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# Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*

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**Summary.** This paper reports the application of the RAPD (random amplification of polymorphic DNA sequence) markers in *Brassica* genetics. Forty-seven arbitrary decamer oligonucletides were used as primers to amplify genomic DNA by polymerase chain reaction. Some of the amplified products were genome specific and could be found in both diploid and derived amphidiploid species. Of a total of 65 such markers, 16 were A genome, 37 B genome, and 12 C genome specific. Of the 37 B-genome-specific markers, 11 were mapped on four independent chromosomes of *B. nigra* with the aid of existing *B. napus-nigra* disomic alien addition lines.

**Key words:** Chromosomes markers – RAPD markers – Cole crops – Rapeseed – Gene mapping

## Introduction

The study of the evolution and genetics of *Brassica*, initiated early this century by U (1935) and Karpechenko (1922) among others, has been recently stimulated by the development of molecular markers (Palmer et al. 1983; Quiros et al. 1987; Song et al. 1988; Hosaka et al. 1990; McGrath et al. 1990) and cytogenetic stocks (Quiros et al. 1987; McGrath and Quiros 1990; This et al. 1990; Hu and Quiros 1991; Chevre et al. 1991). The polymerase chain reaction (PCR) (Saiki et al. 1988) now makes possible the rapid development of chromosome markers in many organisms by disclosure of useful polymorphisms (Ledbetter et al. 1990; Love et al. 1990). The application of this technique was expanded by Williams et al. (1991), who proposed the use of arbitrary primers for random amplification of polymorphic DNA sequences (RAPD markers).

*Brassica* species offer the opportunity to develop chromosome markers by amplification of random and specific DNA sequences because of their following attributes: high levels of polymorphism within each species, distinct genomes in basic diploid and derived natural amphidiploid species, and availability of alien addition lines.

The objective of the present investigation is to develop genome-specific markers by amplification of DNA sequences, and to locate them on their respective chromosomes by existing *B. napus-nigra* disomic alien addition lines.

## Materials and methods

### *Plant materials*

To develop genome-specific markers, we surveyed two accessions of each of the six cultivated *Brassica* diploid and derivied amphidiploid species (Table 1). For chromosomal localization of the B-genome-specific markers, we employed six of the eight possible addition lines corresponding to each of the *B. nigra* chromosomes previously characterized by isozymes, RFLP, and fatty acid content (Chevre et al. 1991). The two parental varieties of the alien addition line series were also included in the study, *B. napus* 'Tandem' (2n = 4x = 38, A and C genome), and *B. nigra* 'Junius' (2n = 2x = 16, B genome) (Janhier et al. 1989). We sampled at least two plants from each of the lines carrying the alien chromosome in a monosomic or disomic condition.

### *DNA extraction*

DNA samples were extracted from young leaves following the protocol described by This et al. (1990). For each accession of the species sampled in the survey and the two varieties, leaves from at least five plants were used. For the alien addition lines, young leaves were collected from individual plants carrying the alien chromosomes. The DNA concentration of each sample was estimated by comparing band intensity with lambda DNA controls of known concentrations on a minigel.

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**Table 1.** List of accessions surveyed for genome-specific markers

| Species | Acc. no. | Variety | Origin |
|---|---|---|---|
| *B. oleracea* | B035 | Kohlrabi 'Castor' | Royal Sluis, Holland |
| *B. oleracea* | B056 | Brussels Sprouts 'Alcazar' | Royal Sluis, Holland |
| *B. napus* | B338 | Rapeseed 'Salamander' | Institut für Pflanzenbau, Germany |
| *B. napus* | B430 | Rapid cycling | P. Williams, University of Wisconsin, Madison |
| *B. campestris* | B200 | Rapeseed 'Torch' | Canada |
| *B. campestris* | B233 | Chinese Cabbage 'Kwan Hoo Choi' | Redwood City Seed Co. |
| *B. juncea* | B198 | Mustard 'Domo' | Unknown |
| *B. juncea* | B199 | Mustard | University of California, Davis |
| *B. nigra* | B165 | Black mustard | D. Cohen, McCabee Seed Co. |
| *B. nigra* | B168 | Black mustard | D. Cohen, McCabee Seed Co. |
| *B. carinata* | B204 | Ethiopian Mustard | Unknown |
| *B. carinata* | B284 | Ethiopian Mustard | Unknown |

*PCR technique*

A series of arbitrary decamer oligonucleotides obtained from various investigators at the University of California, Davis, and others purchased from Operon Technologies, Inc. (Alameda/CA, USA) were used for the amplification of random DNA sequences. Two criteria were used to select the primers: (1) 50–70% CG content, and (2) no self-pairing sequence. The protocol reported by Williams et al. (1991) was followed with minor modifications. The volume for each amplification was 25 µl, with genomic DNA concentrations fluctuating between 25 and 60 ng per reaction. *Taq* DNA polymerase and reaction buffer were purchased from Promega Biotec (Madison/WI, USA). For the DNA amplification, a Perkin Elmer Cetus and a Hybaid (Middless, UK) were programmed for 40–45 cycles at 92 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min, for denaturing, annealing, and primer extension, respectively. Tubes containing all the reaction components, except for the genomic DNA, were included as a control when a new primer was used. After the cycling was completed, 10 µl of the samples was run in 1.4% agarose gels in the presence of ethidium bromide (Sambrook et al. 1989). Molecular standards were either the lamda DNA digested by *Eco*RI/*Hind*III or the 1-kb DNA ladder purchased from BRL (Maryland, USA). The gels were photographed under UV light with Polaroid film 655 or 667.

*Marker nomenclature*

The amplified products useful as markers were named by primer origin (UCD or OP) followed by the primer, kit if applicable, number and the size of the amplified product in base pairs (Tables 2 and 3).

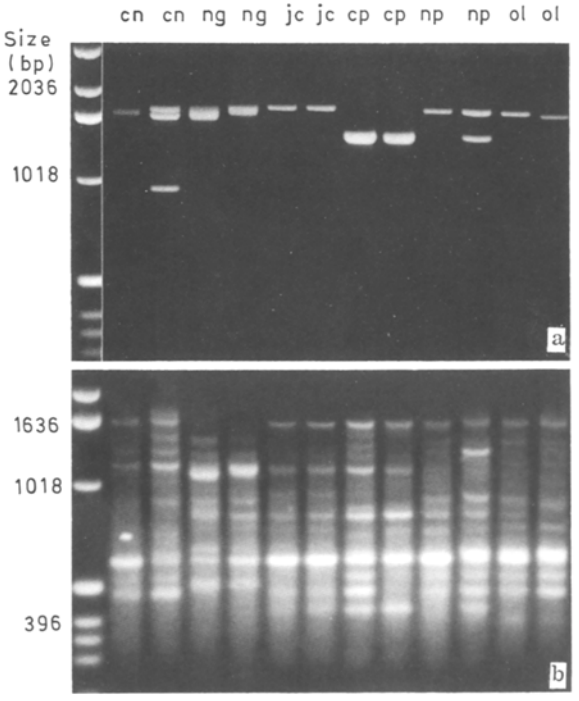


**Fig. 1 a and b.** Examples of number of products amplified by the arbitrary decamer primers. **a** Primer OPA6 only amplified one to two bands; **b** Primer OPA5 produced 15 bands for some accesions (cn – *B. carinata*; ng – *B. nigra*; jc – *B. juncea*; cp – *B. campestris*; np – *B. nigra*; ol – *B. oleracea*)

## Results

*Genome- and species-specific markers*

A survey with 47 arbitrary primers revealed a great amount of polymorphism of amplified DNA fragments ranging in length from 200 to 4,500 bp. The number of bands produced by one primer varied from as few as one (Fig. 1 a) to as many as 15 (Fig. 1 b).

The scorable bands could be classified into three categories: (1) genome-specific bands that were displayed in both the diploid and the derived amphidiploid species, representing the conservative portion of the genome; (2) species-specific bands unique to the two accessions of a single species, representing conserved species sequences; and (3) accession-specific bands displayed by only one of the two accessions of the same species, representing intraspecific variability. Out of a total of 65 genome-specific products, 16 belonged to the A genome, 37 to the B genome, and 12 to the C genome (Table 2; Fig. 2). Most of the primers produced a single genome-specific product for a specific genome. The exceptions were primers UCD57, OPA8, OPA16, and OPB12, which produced two or three specific products for either the A

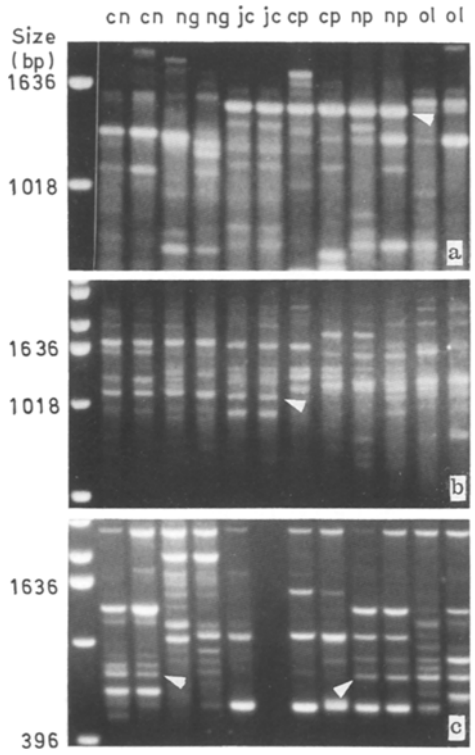


**Fig. 2a–c.** Examples of genome-specific amplification products (*arrows*, species abbreviations same as Fig. 1). **a** A-genome-specific product amplified by primer OPB18 in cp, np, and jc. **b** B-genome-specific product amplified by primer UCD3 in ng, jc, and cn. This was located on *B. nigra* chromosome no. 4 (see Fig. 3a). **a** C-genome-specific product amplified by primer OPA17 in np, ol, and cn

or B genomes. On the other hand, 14 primers produced multiple products specific for more than one genome (Table 2). Approximately half of the primers detected only B-specific markers. No attempt was done to quantify species- and variety-specific products because this was out of the scope of the present study.

*Localization of the B. nigra-specific markers on the respective chromosomes*

The differences in the profiles resulting from the migration of the amplified products for the parental species *B. napus* and *B. nigra* were substantial for all primers used. However, some of the products overlapped in both species, making it impossible to distinguish them from each other. As informative markers for the *B. nigra* chromosomes, we selected B-genome-specific products absent in *B. napus*. From a total of 12 primers tried, 7 were informative, disclosing 11 markers (Table 3) distributed in four of the eight *B. nigra* chromosomes (Table 4). A few instances showed amplification products specific to the *B. nigra* parent that were not found in any of the addition lines.

**Table 2.** List of genome-specific markers

| | A genome | B genome | C genome |
|---|---|---|---|
| | UCD8-2200 | UCD2-1300 | UCD2-1400 |
| | UCD57-450 | UCD3-1050 | UCD57-1300 |
| | UCD58-1200 | UCD6-1330 | UCD58-300 |
| | OPA3-850 | UCD8-800 | UCD60-800 |
| | OPA8-400 | UCD11-1300 | OPA3-1150 |
| | OPA8-800 | UCD11-3700 | OPA9-1000 |
| | OPA9-850 | UCD57-200 | OPA11-400 |
| | OPA12-600 | UCD57-550 | OPA13-700 |
| | OPA16-300 | UCD57-1100 | OPA17-800 |
| | OPB3-1400 | UCD58-400 | OPB14-1100 |
| | OPB6-800 | UCD58-830 | OPB18-600 |
| | OPB11-1700 | UCD60-100 | OPB19-800 |
| | OPB12-300 | UCD62-1150 | |
| | OPB12-500 | OPA2-500 | |
| | OPB18-900 | OPA3-1600 | |
| | OPB19-450 | OPA3-1800 | |
| | | OPA8-300 | |
| | | OPA10-700 | |
| | | OPA12-900 | |
| | | OPA15-800 | |
| | | OPA16-900 | |
| | | OPA16-1400 | |
| | | OPA18-550 | |
| | | OPA18-1050 | |
| | | OPA19-1000 | |
| | | OPB3-850 | |
| | | OPB4-1000 | |
| | | OPB5-650 | |
| | | OPB7-900 | |
| | | OPB9-1200 | |
| | | OPB10-350 | |
| | | OPB10-1100 | |
| | | OPB11-1400 | |
| | | OPB12-450 | |
| | | OPB16-1500 | |
| | | OPB17-600 | |
| | | OPB18-350 | |
| Total | 16 | 37 | 12 |

Primer UCD3 disclosed a *B. nigra* marker of approximately 1,050 bp, which was located in *B. nigra* chromosome 4. Two plants carrying this alien chromosome in the monosomic and disomic condition displayed this marker, which was absent in *B. napus* and in the addition lines carrying other *B. nigra* chromosomes (Fig. 3a).

Primer UCD6 disclosed a single marker of approximately 1,330 bp, which was located in *B. nigra* chromosome 5. The two disomic additions tested for this line had this marker. The profile of both plants displayed a predominance of the *B. nigra* product and poor amplification of the *B. napus* products (Fig. 3b).

Primer UCD11 yielded two products located on independent *B. nigra* chromosomes. The large one of approximately 3,700 bp was present in a disomic plant tested for *B. nigra* chromosome 2, whereas the second product of approximately 1,300 bp was present in two disomic alien addition plants tested for *B. nigra* chromosome 1. In the

**Table 3.** List of arbitrary primers, their origin, sequences, and amplified products mapped on the B genome chromosomes

| Name | Origin | Sequence | Chromosome marker |
|---|---|---|---|
| UCD3 | UC Davis | 5'-GCC GCC ACC A-3' | UCD3-1050 |
| UCD6 | UC Davis | 5'-ACG TAG CGT C-3' | UCD6-1330 |
| UCD11 | UC Davis | 5'-CTG AAG CTA C-3' | UCD11-3700, UCD11-1300 |
| UCD57 | UC Davis | 5'-CGA CAA GCT C-3' | UCD57-1100, UCD57-550, UCD57-200 |
| UCD58 | UC Davis | 5'-CTC GGT GAT C-3' | UCD58-830, UCD58-400 |
| UCD60 | UC Davis | 5'-AGG GAG TCA C-3' | UCD60-100 |
| UCD62 | UC Davis | 5'-ATC TTC CGC C-3' | UCD62-1150 |

**Table 4.** List of markers located on the respective *B. nigra* chromosomes by *B. napus-nigra* addition lines

| Chromosome no. | Markers | |
|---|---|---|
| | Chevre et al. (1991) | (present study) |
| 1 | 6pgdh2, Got5, pBN128$^{-1}$, pB69 (napin), faC18:1, faC18:3 | UCD11-1300, UCD57-200, UCD58-830 |
| 2 | Tpi1, Pgm3 pB488 | UCD11-3700, UCD57-1100, UCD58, UCD58-400 |
| 3 | Pgm1, pBN7, pAF7 (cruciferin) faC20:1, faC22:1 | |
| 4 | Pgm2, Pgi2, pB850 | UCD3-1050 |
| 5 | Adh1, pBN6, pBN14, pBN27, p8B6 (lea) | UCD6-1330, UCD57-550, UCD60-100, UCD62-1150 |
| 6 | pBN128-2 | |

two positive lines, the *B. nigra* markers were added to the expected *B. napus* products (Fig. 3c).

Primer UCD57 disclosed three products distributed in independent chromosomes. All three were confirmed by testig two plants for each of the positive alien chromosomes. The largest product of approximately 1,100 bp was located on chromosome 2, the second product of approximately 550 bp was on chromosome 5, whereas the third product of less than 200 bp was located on chromosome 1.

Primer UCD58 generated two markers located on independent *B. nigra* chromosomes. The first one of approximately 830 bp was located on chromosome 1, while the second one of approximately 400 bp was located on chromosome 2. Both markers were confirmed by testing two disomic alien addition plants for each chromosome.

Primer UCD60 disclosed a product of less than 100 bp located on *B. nigra* chromosome 5. This product was found in two monosomic alien addition plants tested for this chromosome (Fig. 4a).

Primer UCD62 disclosed a product of approximately 1,150 bp also on chromosome 5 in both disomic alien addition plants tested for this chromosome (Fig. 4b).

## Discussion

The polymorphism disclosed by amplification of arbitrary primers is very extensive in *Brassica*. In agreement with other markers based on isozymes (Quiros et al. 1987) and RFLP revealed by both genomic and cDNA probes (Song et al. 1988; Hosaka et al. 1990), the arbitrary primers disclosed genome-specific markers for all three genomes, A, B, and C, of the cultivated diploid species *B. campestris, B. nigra,* and *B. oleracea,* respectively. The fact that approximately half of the primers disclosed only B-genome-specific markers further demonstrates that this genome is less related to either A or C genomes. This agrees with the previous studies based on cytogenetics (Attia and Röbbelen 1986), RFLP (Song et al. 1988) and chloroplast DNA (Palmer et al. 1983). However, the frequent occurrence of shared bands among the diploid species suggests that the three genomes conserve certain homology (Figs. 1 and 2).

The ability of the arbitrary primers to reveal polymorphism between and within species certainly offers researchers another approach to generate genetic linkage maps for each of the *Brassica* species. There is great morphological variation in these species but, because of their complexity, genetic analysis of these traits is difficult to perform without chromosome markers.

The usefulness of RAPD markers for mapping is demonstrated by the identification of alien *B. nigra* chromosomes in the *B. napus-nigra* addition lines. The simplicity and rapidity of the technique now makes it possible to quickly expand the number of markers and genetic maps of the *Brassica* species. The most useful primers were those disclosing two or more markers in all three

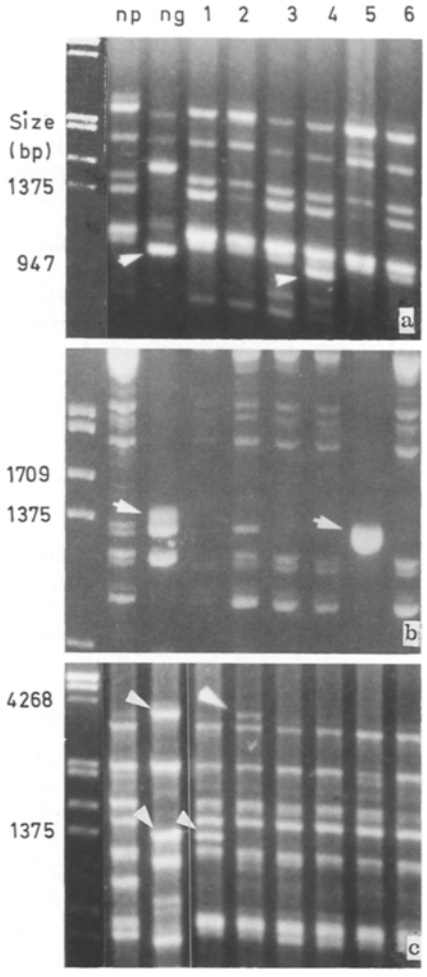


**Fig. 3a–c.** Examples of the B-genome-specific markers mapped on disomic alien addition lines. Numbers at the top correspond to the alien chromsome represented in the lines. **a** UCD3-1050 on *B. nigra* chromosome no. 4; **b** UCD6-1330 on *B. nigra* chromosome no. 5; and **c** UCD11-3700 on *B. nigra* chromosome no. 2 UCD11-1300 on chromosome no. 1

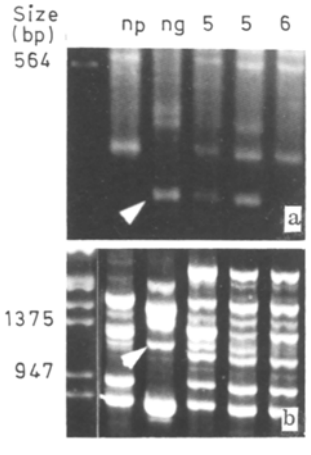


**Fig. 4 and b.** Sample runs of two B-genome-specific markers (*arrow*) present in two plants tested for alien addition line no. 5: **a** UCD60-100; **b** UCD62-1150

genomes, e.g., UCD57. Of the 14 primers producing multiple genome-specific products, 3 revealed possible duplications dispersed in several B genome chromosomes. For example, primers UCD11, UCD57, and UCD58 amplified sequences in chromosomes 1 and 2 of *B. nigra*. In addition, primer UCD57 also amplified sequences on chromosome 5. This is in agreement with our findings, based on RFLP probes, supporting the concept that the *Brassica* genome has undergone extensive duplications followed by chromosomal rearrangements (Quiros et al. 1989; Hosaka et al. 1990; McGrath et al. 1990; Hu and Quiros 1991).

The profiles of the amplification products were highly reproducible when the same amplification program was used in the thermocycler. The *B. nigra* products used as markers of the alien addition lines were always present in the parental variety. In general, the addition lines negative for *B. nigra* markers had the same profile as the parental *B. napus*, except for a few cases where sporadic unique products were evident in some of the plants. These most likely represent polymorphisms within *B. napus*. Similarly, other products present in the *B. nigra* parent but not in any of the addition lines most likely represent polymorphisms within *B. nigra*. Also, they may be markers located in the two alien chromosomes not yet represented in the addition series. Since the DNA of the original plants of both parental species used for extraction of the addition lines was not available, it was impossible to determine their phenotype. However, the DNA pooled from plants of the same accessions provided a fair representation of the genetic make-up of both parents.

The genetic characterization of the *B. napus-nigra* alien addition line series by chromosome markers provides the opportunity to effectively map the B genome and to determine the feasibility of introgressing the B genome into the A and C genomes by interspecific aneuploidy. The markers will also serve to align other sets of alien addition lines for the B genome, such as the *Diplotaxis erucoides-B. nigra* (This et al. 1990) and *B. oleracea-nigra* (derived from *B. carinata*) lines (Quiros et al. 1989), to consolidate all available markers for the B genome chromosomes and complete the B genome series.

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*Note added in proof*

Since submission of the manuscript, seven of the eight B genome chromosomes have been identified by additional RAPD markers.

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