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Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species

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Abstract Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers are being used widely for evaluating genetic relationships of crop germplasm. Differences in the properties of these two markers could result in different estimates of genetic relationships among some accessions. Nuclear RFLP markers detected by genomic DNA and cDNA clones and RAPD markers were compared for evaluating genetic relationships among 18 accessions from six cultivated *Brassica* species and one accession from *Raphanus sativus*. Based on comparisons of genetic-similarity matrices and cophenetic values, RAPD markers were very similar to RFLP markers for estimating intraspecific genetic relationships; however, the two marker types gave different results for interspecific genetic relationships. The presence of amplified mitochondrial and chloroplast DNA fragments in the RAPD data set did not appear to account for differences in RAPD- and RFLP-based dendrograms. However, hybridization tests of RAPD fragments with similar molecular weights demonstrated that some fragments, scored as identical, were not homologous. In all these cases, the differences occurred at the interspecific level. Our results suggest that RAPD data may be less reliable than RFLP data when estimating genetic relationships of accessions from more than one species.

Key words DNA · RFLP · RAPD · *Brassica* · Genetic relationships

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Introduction

Information on genetic relationships among accessions within and between species has several important applications for crop improvement. In plant-breeding programs, estimates of genetic relationships can be useful for organizing germplasm, for the identification of cultivars, assisting in the selection of parents for hybridization, and reducing the number of accessions needed to ensure sampling a broad range of genetic variability. Genetic distance estimates might also be useful for identifying heterotic groups for crops in which this information is important but not currently available. Molecular markers, such as random amplified polymorphic DNAs (RAPDs) and restriction fragment length polymorphisms (RFLPs), provide an efficient method of estimating genetic relationships among genotypes from any organism. These markers can be detected readily throughout plant genomes, they provide discrete data that are less ambiguous than many other types of data, and they can be readily used for statistical analysis.

RFLPs are caused by DNA rearrangements, such as insertions and deletions, or point mutations, and are detected as differences in the lengths of homologous restriction fragments following hybridization of genomic DNA to a labeled probe. RFLP analyses have been used to determine genetic differences among U.S. maize hybrids (Smith and Smith 1991), and among cultivars of *Cucumis melo* (Neuhausen 1992) and *Brassica napus* (Diers and Osborn 1993). They have also been used in phylogenetic and taxonomic studies (Song et al. 1988, 1990; Miller and Tanksley 1990). Genetic-distance estimates based on RFLP data have been employed to assess the relationship between genetic diversity and heterosis in maize (Lee et al. 1989; Melchinger et al. 1990; Smith et al. 1990) and in alfalfa (Kidwell et al. 1993).

RAPD markers (Williams et al. 1990) are based on amplification of DNA by the polymerase chain reaction (PCR) using primers homologous to random target sites in the genome, a method also known as arbitrarily-primed PCR

(Welsh and McClelland 1990). RAPD markers are simpler to detect than RFLPs, requiring only PCR amplification and electrophoresis to generate data. In addition to their ease of detection, RAPDs provide markers in genomic regions that are not accessible to RFLP probes due to the presence of repetitive sequences (Williams et al. 1990). The disadvantages of RAPD markers for phylogenetic studies are that the genomic origin (nuclear or cytoplasmic) of fragments, and the sequence homology of bands with similar mobility in a gel, are not known. Based on studies in soybean (Williams et al. 1990), rice (Welsh and McClelland 1990) and tomato (Klein-Lankhorst et al. 1991), authors have concluded that RAPD markers were appropriate for mapping, as well as DNA fingerprinting and population genetic studies. In cruciferous species, as few as three random primers generated enough information to distinguish between cultivars of broccoli and cauliflower (Hu and Quiros 1991) and, from comparisons of species in the genera *Brassica*, *Raphanus* and *Sinapsis*, Demeke et al. (1992) concluded that RAPD markers might be useful for taxonomic studies.

Although the efficiency of RFLP and RAPD technologies for determining genetic relationships of organisms has been discussed, estimates of genetic relationships based on these two marker types have not been compared. We have, therefore, conducted a study to compare the use of RAPD markers and RFLP markers detected by either genomic DNA or cDNA clones for estimating genetic relationships among accessions within and between cruciferous species. The objectives of this study were to determine whether genetic relationships of these accessions differed based on analyses with the two molecular marker types and to determine the possible causes for any observed differences.

Materials and methods

Plant materials and DNA isolation

Eighteen accessions, representing six cultivated *Brassica* species and one accession of *Raphanus sativus*, were selected for this study (Table 1). *B. oleracea* (diploid species, n=9, genome C), *B. rapa* (diploid species, n=10, genome A) and *B. napus* (amphidiploid species, n=19, genome AC) were represented by four accessions having a range of diversity within each species and one F_1 hybrid between two of the four accessions. *B. carinata* (amphidiploid species, n=17, genome BC), *B. nigra* (diploid species, n=8, genome B) and *B. juncea* (amphidiploid species, n=18, genome AB) were each represented by one accession. Leaves were harvested from single plants or from a bulk of 24 plants of each accession (Table 1). Tissue samples were lyophilized and then stored at –20°C. DNAs were extracted as described by Murray and Thompson (1980).

RFLP analyses

Total genomic DNA of each accession was digested independently with *Eco*RI and *Hin*dIII (Promega, Madison, Wis.). RFLPs were detected as described by Osborn et al. (1987) with the following modifications: nylon membranes (NYTRAN^R from Schleicher and Schuell, Keene, N.H.; or Magna NT from MSI, Westboro, Mass.) were used for Southern blotting (Southern 1975), DNA clones were labeled with α-^{32}P dCTP by random priming (Feinberg and Vogelstein 1983) and the final wash conditions ranged from 0.2 to 0.5×SSC at 60 °C depending on how many times the blots had been re-used. Probes were removed from the membranes after each use by incubation in 0.2 N NaOH for 20 min at room temperature followed by neutralization in 0.5 M Tris (pH 7.5), 0.1 × SSC and 0.1% SDS for 20 min.

The DNA clones used for hybridization were selected from a genomic DNA library, detecting gRFLPs, and a cDNA library, detecting cRFLPs. The genomic DNA library was constructed using total genomic DNA extracted from *B. napus* cv Westar and digested with *Pst*I. Fragments between 0.5 and 2.0 kb were isolated from an agarose gel and cloned into a *Pst*I-digested and dephosphorylated pBluescript SK$^-$ vector (Stratagene, La Jolla, Calif.) and transformed into *E. coli* DH5α as described by Maniatis et al. (1978). Clones were screened by in-situ hybridization (Sambrook et al. 1989) using total genomic DNA from Westar, a mixture of mitochondrial DNAs from *B. rapa*, *B. nigra* and *R. sativus* (provided by C. Makaroff, University of Michigan), and orchid chloroplast DNA (provided by J. Palmer, Indiana University) as probe in order to select clones containing low-copy-number nuclear DNA sequences.

The cDNA library was constructed using RNA extracted from etiolated seedlings of *B. napus* cv Westar. Total RNA was isolated by the phenol-chloroform method as described by Reid et al. (1988). RNA was further purified by adding CsCl to a final concentration of 0.5 M, then pipetting the samples onto a 9.4 ml cushion of 5.7 M CsCl and centrifuging as described by Maniatis et al. (1978) using a Beckman SW41 Ti rotor; mRNA was isolated using an oligo(dT)-cellulose column as described by Perbal (1984). cDNA was synthesized as described in the BRL cDNA synthesis instruction manual (BRL, Grand Island, N.Y.) and column-fractionated with P60 Biogel (Bio-Rad Laboratories, Hercules, Calif.). After precipitation of cDNA with isopropanol, *Eco*RI linker adapters were ligated as described by Promega's 1989/90 protocols and applications (Promega, Madison, Wis.). Unligated adapters were removed using the Geneclean Kit (Bio 101, La Jolla, Calif.). cDNA fragments were ligated into *Eco*RI-digested and dephosphorylated pBluescript SK$^-$. Recombinant plasmids were electrotransformed into *E. coli* DH5α according to the Bacterial Electro-transformation and Gene Pulse Controller Instruction Manual version 2.89 (Bio-Rad Laboratories, Hercules, Calif.). cDNA clones were screened by in-situ hybridization with radiolabeled mRNA according to Sambrook et al. (1989) in order to select clones of low-abundant mRNA sequences.

RAPD analysis

RAPD analyses were performed using 41 primers obtained from J. Carlson, University of British Columbia (primers UBC-101 to 200), and from Operon Technologies Inc., Alameda, Calif. (kit D). Reactions were performed in 10-µl volumes containing 50 mM Tris, pH 8.5, 1 mM $MgCl_2$, 20 mM KCl, 500 mg/ml BSA, 2.5% Ficoll 400, 0.02% Xylene cyanole, 1 unit *Taq* polymerase, 50 ng template DNA, 1 µM primer, and 0.2 m*M* dNTP (50 µM of each deoxynucleotide). A negative control, containing water instead of template, was included in each reaction set. The reactions were loaded and sealed in capillary tubes and placed in a 1605 Air Thermo-Cycler^{tm} (Idaho Technology Inc., Idaho Falls, Idaho). DNA was amplified using a cycling profile of 3 min at 93°C followed by ten cycles of 7 s at 91°C, 25 s at 42°C and 60 s at 71°C, then 40 cycles of 7 s at 91°C, 10 s at 42°C and 60 s at 71°C. After all cycles were completed, the reactions were held at 72°C for 5 min.

Following amplification, the reactions were loaded into a 2% agarose gel prepared in 1× TAE buffer (Sambrook et al. 1989) containing 0.65 µg/ml of ethidium bromide. The amplification products were electrophoresed for 5 to 6 h at 60 V. For each primer, two sets of 20 reactions were performed using DNAs of each of the 19 accessions as template and one negative control (no template). The two replicates of each experiment were conducted at different times using similar reaction conditions.

The electrophoretically-separated RAPD products of 14 primers were Southern blotted and sequentially probed with α-^{32}P-dCTP-labeled chloroplast DNA from orchid, a mixture of *Brassica* and *Raphanus* mitochondrial DNAs, and 15 RAPD fragments selected for different staining intensities. These 15 bands were excised individual-

Table 1 Description of plant materials from *Brassica* and *Raphanus* species used for RFLP and RAPD analysis

Code	Cultivar or accession	Cultivar group or subspecies[a]	Population type[b]	No. of indiv.[c]
Brassica oleracea (C genome)				
1	BI-16	capitata (cabbage)	Inbred	1
2	OSU CR-7	botrytis (broccoli)	Inbred	1
3	F_1 (BI-16 × CR-7)	na	Hybrid	1
4	Bohmerwaldkhol	capitata (cabbage)	OP	24
5	Penca da Povoa	tronchuda (Portug. cab.)	OP	24
Brassica rapa (A genome)				
6	BLC 198	*oleifera* (turnip rape)	OP	24
7	R-500	*trilocularis* (sarson)	OP	24
8	F_1 (Per × R-500)	na	Hybrid	1
9	Per	*oleifera* (turnip rape)	OP	24
10	Flowering Pakchoi	*chinensis* (pakchoi)	OP	24
Brassica napus (AC genome)				
11	Major	*oleifera* (winter oil rape)	OP	24
12	Stellar	*oleifera* (spring oil rape)	DH	1
13	F_1 (Major × Stellar)	*oleifera* (na)	Hybrid	24
14	Quinta	*oleifera* (winter oil rape)	OP	24
15	Hero	*oleifera* (spring oil rape)	OP	24
Brassica nigra (B genome)				
16	WPBS	(black mustard)	OP	24
Brassica carinata (BC genome)				
17	Tex-Sel	(abyssinian cabbage)	OP	24
Brassica juncea (AB genome)				
18	Southern Giant Curl	(leaf mustard)	OP	24
Raphanus sativus				
19	Misato Rose Flesh	(radish)	OP	24

[a] Cultivar groups are listed for *B. oleracea*, subspecies are listed in italics, common names are in parenthesis; na, not applicable because accession is an intergroup hybrid

[b] OP, open pollinated cultivar; DH, doubled haploid

[c] Number of individuals refers to the number of plants used (single or bulk) for DNA extraction

ly from replicate gels and the agarose pieces containing the fragments were melted at 100 °C for 3 to 5 minutes. A small fraction (depending on the intensity of the band contained in the melted sample) was transferred to a microfuge tube and labeled with ^{32}P dCTP as described by Feinberg and Vogelstein (1983), then used as a probe on Southern blots of replicate gels containing RAPD products. The final wash conditions ranged from 0.2 to 2×SSC at 60°C. These probes were also used on Southern blots containing total genomic DNA of the 19 accessions that were digested with either *Eco*RI or *Hin*dIII.

Data collection and analyses

Only RAPD fragments less than 2 kb that were present in both replicates were scored. Bands with similar mobility to those detected in the negative control were not scored. Staining-intensity differences of identical size fragments among individuals may be caused by competition with other sequences within genotypes for a specific primer. Therefore, bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. This was done in order to avoid scoring fragments as identical when they were actually of different sizes. RFLP fragments less than 6 kb were scored, and bands with the same molecular weight were considered to be identical fragments, disregarding hybridization-intensity differences.

The fragment data were entered in a computer file as binary matrices where 0 coded for absence of a band and 1 coded for presence of a band. In order to determine the number of bands necessary to obtain consistent estimates of genetic relationships among the accessions studied, the coefficients of variation were estimated by bootstrap analysis (Efron and Tibshirani 1986). For each data set (gRFLP, cRFLP and RAPD), 200 new matrices (sub-samples) were randomly constructed with sampling sizes ranging from four to the total number of probe/enzyme or primer complexes (sampling unit) available for each marker type. The genetic relationships for all pairwise combinations in each sub-sample were estimated using the complement to the simple matching coefficient (Gower 1985). The overall mean genetic relationships and associated variance were obtained for each accession among all 200 matrices. The mean coefficient of variation was calculated for each sample size and plotted against the mean number of bands included per sampling unit (Tivang 1992).

Additional data analyses were conducted using NTSYS-pc, version 1.70 (Exeter Software, Setauket, N.Y.). Similarities between accessions were estimated using Jaccard and Dice coefficients of similarity (Rohlf 1992), and the resulting pairwise similarities were expressed as distance matrices. Similarity matrices based on different estimators (Jaccard and Dice) and different marker types (RAPD, gRFLP and cRFLP) were compared using the Mantel matrix-correspondence test (Mantel 1967; NTSYS-pc instruction manual). Cluster analyses were conducted on similarity estimates using the unweighted pair-group method, arithmetic average (UPGMA) and the resulting clusters were expressed as dendrograms. In order to obtain estimates of the magnitudes of differences among dendrograms, cophenetic values were computed for each dendrogram followed by the construction of a cophenetic matrix for each marker type. The cophenetic matrices were compared using the Mantel matrix-correspondence test.

Results and discussion

Characteristics of RFLP markers

Two types of clones, genomic DNA and cDNA, were used as probes to detect RFLPs. The genetic difference between these two types of probes is that gDNA clones, theoretically, can detect polymorphisms in DNA sequences anywhere in any of the plant genomes, while cDNA clones de-

tect polymorphisms only in nuclear sequences that are homologous to expressed genes whose transcripts are represented in the cDNA library. All gDNA clones used in this study were pre-screened to select low-copy-number, nuclear DNA sequences. cDNA clones were assumed to detect only nuclear DNA sequences. For gDNA clones, only autoradiographs from clones that hybridized to low-copy-number and polymorphic restriction fragments were scored. A total of 482 fragments were scored for 76 gDNA clones using two restriction enzymes (Table 2). The same criteria were used to select and score fragments on autoradiographs from membranes probed with cDNA clones. A total of 364 fragments were scored for 49 cDNA clones using two restriction enzymes. On average, more polymorphic fragments were scored per cDNA clone than per gDNA clone (Table 2).

Table 2 Number of primers and DNA clones used, total and average number of bands scored for each probe type, and the number of organelle DNA bands identified among 18 accessions of *Brassica* and one accession of *Raphanus*

Probe type	Probes (total)	Bands (total)	Bands/ probe	Organelle DNA detected[a]	
				mtDNA	cpDNA
Primers	41	432	10.54	7 (4.9%)	1 (0.2%)
gDNA clones	76	482[b]	6.34[b]	0	0
cDNA clones	49	364[b]	7.43[b]	–	–

[a] Sample tested: 14 primers (142 bands) and 76 gDNA clones. cDNA clones were assumed to be nuclear sequences
[b] Number of bands scored for two restriction enzymes (*Eco*RI and *Hin*dIII)

Characteristics of RAPD markers

The DNAs of the 19 accessions used for RFLPs analyses were amplified using 41 10-base oligonucleotides as primers to generate RAPD markers. RAPD markers from the 19 accessions amplified with primer UBC-180 are shown in Fig. 1. This primer amplified fragments that were polymorphic between and within species, as well as fragments associated with specific genomes. For example, some fragments of identical size were amplified only for species containing the C genome, *B. oleracea* (Fig. 1, lanes 1–5), *B. napus* (Fig. 1, lanes 11–15) and *B. carinata* (Fig. 1, lane 17), and others only in species containing the B genome, *B. nigra* (Fig. 1, lane 16), *B. carinata* (Fig. 1, lane 17) and *B. juncea* (Fig. 1, lane 18).

Since total genomic DNAs were used as templates, the genomic origin of the RAPD fragments amplified in these accessions is not immediately apparent. Southern blots of RAPD products from 14 primers containing 142 scored fragments were sequentially hybridized with ^{32}P-labeled mitochondrial and chloroplasts DNAs. The results indicated that among the 142 scored bands, seven were homologous to mitochondrial sequences, whereas only one band was homologous to chloroplast sequences. On average, more bands could be scored per primer than per gDNA or cDNA clone (Table 2).

The amplification of total genomic DNA with random primers produced fragments with different staining intensities within and between genotypes (see Fig. 1). Intensity differences may be associated with the degree of homology between the primer and a given priming site on the template DNA or the number of amplified sequences in the plant genome. In order to investigate the cause of these differences, 12 RAPD bands, nine with strong and three with moderate intensity on agarose gels stained with ethidium bromide, were excised from the gels and the fragments were hybridized to Southern blots containing total genomic DNAs from the 19 accessions, digested with either *Eco*RI or *Hin*dIII restriction enzymes. Two of the three moderate-intensity fragments hybridized to low-copy-number sequences, while the third fragment hybridized to a highly-repeated sequence, producing a smear on the autoradiograph. For the nine strong-intensity bands, three hybridized to highly-repeated sequences and the other six hybridized to low-copy-number sequences (data not shown). These results suggest that RAPD marker intensity is not associated with the genomic copy number of the amplified sequences; however, it might be associated with the degree of homology between primer and template or with the amplification of other fragments in the sample.

Comparison of genetic relationship estimates

An important question in any numerical taxonomic study is what number of characters is required to obtain a stable classification of genotypes (Sneath and Sokal 1973). In the present study, the number of characters (i.e., the number of bands) necessary to obtain a stable classification of all accessions was estimated using bootstrap analysis. The mean coefficient of variation decreased as more bands were added to the data set (Fig. 2). Natural-log transformation of the scales resulted in a linear relationship between mean CVs and sample size (data not shown). The number of bands required for a mean CV of 10% was 327, 294, and 288 for RAPD, gRFLP and cRFLP, respectively (Fig. 2). These estimates were obtained by using the complement of the simple matching coefficient (Gower 1985) as the estimator of genetic relationships. This estimator was selected because Jaccard's estimator is not well suited for this type of comparison (Tivang 1992). It is important to realize that the number of bands giving a particular CV may vary in other comparisons, since it might depend on the nature of the genotypes analyzed in each study. Although RAPDs required a greater number of bands than RFLPs in order to reach a 10% CV, this level of precision was obtained with fewer primers than RFLP probes, since more bands were scored per primer than per RFLP probe (Table 2).

The similarity matrices obtained using Jaccard and Dice coefficients for each marker type were compared by the Mantel test statistics for matrix comparison. Mantel (1967) developed a test for matrix correspondence that takes two matrices and plots one against the other, element by ele-

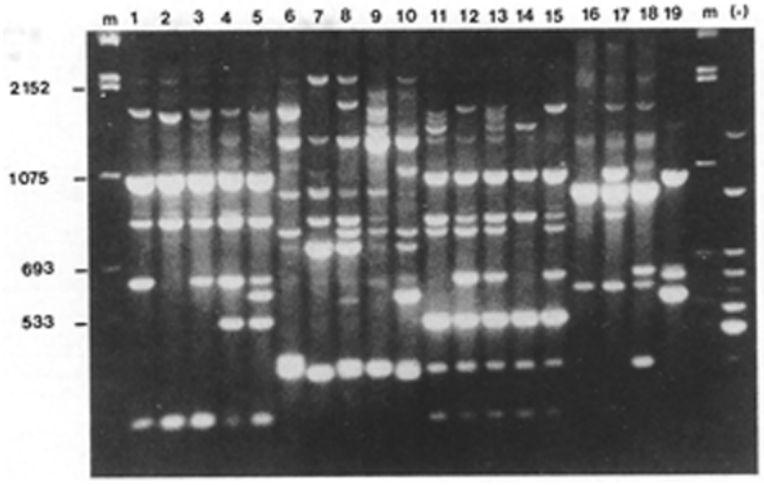

Fig. 1 DNAs of 19 accessions amplified by primer UBC-180. *Lane m* contains λ DNA digested with *DraI* and the sizes of fragments in base pairs are on the left. *Lanes 1–19* contain RAPD products from DNA templates listed in the same order as in Table 1. *Lane (–)* contains the negative control. Since no template was added to the negative control, amplification products were assumed to result from contaminant DNA in the *Taq* polymerase

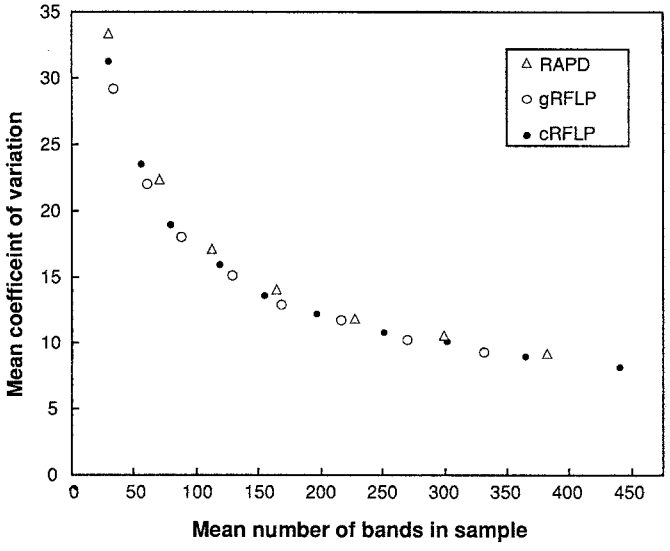

Fig. 2 Plot of the mean coefficient of variation of the complement of simple matching coefficient among all accessions estimated by bootstrap analysis for subsamples with different numbers of probes (primers for RAPDs or clones for RFLPs). The result was expressed as the mean number of bands. The number of bands necessary to obtain a mean CV of 10% was 327, 294 and 288 for RAPDs, gRFLPs and cRFLPs, respectively. These values correspond to 32, 47 and 39 probes, respectively

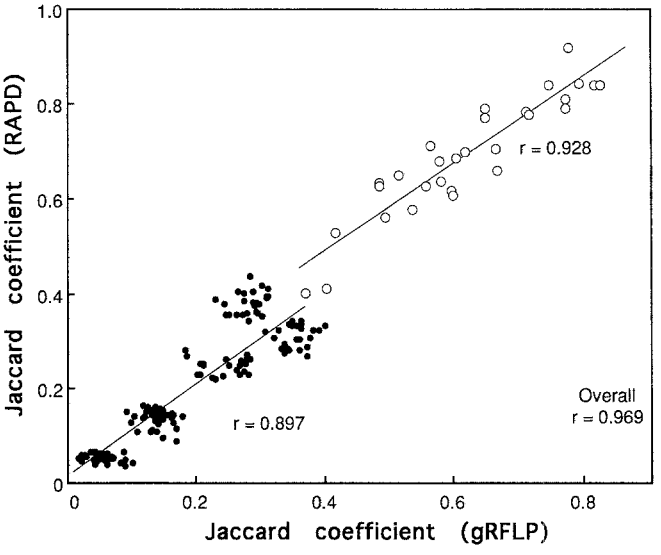

Fig. 3 Plot of genetic-similarity estimates based on RAPD and gRFLP data sets. Each dot in the graph corresponds to a pairwise genetic-similarity estimate calculated using Jaccard's coefficient. ●, interspecific comparisons; ○, intraspecific comparisons. These values were used in the Mantel test for matrix correlation

ment (the diagonal elements are not computed in the correlation). This test gives the product-moment correlation, r, and a statistic test, Z, to measure the degree of relationship between two matrices. Rohlf (1990) suggests that the degree of fit can be interpreted subjectively as follows: $r \geq 0.9$, very-good fit; $0.8 \leq r < 0.9$, good fit; $0.7 \leq r < 0.8$, poor fit; $r < 0.7$, very-poor fit. The matrices generated by Jaccard and Dice coefficients of similarity were highly correlated (r=0.984 for RAPD data, 0.988 for cRFLP data, and 0.985 for gRFLP data). High correlations were also obtained when matrices based on different marker types were compared using the Jaccard coefficient of similarity (r=0.969 for RAPD vs gRFLP, see Fig. 3; r=0.949 for RAPD vs cRFLP, and r=0.965 for gRFLP vs cRFLP), suggesting that these marker types give similar estimates of genetic relationships among the accessions tested. For both portions of matrix-comparison plots corresponding to inter- or intra-specific comparisons, r values were very high (see Fig. 3).

Genetic-similarity estimates (Jaccard coefficient) based on each marker type were used for cluster analyses in order to present genetic relationships as dendrograms (Fig. 4). For all three marker types, the accessions were grouped according to their species. Similar distributions of accessions within species were detected on all three dendrograms; however, major differences were observed in the relative positions of the species to each other. In both dendrograms based on RFLP data, *B. napus* was grouped closer to *B. rapa* than to *B. oleracea;* however, in the RAPD-based dendrograms, this species was closer to *B. oleracea.*

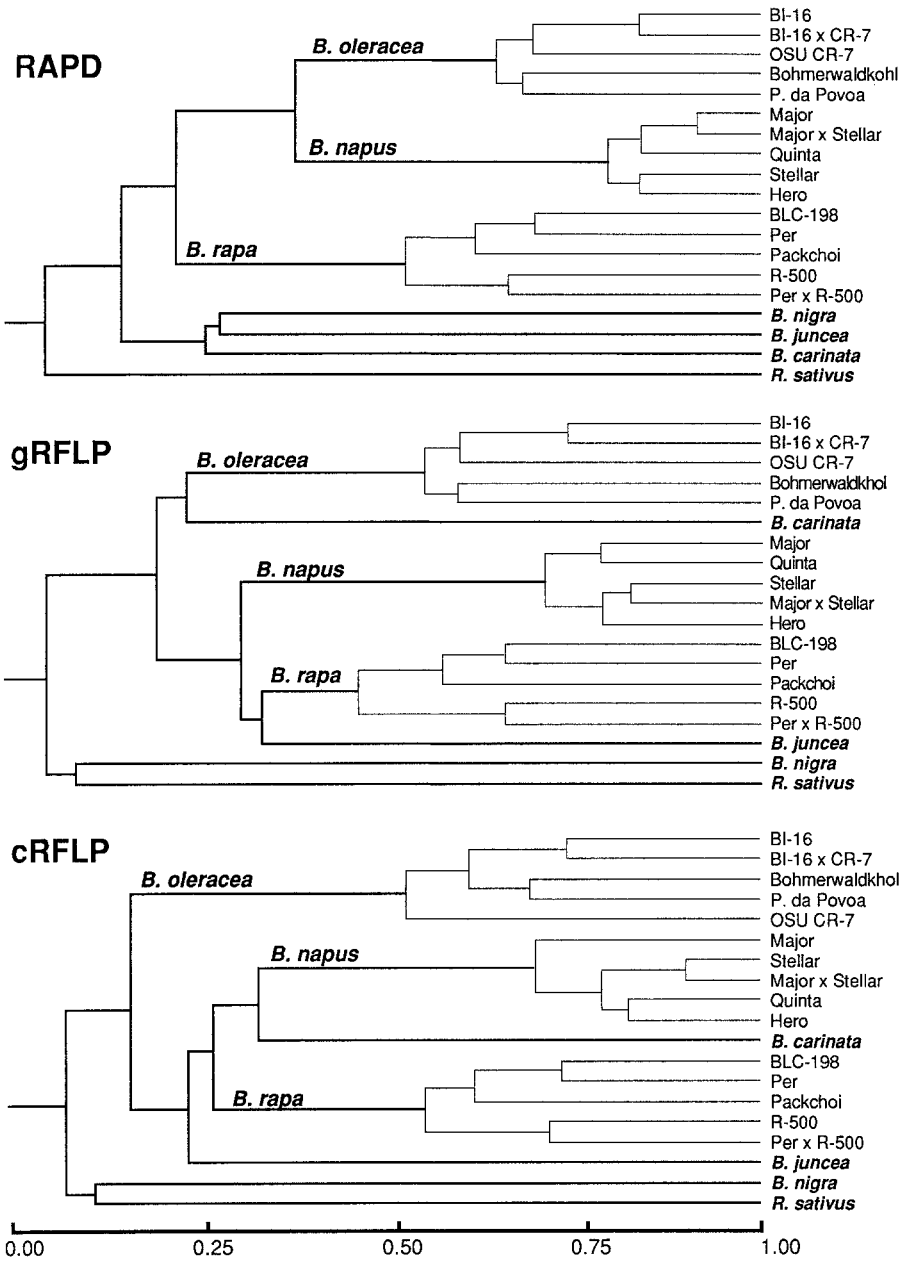

Fig. 4 Dendrograms for *Brassica* and *Raphanus* accessions based on cluster analysis (UPGMA) of genetic-similarity estimates (Jaccard's coefficient) from RAPD, gRFLP and cRFLP data

Another major difference between dendrograms was in the relative position of *B. nigra*. The dendrogram based on RAPD markers placed *B. nigra* (B genome) closest to *B. juncea (AB genome) and B. carinata* (BC genome). In the dendrograms based on RFLP markers (gRFLP and cRFLP), *B. nigra* was placed closer to *R. sativus* than to any other *Brassica* species (Fig. 4). A close relationship between *B. nigra* and *R. sativus* was also observed previously by Song et al. (1990) using nuclear RFLP markers to investigate genetic relationships among cruciferous species.

Despite these initial observations, it is quite difficult to visually determine how significant the differences are between these dendrograms. In order to estimate the similarity between dendrograms, a new set of matrices based on the cophenetic values were constructed and compared using the Mantel test for matrix correspondence. The correlation between matrices of cophenetic values from the dendrograms based on gRFLP and cRFLP was very high (r=0.930). However, when comparing the dendrograms based on cRFLP and gRFLP to the one obtained for RAPD data, lower correlations were obtained (r=0.870 and 0.877, respectively). Although these values are high, indicating good overall correspondence between dendrograms based on RAPD and RFLP data, they are lower than the correlation obtained between dendrograms based on the two types of RFLP markers. The correlation between gRFLP and cRFLP was high for both intra- and inter-specific comparisons (r=0.872 and 0.860, respectively). However, for gRFLP and RAPD data, the intraspecific correlation was high (r=0.932), while the interspecific correlations were very low (r=0.364) (see Fig. 5). Comparing the cophenetic

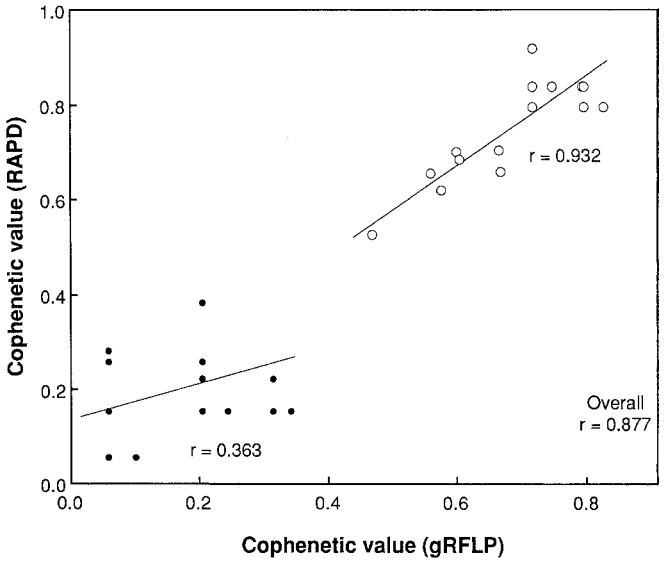

Fig. 5 Plot of the cophenetic values of dendrograms based on gRFLP and RAPD data. ●, interspecific comparisons; ○, intraspecific comparisons

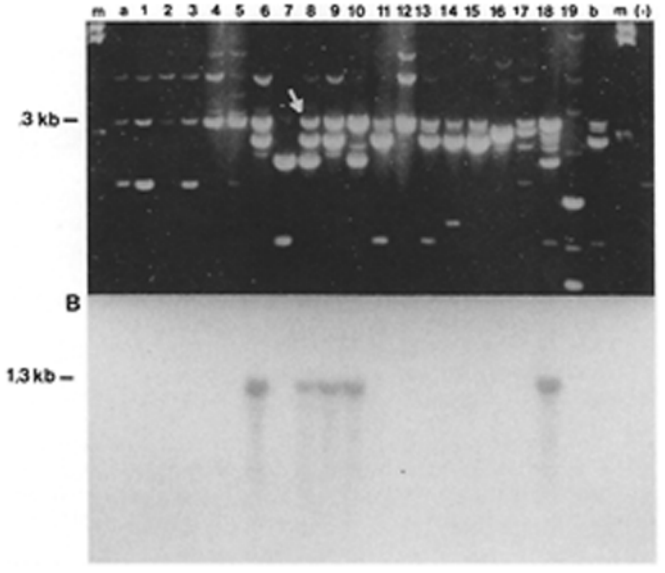

Fig. 6 A, B Homology comparison of RAPD products with similar molecular weights. **A** Agarose gel of RAPD products amplified using primer UBC-127. **B** Autoradiograph of a Southern blot of the gel in **A** following hybridization to a fragment excised from a replicate gel (*arrow* in **A**), washed with 0.2×SSC at 60°C. *Lane* **a** contains DNA from F_1 hybrid between CR-7 and BI-16; *lane* **b** contains DNA from F_1 hybrid R-500 and Per. *Lanes 1–19* contain RAPD products from DNA templates listed in the same order as in Table 1

values of RAPD and cRFLP data sets, lower correlations were obtained (r=0.754 and 0.202 for intra- and inter-specific correlations, respectively).

Since both RFLP data sets were based exclusively on nuclear genome information, while RAPDs included information from all three genomes, one hypothesis to explain differences in the clustering arrangements between marker types was the influence of organelle DNA on the RAPD results. This was investigated by constructing dendrograms using matrices from the data set generated by 14 primers that were tested for organelle DNA bands. Two dendrograms were constructed, one based on a similarity matrix from 142 fragments tested for cytoplasmic DNA fragments and another based on a matrix without the eight identified organelle fragments (Table 2). The arrangements of accessions in these two dendrograms were identical (data not shown), suggesting that the differences between dendrograms based on RAPD and RFLP markers were not due to organelle genomes.

A second hypothesis is that the differences between dendrograms based on RAPD and RFLP markers could be due to the scoring of non-homologous RAPD fragments as identical. A major difference between RAPD and RFLP markers is that RFLP bands are detected based on homology of the entire sequence of the probe with the genomic DNA, while RAPD markers require some degree of homology between only a ten-nucleotide primer and a template DNA. Even though identical-size RAPD fragments have some homology in the priming region, the internal sequences of the amplified fragments may not be homologous. In order to investigate the homology of bands with the same mobility in a gel, 15 RAPD bands, whose fragments were amplified by 14 primers, were excised from the gels, ^{32}P-labeled and hybridized to Southern blots of the replicate gels. Three RAPD probes out of 15 (20%) did not hybridize to all bands with the same mobility from all species. For all three of these RAPD probes, lack of homology was detected only between species. Within species, fragments with the same mobility in all accessions either hybridized or did not hybridize to the probes and appeared to be homologous sequences. For example, primer UBC-127 amplified a 1.3-kb fragment in all *Brassica* species but *B. nigra* (Fig. 6a). However, when this fragment from *B. rapa* was excised from the gel, labeled and used as probe on a Southern blot from the replicate gel, it hybridized only to the 1.3-kb fragments in *B. rapa* and *B. juncea* and not with fragments amplified from *B. oleracea* and *B. napus* (Fig. 6b). In order to determine the degree of non-homology between bands with the same mobility as these three fragments, the fragments were re-hybridized to their respective Southern blots followed by low-stringency washes (2× SSC at 60°C). Two of the three probes had the same pattern detected by washes in 0.2 × SSC, suggesting a complete lack of homology between fragments with similar size but from different species. The third fragment hybridized to all similar-size fragments from all species in 2 × SSC at 60°C, but was removed in 0.2 × SSC at 60°C, suggesting partial homology of this fragment to fragments with similar size from other species. Thus, RAPD bands with similar mobility may have varying degrees of homology.

The simplicity of laboratory assays for RAPD markers makes them an attractive method for obtaining intraspecific genetic information. Among the *Brassica* accessions we analyzed, estimates of intraspecific genetic relationships based on RAPD markers were similar to those based

on RFLP markers, and thus, RAPD markers may be appropriate for these types of comparisons. However, estimates of some of the interspecific relationships we analyzed based on RAPD markers were very different from those based on RFLP markers, and these differences may be caused by the non-homology of RAPD fragments scored as identical. In order to determine the homology of RAPD bands, one could probe Southern blots of the RAPD gels (as suggested by Hadrys et al. 1992), but this would be more difficult and time consuming than RFLP analyses. Our results suggest that RFLPs are more reliable than RAPDs for estimating interspecific genetic relationships. This may also be true for some intraspecific comparisons, especially those involving distantly-related accessions. Before making specific recommendations, additional studies are needed to compare RAPD and RFLP data for estimating genetic relationships among various germplasms in different taxa.

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