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Genetic diversity of oilseed *Brassica napus* germ plasm based on restriction fragment length polymorphisms

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Abstract Oilseed rape (*Brassica napus*) is an important oilseed crop worldwide. Cultivars have been developed for many growing regions, however little is known about genetic diversity in *B. napus* germ plasm. The purpose of the research presented here was to study the genetic diversity and relationships of *B. napus* accessions using restriction fragment length polymorphisms (RFLPs). Eighty three *B. napus* accessions were screened using 43 genomic DNA clones which revealed 161 polymorphic fragments. Each accession was uniquely identified by the markers with the exception of the near-isogenic cvs 'Triton' and 'Tower'. The RFLP data were analyzed by cluster analysis of similarity coefficients and by principal component analysis. Overall, there were three major groups of cultivars. The first group included only spring accessions, the second mostly winter accessions and the third, rutabagas and oilseed rape accessions from China and Japan. These results indicate that within *B. napus*, winter and spring cultivars represent genetically distinct groups. The grouping of accessions by cluster analysis was generally consistent with known pedigrees. This consistency included the grouping of lines derived both by backcrossing or self-pollination with their parents.

Key words Oilseed rape · *Brassica napus* · Restriction fragment length polymorphism · Genetic diversity

Introduction

Oilseed rape (*Brassica napus*) is an important oilseed crop grown in cool, moist climates throughout the world. *B. napus* is an amphidiploid (aacc genome, 2n = 38) and is believed to have arisen by interspecific hybridization between the diploid species *B. rapa* (syn. *campestris*) (aa genome, 2n = 20) and *B. oleracea* (cc genome, 2n = 18). The location and time that *B. napus* was synthesized is not well defined. It is thought to have been formed in the Mediterranean region because both wild *B. rapa* and *B. oleracea* originated there. Its origin is probably fairly recent, and it is believed that interspecific hybridization leading to the development of *B. napus* occurred repeatedly involving different genomes (Prakash and Hinata 1980; Palmer et al. 1983). Song and Osborn (1992) found evidence for multiple origins of *B. napus* by restriction fragment length polymorphism (RFLP) analysis of chloroplast and mitochondrial genomes. The *B. napus* accessions they studied could be divided into four cytoplasmic groups and the cytoplasm from each group could be traced to a potential progenitor. However, all but one oilseed accession had the same cytoplasm type, suggesting a common origin for most oilseed cultivars.

B. napus is predominantly self-pollinated with about 30% outcrossing (Rakow and Woods 1987). Most oilseed rape cultivars are developed through pedigree breeding methods and released as open-pollinated populations derived from inbred plants (Downey and Rakow 1987). Winter and spring *B. napus* cultivars are fairly distinct groups, and there has been little intercrossing between these groups in breeding programs. Spring cultivars are grown extensively in Canada, Australia, and Scandinavia whereas winter cultivars are grown in the rest of Europe. Much of the recent increase in *B. napus* production is the result of the development of canola-type cultivars having low levels of both erucic acid and glucosinolates, which has greatly improved the quality of the oil and meal. There also is interest in developing hybrid oilseed rape cultivars because high levels of heterosis have been reported (Sernyk and Stefansson 1983; Grant and Beversdorf 1985).

Information on the genetic diversity in *B. napus* could help breeders and geneticist understand the structure of *B. napus* germ plasm and help them predict which cross combinations would produce the most heterosis or the best

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Table 1 Characteristics of accessions used in this study (*OP* cultivar derived from inbred plants and maintained by open pollination · *DH* microspore-derived doubled haploid lines · *NI* not included in PCA)

Accession	Population type	National origin	Growth habit	Group[a]
Maluka	OP	Australia	Spring	1
Wesbarker	OP	Australia	Spring	2
Taparoo	OP	Australia	Spring	3
Marnoo	OP	Australia	Spring	4
Marnoo-2	S6 inbred	Australia	Spring	5
Argentine	OP	Canada	Spring	S
Oro	OP	Canada	Spring	S
Stellar	OP	Canada	Spring	S
Hero	OP	Canada	Spring	S
Triton	OP	Canada	Spring	S
Tower	OP	Canada	Spring	S
Regent	OP	Canada	Spring	S
Regent-12	S6 inbred	Canada	Spring	S
Westar	OP	Canada	Spring	S
Westar-13	S6 inbred	Canada	Spring	S
Nugyou7	OP	China	Spring	6
Ariel	OP	Denmark	Spring	S
Ariel-12	S6 inbred	Denmark	Spring	S
Bronowski	OP	East Europe	Spring	S
Lergo	OP	Finland	Spring	S
Lergo-40	S6 inbred	Finland	Spring	S
Pactol	OP	France	Spring	S
Drakkar	OP	France	Spring	7
Brutor	OP	France	Spring	S
Cresor	OP	France	Spring	8
R83-11	OP	France	Spring	9
R83-11–16	S6 Inbred	France	Spring	10
Kosa	OP	Germany	Spring	S
Petranova	OP	Germany	Spring	11
Syn1	DH	Great Britain	Spring	NI
Galaxy	OP	Scandinavia	Spring	S
Gulle	OP	Sweden	Spring	S
Gulliver	OP	Sweden	Spring	S
Topas	OP	Sweden	Spring	S
Karat	OP	Sweden	Spring	S
Karat-6	S6 inbred	Sweden	Spring	S
Ariana	OP	France	Winter	W
Bienvenu	OP	France	Winter	W
Tapidor	OP	France	Winter	W
Jet Neuf	OP	France	Winter	W
Darmor	OP	France	Winter	W
Samouri	OP	France	Winter	W
Major	OP	France	Winter	W
Primor	OP	France	Winter	W
Kenton	OP	France	Winter	W
Kentanova	OP	France	Winter	W
Falcon	OP	Germany	Winter	W
Cobra	OP	Germany	Winter	W
Libravo	OP	Germany	Winter	W
Lembkes (Lenora)	OP	Germany	Winter	W
Quinta	OP	Germany	Winter	W
Rubin	OP	Germany	Winter	W
Santana	OP	Germany	Winter	W
Arabella	OP	Germany	Winter	W
Ceres	OP	Germany	Winter	W
Lictor	OP	Germany	Winter	W
Susana	OP	Germany	Winter	W
Mikado	DH	Great Britain	Winter	W
Capricorn	DH	Great Britain	Winter	W
Corvette	DH	Great Britain	Winter	W
Score	DH	Great Britain	Winter	W
CPB87/5	DH	Great Britain	Winter	W
Bronco	OP	Great Britain	Winter	W
88–159-H	OP	Great Britain	Winter	W
88–163	OP	Great Britain	Winter	W
Beaver	OP	Great Britain	Winter	W
Link	OP	Great Britain	Winter	W
Izuzu	OP	Japan	Winter	12
Norin 1	OP	Japan	Winter	13
Norin 8	OP	Japan	Winter	14
Norin 25	OP	Japan	Winter	15
Norin 39	OP	Japan	Winter	16
VDH 1072–87	OP	Netherlands	Winter	W
VDH 1055–88	OP	Netherlands	Winter	W
VDH 1034–87	OP	Netherlands	Winter	W
VDH 1065–88	OP	Netherlands	Winter	W
VDH 1093–88	OP	Netherlands	Winter	W
Skrzeszowicki	OP	Poland	Winter	W
Bolko	OP	Poland	Winter	W
Jantor	OP	Poland	Winter	W
Panter	OP	Sweden	Winter	W
Norde	OP	Sweden	Winter	W
Jupiter	OP	Sweden	Winter	W
Laurentian	OP	–	Rutabaga	17
American Purple Top	OP	–	Rutabaga	18

[a] Groupings based on principal component analysis (see Fig. 2)

offspring. RFLP markers have been useful for studying genetic diversity in *Brassica*. Figdore et al. (1988) reported a high degree of polymorphism within *Brassica* for RFLP markers. Song and Osborn (1992) surveyed 19 oilseed *B. napus* accessions with nuclear RFLP markers. The markers divided the accessions into two major groups with one containing mostly winter cultivars and the other mostly spring cultivars. In the study described here, we survey more accessions than Song and Osborn (1992) to study genetic diversity and relationships within oilseed *B. napus*. The data from this survey were analyzed to obtain information on the overall groupings of oilseed *B. napus* accessions and to compare relationships revealed by RFLP data to reported pedigrees of the lines.

Materials and methods

Germ plasm

Of the oilseed *B. napus* accessions used in this study 61 were either selected by seed companies or were selected by the authors because they were important cultivars or interesting accessions. These accessions were mostly named cultivars from throughout the world and included both spring and winter types either with or without canola quality (Table 1). Most of the cultivars were represented by open-pollinated seed, although a few were from microspore derived double haploid lines. Also included were a group of 7 oilseed cultivars with a S6 inbred derived from each cultivar (Brandle and McVetty 1989a), and three sets of recurrent parents and backcross-derived lines. Finally, two accessions of rutabaga were included for comparison to oilseed accessions.

Molecular methods

Total DNA for each accession was isolated from 200- to 500-mg samples of lyopholyzed leaf tissue collected from 10 plants. DNAs were isolated using a CTAB precipitation of the DNA according to the procedure outlined by Kidwell and Osborn (1992). The DNA was quantified using a fluorometer and digested using the restriction endonucleases *Eco*RI or *Hin*dIII. The procedure for the DNA digestion, gel electrophoresis, Southern blotting, and hybridization have been described previously (Osborn et al. 1987) with the exception that the hybridizations were done using 25–35 ml of hybridization solution at 60 °C in glass tubes (29 × 3.5 cm) that were rotating on a rotisserie.

The clones used in the study were from genomic libraries constructed using 0.5- to 2.0-kb fragments of either *Pst*I digested DNA from the *B. napus* cv 'Westar' or *Eco*RI digested DNA of the *B. rapa* cv 'Tobin'. The fragments were cloned according to Song et al. (1988) into pBS+ plasmids and transformed into DH5α strain of *E. coli*. The clones were prepared for radiolabelling by first amplifying the inserts for 30 cycles of polymerase chain reaction (PCR) with an Idaho Scientific thermocycler. Miniprep plasmid DNA of the clones were the templates for the PCR, and the reactions were primed with the oligonucleotides 5′ GTAATACGACTCACTATATGGGCG 3′ and 5′ CGCAATTAACCCTCACTAAAGGG 3′. These primers are the T7 promoter and T3 primer, respectively, for the plasmid with 6 extra base pairs. The reactions were done with the samples in capillary tubes using the reaction solutions suggested by Idaho Scientific and the temperature profile of 5 s at 93 °C, 5 s at 50 °C and 60 s at 72 °C. The PCR amplified clone inserts were then quantified and 100–150 ng of the amplification products were radiolabelled using random priming reactions according to Feinberg and Vogelstein (1983).

Data collection and analysis

Only fragments that were polymorphic among accessions and could be clearly scored were used in the data analysis. Each of these fragments was scored independently. The fragment data were entered onto a spreadsheet to form a 0–1 matrix, with 1 representing the presence of a fragment and 0 representing the absence of a fragment for each fragment-accession combination. A cluster analysis was conducted by converting the data matrix into a similarity matrix using a simple matching coefficient. This coefficient was calculated by dividing the number of matches (0–0 and 1–1) by the total number of comparisons (Sneath and Sokal 1973). A cluster analysis was then done using the unweighted pair-group method, arithmetic average (UPGMA).

The principal component analysis (PCA) was done by first calculating a correlation matrix among the markers. Eigen values and eigen vectors were then obtained from the correlation matrix and these were used to calculate the coordinates of each accession. The accessions were then plotted on the basis of these coordinates. Both the cluster analysis and principal component analysis were done on an Austin 386SX PC using the program NTSYS (Exeter Software, Setauket, N.Y.).

Results and discussion

General groupings

The accessions were evaluated using 43 clones (Table 2). These clones were selected because they revealed polymorphisms among *B. napus* accessions in a preliminary screening. A total of 161 polymorphic fragments were revealed among the accessions screened. The number of polymorphic fragments scored per clone ranged from 1 to 9 (Table 2), and the mean was 3.9. Each accession was uniquely identified by the marker data with the exception of the near isogenic cvs 'Triton' and 'Tower'.

Table 2 Clones and restriction enzymes used for detecting RFLP markers

Clone[a]	Restriction enzyme[b]	Number[c]	Clone	Restriction enzyme	Number
WG1G2	*Hin*dIII	2	WG6B2	*Eco*RI	5
WG1G4	*Eco*RI	2	WG6B10	*Eco*RI	4
WG1G5	*Eco*RI	8	WG6C1	*Eco*RI	4
WG1G6	*Eco*RI	5	WG6D6	*Eco*RI	3
WG1G7	*Hin*dIII	2	WG6F10	*Eco*RI	4
WG1G8	*Eco*RI	8	WG6H1	*Eco*RI	3
WG2A11	*Eco*RI	2	WG7A11	*Eco*RI	3
WG2B7	*Eco*RI	3	WG7B3	*Hin*dIII	4
WG2C3	*Hin*dIII	2	WG7D9	*Hin*dIII	3
WG2D5	*Eco*RI	9	WG7E4	*Hin*dIII	4
WG2D11	*Hin*dIII	3	WG7F3	*Eco*RI	7
WG2E12	*Hin*dIII	5	WG8G4	*Eco*RI	3
WG2G4	*Hin*dIII	3	WG8H5	*Hin*dIII	4
WG3C5	*Eco*RI	5	WG9D5	*Hin*dIII	3
WG3G11	*Hin*dIII	2	TG1C8	*Eco*RI	2
WG3H8	*Eco*RI	6	TG1F8	*Eco*RI	2
WG4B6	*Eco*RI	6	TG1H12	*Eco*RI	1
WG4D5	*Hin*dIII	4	TG2B4	*Eco*RI	5
WG5A1	*Hin*dIII	2	TG2H10	*Hin*dIII	2
WG5A6	*Hin*dIII	3	TG5E11	*Hin*dIII	2
WG5B1	*Eco*RI	3	TG5H12	*Eco*RI	4
WG6A11	*Hin*dIII	4			

[a] Clone names starting with W are from a genomic DNA library of the *B. napus* cv 'Westar' and clone names starting with T are from a genomic DNA library from the *B. rapa* cv 'Tobin'
[b] Enzyme used to cut the plant DNA for RFLP evaluation
[c] Number of polymorphic fragments scored for each clone

The fragment data were used to calculate simple matching coefficients for all pairs of accessions and a phenogram was constructed from these values with the unweighted pair group method using arithmetic averages (UPGMA) (Fig. 1). The analysis placed the accessions, into three main groups. The first group included only spring rape accessions and the second group only winter rape accessions with the exception of 'Cresor' a French spring accession. The third group included accessions from China and Japan and also rutabagas. The only accession not in a major group is 'Syn1', a synthetic *B. napus* formed from a cross between *B. rapa* spp. *chinensis* and *B. oleracea* spp. *alboglabra*.

The RFLP data also were analyzed using principal component analysis (Fig. 2). Principal component analysis is useful for describing the overall structure of the data set, but it is not effective in describing the relationships of closely related lines (Sneath and Sokal 1973). The principal component analysis was conducted without 'Syn1', the synthetic accession, because when it was included in the analysis its separation from the other accessions was a large part of the second principal component, resulting in a close grouping of the other accessions. The first principal component explained 16% of the variation in RFLPs among accessions. This component separated the winter and spring accessions into major groups. The second principal component explained 7% of the variation and separated the rutabagas and Asian oilseed rape cvs from the remaining cvs.

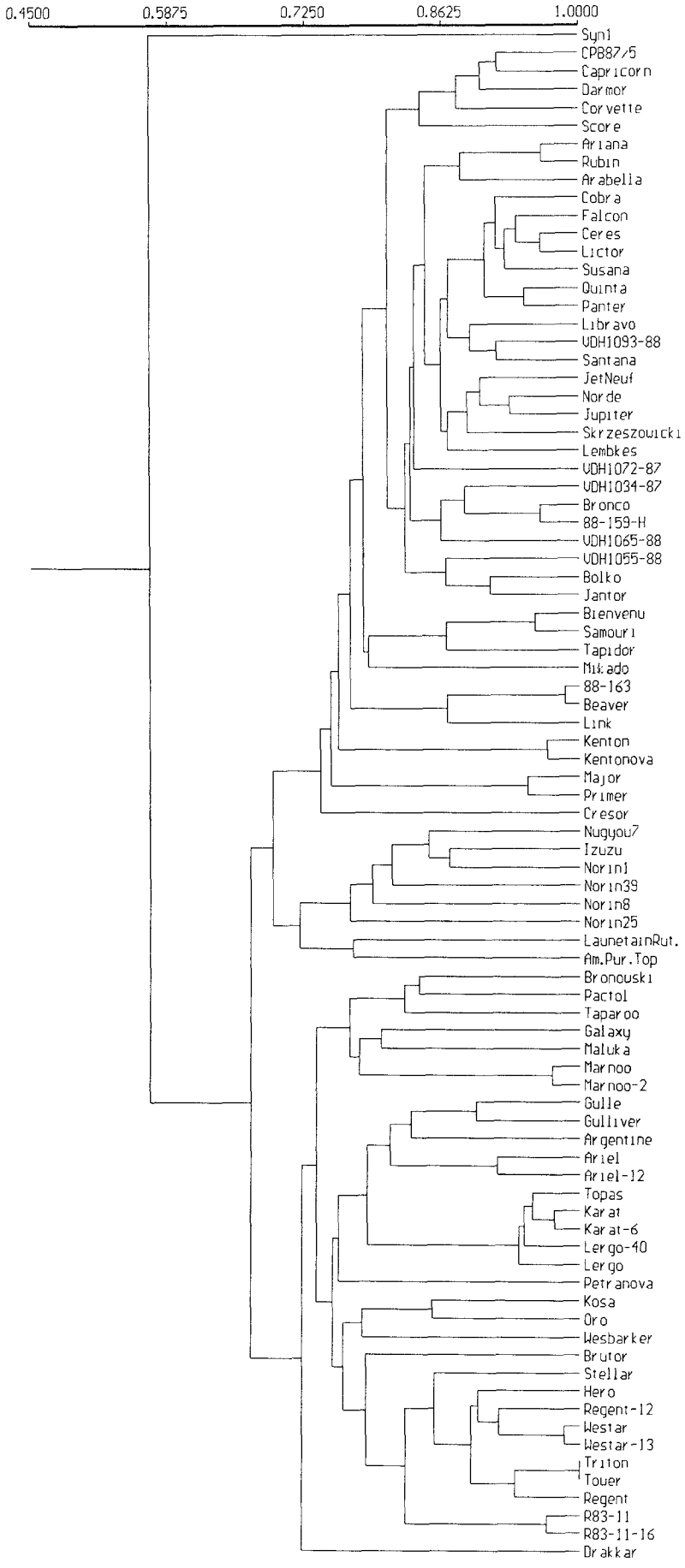

Fig. 1 Phenogram of *B. napus* accessions based on UPGMA cluster analysis of simple matching coefficients calculated from 161 polymorphic fragments. The heading gives the similarity of accessions or groups based on the simple matching coefficient

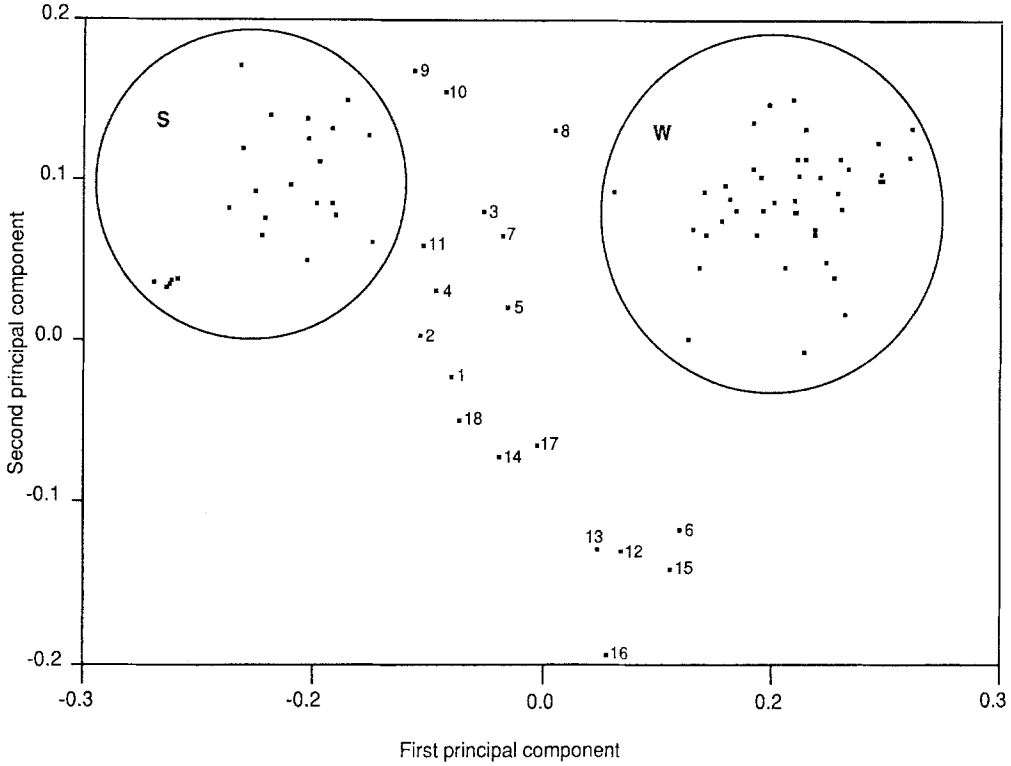

Fig. 2 Plot of the first and second principal components calculated from the correlation matrix of 161 polymorphic fragments. The *numbers* and *letters* correspond to accessions in Table 1

Both the cluster and principal component analyses separated the accessions into three major groups. This is similar to the findings of Song and Osborn (1992) who also separated most of the winter, spring and rutabaga *B. napus* accessions into different groups using another set of RFLP markers, and indicates that the genetic backgrounds of spring and winter oilseed rape accessions are distinct. According to pedigree information (Sernyk 1990), there is little evidence that breeders have made crosses between the winter and spring accessions in cultivar development, except for transferring major genes through backcrossing (e.g. genes controlling low erucic acid and low glucosinolates).

The oilseed rape accessions from China and Japan and the two rutabaga accessions, 'Laurentian' and 'American Purple Top', were grouped together. The Japanese accessions were expected to be distant from other *B. napus* accessions because there has been extensive hybridization between *B. napus* and *B. rapa* in Japanese cv development programs (Erickson et al. 1983). We included 12 *B. rapa* accessions together with the *B. napus* accessions in the germ plasm screen. The Japanese accessions were analyzed to determine if they had fragments that were present at a high frequency in *B. rapa* but at a low frequency in other *B. napus* accessions. The presence of such fragments would provide evidence of introgression from *B. rapa* into the Japanese *B. napus* accessions; however, we did not find any evidence for this introgression.

'Cresor', the only spring type accession that grouped with the winter types in the phenetic analysis (Fig. 1), was positioned between the winter and spring accessions in the principal component analysis (Fig. 2). This is evidence that although the phenetic analysis grouped 'Cresor' together with the winters its association is not very strong. Song and Osborn (1992) also found that 'Cresor' was grouped with the winter accessions based on nuclear RFLP markers.

Comparison of clustering with pedigrees

The grouping of accessions based on the cluster analysis (Fig. 1) was generally consistent with known pedigrees. The recurrent parent-backcross line pairs and the cultivar-inbred pairs were each clustered closely together as expected. The three cvs 'Topas', 'Karat', and 'Lergo', which were developed by Svalof AB of Sweden, were clustered and have the same pedigrees. The pairs 'Tower' and 'Regent', developed by the University of Manitoba, and 'Corvette' and 'Score', developed by Plant Breeding International, Great Britain, each were clustered together and have the same pedigree (Sernyk 1990).

Three backcross lines and recurrent parents were evaluated in this study. The first set included 'Primor', which had low erucic acid backcrossed into it using 'Major' as a recurrent parent and the second set 'Kentanova', which had low erucic acid backcrossed into it using 'Kenton' as a recurrent parent. Primor and Kentanova were each developed using three backcrosses to the recurrent parent (M. Renard, personal communication). The third set included the line 'OAC Triton', which was developed by transferring triazine tolerance from bird rape (*B. rapa*) using 'Tower' as a recurrent parent for six backcrosses (Beversdorf et al. 1980).

There were 8 fragments different between 'Major' and 'Primor' and between 'Kenton' and 'Kentanova'. No fragments differed between 'Triton' and 'Tower'. These results

are consistent with the inheritance of the traits backcrossed into the lines and the number of backcrosses used to develop them. Fewer backcrosses were used to develop 'Kentanova' and 'Primor' than 'Triton', so more of the donor parent genome would be expected in 'Kentanova' and 'Primor' than 'Triton'. Secondly, low erucic acid backcrossed into 'Primor' and 'Kentanova' is conditioned by two nuclear genes (Harvey and Downey 1964), whereas triazine tolerance is conditioned by a chloroplast encoded protein that originated from a weedy bird rape (*B. rapa*) (Souza-Machado et al., 1978). Because the triazine tolerance is cytoplasmically inherited, there should not have been intentional selection of nuclear genes from the donor parent during the backcrossing procedure which would have occurred during the development of the low erucic acid lines.

There were no donor parent fragments in common between the two backcross-derived lines 'Primor' and 'Kentanova'. If fragments were common between the cultivars, it is likely that they would be linked to the genes for low erucic acid because the probability of these fragments occurring twice by chance through separate backcross programs is low.

A set of 7 cultivars and one S6 line developed from each cultivar were also analyzed. These inbreds were developed to study the genetic diversity within cultivars and the combining ability of cultivars compared to inbreds developed from them (Brandle and McVetty 1989a, b). The lines were included to determine the similarity between cultivars and inbreds developed from them using molecular markers.

The marker analysis revealed a range of 2–12 fragments different between the cultivars and inbreds (Table 3). The majority of the differences were extra fragments in the cultivars that were not present in the inbreds. These differences were expected because the cultivars were not completely homozygous or homogeneous, and therefore inbreeding should have resulted in the loss of fragments. Most inbreds also had fragments present that the cultivars did not possess. The most likely explanation for these new fragments in the inbreds is that the plants selected for inbreeding contained fragments from the cultivar that were not present in the 10 plants selected for RFLP analysis.

Table 3 Differences in number of fragments between cultivars and S6 lines derived from each cultivar

Cultivar	S6 Inbred	Unique to cultivar[a]	Unique to inbred[b]
		Number of fragments	
Ariel	Ariel-12	12	0
Karat	Karat-6	4	0
Lergo	Lergo-40	10	1
Marnoo	Marnoo-2	2	2
R83-11	R83-11-16	2	2
Regent	Regent-12	4	4
Westar	Westar-13	1	1

[a] Number of fragments present in cultivar that were absent in inbred
[b] Number of fragments present in inbred that were absent in cultivar

This is possible because the cultivars are somewhat heterogeneous.

Brandle and McVetty (1989a) studied the level of diversity and inbreeding depression in 24 inbred lines from the same 7 cultivars except for 'Lergo', from which 48 inbred lines were derived. Inbreds from 'Ariel', 'R83-11' and 'Lergo' had significant inbreeding depression, determined by measuring the yield reduction of the inbreds relative to the yield of the cultivar from which they were derived. This relative yield decrease of the inbreds was 22% for 'Ariel', 12% for 'R83-11' and 6% for 'Lergo'. Brandle and McVetty (1989a) stated that the cultivars with the highest levels of yield reduction had a greater number of segregating loci with dominance than other varieties. We found that 'Ariel' and 'Lergo' had the highest number of fragments lost between the cultivar and inbred, however, 'R83-11' had lost, only 2 fragments (Table 3). These results suggest that at least part of the reason for the higher levels of inbreeding depression in 'Ariel' and 'Lergo' is that they were more heterozygous than the other cultivars.

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