COMPARISON OF BRASSICA NAPUS AND B. RAPA GENOMES BASED ON RESTRICTION FRAGMENT LENGTH POLYMORPHISM MAPPING

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INTRODUCTION

Restriction fragment length polymorphism (RFLP) linkage maps are built on homology between cloned DNA sequences and restriction enzyme digested genomic DNA fragments that show length polymorphism among different genotypes. Different RFLP loci detected by the same DNA clones possess some degree of sequence homology. By changing the hybridization and/or washing conditions, high levels of sequence homology can be detected (Beltz et al. 1983), suggesting evolutionary relatedness of the detected DNA fragments. Indicative of this are many reports in the literature where heterologous DNA sequences have been used to identify and clone genes in a different plant species (e.g. Rose et al. 1987), as well as several examples of constructing RFLP linkage maps in one species using DNA libraries from another (Tanksley et al. 1988; Bonierbale et al. 1988; Hulbert et al. 1990). Similarily, sequence homology of large pieces of DNA or chromosome fragments, even entire chromosomes, can be implied from linked arrays of RFLP marker loci, if, in each array, the corresponding markers are detected by the same DNA clones.

In this paper we report the current status of RFLP linkage maps in B. napus and B. rapa constructed with a common genomic library, with special emphasis on conserved linkage of marker loci between the two genomes. This provides some insight into genome structure of these two rapeseed species, and allows comparison of RFLP data to cytogenetic features.

MATERIALS AND METHODS

Genomic DNA from leaf tissue of the cultivar "Westar" (B. napus) was digested with the restriction endonuclease PstI, and fragments of 500-3000 base pairs were cloned into the plasmid vector pGEM-7Zf(+) (Promega). The library was screened by colony hybridization (Sambrook et al. 1989) for clones containing low-copy-number-sequence rapeseed DNA.

Plant materials used for map construction are 105 F_2 plants from the cross "BR0002 x BR0019" for B. rapa, and 105 BC_1 plants from the cross "BN0011" x "BN0031" for B. napus. One restriction endonuclease (EcoRI) has been used for the mapping work. Genomic clones detecting simple banding patterns (less than 4 bands) were identified with screening blots containing digested, size-fractionated DNA samples of parental lines in both species, and those detecting polymorphism between the parental lines were then used for mapping. Several multi-copy-sequence clones were also employed. Marker loci detected by the same clone are designated on the linkage map by identical numerical code with different alphabetical suffixes (Fig. 1). For Southern hybridization, the specific activity of the radioactive probes was 1.5×10^8 cpm/µg. The washing stringency was 12.5 mM Na^+ at 65°C (Sambrook et al.

1989), which translates to 95% homology between duplexes.

Computer programs for linkage analysis and distance calculation based on maximum likelihood, as well as for map drawing, were developed at Agrigenetics Company. The total length of any linkage group is the sum of all distances between adjacent loci flanked by the terminal loci in that group.

RESULTS AND DISCUSSION

Both B. napus and B. rapa RFLP linkage maps have been constructed de novo based on a common set of genomic clones from B. napus. Of the 450 clones analyzed, about 66% detect simple banding patterns on DNA samples from parental lines in both species. One half of these low-copy-number clones also detect polymorphism in the parental combination of both species. From segregation data obtained from 130 clones used on the B. napus mapping population, an RFLP linkage map has been constructed, covering 1350 map units, distributed among 19 linkage groups with seven pairs of unassigned markers (Fig. 1b). Also, a map has been constructed in B. rapa with 1150 map units in 10 major linkage groups and 2 unassigned "segments" with data from 160 clones (Fig. 1a). That many clones detect two or more marker loci is reflected in the marker codes used on the maps.

Clones detecting two or more marker loci within each genome can be considered duplicate DNA sequences, given the blot washing stringency. Duplicate blocks of loci (Table 1) suggest common derivations for these linkage segments, supporting the hypothesis that B. rapa evolved as a member of an ascending aneuploid series through mechanisms involving chromosome duplication from a common prototype with a basic chromosome number of 6 (Prakash and Hinata 1980; Song et al. 1990). The B. napus genome is expected to contain numerous duplicate loci and duplicate linkage blocks, as it is an amphidiploid species between B. rapa and B. oleracea, whose genomes share a number of conserved linkage blocks (Osborn, personal communication). In both genomes, data are insufficient to suggest either the autosyndetic pair of chromosomes in B. rapa under haploid condition, or the six possible allosyndetic chromosome pairs between the B. rapa and the B. oleracea genomes as observed in meiosis of haploid B. napus (Prakash and Hinata 1980).

Several major conserved linkage blocks can be identified in the B. rapa and the B. napus genomes by comparing the RFLP linkage maps for the two species (Fig. 1a,b, Table 2). Among the numerous conserved linkage blocks, several cover more than half the length of their respective linkage groups. These major conserved linkage blocks could form bivalents in meiosis of the interspecific hybrid between B. napus and B. rapa (Prakash and Hinata 1980). Although the maximum number of these bivalents is 10, the two smallest linkage groups in the current B. rapa map may not be included, as the conserved region is either absent (#1) or too small (#2, Fig. 1a). The total number of common clones used for both maps is 105, of which 81 detect at least one locus in conserved areas of the two genomes. The conserved linkage blocks make up 58.5% of the total map distances in B. rapa and 54.8% in B. napus (Fig. 1b).

The higher bivalent frequency in haploids of "synthetic" compared to "natural" B. napus has been given as cytogenetic evidence that the constituent genomes in this naturally

occurring allopolyploid have undergone genetic differentiation (Prakash and Hinata 1980). The RFLP data provide molecular genetic corroboration of this theory. Viewing the B. rapa genome as a component of the B. napus genome, most major conserved linkage blocks in B. rapa either show internal marker rearrangement or contain pieces that are parts of different linkage groups in B. napus (Table 2). In the latter category, some locus duplication and deletion are evident. A great deal of joining and breakage of linkage blocks must have occurred during evolution to result in this rearrangement of marker sequence.

Of special interest is the conserved block in linkage group 7 of both maps. The linear order of the seven common loci is preserved, but the computed map distance in B. rapa is about seven times that of B. napus (Table 2). Since other conserved linkage blocks have equivalent sizes, this appears to be just a local effect. A major deletion within this conserved chromosomal segment during the evolution of B. napus may have caused this size difference.

Interspecific crosses between B. rapa and B. napus have often been employed to introduce characteristics between the two species. Since long stretches of conserved linkage between the two genomes are where allosyndetic pairing will most likely occur, they are also the most likely places where genes will be introduced between the two genomes through crossing over. Genes responsible for desirable characteristics will have a better chance of being transferred if they reside in these conserved areas. RFLP provides a means to identify such areas, to localize desirable genes and monitor their transfer between related genomes.

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Table 1. Duplicate Linkage Segments in B. rapa and B. napus Genomes

Linkage Groups Involved and Segment Sizes*						Markers Involved**
B. rapa:						
1	(0.0	- %)	2	(12.1	24.0%)	p98 - p137
1	(27.7	67.9)	3	(21.5	17.0)	p131 - p353
1	(32.0	78.4)	9	(43.1	23.6)	p447 - p353 - p1529
2	(5.5	10.9)	B	(6.7	18.4)	p137 - p404
3	(19.6	15.5)	7	(17.1	11.5)	p336 - p347
5	(9.0	8.4)	7	(9.6	6.4)	p290 - p1555
5	(15.4	14.4)	10	(22.5	13.3)	p290 - p1755
8	(4.3	3.0)	9	(4.1	2.3)	p1529 - p1579
B. napus:						
2	(22.4	41.8)	9	(12.7	21.2)	p226 - p447
4	(11.3	8.5)	5	(13.4	7.1)	p236 - p306
4	(22.6	16.9)	5	(12.2	58.7)	p350 - p1587
5	(0.0	-)	7	(1.9	9.1)	p329 - p1735
6	(7.6	15.1)	13	(17.7	16.7)	p1588 - p1759
10	(2.8	3.6)	A	(1.4	100.0)	p499 - p573
11	(27.6	23.9)	12	(27.1	57.5)	p1582-p1526-p409-p1587
16	(0.0	-)	E	(19.8	100.0)	p268 - p334
18	(29.3	100.0)	19	(25.4	100.0)	p376 - p1705
C	(4.9	100.0)	D	(6.9	100.0)	p434 - p1588

*Total distance covered by the loci involved and its ratio to the whole linkage group are shown in parentheses following linkage group number.
**Only two or more markers involved in duplicate linkage blocks within reliable distance (30 map units) are listed.

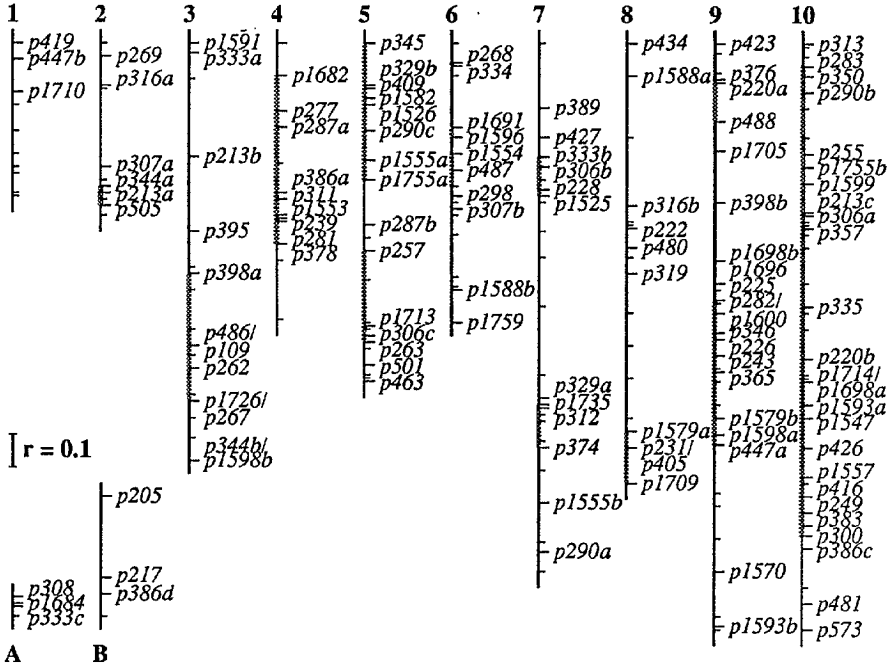

Fig. 1a. Brassica rapa RFLP Linkage Map Showing Conserved Linkage
(Indicated by shaded lines)

Table 2. Conserved Linkage Segments between B. rapa and B. napus Genomes

Linkage Groups Involved and Segment Sizes						Number of Loci	Order of Markers**
B. rapa		Size		B. napus	Size*		
2	(6.7	12.5%)	8	(22.7	58.8%)	4	rearranged
3	(105.8	83.6)	1	(51.0	42.4)	8	rearranged
4	(51.1	60.9)	14	(75.6	87.0)	9	rearranged
5	(24.5	22.7)	11	(31.1	26.9)	4	preserved
5	(25.5	23.6)	12	(10.4	22.1)	4	preserved
5	(33.1	30.7)	11	(10.4	9.0)	2	-
5	(25.1	23.3)	5	(20.8	11.6)	2	-
6	(37.2	48.2)	16	(40.6	100.0)	3	preserved
6	(37.2	48.2)	E	(19.8	100.0)	2	-
6	(3.0	3.9)	3	(11.3	30.6)	2	-
6	(44.3	57.4)	13	(34.4	32.5)	3	preserved
7	(83.7	54.3)	7	(12.2	58.7)	7	preserved
8	(73.1	50.5)	10	(77.5	100.0)	5	rearranged
9	(23.3	12.6)	18	(29.3	100.0)	2	-
9	(23.3	12.6)	19	(35.9	62.0)	3	preserved
9	(43.4	23.4)	2	(53.6	100.0)	7	preserved
9	(71.4	38.5)	9	(45.4	75.8)	4	preserved
10	(114.1	64.0)	4	(108.4	81.1)	11	rearranged
10	(28.4	15.9)	5	(37.1	19.6)	5	rearranged
10	(31.8	17.8)	15	(42.4	100.0)	2	-

*Total distance (in map units) covered by the loci involved and its ratio to the whole linkage group shown.

**Minor discrepancies in the order of tightly linked loci were taken as sampling error present in the segregation data.

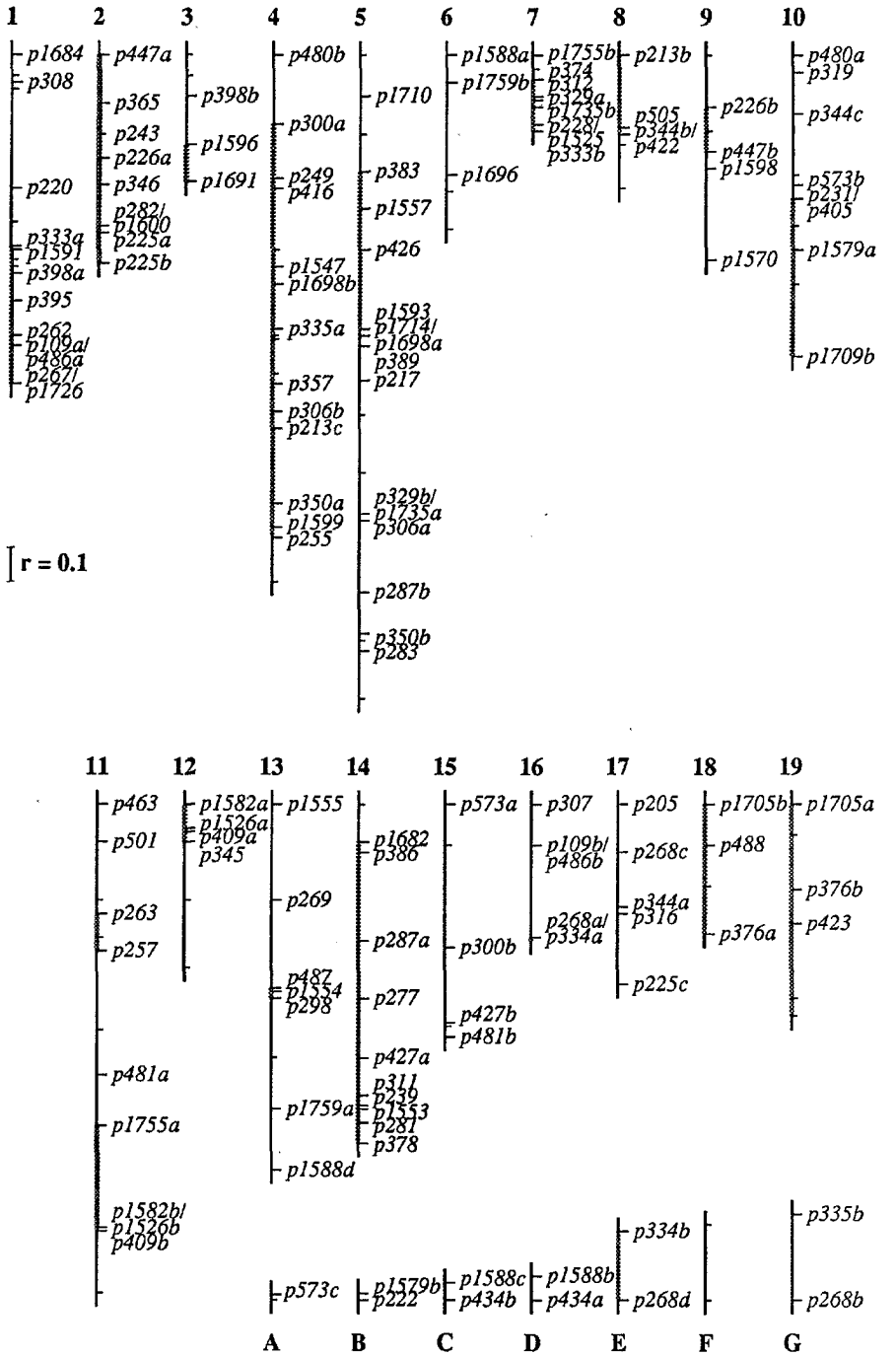

Fig. 1b. Brassica napus RFLP Linkage Map Showing Conserved Linkage
(Indicated by shaded lines)