0.77**		I	OPM07.730 + 0	DY6	10.3	1.00	
	OPO13.490 + 0	DY9	+ 3.8	0.73			**OPP05.1375+2**	**DY3**	**+2.8**	**0.49**	
	OPP05.1375+4	**DY3**	**+ 3.2**	**0.70**			**OPL08.1190+18**	**DY2**	**+2.6**	**−0.54**	
	OPW11.1890 + 6	DY5	+ 2.9	0.54			OPE11.2150 + 2	DY8	+2.7	0.43	
	OPK08.2130 + 6	DY11	+ 2.9	0.52			OPC02.860 + 12	DY1	+2.3	−0.43	50.7
	OPU01.690 + 2	DY10	+ 2.8	0.54							
	OPW05.750 + 8	DY6	+ 3.2	0.54	57.1						
P	1ND2a + 0	**DY5**	**2.8**	**6.4**		P	**OPE02.1200+8**	**DY11**	**4.2**	**12.1**	
	OPR10.830 + 0	DY2	+ 2.7	10.0			**OPL12.980+6**	**DY5**	**+ 3.0**	**10.0**	22.8
	OPC02.1375 + 2	DY10	+ 2.3	10.0							
	OPP07.1665 + 6	DY13	+ 2.7	16.9							
	OPE02.1200+8	**DY11**	**+ 3.1**	**10.0**							
	OPA08.960 + 0	DY9	+ 2.2	4.9	41.4						

The major QTL detected in 1996 is linked to the dwarf gene (*bzh*) situated on the DY6 group. It has the largest R^2 value, explaining individually 28% of the genotypic variation. In 1995, a region linked to this major QTL by about 10 cM was also identified.

From the square root of lost plants data ($\sqrt{P}$), 6 QTL were detected in 1995 and only 2 in 1996, explaining, respectively, 41% and 23% of the genotypic variation. The 2 QTL revealed in 1996 on the DY5 and DY11 groups were common to 1995.

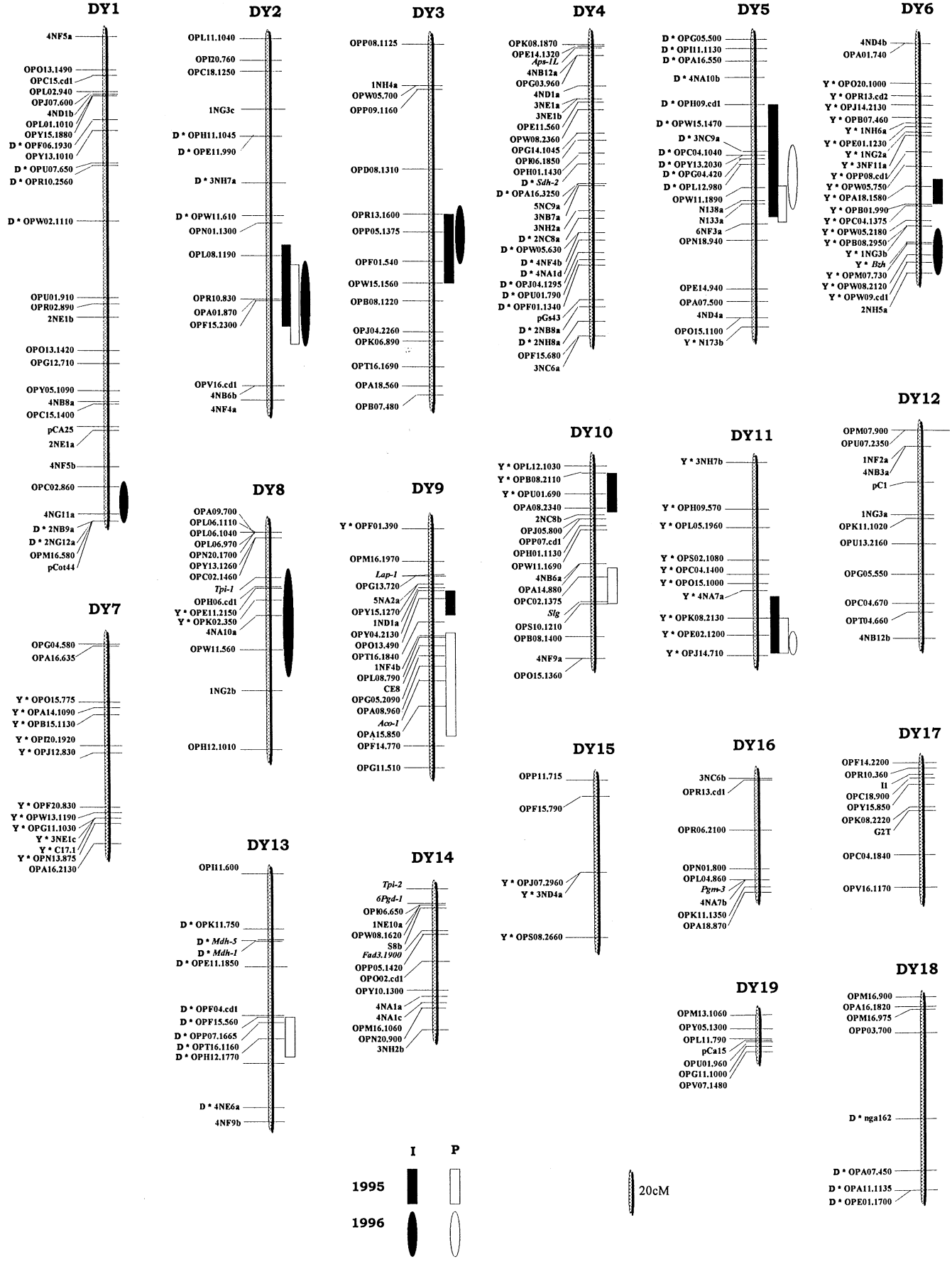
In 1995, 3 genomic regions, localized on the DY2, DY5 and DY11 groups, were common to I and $\sqrt{P}$ for their positions and the sign of their effects. On the DY9 and DY10 groups, QTL were also identified for the two resistance criteria but not at the same positions: $\sqrt{P}$ QTL is linked to the I QTL by about 10cM and 20cM on the DY9 and DY10, respectively. Two other genomic regions on the DY3 and DY6 groups were revealed for I but not for $\sqrt{P}$. In 1996, no QTL was common to both I and $\sqrt{P}$. Correlations between I and $\sqrt{P}$ were lower in 1996 (0.28) than in 1995 (0.59).

Discussion

Blackleg resistance assessment

We have chosen a field test to assess adult resistance to *L. maculans* rather than a test conducted under controlled conditions because a good representativeness of field resistance can not always be demonstrated in greenhouse tests (Gugel et al. 1991). Under field conditions, Van Den Berg et al. (1993) showed that various resistance criteria used in previous studies were all statistically associated: in field tests, scoring all these criteria did not seem to be more informative than scoring one judiciously chosen component. Thus, one global index (I) has been used in our present study, to assess blackleg resistance. The percentage of losses (P) mainly due to *L. maculans* was also of great interest: it corrects the overvalue of the resistance assessed by I from the remaining plants. It was deemed better to consider I and P separately since we could not be sure that all the losses were due to *L. maculans*. It can not be excluded that a P QTL could be involved in the variation of another quantitative trait, thereby also causing losses. In 1995, 3 I and P QTL were colocalized, suggesting that a part of losses was due to *L. maculans*. In 1996, there was no region common to I and P, but the 2 QTL detected for P explained a small part of the total variation. The P genotypic values could not be evaluated with as much precision as the I genotypic values, leading to lower P heritabilities estimates and QTL detection. This could partially explain the lower number of QTL detected for P than for I, especially in 1996.

Fig. 2 Linkage map locations of the putative QTL contributing to adult blackleg resistance identified in the cross 'Darmor-*bzh*' × 'Yudal', assessed by the mean disease index (*I*) and the proportion of lost plants (*P*). The QTL length is the confidence interval where the likelihood of the presence of a QTL is within tenfold (1 LOD) of its maximal value. The names of the 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)


DY1
4NF5a
OPO13.1490
OPC15.cd1
OPL02.940
OPJ07.600
4ND1b
OPL01.1010
OPY15.1880
D * OPF06.1930
OPY13.1010
D * OPU07.650
D * OPR10.2560
D * OPW02.1110
OPU01.910
OPR02.890
2NE1b
OPO13.1420
OPG12.710
OPY05.1090
4NB8a
OPC15.1400
pCA25
2NE1a
4NF5b
OPC02.860
4NG11a
D * 2NB9a
D * 2NG12a
OPM16.580
pCot44
DY2
OPL11.1040
OPI20.760
OPC18.1250
1NG3c
D * OPH11.1045
D * OPE11.990
D * 3NH7a
D * 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
D * Sdh-2
D * OPA16.3250
5NC9a
3NB7a
3NH2a
D * 2NC8a
D * OPW05.630
D * 4NF4b
D * 4NA1d
D * OPJ04.1295
D * OPU01.790
D * OPF01.1340
pGs43
D * 2NB8a
D * 2NH8a
OPF15.680
3NC6a
DY5
D * OPG05.500
D * OPH11.1130
D * OPA16.550
D * 4NA10b
D * OPH09.cd1
D * OPW15.1470
D * 3NC9a
D * OPC04.1040
D * OPY13.2030
D * OPG04.420
D * OPL12.980
OPW11.1890
N138a
N133a
6NF3a
OPN18.940
OPE14.940
OPA07.500
4ND4a
OPO15.1100
Y * N173b
DY6
4ND4b
OPA01.740
Y * OPO20.1000
Y * OPR13.cd2
Y * OPJ14.2130
Y * OPB07.460
Y * 1NH6a
Y * OPE01.1230
Y * 1NG2a
Y * 3NF11a
Y * OPP08.cd1
Y * OPW05.750
Y * OPA18.1580
Y * OPB01.990
Y * OPC04.1375
Y * OPW05.2180
Y * OPB08.2950
Y * 1NG3b
Y * Bzh
Y * OPM07.730
Y * OPW08.2120
Y * OPW09.cd1
2NH5a
DY7
OPG04.580
OPA16.635
Y * OPO15.775
Y * OPA14.1090
Y * OPB15.1130
Y * OPI20.1920
Y * OPJ12.830
Y * OPF20.830
Y * OPW13.1190
Y * OPG11.1030
Y * 3NE1c
Y * C17.1
Y * OPN13.875
OPA16.2130
DY8
OPA09.700
OPL06.1110
OPL06.1040
OPL06.970
OPN20.1700
OPY13.1260
OPC02.1460
Tpi-1
OPH06.cd1
Y * OPE11.2150
Y * OPK02.350
4NA10a
OPW11.560
1NG2b
OPH12.1010
DY9
Y * 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
Y * OPL12.1030
Y * OPB08.2110
Y * 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
Y * 3NH7b
Y * OPH09.570
Y * OPL05.1960
Y * OPS02.1080
Y * OPC04.1400
Y * OPO15.1000
Y * 4NA7a
Y * OPK08.2130
Y * OPE02.1200
Y * 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
D * OPK11.750
D * Mdh-5
D * Mdh-1
D * OPE11.1850
D * OPF04.cd1
D * OPF15.560
D * OPP07.1665
D * OPT16.1160
D * OPH12.1770
D * 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
Y * OPJ07.2960
Y * 3ND4a
Y * 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
D * nga162
D * OPA07.450
D * OPA11.1135
D * OPE01.1700
DY19
OPM13.1060
OPY05.1300
OPL11.790
pCa15
OPU01.960
OPG11.1000
OPV07.1480
I
P
1995
1996
20cM

Blackleg resistance as a quantitative trait

The number of QTL detected emphasizes the polygenic nature of the adult resistance to *L. maculans*. 'Darmor-*bzh*' and 'Yudal' are two parents genetically very distant, which favors the segregation of a high number of QTL and the detection of QTL with strong effect (Gallais and Rives 1993).

The detected QTL explain a maximum of 57% of the genotypic variation. The unexplained variation could be due to non-detected additive QTL because of their too weak effects or because of incomplete map coverage. We can also suppose that some epistatic interactions could significantly contribute to the variation unexplained by the additive effects of the QTL detected. In the interaction pepper/*Phytophtora capsici*, epistasis effects were tested between all markers (Lefebvre and Palloix 1996): the global-value R^2 due to epistasis was almost similar to that due to additivity. Because of epistasis, it is important to study QTL in other genetic backgrounds.

Two mapping studies concerning the *B. napus*/*L. maculans* interaction have been published. The first one (Ferreira et al. 1995) was performed on 105 DH lines from a cross between the Winter cultivar 'Major', resistant to the PHW1245 (PG2) *L. maculans* isolate, and the susceptible Spring cultivar 'Stellar'. The resistance was assessed on cotyledon and stem tissues under controlled and field conditions. Overall, 7 genomic regions were associated with resistance. Only 2 of them were linked to field resistance, and these were different from those identified in cotyledon- and stem- evaluated plants. They explained a low percentage of variation (19% and 15%, respectively), implying a polygenic control of the field resistance. In a second mapping study (Dion et al. 1995), 98 DH lines from the cross between 'Crésor', a late Spring French line resistant in Canada, and 'Westar', an early susceptible line, were assessed for crown canker in the field in four different environments. Results from the mapping strongly indicated the presence of a specific gene responsible for most of the adult plant resistance and a second environmentally-dependent minor region. The specific gene was named $LmFr_1$ and explained 57–84% of the variation, depending on the environment. A discrepancy between this last study and ours exists since we have identified many more regions involved in blackleg resistance. However, it is important to indicate that Canadian *L. maculans* isolates would be predominantly PG2 types and French isolates predominantly PG3 and PG4 types (Mengistu et al. 1991). Work is in progress to characterize *L. maculans* isolates in our trials at Le Rheu. Moreover, the cultivar 'Crésor', resistant to blackleg in Canada, is very susceptible in our French field conditions (Brun, personal communication).

QTL stability

In this present work, a total of 13 genomic regions were detected in 1995 and/or 1996, distributed across 10 of the 19 linkage groups of the map. Four regions, located on the DY2, DY3, DY5 and DY11, were identified from 1995 and 1996 notations of I and P. Among the quantitative resistance mapping studies treating QTL stability in different environments (such as Schön et al. 1993; Thomas et al. 1995), Zhikang et al. (1995) identified field resistance QTL to sheath blight in cultivated rice: 3 of the 6 QTL detected were common over 2 years. In the present study, we also observed a 'year' effect on QTL expression. The 4 common QTL detected had major additive effects during the 2 years. These common QTL seem to be essential for resistance expression. One of them, located on the DY2 group, had a strong negative effect, suggesting that even the susceptible parent has genes for resistance. Many authors have pointed out the contribution of the susceptible parent to the resistance, but this effect was often weak (Schön et al. 1993; Thomas et al. 1995; Camargo et al. 1995; Lefebvre and Palloix 1996). In the 'Darmor-*bzh*' × Yudal' DH population tested, only a few transgressive lines were observed despite the reverse effect of the DY2 QTL. It is expected that the exploitation of the 'Yudal' resistant allele will be interesting since it makes it possible to select segregants more resistant than 'Darmor-*bzh*'.

Another region, close to the dwarf '*bzh*' gene on the DY6 group, could also be common to 1995 and 1996. The 2 regions, detected for the 2 years on the DY6 group, were not located exactly at the same position. They did not have the same positive effect either: in 1996, this QTL had the strongest effect among all QTL identified whereas it had the smallest effect in 1995, which may have contributed to the difference in mapping position. It seems that the dwarf trait acted upon the expression or the evaluation of the resistance in 1996 field conditions, masking other QTL detection. Thomas et al. (1995) also observed that, in Spring barley, the large effect of the *denso* dwarfing gene locus was found to mask other QTL controlling other characters. Plant morphology can be an important component of resistance to *L. maculans*, particularly for field resistance (Hammond and Lewis 1986). In this work, we observed that the dwarf trait not only affected the blackleg resistance trait but also other agronomic traits like earliness, glucosinolate content (Foisset 1995). The effect of the dwarf trait on blackleg resistance could be eliminated only by studying other crosses without the '*bzh*' gene. We also noticed that QTL on the DY2 and DY13 groups were colocalized with earliness QTL (unpublished data). This indicates a potential interaction between the two traits although the notation stage was determined taking earliness into account.

In 1995 and 1996, we also revealed year-specific QTL. Their number was dependent on the year: QTL on 3 linkage groups (DY9, DY10 and DY13) were specifically expressed in 1995. Due to possible biased confidence intervals, it is difficult to conclude if putative close I and P QTL revealed on the DY9 and DY10

groups could correspond to the same region or if they were distinct ones. Two other regions, located on the DY1 and DY8, were specific to 1996. Most of these specific QTL identified for the 2 years had minor effects compared to the year-common regions.

The number of specific QTL observed in 1995 and 1996 could be attributed to inter-year differences in environmental conditions, which would have generated variations in the expression or evaluation of blackleg resistance. Particularly, we have shown that the level of contamination was lower in 1996 than in 1995 due to climatic conditions more favorable to the extension of the fungus in 1995. This hypothesis is supported by results obtained from a preliminary trial performed in 1994 under natural conditions of contamination. In 1994, the low inoculum pressure involved higher levels of estimated resistance: the mean genotypic I index was 4.0 for the DH population and 1.7 for the control 'Darmor-*bzh*'. Consequently, we only detected for the I index 2 QTL explaining together 13% of the genotypic variation: the first one was located on the DY6 group close to the '*bzh*' gene and the second one, on the DY5 group. These 2 regions were also revealed in 1995 and 1996 and seem to belong to the "stable QTL" group.

This preliminary work provides a sound basis for further mapping studies from crosses between relevant breeding material. At present progenies of such crosses are being developed, with the objective of studying QTL stability in various genetic backgrounds and detecting useful I and P QTL in rapeseed breeding. It would be of great interest to test these progenies at several sites in order to analyze QTL stability in different environments.

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