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Evaluation of RFLP and RAPD markers in a comparison of *Brassica napus* breeding lines

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Abstract RFLP and RAPD markers were evaluated and compared for their ability to determine genetic relationships in a set of three *B. napus* breeding lines. Using a total of 50 RFLP and 92 RAPD markers, the relatedness between the lines was determined. In total, the RFLP and the RAPD analysis revealed more than 500 and 400 bands, respectively. The relative frequencies of loci with allele differences were estimated from the band data. The RFLP and RAPD marker sets detected very similar relationships among the three lines, consistent with known pedigree data. Bootstrap analyses showed that the use of approximately 30 probes or primers would have been sufficient to achieve these relationships. This indicates that RAPD markers have the same resolving power as RFLP markers when used on exactly the same set of *B. napus* genotypes. Since RAPD markers are easier and quicker to use, these markers may be preferred in applications where the relationships between closely-related breeding lines are of interest. The use of RAPD markers in fingerprinting applications may, however, not be warranted, and this is discussed in relation to the reliability of RAPD markers.

Key words *Brassica napus* · RFLP markers · RAPD markers · Genetic distance

Introduction

The use of restriction fragment length polymorphism (RFLP) markers in plant breeding is well established (Tanksley et al. 1989). For example, the construction of RFLP linkage maps in many different species has made it possible to directly utilize molecular markers in tagging genes and quantitative-trait loci of high breeding value. The subsequent use of these markers in breeding programmes has allowed the rapid introgression of many interesting traits into new genotype backgrounds. In addition, RFLP maps have become a new tool in comparative investigations of the structure, organization and evolution of plant genomes (Tanksley et al. 1992; Whitkus et al. 1992).

Recently, a technique involving arbitrary priming of polymerase chain reactions using short oligonucleotides of random sequence has been developed (Welsh and McLelland 1990; Williams et al. 1990). These markers are termed random amplified polymorphic DNAs (RAPDs) and arbitrarily primed-PCRs (AP-PCRs), respectively. For some applications, these markers are more suitable than RFLP markers. For example, the use of RAPD markers has alleviated some of the serious limitations of the use of molecular markers in conifer species (Grattapaglia et al. 1992; Tulsieram et al. 1992). Since the RAPD assay is PCR based, and since RAPD markers are in most cases dominant, these markers are efficiently used in cases where only limited amounts of haploid tissue from the megagametophytes are available. RAPD markers are also conveniently used in large-scale screening of bulked segregant individuals (Michelmore et al. 1991). In applications such as genotype comparisons, the use of RAPD markers has several distinct advantages compared to the use of RFLP markers. No initial cloning and characterization steps are necessary, since a general set of primers is available. A RAPD assay is rapid and easy to perform, and also requires only limited amounts of DNA. Recently, within the genus *Brassica*, taxonomic data on the species relationships within the U triangle has been generated from RAPD markers (Demeke et al. 1992). Similarly, different *B. oleracea* cultivars have been compared using RAPD markers (Hu and Quiros 1991; Kresovich et al. 1992).

Serious concern regarding the reliability of RAPD markers has, however, been raised. Thormann and Osborn (1992) observed that RAPD and RFLP markers produced different results in a comparison of the genetic relationships between different *Brassica* species, whereas no differences were detected within a species. Echt et al. (1992) and Reiter et al. (1992) noted that only 76% and 57% of the polymorphic DNA

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fragments found in their mapping populations segregated as dominant Mendelian markers. Furthermore, Riedy et al. (1992) reported an excess of non-parental bands in the offspring from known primate pedigrees, whereas Heun and Helentjaris (1993) detected deviations from the expected dominant inheritance among 16 maize F_1 hybrids compared with their parental lines.

The aim of the present study has been to evaluate and compare the usefulness of RFLP and RAPD markers for determing genotype relationships in a *Brassica* breeding context. Three different related *B. napus* breeding lines with known pedigrees were seleced. These lines represent a level of similarity which is likely to be encountered in many plant breeding contexts when using molecular markers.

Materials and methods

Plant materials and DNA extraction

The three different lines were extracted directly from a *Brassica napus* breeding programme. All lines are doubled haploids of spring type. One of the lines, breeding line Sv 02288 (abbreviated as line 1), was produced by anther culture, while the remaining two lines originated as spontaneous haploids; cv Korall (line 2) and breeding line Sv 02360 (line 3). Line 2 is a selection from cv Topas and line 3 is derived from a cross between Topas and another breeding line. Consequently, these two lines are closely related. Line 1 is less related to the other two lines, but has several ancestors in common with lines 2 and 3. All lines were diploidized by colchicine treatment. Production of lines in this manner guarantees that each line is homozygous for all loci. Leaf material was harvested from 6- to 8-week-old greenhouse grown plants, and stored as freeze-dried samples. Isolation of total genomic DNA was carried out according to the method of Saghai-Maroof et al. (1984), with some modifications as described in Hjerdin et al. (1994).

RFLP analysis

Restriction endonuclease digestion, gel electrophoresis, Southern blotting, and hybridization with labelled probes were as described in Hjerdin et al. (1994). A clone collection compiled from three genomic DNA libraries was kindly supplied by Dr. Mary Slocum, NPI, Salt Lake City.

The three genomic DNA libraries were constructed using "Early White" (*B. oleracea*, EW) cauliflower,"Wisconsin Golden Acres" (*B. oleracea*, WGA) cabbage, and "WR 70 days" (*B. campestris*, WR) Chinese cabbage as sources of DNA (Figdore et al. 1988; Slocum et al. 1990). The libraries were produced from *Pst*I-digested and size-fractionated (500–2 000 bp) DNA fragments which were cloned into plasmids pUC 19 (EW clones) and pTZ18R (WGA and WR clones). The DNA clones originating from *B. oleracea* (28 clones) had RFLPs that map to at least eight of the nine different linkage groups of this species, whereas the map positions of the DNA clones from *B. campestris* (22 clones) were all unknown.

The clone inserts were amplified from bacterial colonies using PCR and appropriate primers (Güssow and Clackson 1989). The amplified DNA fragments were then isolated from low-melt agarose gels and labelled with α-^{32}P-dCTP (3000 Ci/mmol, Amersham) using random nonanucleotide priming (Megaprime, Amersham).

RAPD analysis

A set of 92 decamer oligonucleotides from kits A–F (Operon Technologies Inc, Alameda, Calif., USA) was used in the analysis. PCR reaction buffer was as described by Williams et al. (1990). For the DNA amplification, a Perkin Elmer Cetus 9600 was programmed for 45 cycles as described by Williams et al. (1990), with the following modification; a defined ramping of 0.4 °C/s was used for the transition between annealing and elongating temperatures. Numerous other reaction and run conditions were tested in order to optimize the resolution and reproducibility of the process. DNA fragments were separated in 1.4% agarose gels run in 1 × TAE at 4 V/cm for 5 h, stained in ethidium bromide and documented under UV-light.

Data analysis

A direct comparison between RFLP and RAPD data was made by estimating the number of loci with allele differences revealed by the two methods. Since the majority of RAPD markers are dominant, the RAPD bands present at a certain band level were assumed to represent all detectable alleles at that locus. In this study, 262 out of a total of 434 bands were monomorphic among the three genotypes. These data indicate that there is a high probability of sequence identity for two genotypes showing absence of amplification for a specific band. Consequently, two lines lacking a band at a certain band level were assumed to carry the same allele at that locus. The number of loci with allele differences was thus calculated as the number of band differences divided by the number of comparisons. This estimate is identical to the genetic distance measure, GD, presented by Skroch et al. (1992).

The relative frequencies of loci with allele differences were calculated in a different way for the generally codominant RFLP markers. It is assumed that a band which is present in all three genotypes is the only allele present at that locus, since each of the breeding lines is completely homozygous. When there is more than one allele among the three lines at a particular locus, the situation becomes more complex. For each additional allele at this locus, a band at an additional band level will appear. In the two-allele case, bands are expected at two band levels and, generally, for n alleles at a locus, n band levels are expected. Thus, for loci with n alleles the information from each band level should be weighted by a factor $1/n$. Since our breeding lines are closely related and homozygous, we assume that there are never more than two alleles at any locus among the three lines. Therefore the index is formulated as follows:

$$\text{Relative frequency of loci with allele differences} = 0.5\, z/(x + 0.5y)$$

where x is the number of band levels at which a band is present for all three genotypes, y is the number of polymorphic band levels among the three genotypes, and z is the number of polymorphic band levels between the two genotypes compared. It should be noted that if there are reasons to believe in the existence of more than two alleles per locus, e.g., if many genotypes are compared, this index will be biased. In such cases the alternative approach would be to make all comparisons on a pairwise basis, in which case the index will be identical to the index of Nei and Li (1979).

The robustness of the locus-dissimilarity estimates was analysed using a bootstrap technique (Efron and Tibshirani 1991). For sample sizes varying from one to the maximum number of probes and primers used, the relative frequencies of loci with different alleles were repeatedly calculated (300 runs) from randomly-chosen probes and primers. For each sample size, the coefficient of variation was determined. To illustrate the effect of differing numbers of markers on the repeatability of the detected relationships, we scored for two types of errors for each run of the bootstrap analysis (5 000 runs). First, we noted if any of the three distances was zero. In such cases, the analysis failed to uniquely identify the three lines, i.e., the resolution was lost. Given that all lines were uniquely identified, we noted whether the distance between lines 2 and 3 exceeded any of the other distances. This is equivalent to a reversed ordering of the lines.

In the RFLP analysis, the Spearman rank correlation test was used to determine whether the calculated differences generated by the three different restriction enzymes were correlated. The calculated differences for each probe were used to calculate the pairwise comparison matrix employed to estimate the correlation coefficients and the P-values. The corresponding correlations were calculated for the arbitrarily-generated sets of long and short DNA fragments.

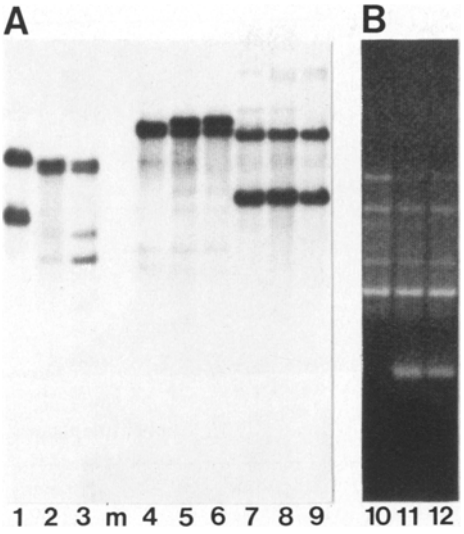


Fig. 1 **A** RFLP and **B** RAPD analysis of total *B. napus* DNA. Line 1 is represented in *lanes 1, 4, 7,* and *10*; line 2 in *lanes 2, 5, 8,* and *11*; line 3 in *lanes 3, 6, 9,* and *12*. *Lanes 1–3*, *Hin*dIII digest; *lanes 4–6*, *Eco*RI digest; *lanes 7–9*, *Eco*RV digest; *lanes 10–12*, RAPD analysis using primer OPE-07. DNA fragments were separated on 0.8% and 1.4% agarose gels, respectively

Results

RFLP analysis

The *B. napus* breeding lines Sv 02288, cv Korall, and Sv 02360 (abbreviated 1, 2, and 3, respectively) were compared by RFLP analysis. DNA samples were digested with the restriction endonucleases *Hin*dIII, *Eco*RI and *Eco*RV, and analysed using a selection of 50 probes with genomic origins from *B. oleracea* and *B. campestris*. An example of a typical autoradiograph is shown in Fig. 1 A. The restriction enzyme *Hin*dIII produced a total of 255 different bands, while *Eco*RI and *Eco*RV produced 165 and 227 different bands, respectively (the corresponding estimates for the number of loci are 209.5, 122.5, and 187). All three genotypes produced a similar total number of bands (line 1, 523 bands; line 2, 514 bands; line 3, 510 bands), whereas the lines showed widely-different numbers of unique bands (line 1, 109 bands; line 2, 10 bands; line 3, 19 bands). In total, monomorphic band patterns were detected for 26% of the probes.

The relative frequencies of loci with allele differences were calculated for each restriction enzyme and each pair of genotypes (Table 1). The breeding lines 2 and 3 are much more similar to each other than either of them are to line 1. A similar pattern is shown by all three restriction enzymes. However, *Eco*RI analysis detects a higher proportion of variable loci for the comparison of line 1 with the two other lines, indicating that this restriction enzyme detects more differences in these comparisons. When separate analyses were made for the probes originating from *B. oleracea* and *B. campestris* the same pattern was observed irrespective of probe origin (data not shown).

Spearman rank correlation coefficients were calculated to investigate whether the RFLP patterns generated by different restriction enzymes give independent information about dissimilarities between the lines (Table 2). The correlation coefficients ranged between 0.30 and 0.76, with most values in the range 0.50–0.65. Highly-significant coefficients of correlation were obtained in all cases but one. These values indicate that the different types of restriction fragments only partly contain unique information about the relationships between the three breeding lines.

To determine whether the length of the restriction fragments influences the frequency of dissimilarities found between the lines, the data sets for each probe/restriction enzyme combination were divided into an upper and a lower half. In this way, arbitrary sets of long and short DNA fragments were generated for separate analyses. Longer DNA fragments revealed higher degrees of dissimilarities than shorter DNA fragments (Table 3). Thus, the frequencies of loci with allele

Table 1 Relative frequencies of loci with allele differences between the three breeding lines. Values are shown for the different types of RFLP and RAPD fragments analysed. Normalized differences in per cent are given within brackets

Type of fragments	Compared breeding lines		
	1–2	1–3	2–3
RFLP, *Hin*dIII	0.18 (41%)	0.20 (46%)	0.06 (14%)
RFLP, *Eco*RI	0.31 (44%)	0.33 (48%)	0.06 (8%)
RFLP, *Eco*RV	0.19 (44%)	0.21 (48%)	0.04 (8%)
RAPD	0.34 (43%)	0.36 (45%)	0.10 (12%)

Table 2 Relative frequencies of loci with allele differences compared by Spearman rank correlation coefficients. Pairwise comparisons were made for RFLP fragments generated by *Hin*dIII, *Eco*RI, and *Eco*RV over all probes. The corresponding probability values for a test of independence between the allele differences are shown within brackets

Compared breeding lines	Compared types of RFLP fragments		
	*Hin*dIII/*Eco*RI	*Hin*dIII/*Eco*RV	*Eco*RI/*Eco*RV
1–2	0.56 (0.0007)	0.53 (0.0001)	0.64 (0.0001)
1–3	0.30 (0.0906)	0.54 (0.0001)	0.52 (0.0018)
2–3	0.60 (0.0002)	0.54 (0.0001)	0.76 (0.0001)

Table 3 Relative frequencies of loci with allele differences between the breeding lines. Values are shown for the different types of RFLP and RAPD fragments and the different fragment size classes

Type of fragments	Fragment size class	Compared breeding lines		
		1–2	1–3	2–3
RFLP, *Hin*dIII	Short	0.13	0.15	0.05
	Long	0.22	0.25	0.08
RFLP, *Eco*RI	Short	0.28	0.29	0.06
	Long	0.33	0.38	0.06
RFLP, *Eco*RV	Short	0.14	0.14	0.03
	Long	0.24	0.28	0.04
RAPD	Short	0.35	0.35	0.12
	Long	0.34	0.37	0.07

differences detected in the RFLP assay are dependent on the size range of the analysed DNA fragments.

RAPD analysis

A set of 92 decamer primers was used in PCR reactions to amplify DNA fragments from the three *B. napus* breeding lines. The DNA fragments were separated by gel electrophoresis (Fig. 1 B). A total of 434 bands was digitized and analysed for dissimilarities between the breeding lines. In this case, the number of bands equals the estimated number of loci. All three genotypes had very similar total numbers of bands (line 1, 341 bands; line 2, 359 bands; line 3, 353 bands), and showed widely-different numbers of unique bands (line 1, 57 bands; line 2, 10 bands; line 3, 10 bands). Monomorphic band patterns were observed for 24% of the primers. The relative frequencies of loci with allele differences were calculated as described in Materials and methods (Table 1). Lines 2 and 3 were very similar to each other (0.10), while line 1 was more distantly related to the others (0.34, 0.36).

Two arbitrary sets of long and short DNA fragments were generated by dividing the digitized data set obtained for each primer into two halves which were analysed separately. Long and short DNA fragments revealed similar frequencies of dissimilarities between the lines (Table 3). Thus, the frequencies of loci with allele differences detected in the RAPD assay are not dependent on the size range of the analysed DNA fragments.

Comparison of RFLP and RAPD assays

The RFLP markers detected 4.2, 2.4 and 3.7 loci per probe in assays using *Hin*dIII, *Eco*RI and *Eco*RV, respectively. The RAPD markers detected an average of 4.7 loci per primer over the three genotypes screened. Thus, RFLP and RAPD assays detect roughly the same number of loci per probe and primer, respectively.

The RFLP (*Eco*RI) and RAPD analyses detected slightly more allele differences among the three lines than the RFLP analyses using *Hin*dIII and *Eco*RV (Table 1). However, the overall pattern of dissimilarities detected for each set of breeding line comparisons is very similar. In all four comparisons, lines 2 and 3 were more closely related to each other, than either of them were to breeding line 1. Normalization of all values gives a result that is independent of method. The normalized values are shown within brackets in Table 1. Regardless of the type of assay, the normalized distances between line 1 and lines 2 and 3 are approximately 40–50%, while they were only 8–14% between line 2 and line 3.

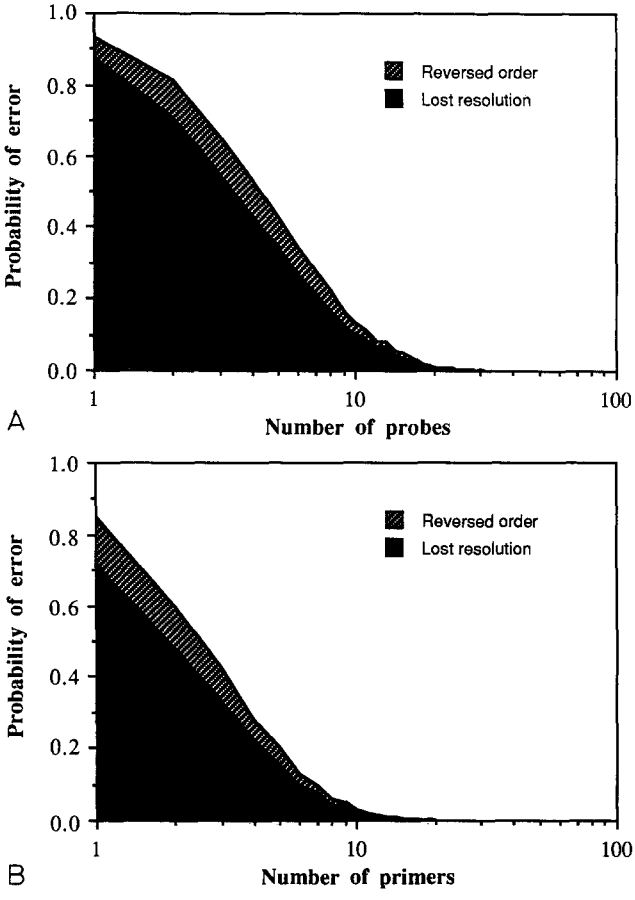
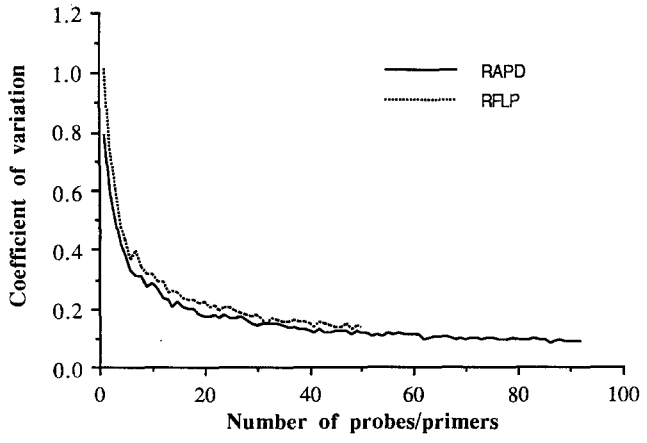


Fig. 3 Probabilities of lost resolution and reversed order of the three genotypes for different numbers of (**A**) RFLP (*Hin*dIII) probes, and (**B**) RAPD primers. The probability of reversed order is added on top of the probability of lost resolution

Fig. 2 Bootstrap plot of coefficient of variation (CV) vs number of probes/primers for the RAPD data (*solid line*) and RFLP (*Hin*dIII) data (*dotted line*). In both cases the comparison is made for line 1 versus line 2



To investigate the effect of the number of markers on sampling error, coefficients of variation (CVs) were calculated by the bootstrap technique. The results for both RFLP and RAPD markers are shown in Fig. 2. The CV falls below 0.2 at 20–30 probes/primers while it reaches 0.1 around 80 primers. ven though the RFLP markers show slightly higher CV values than the RAPD markers, the two methods are compa- rable for the same number of probes and primers. Similar results have been obtained for RAPD markers by Skroch et al. (1992). Their analysis was based on bands rather than primers, so a direct comparison is unfortunately not possible.

A bootstrap analysis was also used to estimate the probabilities of two types of errors; lost resolution and reversed order. The results are shown in Fig. 3, where the probabilities

of the two types of errors are shown as a function of the number of probes/primers. The residual probability, illustrated by the white area, is the probability that all lines are uniquely identified and placed in the correct order. By correct order we mean that lines 2 and 3 are more closely related than either of them are to line 1. Figure 3 shows that when 20 or more probes and primers are used, the probability of any of the two errors is very low. The results of the computer runs show that for the RAPD analysis, the probability of either of the two errors falls below 1/100 at 10–12 primers and below 1/1 000 at 20–22 primers. The RFLP analysis shows slightly higher probabilities of errors than the RAPD analysis. The probability of reversed order falls under 1/100 at 20 probes and under 1/1 000 at 35 probes. For lost resolution, the probability falls under 1/100 at 15 probes and under 1/1 000 at 29 probes.

Discussion

We have evaluated RFLP and RAPD markers as tools for determining the relationships between three *B. napus* breeding lines showing varying degrees of relatedness. The RFLP and RAPD assays detected highly similar relationships between the genotypes. These results are also consistent with the *a priori* knowledge of the pedigree relationships between the lines. Furthermore, bootstrap analyses were carried out to determine the number of probes or primers necessary to achieve the expected relationships between the lines. The probabilities of lost resolution and reversed order were shown to decrease quickly with an increase in the numbers of probes or primers used in the analyses. With 30 probes or primers, there is virtually no risk of the specified errors occurring. The probability of errors depends of course on the numbers and relationships of the investigated lines. With a larger number of closely related lines, the risk of errors is higher. Furthermore, given that no errors occur, variance in the estimates of the genetic distances still exists. This variation decreases as the numbers of probes or primers used in the analysis increases. It would be possible to create criteria for the relatedness between the lines that take this variation into account, but any such criterion would have to be related to the specific questions under investigation.

The proportion of loci with allelic differences detected with the RFLP markers is not identical to that detected with the RAPD markers. This may be explained by the different molecular properties of the two methods. Each DNA band detected by Southern analysis using a restriction enzyme with a six base-pair recognition sequence evaluates the template DNA for differences at 12 base-pairs. On the other hand, each DNA band amplified using a single decamer primer is a result of an interaction between primer and template DNA at up to 20 base-pairs. The fragments under study in a typical RAPD analysis are, however, much shorter than the fragments generated in a typical hexacutter RFLP analysis. Thus, RFLP analyses are expected to reveal more rearrangements than do RAPD analyses, whereas point mutations are expected to be more easily identified using RAPD markers. In our data, the observation that long RFLP fragments revealed more differences than did short RFLP fragments indicates that some structural rearrangements were detected. The fact that long and short RAPD fragments showed similar levels of variation and that the RAPD analysis showed more variation than the RFLP analysis indicates that most of the observed dissimilarities are due to differences at the base-pair level rather than to rearrangements. It should be pointed out, however, that the dynamics of the PCR process are not fully understood. The possibility cannot be excluded that RAPD markers yield apparent variation that is not due to point mutations in the recognition sites but rather to competition between different PCR products, as postulated by Heun and Helentjaris (1993). In the RFLP analysis, use of the restriction enzyme *Eco*RI revealed more variation than *Eco*RV and *Hin*dIII. Since these restriction enzymes have different recognition sequences and generate DNA fragment sets with different mean lengths, such differences are to be expected.

The Spearman rank correlations show intermediate positive correlations between the dissimilarities generated with the three different restriction enzymes in the analysis of the RFLP data. We believe that two biological phenomena contribute to the positive correlation coefficients; (1) structural rearrangements that are detectable with several restriction enzymes, and (2) situations where the two lines under comparison contain regions which are significantly more similar than the average level of relatedness between them. The latter could, for example, result from the introgression of identical regions through crosses with a specific line. The higher correlation coefficients detected in the comparison of lines 2 and 3 may be indicative of this phenomenon, since line number 2 is a selection from cv Topas, and line number 3 is derived from a cross between Topas and a breeding line. (If all regions compared between two genomes have the same relatedness, and all differences are due to point mutations, then the correlation coefficients are expected to be zero.) One consequence of these results is that the gain of information by using more restriction enzymes is reduced by the size of the correlation coefficient. In our case, where the correlation coefficients are approximately 0.5, the variance of the mean genetic distance would be reduced only by half of what would have been achieved if the genetic distances estimated for the different restriction enzymes were uncorrelated.

A number of mapping studies have shown that many RAPD markers deviate from their expected patterns of inheritance (Reiter et al. 1992; Tulsieram et al. 1992; Echt et al. 1993). It has been argued (Heun and Helentjaris 1993) that these problems might have a common basis in competition between different priming sites. They postulate that competition would give rise to selective amplification of a set of RAPD fragments from a larger number of candidate priming sites, and that this selective amplification might be influenced by other regions in the genome. Consequently, RAPD markers should be used with caution, especially in estimations of genetic distances. Although mapping can be carried out efficiently using RAPD markers on doubled haploid lines, the use of RAPD markers in breeding programmes will be limited by their potential unreliability and their non-codominant nature. Ideally, the reliability of the RAPD markers should be confirmed in every experimental system. The problem of

reliability was recently addressed by Thormann and Osborn (1992) who found that RFLP and RAPD markers gave different results when comparing genetic relationships among different *Brassica* species. They interpreted this discrepancy as being due to non-homology of identically-sized RAPD fragments from different species. Comparisons of accessions from the same species, however, showed very similar results for RFLP and RAPD analysis. In this study, RFLP and RAPD markers detected highly similar genetic distances among a set of *B. napus* genotypes. This indicates that the estimation of relationships between closely related *B. napus* genotypes is possible using RAPD markers.

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References

Demeke T, Adams RP, Chibbar, R (1992) Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. Theor Appl Genet 84:990–994

Echt CS, Erdahl LA, McCoy TJ (1992) Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. Genome 35:84–87

Efron B, Tibshirani R (1991) Statistical data analysis in the computer age. Science 253:390–395

Figdore SS, Kennard WC, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica*. Theor Appl Genet 75:833–840

Grattapaglia D, Chaparro J, Wilcox P, McCord S, Werner D, Amerson H, McKeand S, Bridgwater F, Whetten R, O'Malley D, Sederoff R (1992) Mapping in woody plants with RAPD markers: application to breeding in forestry and horticulture. In: Proc Conf Appl RAPD Tech Plant Breed. Minneapolis, pp 37–40

Güssow D, Clackson T (1989) Direct clone characterization from plaques and colonies by the polymerase chain reaction. Nucleic Acids Res 17:4000

Hjerdin A, Säll T, Tuvesson S, Halldén C (1994) RFLP markers in the genus *Beta* (L.). Characterization of DNA sequences from a *Beta vulgaris* library. (Submitted)

Heun M, Helentjaris T (1993) Inheritance of RAPDs in F_1 hybrids of corn. Theor Appl Genet 85:961–968

Hu J, Quiros CF (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Kresovich S, Williams JGK, McFerson JR, Routman EJ, Schaal BA (1992) Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor Appl Genet 85:190–196

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc Natl Acad Sci USA 76:5269–5273

Reiter RS, Williams JGK, Feldmann KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Riedy MF, Hamilton WJ, Aquadro CF (1992) Excess of non-parental bands in offspring from known primate pedigrees assayed using RAPD PCR. Nucleic Acids Res 20:918

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Sci USA 81:8014–8018

Skroch P, Tivang J, Nienhuis J (1992) Analysis of genetic relationships using RAPD marker data. In: Proc Conf Appl RAPD Tech Plant Breed. Minneapolis, pp 26–30

Slocum MK, Figdore SS, Kennard WC, Suzuki JY, Osborn TC (1990) Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor Appl Genet 80:57–64

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257–264

Tanksley SD, Ganal MW, Prince JP, de Vincente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Thormann CE, Osborn TC (1992) Use of RAPD and RFLP markers for germplasm evaluation. In: Proc Conf Appl RAPD Tech Plant Breed. Minneapolis, pp 9–11

Tulsieram LK, Glaubitz JC, Kiss G, Carlson JE (1992) Single-tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. Bio/Technology 10:686–690

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Whitkus R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132:1119–1130

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535