Theor Appl Genet (1991) 81:43–49

THEORETICAL AND APPLIED GENETICS

Characterization of disomic addition lines *Brassica napus-Brassica nigra* by isozyme, fatty acid, and RFLP markers

A. M. Chevre [1], P. This [2], F. Eber [1], M. Deschamps [1], M. Renard [1], M. Delseny [2] and C. F. Quiros [3]

[1] INRA, BP 29, F-35650 Le Rheu, France
[2] UA 565 du CNRS, Laboratory of Plant Physiology, Avenue de Villeneuve, F-66025 Perpignan Cedex, France
[3] Department of Vegetable Crops, University of California, DAVIS, CA 95616, USA

Received July 5, 1990; Accepted July 13, 1990
Communicated by H. F. Linskens

Summary. Six *Brassica napus* – *B. nigra* disomic addition lines were characterized by isozyme, fatty acid, and RFLP markers. The markers were arranged in six synteny groups, representing six of the eight chromosomes present in the *B. nigra* genome. Synteny group 1 displayed high levels of linoleic and linolenic acids in the seeds of the *B. nigra* parent. Synteny group 3 accumulated higher levels of eicosenoic and erucic acid than *B. nigra*. Three of the lines transmitted the alien chromosome to 100% of the progeny. The rest had variable transmission rates but all were above 50%. Most of the lines produced disomic addition plants in their progeny, suggesting pollen transmission of the alien chromosome. In addition to the marked lines, six others remained unmarked. These could be grouped into two classes according to their alien chromosome transmission. It is likely that they represent the two other *B. nigra* chromosomes that remained uncharacterized by the markers. No diploid individuals carrying *B. nigra* genome-specific markers were detected in the progenies studied.

Key words: *Brassica* – Genome – Isozymes – RFLP – Fatty acids

Introduction

Brassica cytogenetics has been an acitve field of research for many years, starting with the classical work of U (1935), who established the allotetraploid origin of the three species, *Brassica napus* L. (AACC, 2n = 38, *B. juncea* (L.) Czern (AABB, 2n = 36), and *B. carinata* A. Br (BBCC, 2n = 34). This early work set a solid foundation for modern research activities, stimulated by the increasing importance of *Brassica* species as oil seed crops and vegetables.

Very little is known about the evolution of diploid species. Consequently, an area of increasing activity is the creation of cytogenetic stocks as tools for genetic analysis and evolutionary studies. These include mostly alien addition lines (Fantes and Mac Kay 1979; Kaneko et al. 1987; Quiros et al. 1987; Jahier et al. 1989; McGrath 1989; This et al. 1990) and a few monosomic stocks (Chang et al. 1987). The plasticity of the *Brassica* genomes and the existence of natural amphidiploids has made possible the construction of a number of alien addition lines dissecting the *B. oleracea* (Fantes and Mac Kay 1979; Kaneko et al. 1987; Quiros et al. 1987) and *B. nigra* genomes (Jahier et al. 1989; This et al. 1990). In a previous paper, we reported on the production of disomic addition lines *B. napus* – *B. nigra*, in which single pairs of homologous chromosomes from black mustard were added to oil seed rape (Jahier et al. 1989). In the present paper we report on the genetic characterization of these lines by biochemical and molecular markers, and the stability of the alien chromosomes.

Materials and methods

Plant material

Of the 34 disomic addition lines previously obtained, 24 derive from different BC_2 plants (Jahier et al. 1989). Selfed progenies from 20 of these were included in this study. The two parental varieties "Tandem", rapeseed line 00 (*B. napus*) and "Junius", a German cultivar of black mustard (*B. nigra*), were used as controls.

Cytogenetical studies

Flower buds were fixed in Carnoy's solution (ethanol:chloroform:acetic acid, 6:3:1) for 24 h and stored in 50% ethanol.

Anthers at the MI stage of meiosis were squashed and stained in a drop of 1% acetocarmine solution.

Isozyme markers

Crude extracts of young leaves, half seeds, roots, or pollen leachates (Weeden and Gottlieb 1980) were prepared in a TRIS-HCl 0.1 *M* (pH 7.5) buffer containing 1% glutathione. The tissue employed depended on the enzyme assayed (see Results). Horizontal starch gel electrophoresis was used to separate the enzymes following the procedures reported by Quiros and McHale (1985) and buffer systems described by Shields et al. (1983). Enzymes malate deshydrogenase (MDH), isocitric deshydrogenase (IDH), leucine amino-peptidase (LAP), 6-phosphogluconate deshydrogenase (6-PGDH), aconitase (ACO), and phosphoglucoisomerase (PGI) were separated on G buffer system (pH 6.1); triose phosphate isomerase (TPI) and glutamate oxaloacetate transaminase (GOT) on C buffer system (pH 8.3); and phosphoglucomutase (PGM) and alcohol deshydrogenase (ADH) on E buffer system (pH 7.0). The staining procedures used were those reported by Vallejos (1983).

The genetic determination and cellular localization of some of these enzymes in *Brassica* species has been reported by Arus and Orton 1983; Quiros 1987; Quiros et al. 1987, 1988.

RFLP

Total genomic DNA was isolated from young growing leaves collected from single plants of the parental species and disomic addition lines. DNA was extracted according to the protocol of This et al. (1990) or according to the following protocol: 10–20 g of leaves was ground in a mortar to fine powder in liquid nitrogen and afterwards for 1 min on high speed in a blender containing 100 ml of H buffer [0.5 *M* sucrose, 80 m*M* TRIS-HCl (pH 9.45), 10 m*M* EDTA, 4 m*M* spermidine, 1 m*M* spermine, 0.2% v/v mercaptoethanol]. The solution was filtered through one layer of miracloth and two layers of cheesecloth into a 250-ml centrifuge flask and centrifuged for 30 min at 4,000 rpm in a Beckman JA14 rotor. The supernatant was discarded and the pellet was resuspended in 40 ml of H buffer, transferred to a 50-ml centrifuge tube, and centrifuged again for 30 min at 4,000 rpm in a JS13.1 rotor. The pellet was resuspended in 4 ml of H buffer. Seven milliliters of lysis buffer [10 m*M* TRIS-HCl (pH 9.4), 40 m*M* EDTA, 6% w/v N-lauroylsarkosine] and 11.6 g of CsCl were added and the tube was incubated at 60 °C for 30 min. The tubes were then centrifuged for 1 h at 11,000 rpm in a JS13.1 rotor. The supernatant was filtred through one layer of miracloth into an ultracentrifuge tube containing 150 µl of ethidium bromide solution (10 mg/ml). The centrifugation was carried out for 16 h at 45,000 rpm at 25 °C and the DNA band was recovered with a syringe. DNA was extracted with TE [10 m*M* TRIS-HCl, 1 m*M* EDTA (pH 8.0)] plus 5 *M* NaCl-saturated isopropanol, diluted twice, and precipitated with 2 vol. of ethanol. After a 30-min centrifugation at 9,000 rpm, the DNA was resuspended in TE buffer.

For RFLP analysis, 2–5 µg of DNA for each line was digested with a series of restriction endonucleases, according to the manufacturer's intructions (BRL, Bethesda/MD). DNA was fractionated in a 0.8% agarose gel and transferred to a Hybond-N nylon membrane by capillarity under akaline conditions (Reed and Mann 1985). Pre-hybridization was carried out for 16 h at 65 °C in 50 ml of 4× SSPE [1× SSPE = 150 m*M* NaCl, 10 m*M* Na_2PO_4, 1 m*M* EDTA (pH 7.5)], 1% SDS, 5× Denhard's (0.1% Ficoll, 0.1% polyvinyl pyrrolidine, 0.1% BSA – fraction v), 0.5% nonfat dry milk, and 10 µg/ml of sonicated denatured salmon sperm DNA. Hybridizations were carried out for 16 h in 10–25 ml of prehybridization solution under the same conditions in the presence of oligolabelled radioactive probes (Feinberg and Vogelstein 1983). After hybridization, membranes were washed once at room temperature in 2× SSPE, 0.1% SDS, once at 65 °C in 1× SSPE, 0.1% SDS, and once at 65 °C in 0.5× SSPE, 1% SDS. Membranes were then exposed to Kodak XAR- X4 film in cassettes provided with intensifiers.

Probes were extacted from *B. napus* cDNA and genomic libraries (Hosaka et al. 1990) or radish cDNA libraries (Raynal et al. 1989, 1990). We selected the combinations of enzyme/probe giving the best separation of the fragments for the parental lines and tried them on addition lines. Most of these were anonymous DNA sequences, except for pB69 (napin), pAF7 (cruciferin), and p8B6 (late embryogenesis abundant mRNA).

Fatty acid analysis

Fatty acid analysis was performed on 5–30 seeds of each addition line obtained by selfing single plants. For extraction, the half-seed technique previously described by Thies (1974) was used. For the separation of the fatty acids, we used 10% BDS columns and HL as carrier gas. After a Bliss angular transformation of percentages, a two-way analysis of variance followed by Duncan's multiple range test was performed for each fatty acid. ANVARM of the AMANCE software package (Bachacou et al. 1981) was applied.

Results

Isozyme markers

Initially, isozyme markers were used for the identification of the addition lines, because of the possibility they afforded for rapidly assaying a large number of plants.

Of the ten enzyme systems analyzed in the parental accessions, six/6-PDGH, GOT, TPI, PGM, PGI, ADH) were found to discriminate between *B. nigra* and *B. napus*. Therefore, loci coding for these enzymes were useful for the identification of the addition lines.

6-PGDH. The most anodal zone of activity, *6-PGDH-1*, corresponding to isozymes of plastid localization, was composed of six bands in *B. napus* and three equidistant bands in *B. nigra*. The *6-PGDH-1 B. nigra* bands were difficult to distinguish from the three most anodal bands of *B. napus* because of their similar migration. Therefore, they could not be used for the identification of the addition lines. On the other hand, the isozymes expressing in the more cathodal activity zone, *6-PGDH-2*, corresponding to cytosolic isozymes, could be clearly distinguished in both species. *B. napus* was characterized by a single, monomorphic *6-PGDH-2* band, whereas *B. nigra* displayed multiple bands of variable mobility, suggesting the presence of duplicated loci. The *6-PDGH-2 B. nigra*-specific isozymes were observed in progenies of two of the addition lines (Fig. 1).

GOT. The isozyme profile for this enzyme was complex. However, *B. nigra* could be differentiated from *B. napus* by the presence of a band that we denominated *GOT-5*. This band was present in the same addition lines identi-

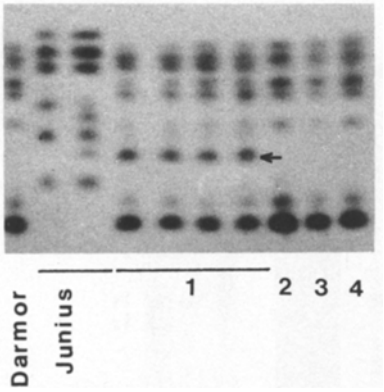

Fig. 1. 6-PGDH zymograms for *B. napus* "Darmor", *B. nigra* "Junius", addition lines of group 1 carrying *6-PGDH-2 B. nigra* chromosome and of groups 2, 3, 4

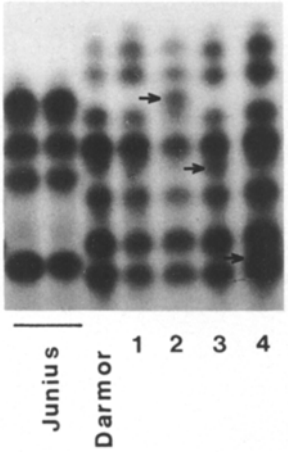

Fig. 2. PGM zymograms for *B. nigra* "Junius", *B. napus* "Darmor", addition lines of groups 1, 2, 3, and 4 carrying no marker, *PGM-3, PGM-1,* and *PGM-2* genes of *B. nigra*, respectively

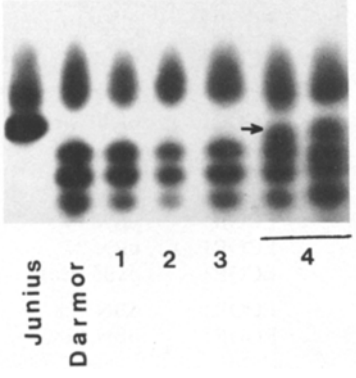

Fig. 3. PGI zymograms for *B. nigra* "Junius", *B. napus* "Darmor", and addition lines of groups 1, 2, 3, and 4, this last one carrying *PGI-2 B. nigra* chromosome

Table 1. Isozyme markers in progenies derived by selfing disomic addition lines

Group	Isozyme markers	Number of parental plants	Number of plants	
			Studied	With marker
1	$6\text{-}PGDH_2$–GOT_5	2	29	29
2	TPI_1 –PGM_3	1	14	14
3	PGM_1	1	1	1
4	PGM_2 –PGI_2	3	48	24
5	ADH_1	3	23	23
6	No marker	10	144	

fied by the *6-PGDH-2* (Table 1). Thus, *6-PGDH-2* and *GOT-5* formed part of the same synteny group.

TPI. The isozymes of locus *TPI-1,* expressing in the most anodal portion of the gel, distinguished *B. napus* and *B. nigra*. Thus, this locus served to identify another set of addition lines. Conversely, the isozymes for locus *TPI-2* overlapped in both species, making it impossible to distinguish them.

PGM. The three activity zones detected for this enzyme, *PGM-1, PGM-2,* and *PGM-3,* expressed isozymes diagnostic for the identification of three independent addition lines (Fig. 2). *PGM-3* was present on the same addition line identified by *TPI-1*. Thus, *TPI-1* and *PGM-3* formed part of the same synteny group (Table 1).

PGI. Locus *PGI-2,* expressing isozymes of cytosolic localization, distinguished the parental species. The addition lines identified by *PGM-2* carried also the *PGI-2 B. nigra*-specific isozymes (Table 1, Fig. 3).

ADH. The isozymes of activity zone *ADH-1,* expressing in the most anodal part of the gel, were diagnostic in identifying another set of addition lines (Fig. 4). The isozymes for *ADH-1* expressed in seed. The isozymes of a second activity zone, *ADH-2,* expressing in roots and seeds, were not diagnostic.

RFLP markers

These markers served to confirm the six different addition lines disclosed by isozymes and supplied additional markers to the *B. nigra* chromosomes.

Twenty *B. napus* and four radish probes were tested on some of the addition lines, and ten probe-endonuclease combinations disclosed *B. nigra*-specific fragments useful for identification of the addition lines (Table 2). Because of fragment size overlapping, highly repeated sequences such as rDNA failed to reveal differences between *B. napus* and *B. nigra* (Delseny et al. 1990). Most

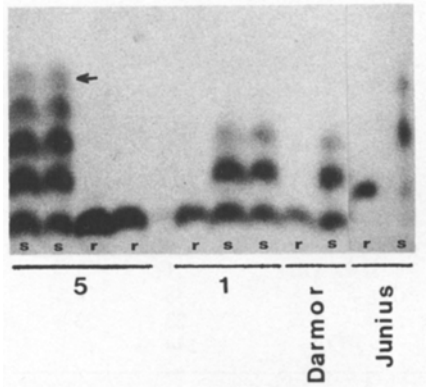

Fig. 4. ADH zymograms from roots (r) or seeds (s) for *B. nigra* "Junius", *B. napus* "Darmor", and addition lines of groups 1 and 5, this last one carrying *ADH-1 B. nigra* chromsome

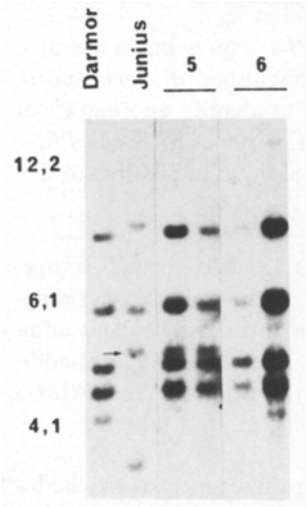

Fig. 5. Characterization of group 5 by RFLP marker pBN6. Autoradiogram of HindIII-digested genomic DNA hybridized with probe pBN6. Genomic DNA was extracted from several plants of *B. napus* "Darmor", *B. nigra* "Junius", and two disomic addition lines of groups 5 and 6. *Arrow* indicates main *B. nigra* fragment. Size in kb

of the other probes displayed simple patterns for *B. nigra* and were mapped on single chromosomes (e.g. probe pBN 6, Fig. 5). For probe pBN 128, however, several loci were located on two different chromosomes (Fig. 6a): 1-1 on group 1 and 1-2 on group 6. The former locus was polymorphic in *B. nigra* (Fig. 6c), explaining the size differences for the *B. nigra*-specific fragments observed between the plant used as control and the addition lines in Fig. 6a. None of these fragments was found in *B. napus*. Digestion with BglII probed with pBN 128 confirmed synteny groups 1 and 6 (data not shown). For napin and cruciferin genes, two multicopy gene families, only one locus has been located thus far on a *B. nigra* chromosome, but we did not test all the progenies for these two probes. Furthermore, only one cruciferin subfamily was assayed.

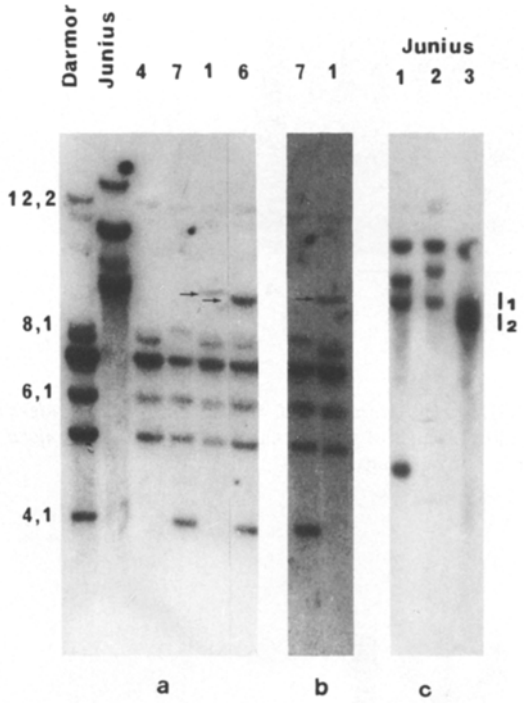

Fig. 6a–c. Characterization of groups 1 and 6 by RFLP marker pBN128. **a** Autoradiogram of EcoRI-digested genomic DNA for *B. napus* "Darmor", *B. nigra* "Junius" (several plants), and disomic addition lines of groups 1, 4, 6, and 7 (single plant) hybridized with probe pBN128. **b** Overexposure of **a** for lines 7 and 1, showing faint band on line 1. **c** EcoRI pattern for three *B. nigra* plants showing variation for the locus localized on group 6. *Arrow* indicate main *B. nigra* band on additional chromosome. Size in kb

Table 2. RFLP markers on plants carrying additional chromosomes in progenies derived by selfing disomic addition lines

Group	Mother plant	Number of plants tested	RFLP markers	
			Enzyme	Probe
1	43.1	4	ECORI	pBN 128
		2	ECORI	pB69 (napin)
2	45.1	1	ECORI	pB 488
3	57.1	1	ECORI	pBN 7
		1	ECORI	pAF 7 (cruc.)
4	74.1	3	ECORV	pB 850
5	100.2	1	HindIII	pBN 6
		1	ECORI	pBN 14
		1	ECORI	pBN 27
		2	ECORI	p8B6 (lea)
6	43.2	3	ECORI	pBN 128
	111.2	4	ECORI	pBN 128
7	27.1	3	–	–
	100.3	1	–	–
	100.4	1	–	–

Table 3. Fatty acid content progenies derived by selfing disomic addition lines

Groups	Number of mother plants	Number of seeds tested	Fatty acids (percent wt)						
			C 16:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:1	C 22:1
1	1	10	6.3	0.9	36.2	37.9	18.5	0.2	–
2	1	10	5.9	1.3	67.6	14.7	9.0	1.0	–
3	1	5	4.3	1.5	35.7	19.5	9.2	25.5	3.9
4	2	30	7.7	1.1	52.3	26.0	12.2	1.1	–
5	3	24	5.9	1.7	48.7	27.6	15.3	1.1	–
6	2	67	5.6	1.2	41.8	22.1	14.4	1.5	–
7	8	91	5.3	1.2	55.1	22.8	13.6	1.3	–
B. napus									
var. Tandem	–	10	5.3	1.4	66.0	18.3	7.3	1.3	–
var. Major	–		4.7	1.1	15.8	12.6	6.6	13.5	44.4
B. nigra									
var. Junius	–	10	5.0	1.4	10.9	23.8	17.0	7.2	31.2

Fatty acid content

No significant differences for the different acids were found between the plants of groups 2, 4, 5, 6, 7, and the rapeseed control, var. "Tandem". Seeds of group 1 as well as *B. nigra* var. "Junius" were characterized by higher linoleic (C 18:2) and linolenic (C 18:3) acid content compared to Tandem (Table 3). Accumulation of eicosenoic acid (C 20:1) and at a lower level of erucic acid (C 22:1) was observed in seeds of group 3 (Table 3).

The isozyme, RFLP, and fatty acid markers identified six independent lines among the plants carrying the additional *B. nigra* chromosomes. However, several plants carrying alien chromosomes remained unmarked by the markers tested.

Alien chromosome transmission

Fifteen progenies derived from selfing of the disomic addition lines were monitored for chromosomes numbers (Table 4). Lines 5, 2 and 1 transmitted the alien chromosome to 100% of the plants in the progeny. The two latter ones produced only disomic addition plants (2n = 40), thus conserving the character of the parental plant. Line 3 produced mostly disomic and a few monosomic additions (2n = 39) in the progeny tested. The transmission rate of the alien chromosome in line 4 was 56%, the lowest value observed. Stability of the alien chromosome was variable in group 6. The unmarked progenies could be divided roughly into two groups, one with a transmission rate of 100%, including both disomic and monosomic additions, and a second group with a transmission rate of ca. 50%. The presence of *B. nigra* markers was always associated with the presence of an alien chromosome for all the plants tested. No diploid individuals carrying *B. nigra*-specific markers were detected in the progenies examined.

Table 4. Chromosome stability in progenies derived by selfing disomic addition lines

Group	Mother plant	Number of plants tested	Number of plants with 2n =			
			40	39	38	Other
1	25.1	4	4	–	–	–
	43.1	24	24	–	–	–
2	45.1	10	10	–	–	–
3	57.1	1	–	1	–	–
4	64.1	8	–	2	6	–
	74.1	15	3	8	2	2
5	100.2	15	13	2	–	–
6	43.2	20	7	1	12	–
	111.2	13	12	1	–	–
7	27.1	15	9	6	–	–
	33.1	8	2	1	2	3
	95.1	7	7	–	–	–
	100.3	18	12	–	6	–
	100.4	9	5	–	4	–
	111.1	8	6	2	–	–

Discussion

Among the different markers studied, the similarity of the RFLP and isozyme profiles observed for *B. napus* (AACC) and *B. nigra* (BB) supports the hypothesis of common phylogenetic origin for the different *Brassica* species (Röbbelen 1960; Song et al. 1988). This resulted in a high number of uninformative markers for the identification of the addition lines. Another reason for this could be the origin of the addition lines: they were extracted from a single BC_1 plant (Jahier et al. 1989). Consequently, of the large variability often observed for most markers on the parental species *B. nigra* cv "Junius",

only a single copy of the B1 genome was represented in the addition lines. This may not have carried the most discriminant alleles.

Eight isozyme loci, seven RFLP probes, and four fatty acids allowed us to define at least six independent addition lines of the eight that could be theoretically extracted from the *B. nigra* genome. However, the two missing lines may be present among the progenies of unmarked plants studied for chromosome transmission.

In rapeseed, eicosenoic acid (20:1) and erucic acid (C22:1) are formed by chain elongation. The main pathway of polyunsaturated fatty acids starts with the desaturation of oleic acid into linoleic acid and subsequently linolenic acid (Laakso 1986). Downey and Harvey (1963) established that inheritance of erucic acid in rapeseed is under embryonic control and is conditioned by two genes (no dominance, additive effect, *e* and *E* alleles). Synthesis of eicosenoic and erucic acid is controlled by the same alleles. In group 3 the gene(s) involved in chain elongation may be present on the *B. nigra* chromosome. This group is characterized by a high C20:1 and a low C22:1 content, compared to the expected value generally observed in rapeseed with one *E* allele (C20:1:3–5%; C22:1:7–15%). However, such a result was obtained from the analysis of only a few seeds harvested in the greenhouse, and therefore must be confirmed in the field on a larger progeny.

The genetic background of polyunsaturated C18 fatty acids is more complex than for erucic acid due to the involvement of maternal effect. Kondra and Thomas (1975) estimated that the minimum number of factors controling C18:1, C18:2, and C18:3 synthesis was two to six for C18:1, three to five for C18:2; and one to four for C18:3. Roy and Tarr (1986) selected a low linolenic acid progeny from an interspecific cross between *B. napus* and *B. juncea*. The B genome chromosome of group 1 characterized by high C18:2 and C18:3 content may be involved in desaturation.

The synteny of some of the markers established in our set of lines could be compared with that observed for other *Brassica* addition lines and with linkages reported in previous studies. For example, the location of *PGM-3* and *6-PGDH-2* on independent chromosomes observed in addition lines *Diplotaxis erucoides* -*B. nigra* (This et al. 1990) was confirmed in our lines. The lack of discrimination between the *B. napus* and *B. nigra* isozymes for *6-PGDH-1* prevented us from confirming the synteny of both *6-PGDH* loci on the same chromosome as reported by Quiros et al. (1986) for the B genome extracted from *B. carinata* and by This et al. 1990. Contrary to the findings of This et al. (1990), we located *PGM-3* and *TPI-1* on the same chromosome. This discrepancy may be due to the presence of duplicated *TPI* loci (Quiros 1987), which may have resulted in the misidentification of the loci coding for this enzyme.

Our mapping data also provides the opportunity to compare syntenic relationships for some of the markers in the three cultivated genomes, A, B, and C. For example, *PGI-2* and *PGM-3* were found on independent chromosomes in the A (McGrath 1989) and B genomes, and linked in the C genome along with *6-PGDH-1* (Arus 1989). In the C genome synteny has been reported for *6-PGDH-2* and *PGM-2* (Quiros et al. 1987), and linkage for *ADH-1* and *PGM-1* (Arus and Orton 1983). Conversely, in the B genome all four loci are located on independent chromosomes. Another difference between the B and C genomes is the location of both sets of *6-PGDH* loci on independent chromosomes in the C genome as well as synteny in the B genome. Thus, our data provides further evidence of extensive chromosomal repatterning in the evolution of the *Brassica* genomes. Furthermore, the presence of multiple fragments for probe pBN128 and their distribution in two chromosomes also supports the existence of duplicated chromosome segments in *B. nigra*.

Because of the small size and the large number of chromosomes in mitosis, no karyotypic characterization of the alien chromosomes was possible. The B genome chromosomes were well tolerated by *B. napus* with a high rate of transmission to the progenies. The large number of disomic additions observed for most progenies indicates high transmission through ovules and pollen. This is in contrast to the addition lines developed in diploid background, where transmission occurs mostly through the female side (Quiros et al. 1987; This et al. 1990). An exception was the addition lines for group 3. The parental disomic line for this chromosome was obtained only by haplodiploidization (Jahier et al. 1989). The selfed progeny of this plant yielded only a single plant carrying the B genome chromosome 3, and this plant was a monosomic addition. Because of the strong incompatibility observed for the *B. nigra* parent, it is tempting to speculate that this chromosome carries the self-incompatibility locus. The alien chromosome representing group 4 also displayed lower transmission rates than the other lines. Since the mother plants for this line exhibit a regular meiotic behavior (Jahier et al. 1989), it is likely that its low pollen transmission was due to pollen competition with an advantage for euploid gametes. This phenomenon has been reported for wheat-rye disomic addition lines (Bernard 1976). Our results indicate that chromosome transmission may serve as another marker for distinguishing the alien chromosomes. Thus, based on this observation, it is possible that the unmarked group 7 may include two different chromosomes.

We observed very little homoeologous pairing between the chromosomes of the B genome and those of the A and C genomes, which is in agreement with the observations of Attia et al. (1987). Therefore, it is unlikely that some of the addition lines carry recombinant alien chro-

mosomes. Since this possibility deserves further investigation, a series of intercrosses among addition lines is in progress in our laboratory. Another aspect that will be followed up is the male and female transmission, specially for the lines displaying lower transmission rates.

Morphological changes in the addition lines caused by the different *B. nigra* chromosomes were not particularly evident. However, the plants of group 1 progeny appeared to have hairy leaves and lighter flower color, whereas the plants of group 2 were less vigorous and had darker leaves. Field evaluations for morphological and fatty acid content traits will be carried out in an attempt to further characterize these lines. Also, the search for additional isozyme and RFLP markers, as well as other *B. nigra* traits, such as the presence of specific fatty acids and glucosinolates and resistance to blackleg, is in progress.

References

Arus P (1989) Linkage analysis of isozyme genes in *Brassica oleracea*. In: Quiros CF, McGuire PE (eds) Proc 5th Cruciferae Workshop, University of California, Davis, p 45

Arus P, Orton TJ (1983) Isozyme and linkage relationships of isozyme loci in *Brassica oleracea*. J Hered 74:405–412

Attia T, Busso C, Röbbelen G (1987) Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome 29:326–330

Bachacou J, Masson JP, Miller C (eds) (1981) Amance 81. Department of Biometry, Publ INRA, pp 1–516

Bernard M (1976) Etude des caractéristiques cytologiques, morphologiques, et agronomiques de six lignées d'addition blé-seigle. Ann Amel Plant 26 [1]:67–91

Chang R, Tai W, Fan Z (1987) Cytogenetic studies of aneuploids in rapeseed. 2. Morphology, fertility, and cytology of self-pollinated progenies of monosomic plants of *Brassica napus* L. Genome 29:174–179

Delseny M, McGrath JM, This P, Chevre AM, Quiros CF (1990) rDNA genes in diploid and amphidiploid *Brassica* and related species: organization, polymorphism, and evolution. Genome (in press)

Downey RK, Harvey BL (1963) Methods of breeding for oil quality in rape. Can J Plant Sci 43:271–275

Fantes JA, Mac Kay GR (1979) The production of disomic addition lines of *Brassica campestris*. Cruciferae Newslett 4:36–37

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Ann Biochem 132:6–13

Hosaka K, Kianian SF, McGrath JM, Quiros CF (1990) Development and chromosomal localization of genome-specific DNA markers of Brassicae and the evolution of amphidiploids and n=9 diploid species. Genome 33:131–142

Jahier J, Chevre AM, Tanguy AM, Eber F (1989) Extraction of disomic addition lines of *Brassica napus* - *B. nigra*. Genome 32:408–413

Kaneko Y, Matsuzawa Y, Sarashima M (1987) Breeding of the chromosome addition lines of radish with single kale chromosome. Jpn J Breed 37 [4]:438–452

Kondra ZP, Thomas PM (1975) Inheritance of oleic, linoleic, and linolenic acid in seed oil of rapeseed (*B. napus*). Can J Plant Sci 55:205–210

Laakso I (1986) An analytical and breeding study on fatty acids in summer turnip rape. J Agric Sci Finland 58:103–141

McGrath JM (1989) Generation of monosomic alien addition lines in *Brassica* and their use in comparative evolutionary studies. PhD thesis, University of California, Davis

Quiros CF (1987) Duplicated isozyme loci and cellular compartementation of their products in *Brassica*. Cruciferae Newslett 12:24

Quiros CF, McHale N (1985) Genetic analysis of isozyme variants in diploid and tetraploid potatoes. Genetics 111: 131–145

Quiros CF, Ochoa O, Kianian SF, Douches D (1986) Evolutionary trends in *Brassica*: gathering evidence from chromosome addition lines. Cruciferae Newslett 11:22–23

Quiros CF, Ochoa O, Kianian SF, Douches D (1987) Analysis of *Brassica oleracea* genome by generation of *B. campestris-oleracea* chromosome addition lines: characterization by isozymes and rDNA genes. Theor Appl Genet 74:758–766

Quiros CF, Ochoa O, Douches D (1988) Exploring the role of the x=7 species in *Brassica* evolution: hybridization with *B. nigra* and *B. oleracea*. J Hered 79:351–358

Raynal M, Depigny D, Cooke R, Delseny M (1989) Characterization of a radish nuclear gene expressed during late seed maturation. Plant Physiol 91:829–836

Raynal M, Depigny D, Grellet F, Delseny M (1991) Characterization and evolution of napin genes in radish and related crucifers. Gene (in press)

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 13:7207–7221

Röbbelen G (1960) Beiträge zur Analyse des *Brassica* Genoms. Chromosoma 11:205–228

Roy NN, Tarr A (1986) Development of near zero linolenic acid (C18:3) lines of rapeseed (*Brassica napus* L.). Z Pflanzenzuecht 96:218–223

Shields CR, Orton TJ, Stuber CW (1983) An outline of general resource needs and procedures for the electrophoresis separation of active enzymes from plant tissue. In: Tanksley SD, Orton TJ (eds) Elsevier, Amsterdam, Oxford, New York, Isozymes in plant genetics and breeding, part A. pp 443–468

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 1. Genome evolution of diploid and amphidiploid species. Theor Appl Genet 75:784–794

Thies W (1974) New methods for the analysis of rapeseed constituents. Proc 4th Int Rapeseed Congr, Giessen, 4–8 June 1974, pp 275–282

This P, Ochoa O, Quiros CF (1990) Dissection of the *Brassica nigra* genome by monosomic addition lines. Plant Breed (in press)

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Elsevier, Amsterdam, Oxford, New York, Isozymes in plant genetics and breeding, part A. pp 469–516

Weeden NF, Gottlieb LD (1980) Isolation of cytoplasmic enzymes from pollen. Plant Physiol 66:400–403