Theor Appl Genet (1997) 94:603–611

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Identification of the different *Brassica nigra* chromosomes from both sets of *B. oleracea-B. nigra* and *B. napus-B. nigra* addition lines with a special emphasis on chromosome transmission and self-incompatibility

Received: 25 September 1996 / Accepted: 18 October 1996

Abstract The F_1 hybrids produced after crosses between *B. nigra* and *B. oleracea* were backcrossed two or three times to *B. oleracea*. Among the 14 plants analysed, five were monosomic addition lines (2n = 19), six were double monosomic addition lines (2n = 20) and three had three or four additional chromosomes. From these lines, 14 isozyme and 80 RAPD loci were localized on the eight chromosomes of *B. nigra*. The comparison between *B. napus-B. nigra*, from which five *B. nigra* chromosomes were already described, and the new set of *B. oleracea-B. nigra* addition lines was performed using five isozyme and 22 common RAPD loci. The homology of the common RAPD loci was confirmed by hybridization of the two sets of addition lines as well as the presence of duplicated loci on different chromosomes. For the five added chromosomes available on the two genetic backgrounds, i.e. *B. napus* and *B. oleracea*, using isozyme markers, the chromosome transmission rate was studied from backcross progeny using the recurrent parent either as male or as female and from the selfing of monosomic addition lines. For each chromosome, no difference was detected between male and female transmission except for chromosome 3. This latter presented a percentage of female transmission of around 20%, close to the ones observed for the other chromosomes, but a very low male transmission (1.3%). The analysis from restriction enzyme digests of PCR products, obtained from primers selected in highly conserved regions of self-incompatible genes, suggested that the chromosome 3 probably carried the SLG-*B. nigra* locus.

Key words *Brassica nigra* · Addition lines · Chromosome map · Chromosome transmission rates · Self-incompatibility

Communicated by H. F. Linskens

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Introduction

The study of addition lines has been shown to be an efficient method of identifying chromosomes carrying different genes controlling agronomic traits. For the two diploid species, *Brassica rapa* L. (AA, 2n = 20) and *B. oleracea* L. (CC, 2n = 18), which are the progenitors of oilseed rape (*B. napus* L., AACC, 2n = 38), addition lines have been obtained from either natural or synthetic AACC amphidiploids backcrossed with one of the constituent diploid species (Fantes and MacKay 1979; Quiros et al. 1987; MacGrath and Quiros 1990; Chen et al. 1992, 1994). In contrast, the other diploid species, *B. nigra* (L.) Koch (BB, 2n = 16), has been analysed from the addition of chromosomes of the B genome either on an allotetraploid genetic background, such as oilseed rape (Jahier et al. 1989; Struss et al. 1991a), or on an unrelated diploid genetic background, *Diplotaxis erucoides* (L.) DC (DeDe, 2n = 14) (This et al. 1990). In the present report, because of its contribution to oilseed rape genomes, diploid *B. oleracea* has been chosen to extract a new set of *B. nigra* addition lines.

B. nigra is one of the interesting species for oilseed rape improvement. Several agronomic traits, such as fatty acid composition (Chèvre et al. 1991; Struss et al. 1991 b) or glucosinolate content (Struss et al. 1991 b), have already been analysed. One of the most interesting characters of this species and of the related amphidiploids, *B. carinata* A. Br. (BBCC, 2n = 34) and *B. juncea* L. Czern. (AABB, 2n = 36), is their blackleg resistance, probably carried by their common B genome.This disease, due to *Leptosphaeria maculans* (Desm) Ces. & De Not., is the most damaging to oilseed rape crops. First

results reported that one (Chèvre et al. 1996) or three chromosomes (Zhu et al. 1993) of the B genome may carry this resistance. Resistant introgression lines were reported from *B. juncea* by Roy (1984) and from *B. nigra* by Struss et al. (1995) and Chèvre et al. (1996).

Breeding programmes would be improved by a better understanding of the structure of the B genome and by knowledge of chromosome behavior on different genetic backgrounds. For this purpose, two sets of addition lines were used in the present study. Five out of the eight chromosomes of *B. nigra* on either a *B. napus* or *B. oleracea* genetic background were identified and analysed. The relationship between the chromosome transmission rates and the localisation of loci implied in self-incompatibility is discussed.

Materials and methods

Plant materials

Reciprocal crosses were performed between a forage kale line, C51, kindly provided by G. Thomas (INRA, Le Rheu, France) and a German black mustard variety, 'Junius'. The following progenies, F_1, BC_1, BC_2 and BC_3, were obtained by backcrossing with the same kale line, C51, used as male parent.

Oilseed rape-black mustard addition lines obtained from a winter double low French oilseed rape variety, 'Darmor', and the German black mustard variety, 'Junius', were previously described by Jahier et al. (1989). From these lines, five chromosomes among the eight of black mustard were well characterized (Chevre et al. 1991; Quiros et al. 1991; Chevre et al. 1996).

Chromosome transmission was studied from monosomic addition lines crossed to the recurrent parent used either as male or as female or selfed.

Ovary culture

For obtaining kale-black mustard F_1 hybrids, and BC_1 and BC_2 progenies after backcrossing to kale, ovaries were excised 6–8 days after hand pollination and in vitro cultured, as previously described by Delourme et al. (1989). The plantlets developed from seeds were transplanted in the greenhouse.

Cytogenetic studies

For meiotic analyses, floral buds were fixed in a Carnoy solution containing 6 ethanol : 3 chloroform : 1 acetic acid at room temperature. After 24 h, buds were stored in 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.

Fertility was assessed as the percentage of pollen stained by the aceto-carmine solution. At least three flowers and 800 pollen grains were observed per plant.

Isozyme analyses

Young leaves were crushed in a Tris-HCl 0.1 M buffer containing 1% of reduced glutathione. Seven isozyme systems were revealed using the standard starch-electrophoresis method. For the latter, gel-electrode buffers were as described by Shields et al. (1983). Aconitase (ACO), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI) and shikimate dehydrogenase (SDH) were separated on histine/tris-citrate buffer pH 7.0 (H 7.0), leucine aminopeptidase (LAP) and 6-phosphogluconate dehydrogenase (6 PGD) on morpholine citrate buffer pH 6.1 (MC 6.1), and glutamate oxaloacetate transaminase (GOT) on tris-citrate/lithium borate buffer pH 8.3 (TC 8.3). The staining procedures and the nomenclature for ACO, PGM, PGI, LAP and 6 PGD used were previously described by Chevre et al. (1995). The staining procedures for SDH and GOT were as reported by Vallejos (1983). Acid phosphatase (APS) was separated on acrylamide gel. The method and nomenclature used were described by Chevre et al. (1995).

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 was 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 Ulysses, France). The samples were run in 1.8% agarose gel at 3V /cm to separate amplified products visualized by ethidium bromide (0.5 µg/ml) staining. The loci are designated by an OP prefix followed by the kit letter, the primer number and the size of the band (in base pairs).

Hybridization of the RAPD profiles with 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 TAE buffer. Amplified bands were cut off from the agarose gel with a sterile cutter. DNA was recovered by 2-min centrifugation through 3MM paper at 10 000 g in a microcentrifuge tube. After butanol-2 concentration up to 15 µl, 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 is 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 the 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.

The amplification reaction for S-specific glycoprotein locus (SLG) sequences was carried out in 100 µl containing 1 × reaction buffer (Boehringer), 2.5 mM of each dNTP, 0.1µM of each primer, 1.25% w/v formamide, 0.25 µg *Brassica* DNA and 2.5 U *Taq* polymerase (Boehringer). Amplification was 30 cycles of 94°C for 20 s, 58°C for 2 min and 72°C for 1.5 min, followed by a final cycle where the extension time was 5 min. The primers were as follows: primer A (5′-AGAACACTTGTATCTCCCGGT-3′) and primer B (5′-CAATCTGACATAAAGATCTTG-3′). Amplified products were analysed by electrophoresis through 1% agarose gels. For restriction digestion, oil was removed from the PCR products with dichloromethane. To 8 µl of PCR product were added 1 µl of the restriction enzymes *Sty*I, *Sal*I, *Pst*I or *Nci*I and 1 µl of appropriate restriction buffer (Brace et al. 1993). Restriction digests were performed for 1 h and were analysed by running through 2% agarose gel.

Results

Production of kale-black mustard addition lines

After hand pollination and ovary culture, only two F_1 hybrid plants were produced using black mustard as female. The reciprocal crosses were unsuccessful (Table 1). The average meiotic behavior and the male fertility of these two F_1 hybrids are reported in Table 2. One of the F_1 hybrids was used as female and crossed with kale to produce the BC_1 progeny. The percentage of BC_1 plants was low compared to the F_1 hybrid production, even using ovary culture (Table 1). The two BC_1 plants obtained were characterized by their meiotic behavior and their male fertility (Table 2). One of these plants was kept as cuttings and used as female for crosses with kale. As no seed was obtained after hand pollination (data not shown), ovary culture was again used to produce the BC_2 progeny (Table 1). Among the 16 plants obtained, 12 were analysed: three of them were monosomic addition lines, six were double monosomic, and the three other ones had three or four additional chromosomes (Table 2). Backcrosses with kale were performed on addition lines carrying more than one chromosome. A BC_3 progeny was obtained from only one of them and two other monosomic addition lines were created (Table 2).

Chromosome map from kale-black mustard addition lines

From the eight isozyme systems used, 14 loci were localized on six different black mustard chromosomes (Fig. 1).

Among the 40 primers used, 37 revealed black mustard-specific loci with a different mobility than those of kale. The number of specific bands per primer selected ranged from 1 to 4, with an average 2.16 loci. By comparison between the different addition lines, taking into account their chromosome number, 80 RAPD loci were localized on the eight chromosomes of black mustard (Fig. 1). Some RAPD bands with the same size were localized on two or three different chromosomes. This was observed for seven fragments revealed from seven primers (Fig. 1). The identity of these loci was confirmed for one of them by hybridization with the band OPA11.1200 on the amplified products of lines carrying chromosome 2 or 3. In all cases, only one fragment of 1200 bp was revealed.

Table 1 Production of Kale black mustard hybrid plants

Progeny	Crosses	Number of ovaries	Embryos		Plants		Genomic structure
			Number	%	Number	%	
F_1	CC × BB	77	0	0			
	BB × CC	88	7	7.9	2	2.3	BC
BC_1	[BC] × CC	258	2	0.8	2	0.8	BCC
BC_2	[BCC] × CC	432	25	5.8	16	3.7	CC + 1–5 B chromosomes

Table 2 Meiotic behavior of the hybrids obtained from each progeny

Hybrids	2n	Number of plants	Number of PMCs	Univalents	Bivalents	*B. nigra* chromosomes	% of male fertility
F_1	17	2	60	11.37 (5–17)	2.82 (0–6)	All	5.9
BC_1	26	2	51	8.74 (8–12)	8.63 (7–9)	All	6.8
BC_2	19	1	6	1	9	1	88.9
	19	1	10	1	9	2	0
	19	1	10	1	9	3	88.9
	20	1	30	2.40 (2–4)	8.80 (8–9)	2 + 4	0
	20	1	20	2	9	3 + 6	29.7
	20[a]	1	20	2	9	5 + 7	69.9
	20	1	20	2	9	1 + 3	27.9
	20	1	30	2.47 (2–4)	8.77 (8–9)	1 + 2	–
	20	1	19	2	9	3 + 7	–
	21	1	10	3	9	2 + 6 + 7	0
	22	1	15	4	9	3 + 5 + 6 + 7	69.9
	22	1	10	4	9	2 + 5 + 6 + 8	–
BC_3	19	1	10	1	9	5	–
	19	1	10	1	9	7	–

[a] Mother-plant of the BC_3 progeny

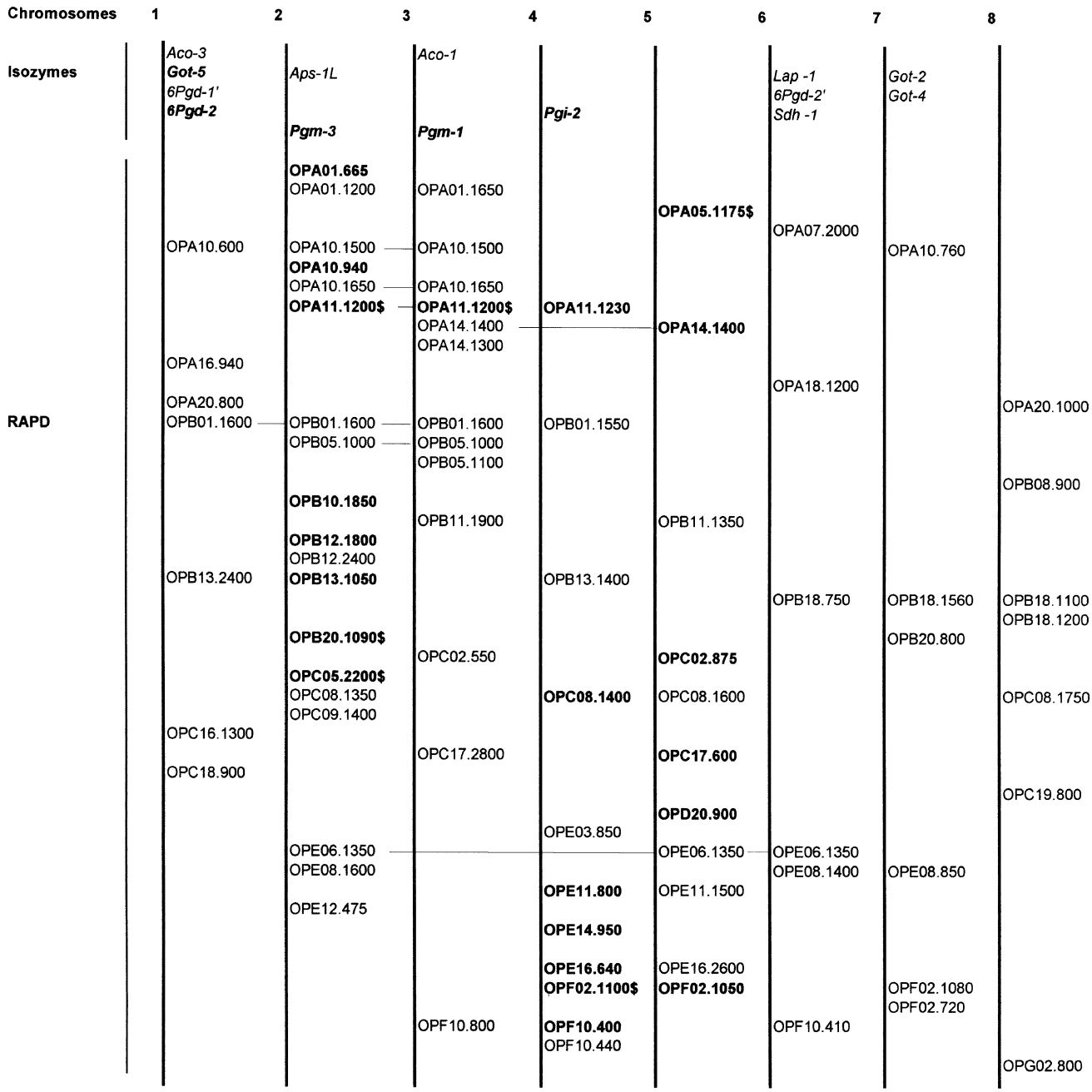

Fig. 1 The *B. nigra* chromosome map established from *B. oleracea-B. nigra* addition lines. Loci in *bold* character correspond to loci common to both sets of *B. napus-B. nigra* and *B. oleracea-B. nigra* addition lines. RAPD loci with *$* were confirmed to be common to the two sets of *B. napus-B. nigra* and *B. oleracea-B. nigra* addition lines by hybridization. Loci *joined with a line* are duplicated on several chromosomes

Establishment of a concensus black mustard chromosome map

The map established was compared to the one described from oilseed rape-black mustard addition lines from which five chromosomes among the eight different chromosomes expected of black mustard were characterized (Chèvre et al. 1991; Quiros et al. 1991; Chèvre et al. 1996). Five common isozyme loci were localized on the two maps and they allowed chromosomes 1, 2, 3 and 4 to be named according to previous data. An example is presented in Fig. 2 for the *Pgi-2* locus of black mustard. In the same way, 22 RAPD fragments revealed from 19 primers presented the same size on the two sets of addition lines (Fig. 1). Except for chromosome 1 without a common marker, 1–8 common markers were found per chromosome (Fig. 3). The similarity of six loci, with at least one locus per

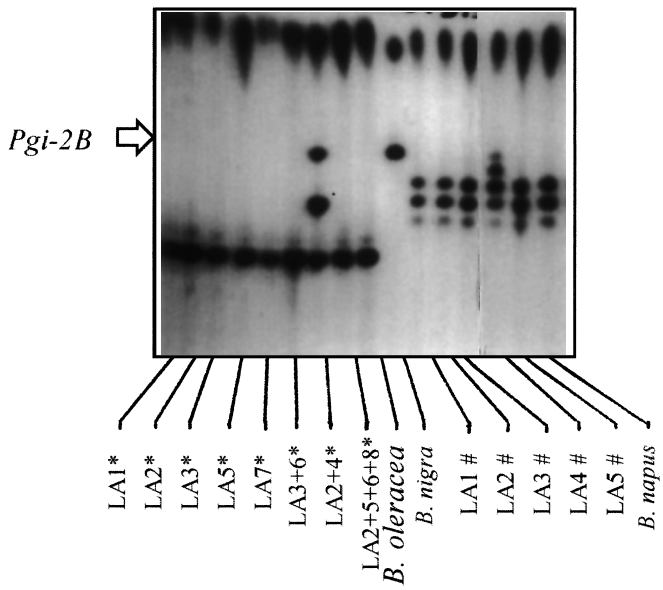

Fig. 2 PGI zymogram obtained from *B. oleracea-B. nigra* (LA*) and *B. napus-B. nigra* (LA #) addition lines giving the identification of the addition lines carrying *B. nigra* chromosome 4 on which the *Pgi-2B* locus was located

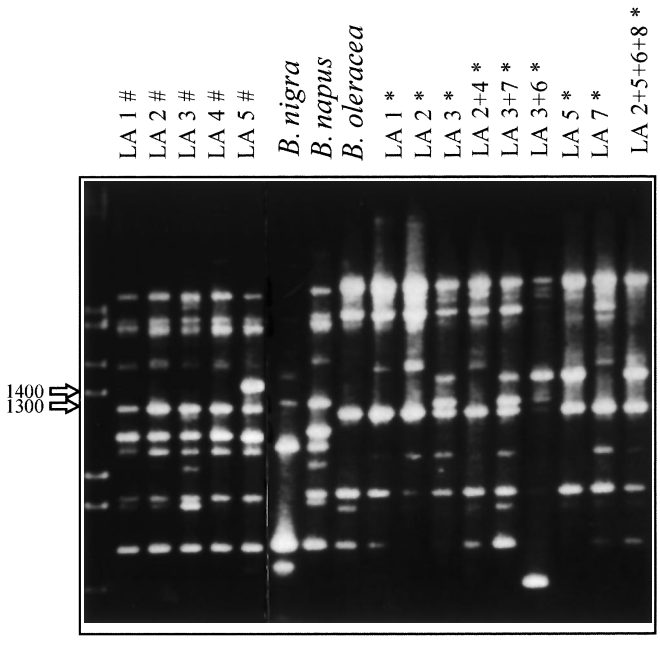

Fig. 3 RAPD profiles obtained from *B. oleracea-B. nigra.* (LA*) and *B. napus-B. nigra* (LA#) addition lines with the OPA14 primer giving the identification of the common OPA14.1400 *B. nigra* locus carried by chromosome 5. This marker is duplicated on chromosome 3 (LA3*) which also carries the OPA14.1300 locus

chromosome, was confirmed by hybridization with the band extracted from one addition line onto the amplified products of the two sets of addition lines carrying the same chromosome. An example for locus OPF02.1100 is presented in Fig. 4.

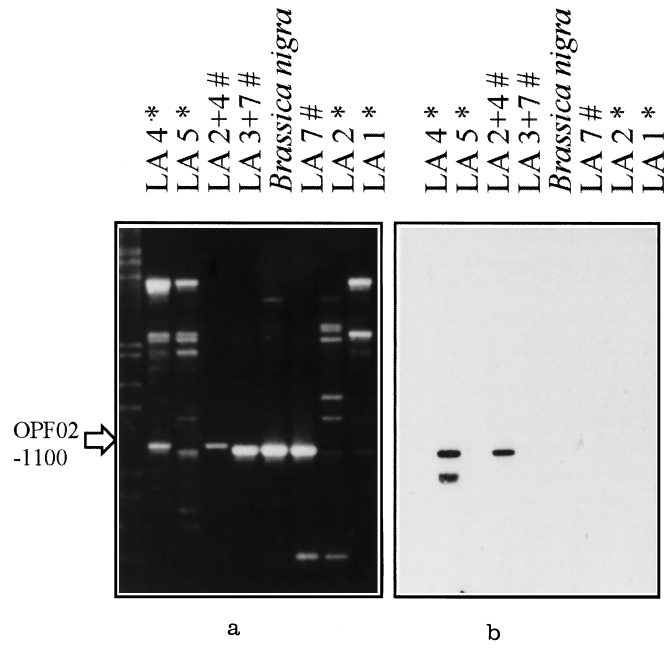

Fig. 4a, b Characterization of the OPF02.1100 locus carried by chromosome 4 on the two sets of addition lines, *B. oleracea-B. nigra.* (LA*) and *B. napus-B. nigra* (LA#). **a** Agarose-gel elctrophoresis profile; **b** corresponding profile after hybridization with the OPF2.1100 band

All these common markers allowed the five chromosomes already described from oilseed rape-black mustard addition lines on the kale genetic background to be identified (Fig. 1).

Analysis of chromosome transmission

Isozyme markers carried by the different black mustard chromosomes were used to assess the chromosome transmission. The poor male fertility and/or the absence of a line at the monosomic stage did not allow us to perform this study using the kale-black mustard addition lines except for female transmission and transmission after selfing the lines carrying chromosomes 1 and 3. However, the different progenies of monosomic oilseed rape-black mustard addition lines with five different black mustard chromosomes were analysed. The results are reported in Table 3.

No difference was detected for female transmission, ranging from 14.1 to 23.8%, whatever the chromosome analysed (chi-square value for contingency = 7.4, P = 11.46%). Similarly, the genetic background, kale or oilseed rape, did not seem to affect chromosome transmission ($\chi^2 = 0.05$ for chromosome 1 and $\chi^2 = 0.04$ for chromosome 3).

No difference was observed for the male transmission of chromosomes 1, 2, 4 or 5 (chi-square value for contingency = 4.32, P = 22.8%), the percentages

Table 3 Transmission rate according to the additional chromosome

Addition lines	Isozyme loci	Male transmission		Female transmission		Transmission from selfing progeny		Theoretical transmission from selfing %
		No. pl. studied	%	No. pl. studied	%	No. pl. studied	%	
LA1#	*6 Pgd-2*	140	28.6	105	23.8	99	39.4	45.6
LA1*	*6Pdg-2 +6Pgd-1′*	–		100	21.0	108	28.7	
LA2#	*Pgm-3*	46	19.6	78	14.1	90	27.8	30.9
LA3#	*Pgm-1*	154	1.3	143	23.8	161	18.0	24.8
LA3*	*Pgm-1 +Aco-1*	–		135	14.8	110	20.9	
LA4#	*Pgi-2*	105	18.1	105	14.3	118	33.9	32.3
LA5#	*Adh-1*	124	28.8	122	15.6	106	37.7	39.9

B. napus-B. nigra addition lines
* *B. oleracea-B. nigra* addition lines

ranging from 18.1 to 28.8%. However, the transmission rate of chromosome 3 was very low, 1.3%.

For each chromosome, no difference was observed between female and male transmission, except for chromosomes 3 and 5.

The theoretical values of chromosome transmission in selfing progeny were calculated from the observed data of the male and female transmission rates. The comparison of this percentage with the one obtained from the selfing progeny of the monosomic addition lines for each chromosome did not reveal any significant difference. Because of the low male transmission of chromosome 3, backcrosses using this addition line as female and selfing gave similar results. For all the other chromosomes, and whatever the genetic background, the transmission rates ranged from 27.8 to 39.4%.

Localisation of the SLG locus

Localisation of the SLG locus was attempted from the *B. oleracea-B. nigra* addition lines. Using A and B primers, it was possible to amplify S-sequences from the DNA extracted from *B. oleracea*, *B. nigra*, as well as from the BC_1 plant selected and the corresponding different addition lines. In all cases, a single PCR product of about 1150 bp was obtained.

When the PCR product from *B. oleracea* was digested with the enzyme *Sty* I, three bands of about 550, 320 and 300 bp were evident (Fig. 5). *Sty*I digestion of *B. nigra* PCR products also showed these bands, together with additional bands at about 620, 340 and 190 bp.These additional bands were also found in the *Sty* I digests of the addition lines carrying chromosome 3 but not in any of the other addition lines (Fig 5). Digestion of the PCR product amplified from *B. oleracea* DNA with the enzyme *Sal* I showed two bands at about 930 and 220 bp. In addition to these two bands, extra bands were found at 740 and 180 bp in *B. nigra*, the BC_1 plant, and all the addition lines carrying chromosome 1, 3 or 4. Digestion of PCR products with the enzymes *Pst*I and *Nci*I showed no variation between samples.

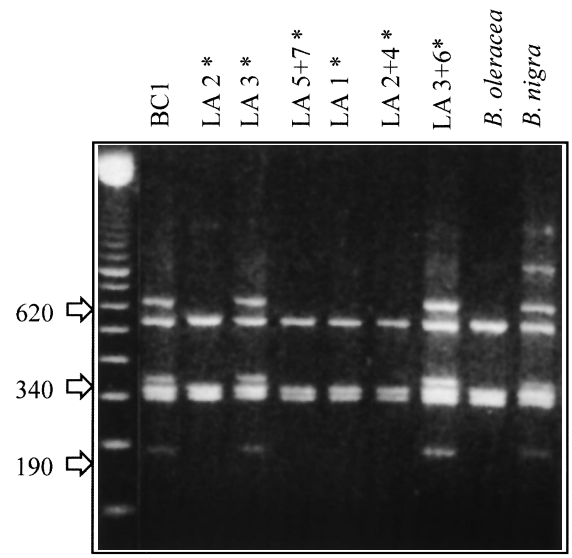

Fig. 5 Profiles obtained from *B. oleracea-B. nigra.* (LA*) addition lines after *Sty* I digestion of PCR products amplified from the A and B primers. Identification of chromosome 3 by the specific 620, 340 and 190-bp digest products of *B. nigra*

Discussion

In order to analyse the B genome of *B. nigra*, addition lines have been created in *B. napus* (Jahier et al. 1989; Struss et al. 1991 a) or in *Diplotaxis erucoides* (This et al. 1990) genetic backgrounds. In the present report, the production of *B. oleracea-B. nigra* addition lines is reported for the first time. They provide the possibility of studying the B genome on a simple diploid genetic background, moreover on a genetic background related to *B. napus*.

The production of F_1 hybrids has been reported by several authors (Prakash and Hinata 1980; Attia and Röbbelen 1986; Song et al. 1993). In the present study, no hybrid was produced using *B. oleracea* as female parent. In fact, Song et al. (1993) suggested that the hybrid yield is higher when the female parent is the same as the cytoplasm donor of the natural amphidiploid, i.e. *B. nigra* the case of *B. carinata*, the natural BBCC amphidiploid. The possibility of producing, at a low frequency, BC_1 plants from female unreduced gametes of F_1 hybrids has been reported several times.

The meiotic behavior of the F_1 hybrid is close to that described by Attia and Röbbelen (1986). It showed a low frequency of chromosome pairing with few bivalents. This result was confirmed by the analysis of BC_1 plants in which it is likely that the eight chromosomes of *B. nigra* remained at the univalent stage since never less than eight univalents were observed. No multivalents was formed and the high frequency of univalents can be related to desynapsis between homologous C chromosomes already reported from diploid and tetraploid kales by Chèvre et al. (1989). *B. nigra* was described as belonging to a lineage other than *B. oleracea* and *B. rapa* from cytogenetical (Attia and Röbbelen 1986) or molecular analyses (Song et al. 1988; Warwick and Black 1991), so no chromosome interchanges probably occurred between the B and C genomes as revealed by meiotic behavior. Among the 12 plants analysed in the following backcross progeny, nine were monosomic or double-monosomic addition lines with a regular meiotic behavior, the added chromosomes remaining as univalents. The male and female fertilities of the addition lines were highly variable depending on the chromosome added. Each time chromosome 2 was present, the *B. oleracea-B. nigra* addition line was male-sterile. The same chromosome on an oilseed rape genetic background did not affect the fertility of the addition line (Jahier et al. 1989; Chèvre et al. 1991). It is likely that an allotetraploid genetic background has a buffer effect and allows the plant to support the depressive characters carried by the additional chromosome. This is confirmed by the absence of distinctive morphological characters between the different addition lines having *B. napus* as genetic background (Jahier et al. 1989; Chèvre et al. 1991; Struss et al. 1991 b). Using also a simple diploid genetic species as recurrent parent, i.e. *Diplotaxis erucoides*, This et al. (1990) reported that two monosomic addition lines were totally male-sterile. Hand pollinations after backcrossing with *B. oleracea*, followed by ovary culture, will be used to maintain this line and to attempt the production of monosomic addition lines from those carrying more than one chromosome. Five among the eight chromosomes of *B. nigra* were extracted at the monosomic stage.

From the different *B. oleracea-B. nigra* addition lines, a chromosome map of the eight *B. nigra* chromosomes was established using molecular markers. From the genetic analysis of isozyme loci within *B. nigra* populations (Chèvre et al. 1995), five single loci among the 14 localized were common with the chromosome map previously published from *B. napus-B. nigra* addition lines (Chèvre et al. 1991, 1996). The identity of the chromosomes in the two sets of addition lines was confirmed by the presence of RAPD markers with the same molecular weight. We have checked that some of these loci hybridized to the two sets of addition lines. In fact, the RAPD bands with identical molecular weight among accessions within a species were previously shown to be homologous by Thormann and Osborn (1992). In spite of the use of the same primers on the two sets of addition lines, *B. napus-B. nigra* and *B. oleracea-B. nigra* carrying five different B-genome chromosomes, only 22 loci were common. From the *B. napus-B. nigra* addition lines (Chèvre et al. 1996), 26 loci were not recovered in the corresponding *B. oleracea-B. nigra* addition lines. This observation has to be related to the high variability within *B. nigra* populations (Chèvre et al. 1996) and it is likely that, even if the same variety was used to produce the addition lines, the B genomes added did not always present the same polymorphism at the same loci. In contrast, 39 new loci were located on the same five chromosomes from the *B. oleracea-B. nigra* addition lines. In this case, the simpler genetic background of *B. oleracea* allowed the detection of more *B. nigra* loci. On the other hand, duplications of RADP loci on different *B. nigra* chromosomes were revealed. These results are in agreement with those reported by several authors (Song et al. 1988; Kianian and Quiros 1992) which estimated that around 50% of RFLP loci are duplicated in the *Brassica* diploid species. Similar observations were made from the establishment of the *B. nigra* genetic maps (Truco and Quiros 1994; Lagercrantz and Lydiate 1995).

From the addition lines it was impossible to order, the 94 loci located on the eight different *B. nigra* chromosomes. The polymorphism between *B. nigra* plants for the loci analysed would require the creation of a segregating population to establish the corresponding genetic map. In fact, few loci are at the same time polymorphic between *B. oleracea* or *B. napus* and *B. nigra* and within *B. nigra* populations. However, from common RAPD markers presenting the same molecular weight, 5 of the 11 linkage groups established by Truco and Quiros (1994) may be attributed to five chromosomes. The verification by hybridization was performed for two specific loci (data not shown) and allowed the B4 and B3 linkage groups (Truco and Quiros 1994) to be allocated to chromosomes 2 and 5 respectively.

For the five B-genome chromosomes isolated on either *B. napus* or *B. oleracea* genetic backgrounds, the chromosome transmission rates were observed and the loci implied in self-incompatibility localised. Morrison (1953) showed that an additional wheat chromosome is

transmitted on average to 25% of the gametes from monosomic addition lines. Our results are close to this observation and to those reported for other *Brassica* addition lines (Quiros et al. 1987; This et al. 1990; Chen et al. 1992). However, this is the first report of a complete analysis performed for the male and female transmission of alien chromosomes in *Brassica* species. The same behavior was observed from the monosomic addition lines carrying chromosomes 1, 2 or 4. Even if a small discrepancy existed between the male and female transmission rates for chromosome 5, the most important difference was reported for chromosome 3. Pollen competition is likely to be responsible for this observation as the male gametes containing chromosome 3 seemed to be unable to compete with oilseed rape pollen because of interactions with loci carried by the A or C genomes. Similar results were reported by Song et al. (1993) who suggested that self-incompatible alleles of diploid *Brassica* genomes might interact. The hypothesis that this chromosome carried the SLG *B. nigra* locus was already supported by the fact that the original disomic addition line for this chromosome, produced by anther culture (Jahier et al. 1989), had a very low seed set after selfing, in spite of a good male fertility. From the few seed produced, only monosomic addition lines were obtained. PCR reactions were performed from primers selected from regions that were highly conserved in different genes implied in self-incompatibility in *B. oleracea*, SLG, and the S-locus related genes, SLR1 and SLR2 (Brace et al. 1993). The profiles obtained from oilseed rape and black mustard did not discriminate between the different addition lines because the bands generated after restriction enzyme digestions showed the same mobility (data not shown) and further analyses were developed from *B. oleracea-B. nigra* addition lines.

The band amplified from *B. oleracea* and *B. nigra* had the expected size indicating that the primers from *B. oleracea* genes are suitable to amplify the corresponding regions in the *B. nigra* genome. After digestion with *Sty* I, *B. nigra* profiles showed three additional bands compared to the *B. oleracea* profile. As the total molecular weight of the additional bands was approximately 1150 bp, this indicated that two sequences had been amplified in *B. nigra*, compared to the single amplified sequence of *B. oleracea*. Based on the *Sty* I digestions, the additional sequence was amplified only from the DNA extracted from addition lines carrying chromosome 3, and not from the other addition lines. This would seem to imply that the SLG locus of *B. nigra* located on chromosome 3. However, digestion of PCR products with *Sal* I showed that the addition lines with chromosomes 1, 3 and 4 had restriction sites similar to those found in *B. nigra* DNA. Thus a further sequence was amplified from addition lines with chromosomes 1 and 4. Since the fertility of these lines was not affected, indeed transmission of chromosomes 1 and 4 was close to the expected ratio, it could be postulated that these are not SLG sequences, but rather are other members of the S multigene family. In fact, Nasrallah et al. (1988) estimated that this multigene family contains around 12 members. Further experiments will be required to support these results, especially the analysis of male transmission in the fertile *B. oleracea-B. nigra* addition lines.

From the *B. oleracea-B. nigra* addition lines created, the eight *B. nigra* chromosomes were well characterized. Our results demonstrated the value of having the same additional chromosome in different genetic backgrounds for analysing the B genome. Several agronomical characters were found to be located on different B genome chromosomes, especially blackleg resistance (Chèvre et al. 1996). If we succeed in inducing chromosome rearrangements between B and C chromosomes, the *B. oleracea-B. nigra* addition lines might be a useful bridge material for the introduction of new characters into oilseed rape varieties.

Acknowledgements R. Delourme, J. Jahier and M. Renard (INRA, Le Rheu, France) are gratefully acknowledged for their critical and helpful reading of the manuscript. We thank J.C. Letanneur for his technical assistance.

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