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Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers

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Abstract It has frequently been suggested to use the resynthesis of rapeseed (*Brassica napus*) from *B. campestris* and *B. oleracea* to broaden its genetic base. The objective of the present study is twofold: (1) to compare the genetic variation within resynthesized rapeseed with a world-wide collection of oilseed rape cultivars, and (2) to compare genetic distances estimated from RFLP markers with distances estimated from a relatively small number of allozyme markers. We investigated 17 resynthesized lines and 24 rapeseed cultivars. Genetic distances were estimated either based on the electrophoresis of seven allozymes, with a total of 38 different bands, or based on RFLP data of 51 probe/enzyme combinations, with a total of 355 different bands. The results of allozyme and RFLP analyses agreed reasonably well. Genetic distances, estimated from two independent sets of RFLP data with 25 and 26 probe/enzyme combinations respectively, were highly correlated; hence about 50 RFLP markers are sufficient to characterize rapeseed material with a large genetic diversity. The cultivars were clustered into three groups: (1) spring rapeseed of European and Northern American origin, (2) winter rapeseed of European and Northern American origin, and (3) rapeseed of Asian origin. Several of the resynthesized rapeseed lines were similar to European winter rapeseed cultivars, whereas others had quite unique patterns. It is concluded, that resynthesized rapeseed is a valuable source for broadening the genetic variation in present breeding material of *Brassica napus*. However, different lines differ widely in their suitability for this purpose.

Key words *Brassica napus* · Resynthesized rapeseed · Genetic distance · Isozymes · Allozymes · RFLP

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Introduction

The genetic diversity of oilseed rape (*Brassica napus*) is small because: (1) rapeseed is of recent origin and extensive rapeseed cultivation and breeding started not more than 50 years ago, and (2) the species has a narrow genetic base. Rapeseed originates from natural hybridization between cabbage (*B. oleracea*) and turnip (*B. campestris*). Such hybridizations may have occurred several times, and rapeseed is probably of polyphyletic origin (Olsson 1960; Song and Osborn 1992). Nevertheless, the present breeding material of oilseed rape is derived from very few interspecific hybrid plants that occurred spontaneously some centuries ago.

The genetic diversity of rapeseed can be increased by its artificial resynthesis from the two parental species. Resynthesized rapeseed has been used for a variety of purposes for about 60 years ago (for review see Olsson and Ellerström 1980; Chen and Heneen 1989; Engqvist and Becker 1994). It generally has a low fertility and many other undesirable characters (Krähling 1987). Most efforts so far to use resynthesized rapeseed have been aimed at introducing one or more genes to improve specific traits, e.g. meal quality (Gland et al. 1981), fibre content (Chen et al. 1988), photoperiodic response (Akbar 1989), or *Plasmodiophora* resistance (Diedrichsen and Sacristan 1991). When using such resynthesized lines in breeding programs, they are backcrossed at least twice with adapted material, and hence the overall genetic diversity of rapeseed is only slightly increased.

We have suggested improving resynthesized rapeseed by recurrent selection without any backcrossing to breeding material (Engqvist and Becker 1994). In this way a gene pool can be established that may be useful in two respects: (1) to broaden the genetic base of rapeseed breeding, and (2) to develop material with a large genetic distance from present breeding lines; this should also be useful for hybrid breeding. The develop-

ment of a new gene pool of resynthesized rapeseed requires considerable time and money. Such a strategy is only justified if resynthesized rapeseed lines contain genetic information that is not available within the world-wide breeding material adapted to agronomic use. The objective of the present study is twofold: (1) to compare the genetic variation of newly resynthesized rapeseed lines with the genetic variation of commercial oilseed rape cultivars, and (2) to compare allozymes and RFLP markers for their suitability to measure genetic distances.

Materials and methods

Materials

The material consists of 24 cultivars and 17 resynthesized lines. The cultivars represent a large range of breeding material from different geographical regions involving spring and winter types, and with various quality characteristics. They are described in Table 1. The resynthesized rapeseed lines cover a wide range of different parental subspecies (Table 2). The lines were provided by Prof. W. Odenbach, Freie Universität Berlin (FUB), Prof. G. Röbbelen, Georg-August-Universität Göttingen (GAU), B. Gertsson, Svalöf Weibull AB (SW), Dr. A. Gertz, Dansk Planteforædling (DP), and Dr. E. Sundberg, Sveriges Lantbruksuniversitet Uppsala (SLU).

Methods

Allozyme analysis

At least two plants from each line were investigated for seven enzymes that were known to be polymorphic; a total of 38 bands were observed. If the two plants differed in pattern, three additional plants were analysed and the most frequent pattern was considered to be representative for the respective line. The following enzymes were assayed: aconitate hydratase (ACO, EC 4.2.1.3), diaphorase (DIA, EC 1.6.4.3), glucosephosphate isomerase (GPI, EC 5.3.1.9), leucine aminopeptidase (LAP, EC 3.4.11.1), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), phosphoglucomutase (PGM, EC 2.7.5.1), and shikimate dehydrogenase (SDH, EC 1.1.1.25). DIA, GPI, and SDH were analysed on starch gels as described by Becker et al. (1992); ACO, LAP, PGD , and PGM were analysed on cellulose acetate according to Herbert and Beaton (1989).

Table 1 Survey of utilized rapeseed cultivars

Code	Name	Origin	Type	Quality[a]
PAN	Panter	Sweden	Winter	+
MAT	Matador	Sweden	Winter	+
CAS	Casino	Sweden	Winter	00
CER	Ceres	Germany	Winter	00
LIR	Lirajet	Germany	Winter	00
MAD	Madora	Germany	Winter	00
JAN	Jantar	Poland	Winter	00
TAP	Tapidor	France	Winter	00
MIK	Mikado	Great Britain	Winter	0
OLI	Olimpiade	Italy	Winter	+
WIN	Winfield	Canada	Winter	00
ASA	Asahinatane	Japan	Winter	+
QUI	Quinyou 2	China	Winter	+
Reg	Regina II	Sweden	Spring	+
Top	Topas	Sweden	Spring	00
Del	Delta	Sweden	Spring	00
Bin	Bingo	Denmark	Spring	00
Var	Varma	Finland	Spring	00
Dra	Drakkar	France	Spring	00
Wes	Westar	Canada	Spring	00
Hyo	Hyola 40	Canada	Spring	00
Bar	Barossa	Australia	Spring	00
Hua	Hua Za 2	China	Spring	0
Qin	Qing 2	China	Spring	00

[a] +, high erucic acid and high glucosinolate; 0, erucic acid free and high glucosinolate; 00, erucic acid free and low glucosinolate

Table 2 Survey of utilized resynthesized rapeseed

Line	Origin[a]	Subspecies used as parents		Mother[b]
		B. oleracea	*B. campestris*	
S 3	FUB	*sabellica*	*rapa*	c
S 5	FUB	*capitata*	*oleifera* 'Lembkes'	c
S 7	FUB	*sabellica*	*oleifera* 'Lembkes'	c
S 9	FUB	*gemmifera*	*pekinensis*	c
S 12	FUB	*sabauda*	*oleifera annua*	o
S 13	FUB	*medullosa*	*oleifera*	c
S 20	SW	*sabellica*	*oleifera annua*	f
S 23	SW	*italica*	*oleifera annua*	o
S 27	DP	*oleracea*	*oleifera* 'Nokanova'	o
S 29	GAU	*sabellica*	*pekinensis*	o
S 30	GAU	*capitata*	*pekinensis*	o
S 31	GAU	*italica*	*pekinensis*	o
S 32	GAU	*sabauda*	*pekinensis*	o
S 33	GAU	*alboglabra*	*pekinensis*	o
S 40	GAU	*gongylodes*	*oleifera*	o
S 65	FUB	*fructicosa*	*trilocularis* 'Yellow Sarson'	c
S 76	SLU	*botrytis*	*oleifera*	f

[a] for explanation see Materials and methods
[b] o, *B.oleracea* used as mother; c, *B. campestris* used as mother; f, protoplast fusion

RFLP

The analyses were performed by Linkage Genetics, Salt Lake City, USA, following standard techniques as described by Song et al. (1990, 1993). DNA was isolated from bulk-leaf samples from at least 20 plants of each line. The probes used were 41 genomic *Pst*I fragments from *B. napus* cv 'Westar' and nine genomic *Eco*RI fragments from *B. campestris* cv 'Tobin'. Each probe was digested with one of the enzymes *Eco*RI, *Eco*RV or *Sst*I. In total, 51 probe/enzyme combinations ('markers') with 355 bands were analysed; all markers were polymorphic. The probe/enzyme combinations are described in Table 3. To evaluate the precision of genetic-distance estimates from RFLP data, the markers were arbitrarily divided into two subsets: set $RFLP_{25}$ consists of markers nos. 1 to 25 and set $RFLP_{26}$ consists of markers nos. 26 to 51.

Statistical analysis

The RFLP patterns were binary coded, i.e. presence or absence of a band was coded by 1 and 0, respectively. Allozyme patterns were coded in the same way. Genetic similarity was measured by the Dice coefficient (Nei and Lee 1979; Rohlf 1993). The Dice coefficient of similarity (DICE) was transformed into genetic distance (GD) as follows: $GD = 1 - DICE$. A genetic distance of 0 means that two lines show identical patterns and a distance of 1 means that they do not share any common band. The results are presented graphically as dendrograms based on UPGMA clusters or as plots of the first two principal coordinates. All analyses were made using NTSYS-pc, version 1.80 (Rohlf 1993).

Table 3 Genomic clones and restriction enzymes used for detection of RFLP markers

Clone[a]	Restriction enzyme	No. Fragments[b]	Clone	Restriction enzyme	No. Fragments
TG1C08	*Sst*I	6	WG3G11	*Eco*RV	7
TG1H12	*Sst*I	5	WG3H08	*Eco*RI	6
TG2C04	*Eco*RV	11	WG4B06	*Eco*RV	4
TG2D06	*Sst*I	3	WG4C05	*Eco*RV	10
TG4D02	*Sst*I	6	WG4D10	*Eco*RV	6
TG5B02	*Eco*RI	8	WG4D11L	*Eco*RV	4
TG5B02	*Sst*I	8	WG4D11U	*Eco*RV	3
TG5D09	*Eco*RI	3	WG4E12	*Eco*RV	12
TG5E11	*Sst*I	6	WG4H05	*Sst*I	7
TG5H12	*Eco*RI	13	WG5A01	*Sst*I	7
WG1D07	*Sst*I	5	WG5B01	*Eco*RV	8
WG1E03	*Eco*RV	9	WG6A01	*Sst*I	5
WG1F06	*Eco*RI	6	WG6B02	*Sst*I	5
WG1G02	*Eco*RI	6	WG6B04	*Sst*I	5
WG1G03	*Sst*I	6	WG6F03	*Eco*RI	6
WG1G04	*Sst*I	6	WG6F10	*Eco*RV	5
WG1G05	*Sst*I	5	WG6G09	*Sst*I	6
WG1G06	*Eco*RV	13	WG7A08	*Eco*RV	11
WG1G07	*Eco*RV	8	WG7A11	*Eco*RV	8
WG1G08	*Eco*RI	8	WG7B06	*Eco*RV	5
WG2A11	*Eco*RI	13	WG7E10	*Eco*RV	11
WG2C03	*Sst*I	3	WG7F03	*Eco*RV	8
WG2C04	*Eco*RV	7	WG7H02	*Eco*RV	11
WG2D05	*Eco*RV	8	WG8G03	*Eco*RV	7
WG2G04	*Eco*RI	2	WG8H05	*Eco*RI	9
WG3F07	*Eco*RV	5			

[a] Clones designated with WG are from a genomic DNA library of *B. napus* cv 'Westar' and clones designated with TG are from a genomic library from *B. campestris* cv 'Tobin'
[b] Numbers of fragments scored for each clone

Results

Table 4 gives a survey on the variation of the genetic distances in the various groups. The genetic variances are most variable among the resynthesized lines and the variability is generally larger for distances estimated from allozymes than for distances estimated from RFLPs. For both the total material and the resynthesized lines, correlations were notably high between allozyme and RFLP data, and also between two independent sets of RFLP markers.

The cluster analysis of genetic distances estimated from the RFLP data revealed three main groups (left part of Fig. 1). Cluster 1 includes all European and Canadian spring rapeseed cultivars, and one resynthesized line. Cluster 2 includes all European and Canadian winter rapeseed cultivars; this group also contains six resynthesized lines. Cluster 3 comprises mainly cultivars from Asia: three Chinese and one Japanese cultivar, and the Australian cv 'Barossa' which has Japanese material in its pedigree. Most of the resynthesized lines lay outside these three clusters and have large genetic distances to all the cultivars and to one another.

The results from allozyme analyses are in reasonable agreement with the RFLP data: also according to allozymes there is a group of winter and spring types, and one resynthesized line (S 20) is included in the spring group. However, the separation between winter and spring types is not as clear as with RFLPs. All resynthesized lines that have a large distance to cultivars according to allozymes also have a large distance according to RFLP. However, some resynthesized lines (e.g. S 76) with a large distance to cultivars based on RFLP data are not detected with allozymes.

Table 4 Mean, minimum, maximum, and standard deviation of genetic distance estimates from allozyme (Allo) and RFLP data and correlations between different data sets for various groups of material

Material[a]	N[b]	Mean		Min		Max		SD		Allo/RFLP correlation	$RFLP_{25}/RFLP_{26}$ correlation[c]
		Allo	RFLP	Allo	RFLP	Allo	RFLP	Allo	RFLP		
Resyn	136	0.31	0.35	0.00	0.15	0.61	0.56	0.133	0.095	0.52**	0.85**
Winter	78	0.18	0.20	0.00	0.07	0.38	0.27	0.088	0.041	0.45**	0.56**
Spring	55	0.22	0.21	0.06	0.12	0.38	0.32	0.074	0.048	0.31*	0.50**
Total	820	0.26	0.29	0.00	0.07	0.69	0.56	0.124	0.092	0.60**	0.82**

*, **: Statistically significant at $P = 0.05$ and $P = 0.01$ respectively
[a] Resyn, 17 resynthesized lines; Winter, 13 winter rapeseed cvs; Spring, 11 spring rapeseed cvs; total, all 41 lines
[b] Number of line combinations in respective group
[c] $RFLP_{25}$, results from markers nos. 1 to 25; $RFLP_{26}$, results from markers nos. 26 to 51

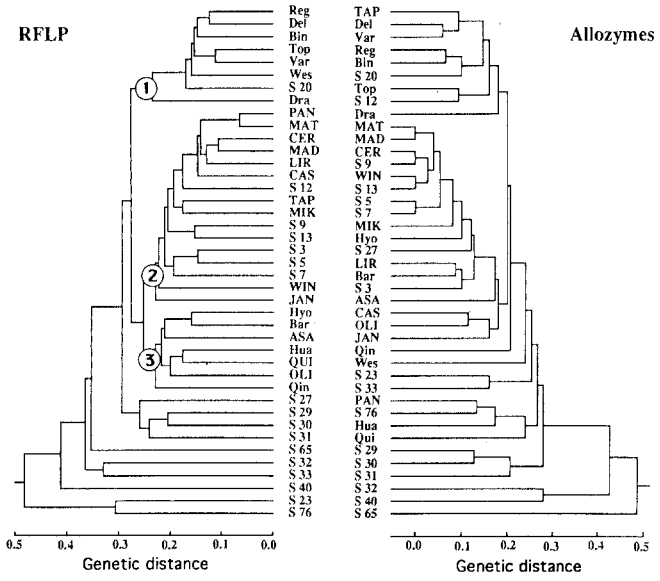

Fig. 1 Associations among 41 rapeseed lines based on RFLP data (left dendrogram) and allozyme date (right dendrogram), respectively

Fig. 2 Associations among 41 rapeseed lines revealed by principal coordinate analysis based on genetic distance estimates, calculated from RFLP data (lower diagram) and allozyme data (upper diagram), respectively

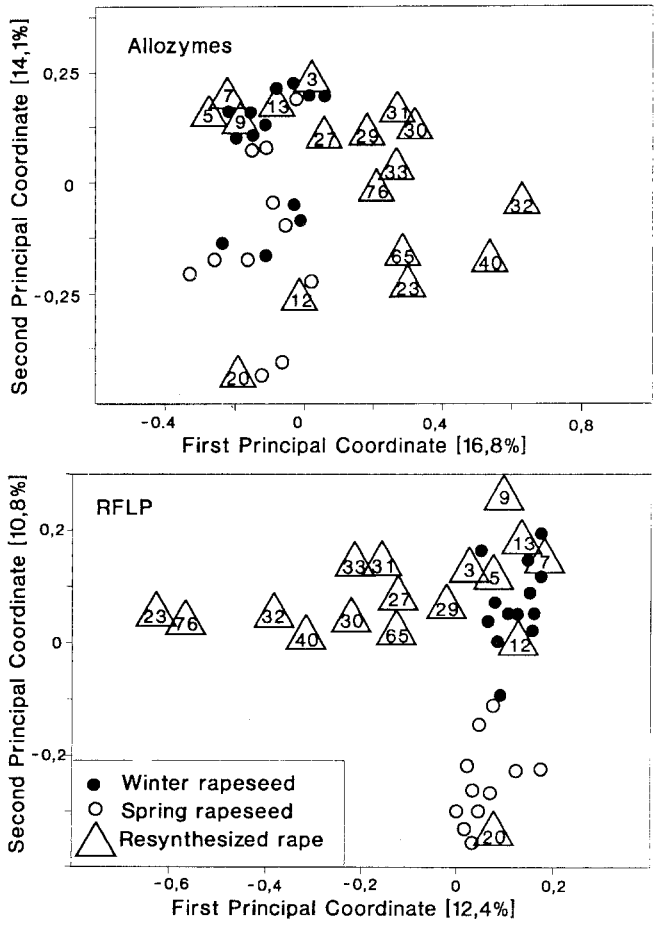

Figure 2 presents the results from principal coordinate analyses. Though this statistical approach is quite different from the clustering procedures presented in Fig. 1, the results agree very well. When looking at the RFLP data (lower part of Fig. 2), winter rapeseed and spring rapeseed cultivars form two distinct groups, which are separated by the second principal coordinate. Most resynthesized lines differ from all cultivars, as indicated by the first principal coordinate. However, some of the resynthesized lines are very similar to winter rapeseed, while line S20 is similar to spring rapeseed.

The results based on allozyme data (upper part of Fig. 2) look somewhat different from the results based on RFLPs. Allozymes from resynthesized lines often gave positive values for the first principal coordinate, whereas RFLPs gave negative values. However, the sign of the principal coordinates is without interest and the essential information is the relative position of the genotypes to each other. Keeping this in mind, the results from allozyme data generally agree with RFLP data, though the separation of the different groups is less distinct.

Discussion

Comparison of allozyme and RFLP data

Allozymes and RFLPs detect genetic variation at different levels: allozymes are proteins that are the product of expressed genes, while RFLPs reveal differences directly at the level of DNA in both coding and noncoding regions. As discussed by Clegg (1989), discrepancies between the two levels of polymorphism may occur. Experimental comparisons of allozyme polymorphism and RFLPs led to contradictory results. Messmer et al. (1991) and McGrath and Quiros (1992) observed a much higher degree of variation for RFLPs as compared to allozymes, whereas Liu and Furnier (1993) and Zhang et al. (1993) did not find any consistent difference. In our study, the genetic distance estimates from allozymes and RFLPs were similar regarding their mean, but the standard deviation of distances estimated from allozymes was much larger (Table 4). The number of loci available for allozyme studies is always very limited. As pointed out by Zhang et al. (1993), there is a considerable heterogeneity in the amount of polymorphism at different loci, and most of the discrepancies between allozyme and RFLP studies can be explained as a result of sampling in which only a small number of allozyme loci have been included.

The correlations between allozymes and RFLP data and between two independent sets of RFLP data with 25 and 26 probes, respectively, are relatively high. In a comparable investigation with maize, Messmer et al. (1991) found much lower correlations: in their study the correlation between allozymes and RFLPs was 0.23 (compared with values between 0.31 and 0.60 in our data, Table 4), and the correlation between two sets of

RFLP data from 54 probes each was only 0.17 (compared with correlations between 0.50 and 0.85 in our material). We have no explanation for the discrepancy between these two studies, for the genetic variation in maize was similar to the variation in spring or winter rapeseed cultivars.

We used the Dice coefficient of similarity to estimate genetic distances. This procedure is widely used, but its application is not without problems. The method is based on the assumption that RFLPs show a diploid co-dominant inheritance and each band represents one allele. If this assumption holds, each homozygous genotype should have the same total number of bands. However, the lines of our material differed widely in the total number of bands, with a range from 121 to 188 bands per line. Possible reasons for this variation are: (1) difficulties and mistakes when scoring weak bands, (2) heterogeneity and heterozygosity of the lines, and (3) allopolyploidy. We suggest that the last mentioned reason is of special importance in rapeseed and needs more consideration. Rapeseed is allotetraploid and might have duplicate loci in the two genomes. If a line is homozygous for the same allele at both loci, the RFLP pattern will show one single band for this marker, while a line that is homozygous for different alleles at the two loci will show two bands. For the same reason, the interpretation of allozyme patterns is more difficult in allopolyploids than in diploids and genotypes homozygous for different alleles at homoeologous loci look like heterozygotes (for discussion see Becker et al. 1992).

Comparison of resynthesized rapeseed and commercial cultivars

In the following, we will concentrate on results from the RFLP data. These results are generally in good accordance with present knowledge on the pedigrees where available. There is a clear general difference between spring and winter rapeseed. This is somewhat surprising, for the difference between annual and biannual behaviour is due to only one major gene (Andersson and Olsson 1961). Moreover, all double low-winter cultivars have spring cultivars in their pedigree. Both zero erucic acid content and low glucosinolate content were introduced from spring rapeseed. In a similar investigation Song and Osborn (1992) observed a clustering into a North American group of spring cultivars and an European group of winter cultivars. Our results suggest that the essential difference between these two groups is not their geographic origin, but rather their vernalization requirement.

The genetic distances between cultivars are generally low compared to other crops. Mailer et al. (1994) also observed genetic distances of below 0.2 when analysing 23 cvs of rapeseed. This may partly reflect the small amount of variation available and the close relatedness of the breeding material. However, even when including resynthesized rapeseed, the maximum distances observed are lower than values found among European flint inbred lines of maize (Boppenmaier et al. 1993) or European faba bean cultivars of the minor type (Link et al. 1995). When comparing different studies, one has to consider that monomorphic bands (i.e. bands present in all investigated genotypes) are treated differently by different authors. In our study, we included monomorphic bands, whereas in some other studies, such bands were excluded (for discussion see Link et al. 1995). However, this problem is of minor importance in our material, for only 10 out of 355 bands were monomorphic.

Most of the resynthesized lines are different from all cultivars. A similar observation was made by Song et al. (1993) who, however, included only two resynthesized lines. There is no relation between the diversity estimates and the parents of the resynthesized lines, e.g. S20 is related to spring rapeseed cultivars, but no obvious explanation for this can be seen from its pedigree (Table 2). Some of the lines in our material show a surprisingly low distance to commercial cultivars. One might question whether this result can be partly explained by experimental mistakes when multiplying the material. That such mistakes can occur is corroborated by the distance estimates for the Swedish cv 'Panter'. According to the reported pedigree, this cultivar is directly derived from a resynthesized plant. However, its RFLP pattern is very similar to the Swedish cv 'Matador', which was the leading variety at the time 'Panter' was released. This suggests that 'Panter' is not an original resynthesized genotype, but an outcross with breeding material. We have started crossing experiments between cultivars and resynthesized lines to investigate whether the estimated genetic distances reflect the heterosis in such crosses.

Conclusions

RFLP markers are a very powerful tool to estimate genetic distances in rapeseed. For a characterization of a material with large diversity, about 50 RFLP markers are sufficient. For a preliminary investigation of allozymes about ten loci are informative.

Resynthesized rapeseed is a valuable source for broadening the genetic base of rapeseed breeding. Different lines differ widely in their suitability for this purpose and some resynthesized lines show an unexpectedly high similarity to breeding material. Winter and spring cultivars are two distinct groups, and using spring rapeseed could be a reasonable long-term strategy to increase the genetic variation in winter rapeseed and vice versa.

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