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A. M. Chèvre · P. Barret · F. Eber · P. Dupuy
H. Brun · X. Tanguy · M. Renard

Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 1. Identification of molecular markers, chromosomal and genomic origin of the introgression

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Abstract A scheme of selection combining selfing and backcross was applied to a *B. napus* line with the blackleg resistance from *B. juncea* in order to transfer this resistance to a winter oilseed rape variety. Cytogenetic analyses combined with cotyledon blackleg resistance tests at each generation allowed us to obtain a recombinant line showing regular meiotic behavior. The resistance is monogenic and is highly efficient under field conditions. Four-hundred RAPD primers were tested on two segregating populations by bulk segregant analysis. Three markers totally linked to the introgression were identified. The analysis of these markers on both sets of *B. napus-B. nigra* and *B. oleracea-B. nigra* addition lines revealed that they are not located on the B4 chromosome of *B. nigra*, which has already been shown to carry a blackleg resistance gene, but rather on the B8 chromosome. We confirmed that the resistance gene is carried by the B genome of *B. juncea*. Based on these data, two hypotheses, one involving chromosome rearrangements between the two B genomes of *B. nigra* and *B. juncea*, and the other based on a more probable digenic control of the resistance within *B. juncea*, are discussed.

Key words *Brassica napus* · *B. juncea* · *B. nigra* addition lines · Blackleg resistance · RAPD markers

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A. M. Chèvre (✉) · P. Barret · F. Eber · P. Dupuy
X. Tanguy · M. Renard
INRA, Station d'Amélioration des plantes, BP 29, 35653 Le Rheu Cedex, France

H. Brun
INRA, Station de pathologie végétale, BP 29, 35653 Le Rheu Cedex, France

Introduction

Blackleg, caused by *Leptosphaeria maculans* (Desm) Ces. & De Not., is one of the most damaging diseases of oilseed rape (*Brassica napus* L., AACC, 2n = 38) crops. Either oligogenic specific resistance or polygenic partial resistance are available within oilseed rape germplasm at the cotyledon and adult stages, respectively. By contrast, all *Brassica* species containing the B genome, i.e. the diploid species *B. nigra* L. Koch (BB, 2n = 16) and the two derived amphidiploids, *B. juncea* L. Czern (AABB, 2n = 36) and *B. carinata* A. Br. (BBCC, 2n = 34), carry complete resistance which remains effective throughout the life of the plant (Rimmer and van den Berg 1992).

The genetic control of this hypersensitive type of resistance has been studied through different methods. Among species containing the B genome, susceptible lines have been described only in *B. juncea*, and segregating populations revealed a digenic mode of control (Hill 1991; Rimmer and van der Berg 1992). The different B genomes were analysed by the creation of addition lines containing one B chromosome on an oilseed rape genetic background (Jahier et al. 1989; Struss et al. 1991). From this material, Chèvre et al. (1996) observed that one chromosome of *B. nigra*, among the five analysed, carried a resistance gene(s) effective at the cotyledon and adult stage. From the complete sets of addition lines of the different B genomes (except for *B. juncea* from which seven different chromosomes were characterized), Struss et al. (1996) reported that two or three B chromosomes were involved in resistance at the stem level. In all cases, the authors suggested either mono- or oligo-genic control for the resistance.

The introduction of resistance genes in the oilseed rape genome has been attempted either by sexual crosses (Roy 1978; Sacristan and Gerdemann 1986; Chèvre et al. 1996; Struss et al. 1996) or by asymmetric

protoplast fusion (Sjödin and Glimelius 1989). From *B. juncea*, Roy (1984) reported the creation of a recombinant line, Onap^{JR}, carrying a *B. juncea* (JR) resistance gene(s) efficient at the adult stage. However, from this same material, other authors reported loss of the introgression (Salisbury et al. 1995) or the production of aneuploid lines (Rimmer and van den Berg 1992). Only Pang and Halloran (1996) have concluded that major genes are involved in resistance at the adult stage.

In the present study, we report the selection of resistant recombinant lines by combining blackleg resistance tests and cytogenetic characterization. We postulated that the resistance gene introduced was the same as the one already described on chromosome 4 of *B. nigra* (Chèvre et al. 1996). To check this hypothesis, molecular mapping of the *B. juncea* resistance gene and of the sets of *B. nigra* addition lines was performed.

Materials and methods

Plant materials

Recombinant lines of spring type *B. napus* L. containing *B. juncea* resistance were provided by Dr. N. N. Roy (department of Agriculture, South Perth, Australia). Among the six seed samples received, one was selected for blackleg resistance under field conditions. Plants were selfed during three generations and then backcrossed four times to the French double-low winter variety 'Samouraï' and selfed to fix the introgression at the homozygous stage.

For the cotyledon test, two winter oilseed rape varieties, 'Shogun' and 'Darmor', were used as susceptible controls, with a German black mustard variety, 'Junius', as the resistant control. For field experiments, the controls chosen were, 'Tanto' (France), 'Marnoo' (Australia) and 'Westbrook' (Australia) for spring oilseed varieties, 'Shogun' (United Kingdom), 'Samouraï' (France), 'Rafal' (France), 'Darmor' (France) and 'Falcon' (Germany) for winter oilseed rape varieties, and brown mustard varieties 'Stoke', 'Zaria', 'Picra'. Two *B. juncea* varieties, 'Picra' and 'Aurea', were used as controls for the molecular analyses.

Addition lines of *B. nigra* were previously described for *B. napus*-*B. nigra* by Jahier et al. (1989) and for *B. oleracea*-*B. nigra* by Chèvre et al. (1997). Five *B. nigra* chromosomes were analysed from both sets of addition lines whereas the three complementary chromosomes were isolated only on a *B. oleracea* genetic background. All of them were obtained from the black mustard variety 'Junius'.

Blackleg resistance tests at the seedling stage

Inoculations were performed as described by Williams and Delwiche (1979). Seeds were sown in a greenhouse and each half cotyledon of 7-day-old seedlings was wounded in the center with a needle and inoculated with a 10-µl droplet of inoculum (10^7 pycnidiospores/ml). One highly virulent isolate (#314) belonging to the A-group and obtained from oilseed rape leaf lesion was used (Brun, personal communication). It was representative of fungal population pathogenicity under our field conditions. After incubation in darkness for 24 h at 20°C in saturated humidity, seedlings were kept in a growth chamber (20°C, 16 h light/8 h night). Symptoms were scored 14 days after inoculation using a 1–9 scale according to lesion size, the occurrence of necrosis or chlorosis, and the presence of pycnidia (Williams and Delwiche 1979). Plants of classes 1 to 5 were considered as resistant and plants of classes 6 to 9 as susceptible. For the plants of this last group, a collapse of tissue with abundant pycnidial formation around the inoculation point was observed. The highest score out of the four inoculation points was considered as representative of plant behaviour. During the selection procedure, 20 plants/genotype were tested at each generation.

Blackleg resistance under field conditions

A randomized complete block design with four replicates was chosen. Naturally infected oilseed rape stubble was scattered on 3–4-leaf seedlings in the field in autumn. Disease levels were scored just before harvest in June using a 6-class scale based on the extent of internal and external necrosis at the crown of the plant (Newman and Bailey 1987). Class 1 corresponded to immune plants and class 6 to broken ones. From the number of plants in each class, a pathological index (PI) was calculated for 30 plants from each plot as follows: (0 × number of plants in class 1) + (1 × number of plants in class 2) + (3 × number of plants in class 3) + (5 × number of plants in class 4) + (7 × number of plants in class 5) + (9 × number of plants in class 6)/total number of plants observed. The PI was surveyed by variance analysis in a factorial experiment including the effect of the genotype and the blocks per year. The genotypes were classified using the Newman-Keuls test ($\alpha = 0.05$).

Cytogenetic studies

The method employed for meiotic analysis was previously described by Jahier et al. (1989). Floral buds were fixed in a Carnoy solution containing 6 vol ethanol : 3 vol chloroform : 1 vol acetic acid at room temperature. After 24 h, buds were stored in a 50% alcohol solution at 4°C. Anthers containing pollen mother cells (PMCs) at the M-I stage of meiosis were squashed in a drop of aceto-carmine solution.

DNA amplification

DNA was extracted from young leaves according to the method of Doyle and Doyle (1990).

For RAPD analyses, 12.5 ng of DNA were used and we followed the protocol described by Hu and Quiros (1991). Random 10-mer primers were purchased from Operon Technologies (Alemada, Calif. USA) and *Taq* polymerase from Eurobio (Les Ulis, France). The samples were run in a 1.8% agarose gel at 3 V/cm to separate the amplified products, which were then visualized by ethidium bromide (0.5 µg/ml) staining. The *Hae*III restriction enzyme (1 unit/12.5 µl) was added for 1 h at 37°C to the amplified products obtained from the primer OPI01. This allowed us to check the inconspicuous differences observed on the initial amplified products after agarose separation. The loci are designated by an OP prefix followed by the kit letter, the primer number, the abbreviation of the restriction enzyme, if used, and the size of the band (in base pairs).

Marker identification was performed by bulk segregant analysis from two backcross progeny; for each of them, an equal amount of DNA of resistant (classes 1–3) plants was pooled and compared to a DNA mixture of susceptible (classes 6–9) plants. For the first progeny (B_3F_1-1), the bulks were constituted from eight resistant and seven susceptible plants and for the second one (B_3F_1-2) from five resistant and nine susceptible plants.

Hybridization of the RAPD profiles with the amplified products was performed using the ECL direct labelling kit (Amersham) as follows. Gels were de-stained in distilled water for 1 h to eliminate the TAE buffer. Amplified bands were cut from the agarose gel with a sterile cutter. DNA was recovered by extraction (Sephaglass band preparation, Pharmacia). The amount of DNA was estimated by electrophoresis, on an agarose gel, of 5 µl of the solution in comparison to different amounts of known concentration of lambda DNA

cut with restriction endonucleases *Eco*RI and *Hin*dIII; 20–50 ng of DNA in 10 µl of water was sufficient for hybridization. The different RAPD profiles were transferred to nylon membranes by the alkaline process. Gels were left in 0.4 M NaOH for 0.5 h and alkaline Southern blotting was carried out using 0.4 M NaOH buffer (Sambrook et al. 1989). Probes were labelled with horseradish peroxidase using the ECL direct labelling system (Amersham). Pre-hybridization of the membranes was performed at 42°C for 1 h using hybridization buffer purchased with the kit containing 0.5 M NaCl. After addition of the probe, hybridization was continued at the same temperature using the same buffer for 2 h. High-stringency washes (0.1–0.2 × SSC, 6 M urea) were performed twice for 20 min at 42°C. After two washes in 2 × SSC, development and exposure of the membranes (2–45 min) were carried out as described by Amersham.

Results

Selection of resistant material

Among the samples received from Dr N. N. Roy, one which had a spring oilseed rape genetic background (Roy 85-2) was tested under field conditions for blackleg resistance. A comparison to a range of oilseed rape spring and winter varieties and to different cultivars of brown mustard revealed that this material was as resistant as brown mustard (Fig. 1-1). All the oilseed rape cultivars, irrespective of their origin or type, were always more susceptible.

The scheme of the breeding procedure developed from Roy 85-2 is presented in Fig. 2. Resistant plants were selected and selfed three times. A cotyledon test was performed with each progeny and resistant plants were kept for the following generation.

At the third selfing generation, a cytogenetical control was applied. The meiotic behavior of 25 plants was established: two had 35 chromosomes (mainly one univalent and 17 bivalents), three had 37 chromosomes (mainly one univalent and 18 bivalents) and 20 had the expected chromosome number, i.e. 38. For the latter,

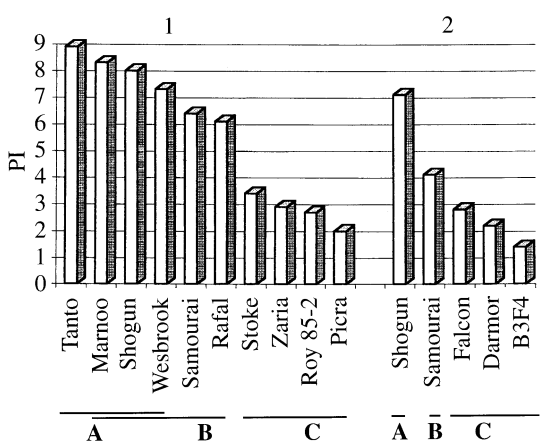

Fig. 1 Pathological index (*PI*) obtained after a blackleg resistance test under field conditions at the beginning (*1*) and at the end (*2*) of the breeding procedure. In respect of both experiments, 1 and 2, lines *with the same letter* were not significantly different at the 5% level

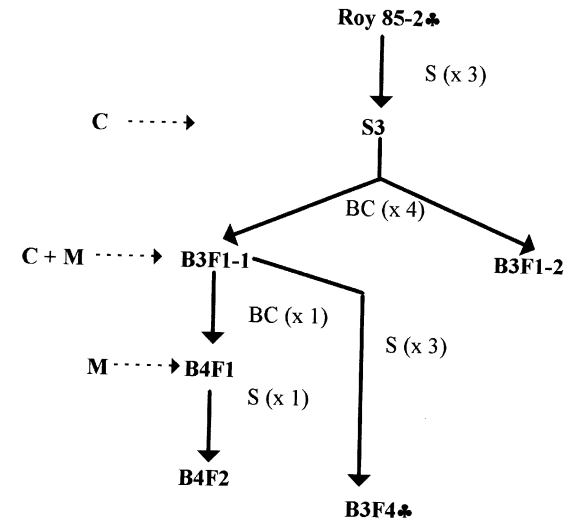

Fig. 2 Scheme of selection for the resistant recombinant lines by selfing (*S*) and backcrossing (*BC*) to 'Samouraï'. The number of crosses is given in *brackets*. Blackleg tests under field conditions (♣) were performed at the beginning and the end of the selection, whereas cotyledon tests were applied at each generation combined with cytogenetic (*C*) and/or molecular-marker (*M*) analyses

the average of univalents ranged from 0.08 to 0.79, according to the plant. One S_3 plant, presenting the most regular meoitic behavior (Table 1) with few univalents and no multivalent, was selected and backcrossed to 'Samouraï'.

At each following generation of backcross to 'Samouraï', a susceptible oilseed rape line, a blackleg resistance test at the cotyledon stage was carried out and the resistant plants selected. From the results reported in Table 2, segregations 1R : 0S in F_1 and 1R : 1S in B_1F_1 and B_2F_1 were observed at each generation, which corresponds to the Mendelian segregation of one gene. Two plants were selected in the second backcross progeny and crossed again with 'Samouraï'. They provided the B_3F_1-1 and B_3F_1-2 generations, respectively. The results of the blackleg resistance test are presented in Table 2. Resistant and susceptible plants were selected among them. They always showed 38 chromosomes and their meiotic behavior, established for most of them (Table 1), was very close to the S_3 initial selected plant.

In order to confirm the genetic control and efficiency of the resistance under field conditions, B_3F_1 plants were either backcrossed to 'Samouraï' (B_4F_1) and the resistant plants selfed (B_4F_2) or selfed three times (B_3F_4) for the selection of homozygous plants. The results from cotyledon tests in the backcross (1R : 1S in B_4F_1) and selfing (3R : 1S in B_4F_2) generations, reported in Table 2, confirmed the monogenic control of the resistance. Homozygous plants B_3F_4 for the resistance gene were tested under field conditions and were as resistant as Roy 85-2 (Fig. 1), indicating that the resistance gene present in the original material was maintained and

Table 1 Meiotic behavior of the plants selected after three selfing progeny (S_3) and after three backcrosses to 'Samouraï' (B_3F_1-1 and B_3F_1-2)

Generation	No. of plants	Blackleg resistance	2n	Cell	Uni-valents	Bi-valents	Tri-valents	Quadri-valents
S_3	1	R	38	23	0.26 (0–2)[a]	18.87 (18–19)		
B_3F_1-1	7	R	38	186	0.24 (0–2)	18.78 (17–19)		0.05 (0–1)
	4	S	38	61	0.11 (0–2)	18.82 (17–19)	0.02 (0–1)	0.05 (0–1)
B_3F_1-2	3	R	38	63	0.19 (0–2)	18.78 (17–19)		0.06 (0–1)
	6	S	38	149	0.13 (0–2)	18.79 (17–19)		0.07 (0–1)
'Samouraï'	1	S	38	50	0.08 (0–2)	18.92 (17–19)		0.02 (0–1)

[a] Range

Table 2 Results from blackleg resistance test at the cotyledon stage of the F_1, the first backcross generations (B_1F_1 to B_4F_1) to the variety 'Samouraï' and the selfing progeny after four backcrosses (B_4F_2)

Generation	No. of resistant plants		No. of susceptible plants	Segregations	
				Expected ratio R : S	χ^2
	(1–3)[a]	(4–5)	(6–9)		
F_1	49	1	0	1 : 0	0*
B_1F_1	5	3	7	1 : 1	0.07*
B_2F_1	10		11	1 : 1	0.05*
B_3F_1-1	10	2	6	1 : 1	2.00*
B_3F_1-2	6	3	10	1 : 1	0.05*
B_4F_1	47	8	63	1 : 1	0.54*
B_4F_2	40		13	3 : 1	0.006*

[a] Range of disease notes
* NS at $\alpha = 0.05$

remained efficient. In fact, the B_3F_4 plants were significantly more resistant than the susceptible recurrent oilseed rape variety, i.e. 'Samouraï' (Fig. 1-2).

Identification of molecular markers

Plants of the two B_3F_1 progeny, characterized by the cotyledon test (Table 2) and by cytogenetic analysis (Table 1), were used to perform a bulk segregant analysis as described by Michelmore et al. (1991). Among 400 RAPD primers tested, three of them, OPG02, OPI01 and OPT01, revealed markers present only in the resistant plants whatever the progeny. For improving the identification of the marker band, amplified products from primer OPI01 were digested by the restriction enzyme *Hae*III (Fig. 3).

Plant-by-plant analysis was performed for 46 resistant plants and 42 susceptible plants of the B_4F_1 progeny (Table 2) obtained from one B_3F_1-1 resistant plant backcrossed to 'Samouraï'. The three markers were totally linked to the resistance gene.

Markers on the B genome

In order to confirm that the *B. juncea* resistance gene was carried by the B genome, amplification was performed from the DNA of *B. nigra* (BB) var. 'Junius', *B. juncea* (AABB) var. 'Aurea', 'Picra' and *B. napus* (AACC) var. 'Samouraï' plants with each of the primers, OPG02, OPI01 and OPT01. Except in the case of primer OPG02 on *B. juncea* DNA, markers were found only from varieties carrying the B genome, i.e. *B. nigra* and *B. juncea*, whereas none of the markers was detected from *B. napus* DNA (Table 3). This result was confirmed by hybridization of the amplified products transferred to a nylon membrane and hybridized with the marker band (Fig. 4).

From previous data, we noted that, among the five *B. nigra* chromosomes characterized from *B. napus*-*B. nigra* addition lines, the chromosome named B4 carried blackleg resistance that was efficient both at the cotyledon and the adult stage (Chèvre et al. 1996). Several RAPD markers were identified as specific for this chromosome 4 (Chèvre et al. 1996, 1997). Ten of the primers used were analysed on the *B. napus*-*B. juncea* recombinant lines and, reciprocally, the three primers revealing markers linked to the *B. juncea* resistant gene were tested on the addition lines carrying chromosome B4 either on an oilseed rape or a kale genetic background. None of the markers were common to both types of material (Table 3).

In order to localize the markers of the *B. juncea* resistance gene on the B genome, we used the two sets of *B. napus*-*B. nigra* and *B. oleracea*-*B. nigra* addition lines from which the eight different *B. nigra* chromosomes were characterized. New markers were detected

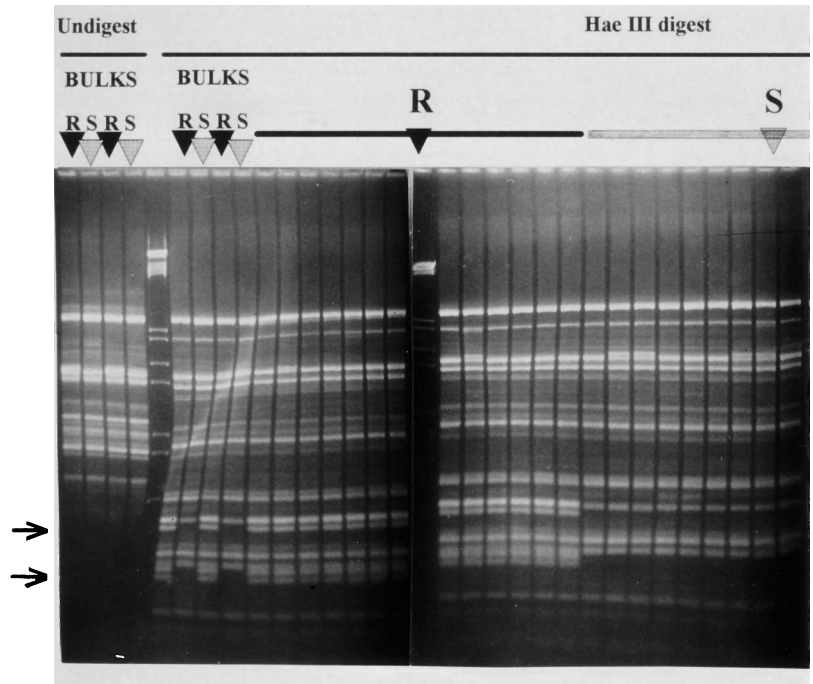

Fig. 3 Amplification patterns obtained with primer OPI01 from resistant (*R*) and susceptible (*S*) bulks without and with *Hae*III digestion, and from individual plants of the bulks after restriction-enzyme digestion

Table 3 Analysis of addition lines, recombinant lines which are resistant (R) or susceptible (S) and of the three *Brassica* species concerned, for the presence (+) or absence (−) of RAPD markers localised either on chromosome 4 of *B. nigra* [carried by *B. napus*-*B. nigra* (LA4*) or *B. oleracea*-*B. nigra* (LA4#) addition lines] or linked to the *B. juncea* resistance gene in the recombinant lines

	Addition lines		Recombinant lines		*B. juncea*	*B. nigra*	*B. napus*
	LA4*	LA4#	R	S			
Markers of chromosome 4 of *B. nigra*							
OPB01.1500	−	+	−	−	+	+	−
OPB05.600	+	−	−	−	+	+	−
OPC08.1400	+	+	−	−	+	+	−
OPE03.850	−	+	−	−	+	+	−
OPE11.800	+	+	−	−	+	+	−
OPE12.690	+	−	−	−	+	+	−
OPE14.950	+	+	−	−	+	+	−
OPE16.640	+	+	−	−	+	+	−
OPF02.1100	+	+	−	−	+	+	−
OPF10.400	+	+	−	−	+	+	−
Markers of the *B. juncea* resistance gene							
OPG02.800	−	−	+	−	−	+	−
OPT01.760	−	−	+	−	+	+	−
OPI01-*Hae*III.430	−	−	+	−	+	+	−
OPI01-*Hae*III.380	−	−	+	−	+	+	−
OPI01-*Hae*III.330	−	−	+	−	+	+	−
OPI01-*Hae*III.280	−	−	+	−	+	+	−

on five out of the eight *B. nigra* chromosomes studied either on one of the sets of addition lines or on both (Table 4). All the markers of the *B. juncea* resistance gene were recovered on chromosome B8 isolated only on a *B. oleracea* genetic background.

Discussion

Our results showed that it was possible to select recombinant oilseed rape lines containing the *B. juncea* blackleg resistance from the material sent by Dr N. N. Roy. This author produced homozygous resistant lines with a regular meiotic behavior similar to that of oilseed rape (i.e. 19 bivalents) after several backcrosses to oilseed rape of the F_1 *B. napus*-*B. juncea* hybrids followed by selfing. He named these lines Onap^{JR} (Roy 1984). Different authors have worked with this material. Rimmer and van den Berg (1992) reported that most of the lines were aneuploid and Salisbury et al. (1995) indicated that their attempts to transfer this resistance to different oilseed rape lines were

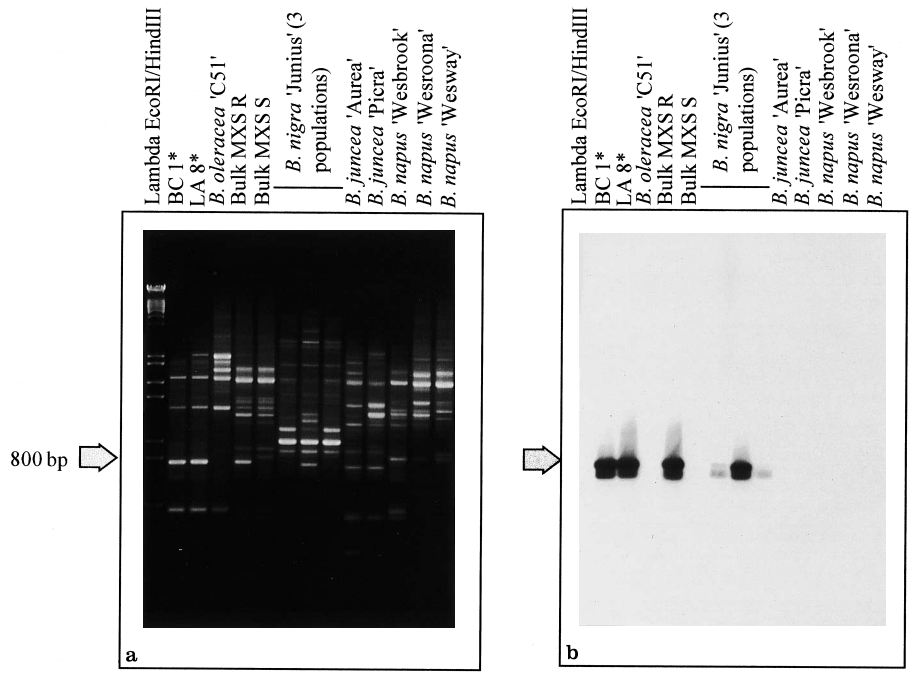

Fig. 4a, b Amplification patterns obtained with OPG02 and hybridization with the marker band. **a** Agarose gel on which the 800-bp band is present on the *B. oleracea-B. nigra* BC_1 hybrid (*BC1**), on the addition line carrying the B8 chromosome on *B. nigra* (*LA8**), on the bulk of B_3F_{1-1} (*MXS R*) resistant plants and on *B. nigra*. **b** Hybridization of the RAPD pattern with the OPG02.800 probe. The presence of the band is confirmed on BC1*, on LA8*, on the bulk of MXS resistant plants and on the B genome of *B. nigra*

Table 4 Analysis of the *B. nigra* chromosomes (B) from *B. napus-B. nigra* or *B. oleracea-B. nigra* addition lines carrying markers, characterized by the size of the band in base pairs, as revealed by the three primers used to detect markers linked to the *B. juncea* resistance gene. The bands underlined were observed only from *B. napus-B. nigra* addition lines, the ones in normal character were identified in *B. oleracea-B. nigra* addition lines, while those in bold type were common to the both sets of addition lines. *: markers of the *B. juncea* recombinant lines

Marker	B2	B3	B5	B7	B
OPG02		1050	950		800*
OPI01-*Hae*III	1500				430*
	300				380*
					330*
					280*
OPT01	**710**			1330	760*

unsuccessfull, most likely due to loss of the introgression. More recently, Pang and Halloran (1996) studied the genetic control of the *B. juncea*-like resistance in segregating populations. Three genes were implied but the genomic origins of these genes was not identified and a hypothesis involving the selection of a disomic substitution line of a *B. juncea* chromosome could not be excluded. In our study, cytogenetic analysis combined with resistance tests allowed us to keep only the resistant plants with 38 chromosomes for the following generations. This double selection was especially necessary at the S_3 progeny where several aneuploids were detected. The regularity of the meiotic behavior of the resistant plants whatever the progeny, backcross or selfing, and the monogenic control of the resistance indicated that the gene involved was carried by one *B. juncea* genome segment introgressed into the oilseed rape. This result was confirmed by the transfer of the resistance by backcrossing to other winter oilseed rape varieties (Renard, personal communication).

From the first field experiment, plants as resistant as *B. juncea* were observed in agreement with the data published by Roy (1984). During the following selection procedure, only cotyledon tests were applied. After more than ten generations, a new field experiment was performed and the same level of blackleg resistance as in the initial material was observed. We conclude that cotyledon tests provide an efficient method to select for the resistance carried by *B. juncea*. This is in agreement with Rimmer and van den Berg (1992) who reported that one gene of *B. juncea* is sufficient to confer resistance at whatever the plant stage tested. By contrast, no correlation between the cotyledon and adult tests was found for the *B. napus* resistance genes described by Salisbury et al. (1995) and Chèvre et al. (1996).

Using the "bulked-segregant analysis" method proposed by Michelmore et al. (1991), three RAPD markers were identified among 400 primers tested. The fact that a large number of primers was necessary to identify few markers, and that the plants showed a regular meiotic behavior, suggested that the size of the introgression was most likely short. In addition, it is probable that, without restriction enzyme digestion, the marker revealed by primer OPI01 would have been missed. However, even if the markers were totally linked to the resistance gene, no information is

available on the size of the introgression because very rare recombination events are possible between homoeologous segments and, as a result, a marked discrepancy may exist between the genetic and the physical distances.

Roy (1984) suggested that the *B. juncea* blackleg resistance gene(s) came from the B genome since all the species carrying this genome showed the same level of resistance. This result was confirmed by the presence of the markers only on the *B. nigra* and *B. juncea* genomes. However, OPG02.800 was not detected on the two *B. juncea* varieties analysed. This most likely is due to the fact that we did not have the original *B. juncea* variety used by Roy (1978) as a control. This observation has to be related to the high variability observed within *Brassica* species and similar results have already been reported from *B. nigra* addition lines (Chèvre et al. 1997). The polymorphism revealed by primer OPG02 among different accessions of *B. juncea* is currently under study.

Markers linked to the *B. juncea* resistance gene were located on chromosome B8 instead of on chromosome B4, previously described as carrying a resistance gene in *B. nigra* (Chèvre et al. 1996). Two hypotheses can be proposed to explain this result: (1) either the *B. nigra* chromosome-4 segment, carrying the resistance gene, had been translocated to chromosome 8 in the *B. juncea* B genome since, as Struss et al. (1996) showed, several chromosomal rearrangements were the cause of the differences observed between the B genomes of *B. nigra*, *B. juncea* and *B. carinata* species, or (2) the resistance is digenic and is carried by two different chromosomes. *B. nigra* chromosome 8 is only available on a kale genetic background and too few seeds were produced to check if this addition line is, or is not, resistant. The production of backcross progeny from this line is currently in progress. However, the presence of two independent resistance genes is supported by the genetic analysis of *B. juncea* blackleg resistance from either segregating populations (Hill 1991; Rimmer and van den Berg 1991) or from addition lines (Struss et al. 1996); all of these authors reported that at least two dominant genes confer resistance and that one gene is sufficient to confer the same level of resistance as in the donor species.

The localization of resistance genes from the recombinant lines obtained from *B. nigra* and *B. juncea* on the oilseed rape genetic map (Foisset et al. 1996) are currently in progress. We have already observed that the two introgressions are independent (data not shown). The closely linked molecular markers will be useful for marker-assisted selection and for further gene cloning.

In addition, studies of the pathogen populations have been performed. From the rare pseudothecia present on a few mustard plants only non-aggressive isolates (B group) of *L. maculans* were obtained from *B. juncea* whereas aggressive isolates (A group) were characterized from *B. nigra*. Mainly A-group isolates were recovered from susceptible *B. napus* varieties, as well as from a few pseudothecia from resistant lines carrying either the *B. nigra* chromosome 4 or the *B. juncea* resistance gene (Brun et al. 1995; Somda et al. 1996). These results suggest that differences exist between the two donor species and that the resistance carried by *B. juncea* was not completely transferred in the resistant recombinant lines selected. In order to check the efficiency of the different resistance genes and their effect on the structure of *L. maculans* populations, field experiment are in progress involving the isolation, characterization and field re-inoculation of fungal populations developed from the few symptoms found on the resistant lines. Genes from *B. nigra* and *B. juncea* will be tested alone and in combination on different oilseed rape genetic backgrounds. These data should allow the creation of durable blackleg-resistant lines.

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