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Structure and organization of the B genome based on a linkage map in *Brassica nigra*

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Abstract We constructed a genetic map on *Brassica nigra* based on a segregating population of 83 F_2 individuals. Three different types of molecular markers were used to build the map including isozymes, restriction fragment length polymorphisms (RFLP), and random amplified polymorphic DNA (RAPD). The final map contained 124 markers distributed in 11 linkage groups. The map covered a total distance of 677 cM with the markers distributed within a mean distance of 5.5 cM. Of the sequences found in the *B. nigra* map, 40% were duplicated and organized into three different types of arrangements. They were either scattered throughout the genome, organized in tandem, or organized in blocks of duplicated loci conserved in more than 1 linkage group.

Key words Linkage map · *Brassica nigra* · RFLP · RAPD

Introduction

Among the three cultivated diploid *Brassica* species, *B. nigra*, commonly known as black mustard, has the lowest chromosome number (2n = 2x = 16, B genome). This species is grown primarily for its seeds, which are used in the elaboration of mustard and other condiments. The range of cultivation of black mustard is quite narrow because of its replacement by *B. juncea* during the past decade (Hemingway 1979). Although *B. nigra* has little economic importance as a crop, it is being used as a significant source of useful genes, such as those coding for disease resistance and oil seed quality, for the improvement of rapeseed (*B. napus*) (Sjodin and Glimelius 1989; Struss et al. 1991a, b; Chevre et al. 1991). More importantly, *B. nigra* is one of the progenitors of two other cultivated allotetraploid species, *B. juncea* and *B. carinata*. Along with the other diploid cultivated species, *B. oleracea* (2n = 2x = 18, C genome) and *B. campestris* (2n = 2x = 20, A genome), *B. nigra* forms part of the U triangle (U 1935). All these species are intimately related in this triangle involving hybridization and polyploidization. Therefore, in order to understand their relationships, it is essential to know their genomic structure and organization. These studies already exist for the A and C genomes (McGrath and Quiros 1991; Song et al. 1991; Chyi et al. 1992; Kianian and Quiros 1992; Landry et al. 1992), however, very little is known about the B genome.

The objective of the present study is to report the construction of a linkage map based on isozyme and molecular markers for *B. nigra*. This map will serve to compare the genomic structure and organization of all three cultivated species and to provide markers for useful genes in rapeseed breeding.

Material and methods

Plant material

A single plant was selected from each of two populations of *B. nigra*, one from Turkey (B1164) and another from India (B1157). They were crossed to produce F_1 plants. A single F_1 individual was used to generate a segregating F_2 population of 83 individuals for the map construction. Bud-pollination was used in the crosses to assure their fidelity and to overcome self-incompatibility of the F_1 individuals. All the plants were grown under standard greenhouse conditions to minimize environmental influences on the observations.

Isozymes

Crude extracts were prepared by crushing young leaves in TRIS-HCl pH 7.5 buffer and 2% glutathione. The samples were subjected to starch gel electrophoresis following the protocol described by Quiros

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et al. (1987). Staining procedures were as in Vallejos (1983). The following enzymatic systems were analyzed: alcohol dehydrogenase (ADH), acid phosphatase (APS), aconitase (ACO), esterase (EST), glutamate oxalacetate transaminase (GOT), isocitric dehydrogenase (IDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), and shikimate dehydrogenase (SDH).

Restriction fragment length polymorphism (RFLP)

DNA was extracted in a CsCl gradient following a protocol from Fisher and Goldberg (1982) modified by Kianian and Quiros (1992). On the basis of the high level of polymorphism reported for *Brassica* species (Landry et al. 1991; McGrath and Quiros 1991; Kianian and Quiros 1992), a single restriction enzyme was used (*Eco*RI in this case), as suggested by Fidgore et al. (1988). In a few cases a second restriction enzyme (*Bam*HI) was used in order to get polymorphism for specific clones. Digested DNA samples were separated in 1% TAE (40 m*M* Tris-acetate, 1 m*M* EDTA) agarose gels and blotted on Hybond-N nylon membranes (Amersham) using alkaline Southern blotting procedures suggested by the manufacturer. Hybridization was conducted on 5 × SSPE (180 m*M* NaCl, 10 m*M* NaH_2PO_4. H_2O), 1 m*M* EDTA, 7 × Denhardt's solution, 0.5% SDS, and ssDNA overnight at 65 °C. The washing procedure was that suggested by the membrane manufacturer with some modifications: we used 30 min at room temperature with 2 × SSPE 0.1% SDS, and 15–30 min on 0.1 × SSPE, 0.1% SDS at 65 °C. The membrane was exposed to an X-ray film and an intensifier screen (Dupont Cronex, Lightning Plus II Ref. 300070) at −80 °C for 5 days.

Several probes were used in this study including genomic clones isolated from a *B. napus* library, designated pB (Hosaka et al. 1990); anonymous clones from a cDNA library from *B. napus* provided by J.J. Harada (Dept. of Botany, University of California, Davis), designated pBN, p1, or p2; a few clones of known function, such as rDNA genes from wheat (pTA71; Gerlach and Bedbrook 1979); isocitrate lyase and malate synthase (pBSIL9 and pBSMS respectively; Comai et al. 1989); genes expressed during seed germination and postgerminative development in *B. napus* (pLEA, pGS, pCOT; Harada et al. 1988); a gene for self-incompatibility in *B. oleracea* (pBOS5; Nasrallah et al. 1985); probes corresponding to the major storage proteins in *Brassica*, napin (pN2; Crouch et al. 1983) and cruciferin (C1; Simon et al. 1985); and phosphoenol pyruvate carboxylase kinase from *B. napus* (PEP; M. Delseny, unpublished). Almost all of the probes were polymorphic in the segregation population for *Eco*RI digests. Oligolabeling of the probes was accomplished by random priming with the kit "multiprime" (Amersham, UK RPN. 1601Z) based on the method of Feinberg and Vogelstein (1983). Non-incorporated nucleotides were eliminated by elution through sephadex-50 columns (Maniatis et al. 1982).

Random amplified polymorphic DNA (RAPD)

Approximately 12 ng of DNA was amplified following the protocol described by Hu and Quiros (1991). Random primers were from Operon Technologies (Alameda, Calif.) kits A–E; *Taq* polymerase from Promega (Madison, Wis.). The amplification was conducted in a Perkin-Elmer thermocycler, and the amplified bands were separated on 2% TAE agarose gels and visualized by ethidium bromide staining.

Only those primers that gave a clear pattern of bands were considered in the study. Since more than one run was necessary to study the entire F_2, comparisons between runs were used to discard those primers yielding irreproducible patterns of bands. Amplified bands segregating in the F_2 population were designated with the name of the primer (kit letter and number) and the size of the fragment in base pairs.

Map construction and linkage analysis

The F_2 linkage map was constructed using the Mapmaker computer program (Lander et al. 1987; Lincoln et al. 1992). Linkage 1 (Suiter et al. 1983) was used to calculate chi-square goodness-of-fit estimates of the loci included on the map (Table 1).

Table 1 Goodness-of-fit estimates for those RFLP and RAPD loci segregating in the F_2 *B. nigra* population and used in the linkage map construction with χ^2 estimates that had distorted segregations from expected Mendelian ratios

Locus	Observed F_2 segregation	Expected ratio	χ^2	Probability
BN7-2	7:46:22	1:2:1	9.85**	0.007
BN14-2	50:29	3:1	5.78*	0.016
BN113	18:56:9	1:2:1	12.1**	0.002
BN128-1	28:42:12	1:2:1	6.29*	0.04
BN128-3	33:28:21	1:2:1	11.8**	0.003
N2-1	4:16:17	1:2:1	9.81**	0.007
BOS5	54:29	3:1	4.37*	0.04
A16-1020	77:6	3:1	13.98**	0.00
A20-1000	51:31	3:1	7.17**	0.007
B10-1300	44:39	3:1	21.4**	0.00
C08-900	73:10	3:1	7.42**	0.006
C18-1300	75:7	3:1	11.9**	0.001
D11-875	71:12	3:1	4.92*	0.03
D13-800	54:29	3:1	4.37*	0.04
D16-2000	53:30	3:1	5.50*	0.02
D16-1018	76:7	3:1	12.15**	0.00
E05-1150	38:45	3:1	35.92**	0.00

** $P \leq 0.01$; * $0.05 \geq P \geq 0.01$

Mapmaker considers multipoint analysis and maximum likelihood estimations in the calculation of map distances. Linkage groups were assembled using two-point data with a linkage criteria of ≤ 0.3 for the recombination frequency and a log likelihood (LOD) of ≥ 3.8. The order of the markers within a linkage group was determined using three-point analysis with a LOD ≥ 3.0 for the frame markers and LOD 2.0 or 1.0 for the rest of the markers. If a gap of more than 30 cM was found between adjacent markers after ordering the markers in a given linkage group, the linkage group was split into 2 different linkage groups. The order of the markers in a linkage group was confirmed by comparing the likelihoods of the original map order with those found when the order of neighboring markers was permuted. The log-likelihood difference considered was 4.0.

Assignment of linkage groups to chromosomes

We attempted to assign linkage groups to chromosomes using two sets of alien addition lines, *B. napus-B. nigra* (Chevre et al. 1991; Quiros et al. 1991; This 1992) and *B. oleracea-B. nigra* (A. M. Chevre, personal communication). For some of the RAPD markers, homology between addition lines and F_2 linkage map markers was confirmed by hybridization. DNA samples from addition lines and segregating F_2 population were amplified (Hu and Quiros 1991), separated in 2% TAE agarose gels and transferred to nylon membranes (Hybond +) by alkaline blotting. Segregating amplified products corresponding to mapped loci in the *B. nigra* linkage map were isolated from the agarose gel and used as probes. Membranes were exposed to X-ray film at room temperature for approximately 1 h.

Results

We constructed our genetic map using 136 markers (Fig. 1). These markers comprised 4 isozyme, 47 RFLP and 85 RAPD loci.

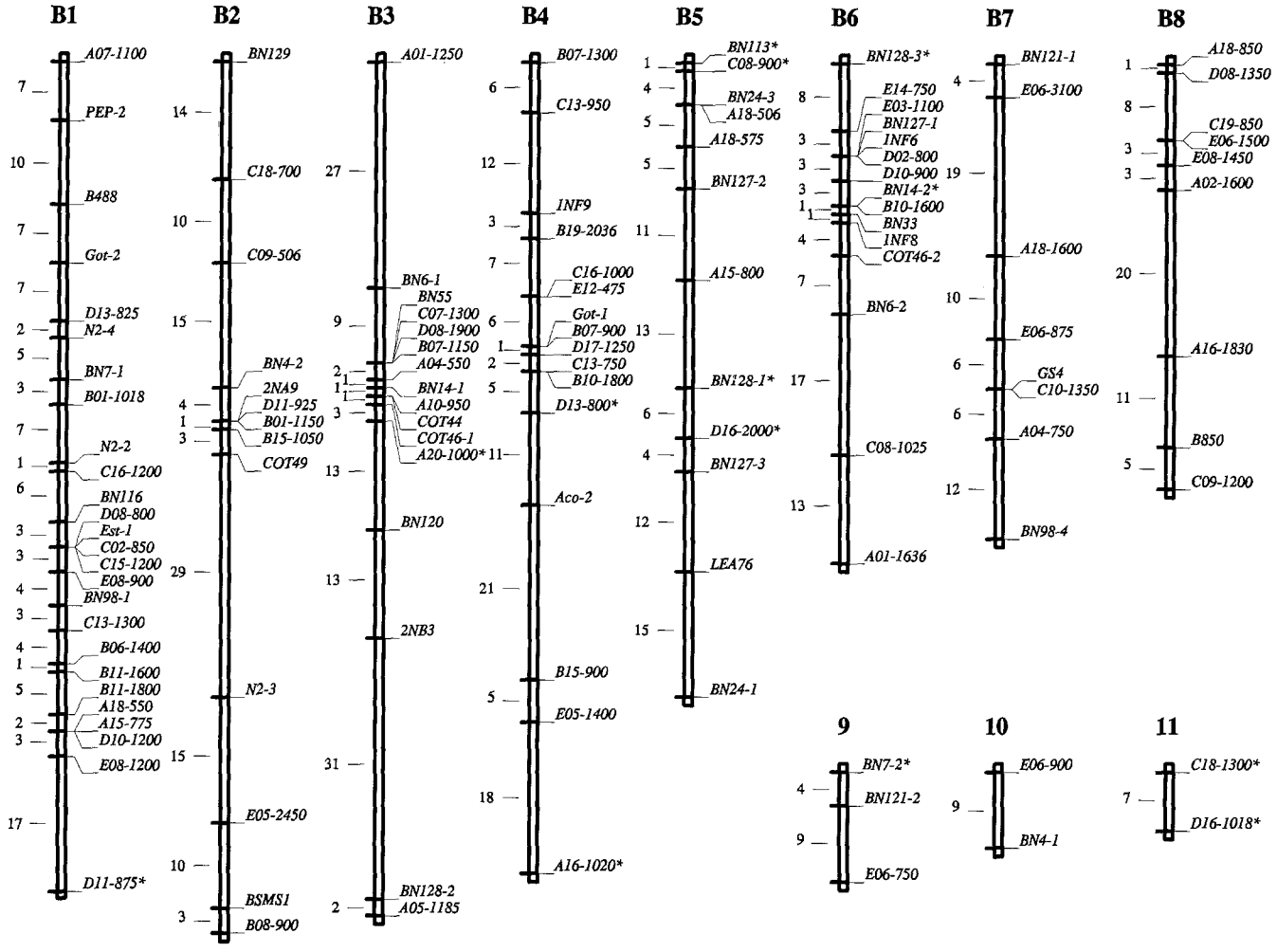

Fig. 1 Linkage map in *Brassica nigra*. Linkage groups were designated arbitrarily from *B1* to *B8* corresponding to the eight chromosomes in *B. nigra*. Additional linkage groups were designated with *numbers*. Distances between markers are in cM. Markers with a* had segregations skewed from expected Mendelian ratios ($P < 0.05$). Linkage groups B4 and B3 have been assigned to chromosomes 2 and 5 in addition lines *B. napus-B. nigra* (Chevre et al. 1991; This 1992) and *B. oleracea-B. nigra* (A. M. Chevre, personal communication). In the same set of addition lines, linkage group B1 has been assigned to chromosome 1

Isozymes

Polymorphism

From the ten systems we assayed, only three were polymorphic in the segregating population, namely aconitase (ACO), glutamate oxalacetate transaminase (GOT) and esterase (EST). GOT disclosed two segregating loci, whereas EST and ACO revealed a single segregation locus each. Of the 4 isozymatic loci that were polymorphic, 2 of them, *Got-1* and *Aco-2*, were codominant, each segregating for two different alleles. *Est-1* and *Got-2* resolved only a single allele at each locus. In *Got-2*, the unresolved alloenzyme was probably null or migrated to the same location as an intergenic hybrid band. In *Est-1*, one of the alleles was null. Markers for both loci were scored as dominant, segregating in a 3:1 ratio.

Degree of duplication

Only GOT was segregating for more than 1 locus. The 2 GOT genes were located on independent linkage groups, B4 and B1. Potential duplication for the other segregating isozyme in the experimental population was possible, since additional non-segregating bands were found in ACO and EST zymograms, indicating monomorphic loci.

RFLPs

Polymorphism

We analyzed a total of 50 clones. Five of them corresponded to genomic clones from a *B. napus* library; 37 corresponded to anonymous cDNA clones from a second *B. napus* library; and 8 corresponded to the specific genes described in the Material and methods. Thirty-one (62%) of these disclosed 47 segregating loci; the

other 19 clones (38%) gave band patterns that were either monomorphic or difficult to read. On the basis of the hybridization patterns of the parents and F_2 individuals, 13 (28%) of the loci had dominant alleles; the rest had codominant alleles. Significant deviations ($P \leq 0.05$) from the segregation ratios were found for 7 (14.5%) of the RFLP loci (Table 1), with 5 of these being distributed on 3 different linkage groups (B5, B6 and 9), clustered in 2 groups of 2 markers and a single one of 1 marker.

Degree of duplication

Almost 40% of the RFLP clones we used in this study represented duplicated sequences that detected 2–4 polymorphic loci. The remaining 60% of the clones corresponded, in most cases, to low-copy sequences disclosing a single polymorphic locus and a fixed band that might correspond to a non-segregating locus (Fig. 2). Truly single copy genes were rare (Fig. 3); only 4% of the clones gave a pattern showing only 1 or 2 bands.

Intragenomic homology of chromosomal segments between linkage groups was evident by the distribution of the duplicated loci (Figs. 1 and 4). Intrachromosomal duplications were illustrated by linkage group B1, which carried two copies of pN2 interspaced among other unrelated loci. Similarly, linkage group B5 had two sets of duplicated sequences, pBN24 and pBN127, each with 2 loci. The *BN127* loci at 34cM from each other fell in between the two *BN24* loci, whose distance was 71 cM. Duplicated sequences of clones pBN4, pBN6, BN14, pBN98, pBN128, pCOT46, and Got were dispersed on different linkage groups; however, conserved associations of these loci with other duplications were also detected. For example, associations between *BN127* and *BN128* loci were conserved in linkage groups B5 and B6 (Fig. 4), and the map segment comprised of pCOT46, pBN6, pBN14, and pBN128 was conserved between linkage groups B3 and B6 (Fig. 4). The two copies of pBN4, pBN7, pBN98, and pBN121 were dispersed on 5 linkage groups, keeping no conserved association with any other duplicated loci. The known gene clones napin (pN2) and phosphoenol pyruvate carboxylase kinase (PEP) were also duplicated. pN2 segregated for 4 different loci; 3 of them mapped in linkage groups B1 and B2. From the 2 segregating loci disclosed by PEP, one was mapped in linkage group B1 and the other did not link to any group. Malate synthase (pBSMS) and self-incompatibility (pBOS5) clones, although presenting hybridization patterns disclosing more than 1 band for *Eco*RI digests, segregated for a single locus each. Five additional bands were present in pBOS5 blots, and an additional single band in pBSMS blots, suggesting at least 1 more locus for malate synthase and additional monomorphic loci for the self-incompatibility probe. Isocitrate lyase (pSIL9), rDNA (pTA71), and cruciferin (C1) were not polymorphic for either of the two restriction enzymes assayed.

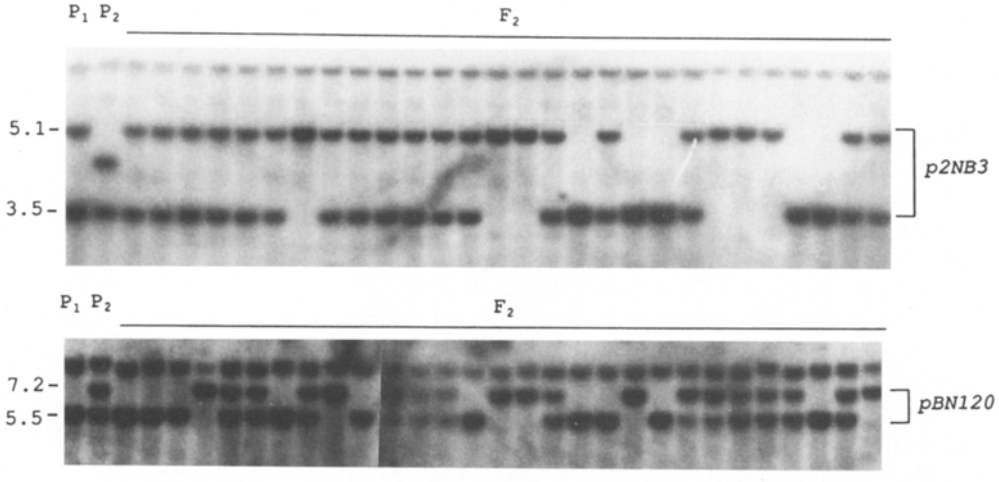

Fig. 2 Hybridization patterns for probes p2NB3 and pBN120 on the F_2 *B. nigra* population and its parents (P_1 and P_2). Although these probes were segregating for a single locus, additional monomorphic bands were detected indicating possible duplications. Sizes of the segregating DNA fragments are in kb

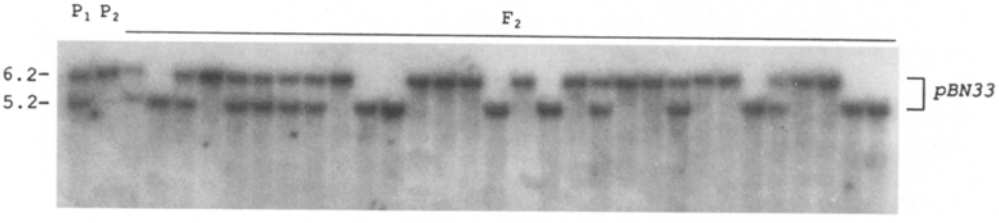

Fig. 3 Hybridization pattern for clone pBN33 disclosing a single-copy locus on the F_2 *B. nigra* population and its parents (P_1 and P_2). Sizes of the segregating fragments are in kb

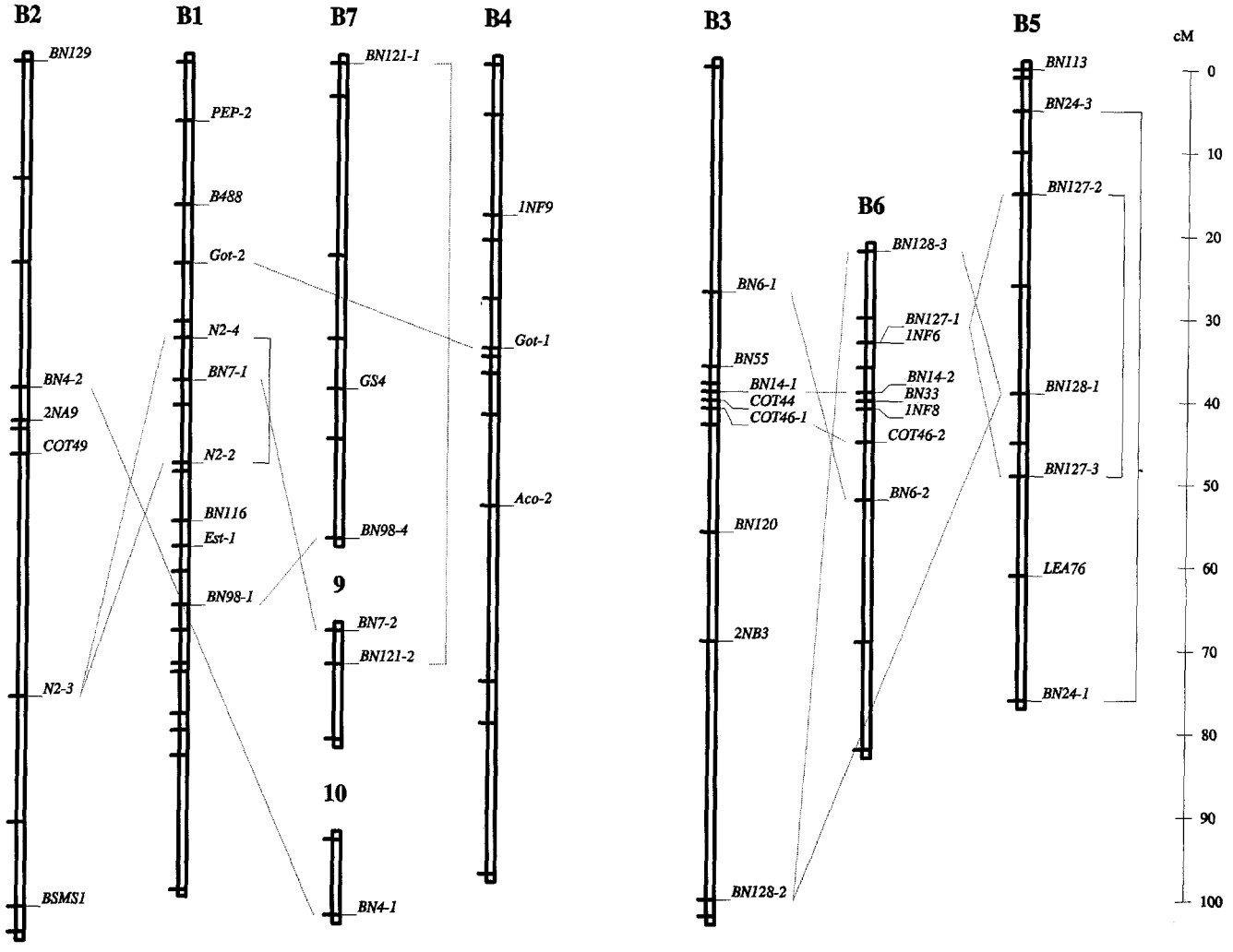

Fig. 4 Distribution of duplicated loci on the linkage groups. *Brackets* designate duplicated loci located in the same linkage group. *Dashed lines* are between duplicated loci located in different linkage groups

RAPDs

Polymorphism

Only the two parents and the F_1 hybrid were surveyed for polymorphism of the RAPD markers. From a total of 100 primers assayed, 58 were excluded because they either lacked polymorphism or the amplification pattern was irreproducible. Thus, we selected 42 useful primers that disclosed 85 polymorphic loci (Fig. 5).

Almost all of the loci revealed by RAPDs were dominant, segregating for the presence or absence of a specific band in a 3 to 1 ratio. For only 3 loci (*A05-1185*, *A16-835*, and *E05-2450*) could we identify 2 bands that were segregating as codominant alleles in a single locus (Fig. 5). For these 3 loci the 2 segregating bands fit a 1:2:1 segregation ratio and no double-null phenotypes were encountered. Additionally, when the 2 bands were scored as independent loci segregating in a 3:1 ratio and checked for linkage, a tight linkage relationship was always found. Allelic relationship was confirmed by homology in Southern blot hybridization only for the 2 bands segregating in *A05-1185*. Significant distortions of the segregation ratio ($P \leq 0.05$) were found in 11.7% of RAPD loci (Table 1). These markers were distributed in 5 linkage groups (B1, B3, B4, B5 and 11). Their location on the map had no relation with any distorted RFLP marker, except in the case of *BN113* and *C08-900*, and *D16-2000* and *BN128-1*. *BN113* and *C08-900* were located at one end of linkage group B5, separated by 1 cM, and *D16-2000* and *BN128-1*, also in linkage group B5, were separated by 6 cM (Fig. 1).

Number of amplified loci

Twenty-six (62%) of the primers revealed more than one polymorphic locus, 14 (54%), 7 (27%), 2 (8%), and 3 (11%) resulted in the amplification of 2 and 5 loci, respectively.

Linkage map

The resulting linkage map consisted of 136 molecular markers (Fig. 1). Of these markers, 122 (90%) were linked on 11 linkage groups, with a total distance of

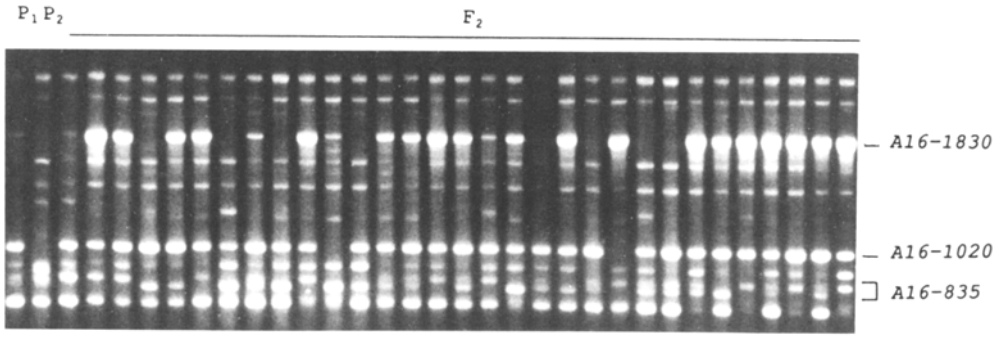

Fig. 5 Amplification pattern for primer A16 in the F_2 *B. nigra* population and parental plants (P_1 and P_2). Loci *A16-1830* and *A16-1020* segregated as independently dominant markers in a 3:1 ratio. Locus *A16-835* segregated 1:2:1 for two codominant bands

677 cM and an average map density of 5.5 cM per marker. The range of the distances was from 0 to 31 cM. Of the 11 linkage groups, 8 contained the majority of markers (94%), which tallies with the number of chromosomes in *B. nigra*.

Initially, linkage groups B4, B6, and B7 formed part of the same linkage group when using the linkage criteria of ≤ 0.3 for the recombination frequency and a log likelihood (LOD) of ≥ 3.8. After ordering the markers within this large linkage group, two gaps of more than 30 cM were encountered; therefore, we split the linkage group into these 3 separated linkage groups, each containing only markers with adjacent distances of less than 30 cM.

Assignment of linkage groups to chromosomes

Seven of the probes we used in the construction of the linkage map gave fragments that were located on the chromosomes of the addition line *B. napus-nigra* (Chevre et al. 1991; This 1992); these were pB488, pB850, pBN6, pBN7, pBN14, pBN120, and pBN128. Of these, only pBN128 gave a RFLP fragment the same size as those that were segregating in the F_2 population used for constructing the linkage map. This fragment, located on chromosome 6 (This 1992), was similar to *BN128-2* mapped in linkage group B3 (Table 2). The rest of the markers gave fragments that were either of different sizes from those segregating in the F_2 population or were generated with a different restriction enzymes; thus, no comparison was possible because of the multiple band patterns disclosed by these clones.

Two RAPD markers, amplified by primers A05 and E12, segregating in the F_2 population and present in the addition lines, permitted the assignment of linkage group B3 to chromosome 5 and B4 to chromosome 2 (Table 2). The homology of these bands was confirmed by hybridization. Two other RAPD markers, *B10-1800* and *C13-750* confirmed the location of B4 on chromosome 2.

Table 2 Assignment of linkage groups to chromosomes using addition lines

Marker	Linkage group	Addition lines	
		B. napus-B. nigra	*B. oleracea-B. nigra*
BN128-2	B3	6	
A05-1175	B3	5	5
E12-475	B4		2
B10-1800	B4	2	2
C13-750	B4	2	
C16-1300[a]	B1		1

[a] This marker was identified by A. M. Chevre

Discussion

The final map linked 124 molecular markers in 11 groups. Of the groups, 8 had the majority of the markers, probably corresponding to the 8 chromosomes in *B. nigra*. Markers were distributed evenly throughout the map with some clusters that were not normally situated in distal positions.

When only RFLP analysis is considered, 62% polymorphic markers were identified in the *B. nigra* map. This value is within the range of those reported in other diploid *Brassica* species such as *B. oleracea* (Landry et al. 1992; Kianian and Quiros 1992) and *B. campestris* (McGrath and Quiros 1991; Chyi et al. 1992). In general, *Brassica* intraspecific polymorphism is high when compared with other crop species such lettuce (11.1–27.4%, Landry et al. 1987a), tomato (14%, Helentjaris et al. 1986; Bernatzky and Tanksley 1986), barley (28%, Graner et al. 1991), or sugar beet (47%, Pillen et al. 1992), but it has similar values when compared to those reported for rice (78%, McCouch et al. 1988), potato (80%, Gebhardt et al. 1989), and maize (79%, Helentjaris 1985).

Few skewed allele segregations were found in our analysis. In some cases, distorted segregations seem to be the result of genetic divergence of the parents (Durham et al. 1992; Helentjaris et al. 1986; Paterson et al. 1990; Havey and Muehlbauer 1989; Gebhardt et al. 1989; Chyi et al. 1992; Kianian and Quiros 1992). In other cases, the distorted ratios have been attributed to linkages between markers and factors under selection (Zamir and Tadmor 1986; Landry et al. 1991). In most of these cases, the markers involved were clustered and the

distortion was towards one of the parental alleles in the whole cluster, indicating selection pro or against the allele (Jarrell et al. 1992). That might be the case for markers *BN128-1*, *BN113*, and *C08-900* whose segregation was distorted towards the same parental type (female) or for markers *A16-1020* and *D13-800* in linkage group B4 that were also distorted towards the female parent.

In this study, *B. nigra* showed almost 40% duplicated loci when both RFLP and isozymes markers were considered. This value is probably underestimated since in both isozymes and RFLPs it was possible to identify additional non-polymorphic bands that probably corresponded to other monomorphic loci in the F_2 population. The three diploid cultivated species of the genus *Brassica* seem to have the same number of duplicated loci for different isoenzymatic systems (Arús et al. 1991). GOT has been reported to have at least 5 loci in *B. campestris* (Truco 1986), which raises the possibility of additional copies of GOT in *B. nigra*. Surveying different F_2 populations or increasing the number of restriction enzymes in the RFLP analysis might increase the number of duplicated loci (Kianian and Quiros 1992). The value of 40% duplicated loci in *B. nigra* is clearly within the range of the other *Brassica* species, where duplications have been frequently reported (Slocum et al. 1990; McGrath and Quiros 1991; Song et al. 1991; Kianian and Quiros 1992). The degree of gene duplication in the genus *Brassica* is high when compared with other species such as tomato (Bernatzky and Tanskley 1986), lentil (Havey and Muehlbauer 1989), *Arabidopsis thaliana* (Chang et al. 1988), rice (McCouch et al. 1988), and lettuce (Landry et al. 1987b), where mostly single-copy sequences have been found. *Brassica* species have a frequency of duplicated genes similar to the range reported for maize (Helentjaris et al. 1988) and wheat (Anderson et al. 1992), for which a polyploid origin has been postulated.

Diploid *Brassica* species are considered to be secondary polyploids (Prakash and Hinata 1980), possibly derived by the polysomy or duplication of whole chromosomes of an extinct common ancestor with six chromosomes. This ancestral genome would have given origin to the present genomes that range from $n = 7$ $n = 12$ chromosomes (Richaria 1937a, b; Robbelen 1960; Venkasteswarlu and Kamala 1971). This distribution of duplicated sequences seems to be the result of duplication followed by extensive rearrangements rather than the simple reiteration of whole chromosomes from an ancestor with six chromosomes (Richaria 1937a, b; Robbelen 1960; Venkasteswarlu and Kamala 1971). On the basis of our limited number of duplicated sequences, the chromosome corresponding to group B1 may have resulted from an exchange of chromosomes B2 and B4. B6 may also derive from B3 and B5. Linkage groups 9 and 10 were too small for any meaningful inference. These findings agree with the possible existence of an ancestral genome of at least 5 founder chromosomes.

It is interesting that *B. nigra*, the diploid cultivated species with the lowest chromosome number in the genus ($n = 8$), has a percentage of loci duplication similar to that of *B. oleracea* ($n = 9$) and *B. campestris* ($n = 10$). These three diploid cultivated species have DNA contents per diploid nuclei of 0.97–1.07 pg (468–516 Mbp) for *B. campestris*, 1.24–1.37 pg (599–662 Mbp) for *B. oleracea*, and 0.97 pg (468 Mbp) for *B. nigra* (Arumuganathan and Earle 1991). These values are similar, with a slightly larger value for *B. oleracea*; therefore, it is not surprising that these species have comparable percentages of loci duplication, regardless of chromosome numbers. Undoubtedly, the amount of genetic information in these three species is similar, although its organization and distribution on the chromosomes is different.

Duplicated sequences in *B. nigra* were organized in three different types of arrangements; scattered through the genome, organized in tandem (gene families), and organized in blocks of duplicated loci conserved in more than one linkage group. The first class of duplications may result from tandem duplications followed by dispersal of the copies (Helentjaris et al. 1988) or by breakage of DNA fragments followed by reinsertion of the fragments in regions of sequence homology (Pichersky 1990). The case of napin (tandem duplication in linkage group B1, *N2-2*, and *N2-4*) could be an example of the second type of arrangements. They are probably the result of unequal crossing-over events. In our map, *COT44* was linked to *COT46-1* in group B3 (Fig. 1). The expression of these 2 clones is coordinately regulated during seed germination and postgerminative development (Harada et al. 1988). These loci have also been reported to be linked in *B. napus* (Landry et al. 1991) and *B. oleracea* (Kianian and Quiros 1992). Duplicated sequences for the self-incompatibility and malate synthase clones have been observed in *B. oleracea* (Kianian and Quiros 1992). Since a single locus of pBOS5 and pBSMS was segregating in the *B. nigra* population, no comparison was possible with the organization reported in *B. oleracea*. *COT46*, *BN14* and *BN6*, or *BN127* and *BN128* represented the third group of duplicated sequences. Blocks of duplicated sequences in independent linkage groups may be the result of overlapping reciprocal translocations or insertional translocations (Gottlieb 1983). This would result in amphi-duplications which, if followed by selfing, would fix the new gene combinations in a homozygous condition (Pichersky 1990). This seems to happen at the initial stages of amphidiploid formation (Quiros et al. 1989). Duplication may also result from interspecific aneuploidy (Helentjaris et al. 1988). Duplications have the evolutionary advantage of allowing change in one of the sequences while the other copy of the gene remains functional (Ohno 1970). Evidence of the same type of organization has been reported in *B. oleracea* (Slocum et al. 1990; Kianian and Quiros 1992; Landry et al. 1992) and *B. campestris* (Song et al. 1991; Chyi et al. 1992).

We were able to locate 3 linkage groups into their respective chromosome using two sets of addition lines, *B. napus-B. nigra* (Chevre et al. 1991; This 1992) and *B. oleracea-B. nigra* (A.M. Chevre, personal communication). Most of the comparable markers were RAPD markers. RAPD markers are sensitive to reaction conditions, which sometimes makes repeatability difficult among laboratories (Kesseli et al. 1992). For primers A05 and E12, homology between bands amplified in the F_2 population and the addition lines was corroborated by Southern hybridization. Those comparisons would assign linkage group B3 to chromosome 5 (marker *A05-1175*) and linkage group B4 to chromosome 2 (marker *E12-475*). The ambiguous location of group B3 to chromosoem 5 (marker *A05-1175*) and chromosome

6 (*BN128-2*) may be due to the presence of sequences homologous to pBN128 in more than 1 chromosome. One additional RAPD marker (*C16-1300*) assigned chromosome 1 to B1 (A.M. Chevre, personal communication).

This map as the first linkage map reported for *B. nigra* is a initial step in the understanding of the organization and evolution of the B genome in the *Brassica* genus. Both types of molecular markers, RFLP and RAPD, proved to be useful in the construction of the linkage map in *B. nigra*. Codominant markers like RFLP were more efficient in the estimation of genetic distance, but the case of generating markers with the polymerase chain reaction was considered an advantage.

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