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Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers

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Abstract. RAPDs (Randomly Amplified Polymorphic DNAs) were used to discriminate among 23 cultivars of oilseed rape (*Brassica napus*) selected from several breeding programs. A set of 100 random sequence 10-mer primers were tested, of which 70 produced bands and 22 showed evidence of polymorphism. A selection of six primers produced 23 polymorphic bands of between 300 to 2200 base pairs in size, sufficient to distinguish between the cultivars. An analysis of seed of five cultivars obtained from four different sites showed stability of banding pattern over source of seed. The analysis was repeated using four different thermocyclers, each of which produced the same band pattern. UPGMA cluster analysis indicates that the relationships among some of the cultivars is closer for those from the same breeding program than for those from different programs. The results of this study show that RAPDs can be used as a method of identification for oilseed rape cultivars.

Key words: *Brassica napus* – Cultivar identification – RAPDs – Rapeseed – Taxonomy

Introduction

Identification of plant cultivars has become increasingly important with the requirement of Plant Breeders Rights (PBR) to demonstrate distinctness, uniformity and stability (DUS) (Plant Varieties Journal 1991) for each new cultivar. Phenology and morphological characteristics may not be significantly distinct and usually require growing plants to full maturity prior to classification. The need for alternative methods of cultivar discrimination is reflected in the number of techniques developed over recent years. In a survey by the International Seed Testing Association (ISTA) (Van den Burg and Van Zwol 1991), the most common rapid tests employed by laboratories were biochemical and electrophoretic analyses. Electrophoresis, restriction fragment length polymorphisms (RFLPs), and high performance liquid chromatography (HPLC) were described as emerging technologies, requiring the development of simple and standardised laboratory protocols before being adopted as standard tests.

The phenological and morphological characteristics used for cultivar identification include plant growth period (Halligan et al. 1991) and seed shape (Barker et al. 1992). Electrophoresis of seed proteins has been a prominent test for cultivar identification in crops such as wheat, *Triticum aestivum* L. (Wrigley et al. 1982), cotton, *Gossypium* spp. Rao et al. 1990), and pasture legumes, *Trifolium* spp. (Gardiner and Forde 1988). In many cases, high-performance liquid chromatography (HPLC) has replaced electrophoresis for soybean, *Glycine max* L. (Buehler et al. 1989) maize, *Zea mays* L. (Smith 1988) rice, *Oryza sativa* L. (Huebner et al. 1990) and wheat (Marchylo et al. 1988). Although most electrophoretic work has been on predominantly autogamous (selfing) species, the technique has also been shown to be useful for predominantly allogamous (outcrossing) species (Gilliland 1989).

The interest in cultivar discrimination of *Brassica* spp. has resulted in many specialised tests based on seed or plant composition, such as fatty acid in oilseed rape (*B. napus* L.) (White and Law 1991), volatile

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hydrolysis products in swede (Cole and Phelps 1979), and glucosinolate content of the vegetable forms, e.g., brussels sprouts (*B. oleracea* L. var. *gemmifera* Zenker.) (Heaney and Fenwick 1980) and swede [*B. napus* L. *var. napobrassica* (L.) Peterm.] (Adams et al. 1989).

Restriction fragment length polymorphisms (RFLPs) have been used by Figdore et al. (1988) and Song et al. (1988) for the purpose of studying genome evolution among *Brassica* species. Although the methodology for RFLPs is now well established, the technique has several disadvantages including the time and labour requirements and the need for the use of radioactive material.

The recent development of the polymerase chain reaction (PCR) to amplify DNA, and the use of randomly amplified polymorphic DNA (RAPD, Williams et al. 1990) or arbitrarily primed PCR (AP-PCR, Welsh and McClelland 1990), has resulted in a potentially useful tool for cultivar discrimination. RAPD involves the amplification of DNA segments using random sequence primers, generally of ten bases, to find polymorphic regions within the genome defined by the primer sequence. The products formed and separated by agarose-gel electrophoresis reveal sequence variation in the form of variable numbers of bands of variable length which may be characteristic of species and/or cultivars within species. RAPD requires no previous sequence information for the fingerprinting of cultivar genomes.

This study investigates the application of the discrimination of oilseed rape (*B. napus*) cultivars.

Materials and methods

Plant material

The *B. napus* cultivars Argentine and Bronowski, selected because of their different backgrounds, were used to optimize the RAPD method and select suitable primers which exhibit polymorphisms between the two cultivars. Bronowski is a Polish cultivar and is the source of the low glucosinolate characteristic of all low-glucosinolate oilseed rape cultivars. Argentine, although not a true variety, is a landrace from Argentina which has been used in the early development of many Canadian oilseed rape cultivars.

The method was then used to investigate the possibility of employing RAPD to differentiate among 23 cultivars of *B. napus*. Breeders seed was obtained for the study from: Pioneer Hibred International and Svalöf Seeds Ltd., Ontario, Department of Plant Science, University of Manitoba and ICI Seeds Canada, Manitoba, Agriculture Canada Research Station, Saskatoon, Saskatchewan and the New South Wales Department of Agriculture, Australia (Table 1). One seed sample of *B. rapa* cv Horizon was included to observe the differences between the species.

The possibility of variability in seed samples, due to contamination or outcrossing of seed, interfering with cultivar discrimination was investigated using seed samples of five cultivars harvested from trials at five diverse sites in Saskatchewan and Manitoba in 1991.

Table 1. Cultivars and seed suppliers used for method development (all cultivars are *B. napus* var. *oleifera*, summer types, "annua", unless otherwise specified). The sample numbers are used elsewhere in the paper to identify cultivars: I, *Agriculture Canada*; II, *ICI Seeds, Canada*; III, *Department of Plant Science University of Manitoba*; IV, *Pioneer Hibred International*; V, *Svalöf Seed Ltd., Ontario*; VI, *New South Wales Department of Agriculture, Australia*. [*Y*], *B. rapa* var *oleifera* "annua"; [*Z*], *B. napus* var *oleifera* "biennis"

Cultivar	Source	Cultivar	Source
1 Stellar	III	13 Horizon [Y]	V
2 Westar	I	14 Crystal [Z]	V
3 Hero	III	15 Legend	V
4 Regent	III	16 Vanguard	V
5 Delta	IV	17 Global	V
6 Excel	I	18 Touchdown [Z]	IV
7 Midas	I	19 Winfield [Z]	IV
8 Tristar	I	20 Bounty	IV
9 Argentine	I	21 Hyola-40 (Hybrid)	II
10 Bronowski	Polish	22 Tower	III
11 Profit	I	23 Oscar	VI
12 Stallion	V	24 Yickadee	VI

Reproducibility of the RAPD analytical procedure was investigated with repeated analyses of samples using a Techne MW-2 thermocycler. To determine if the method was instrument-dependent, samples were also tested on a range of thermocyclers, incorporating identical operating conditions but with each instrument's unique method of cooling and/or sample holder. The instruments included: Techne Multiwell 2.01 with a 96-well microtitre tray, circulating-refrigerated-water-cooled, heating block; Techne PHC-2 1.05 A with 0.5 ml centrifuge tubes, circulating-refrigerated-water-cooled heating block; Thermolyne Temp. Tronic refrigerated block with 0.5 ml centrifuge tubes (no water circulation); and Ericomp Ez-Cycler with 0.5 ml centrifuge tubes, ambient-temperature-water-cooled heating block. Due to the different methods of cooling, the cycing rate varied considerably among instruments.

Sample preparation

Twenty seeds of each cultivar were grown to the two-to-three-leaf stage (2 weeks) in a controlled environment. The true leaves of each cultivar were excised and placed into separate sealed plastic bags on ice, samples of each cultivar being bulked together. The bags were placed directly into a freeze drier and lyophilised for 48 h. After flattening the bags to remove air, they were sealed into air-tight containers with desiccant and stored at 4 °C.

DNA isolation

DNA was extracted from the lyophilised leaf tissue (300 mg) using the method of Kidwell and Osborn (1992). Precipitated DNA was hooked out using a hooked glass rod, rinsed several times in ethanol (75%)/ammonium acetate (10 mM) and dried under vacuum. The dried DNA was redissolved in Tris/EDTA (TE) buffer (500 µl) and centrifuged (13,000 g, 10 min).

Ribonuclease (1.5 µl 10 µg/ml, Sigma Chemicals R4875 Type 1-A) was added and the solution was incubated at 37 °C for 3 h. DNA was reprecipitated in 3 M sodium acetate (1/10 vol) and isopropanol (1 vol), held at −20 °C for 2 h, centrifuged (5,000 g, 20 °C, 30 min) and washed three times with 70% ethanol

(−20 °C). The DNA was dissolved and stored in TE buffer (1 ml). Concentration was measured using a UV spectrophotometer at 260 nm. To check for fragmentation of the DNA, the samples were loaded into a 1.4% agarose gel in 1 × TAE buffer (tris/sodium acetate/EDTA pH 7.8) and separated electrophoretically.

The DNA was of high molecular weight with little fragmentation and free from RNA as indicated by UV spectra and gel electrophoresis (data not shown).

RAPD amplification

Techniques for the generation of RAPD markers are well documented (Rafalski et al. 1991). A single primer is used in each reaction. The primer is generally ten nucleotides long (10-mers); short enough to ensure adequate numbers of binding sites on the template DNA which will generate sufficient bands for study. Commercial sets of these 10-mer primers are now available. A set of 100 10-mer primers was obtained from J. Carlson,University of British Columbia. Concentrations of primer, template and *Taq* (*Thermus aquaticus* DNA polymerase, Promega M186A) were optimized to give maximum band intensity with minimum quantities of polymerase in order to conserve costs. The final reaction mixture included 1 × Promega reaction buffer, 1.5 mM $MgCl_2$, 0.2mM dNTP (deoxyribonucleoside 5′-phosphates), 0.25 µM primer, 0.75 units of *Taq* polymerase, 50–100 ng of genomic DNA made to a final volume of 25 µl with sterilized double-distilled water. Amplification of the DNA was carried out in microtitre plates (Mandel Scientific) using a Techne MW-2 thermocycler programmed for 1 min at 94 °C followed by 45 cycles of 1min at 94 °C (denaturation), 1 min at 37 °C (annealing), and 2 min at 72 °C (elongation) and a final stage of 10 min at 72 °C. Initially only 25 cycles were used but extra cycles produced more intense bands. Although 45 cycles may be excessive, there was no detrimental effect in terms of altered band numbers and, as the reaction was carried out overnight, the time factor was not important.

The RAPD products (15 µl) plus blue mix (3 µL, stop buffer/0.5% bromophenol blue/glycerol: 200/400/200), were separated by electrophoresis using 1.4 % agarose in 1 × TAE buffer. A 1-kb ladder (Bethesda Research Laboratories, BRL) was included as a size marker, and the bands detected with ethidium bromide staining [2.5 µl (10 mg/ml)/100 ml].

Selection of primers

Initially the entire set of 100 primers were screened using the DNA from the cultivars Bronowski and Argentine. The primers which exhibited polymorphisms among these two cultivars were tested against the remaining cultivars identified in Table 1.

Cluster analysis

Cluster analysis is a method for determining the similarity among samples. The form most frequently used is the unweighted pair group arithmetic average clustering (UPGMA; Sneath and Sokal 1973), developed by Sokal and Michener (1958). Average linkage cluster analysis is the average distance between pairs of observations, one in each cluster.

Fragment sizes of RAPD were estimated from the gel by comparison with a 1-kb ladder marker. The bands were recorded as present (1) or absent (0) and assembled in a data matrix table. Pairwise comparisons were calculated from the data matrix using a simple matching coefficient (Nei and Li 1979) and NEIRAPD software and a similarity matrix was constructed. The coefficient was calculated from:

$$F = 2n_{XY}/(n_X + n_Y)$$

in which n_X and n_Y are the number of fragments in populations X and Y respectively, whereas n_{XY} is the number of fragments shared by the two populations (Nei and Li 1979). Cluster analysis (UPGMA) of the similarity indices was then carried out, using NT-SYS software (Rohlf et al. 1971).

Results

Primer selection

Of the 100 primers (oligonucleotides) tested, 70 produced some product and 22 of these showed evidence of polymorphisms. Six primers were ultimately selected for further evaluation which produced a total of 43 clear, easily-detectable bands, 23 of which were polymorphic among the *B. napus* cultivars (see Table 3). Easily-detectable, well-resolved bands were those which were reproducible over repeated runs, with sufficient intensity to determine presence or absence in samples with the same relative band intensity. The sequences of primers are shown in Table 2. Other polymorphic primers produced patterns which were either faint and hard to detect or not reproducible. The total number of bands produced per primer varied from 6 to15 although only 1–7 of these were polymorphic and useable. The size of bands ranged from 350 to 2200 base pairs (bp) (Table 3).

Cultivar identification

Examples of polymorphic bands used for discrimination within the sample set are shown in Fig. 1. Comparison of band patterns among 23 *B. napus* cultivars using six primers, indicated discrimination of all but the two cultivars, Legend and Vanguard. There was a difference of two bands between the two Australian cultivars, Oscar (cultivar 23 in Table 1) and Yickadee (24), and up to ten band differences among all the others, e.g., Tower (22) and Oscar (23). *B. rapa* cv Horizon (13) differed from the other cultivars by up to 37 of the total 43 bands.

Table 2. Primers obtained from University of British Columbia (UBC) and used for RAPD analysis and discrimination of *B. napus*

UBC prime number	Nucleotide sequence
302	CGG CCC ACG T
303	GCG GGA GAC C
329	GCG AAC CTC C
341	CTG GGG CCG T
346	TAG GCG AAC G
354	CTA GAG GCC G

Table 3. Data matrix of RAPD data for 24 cultivars of *B. napus*. Primers are identified with UBC (University of British Columbia) code numbers. Marker size is in kilobases

Primer (UBC)	Marker size	Cultivar 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
302	2.2	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	2.0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	0	0	1	1	1	1	0
	1.3	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	1.0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	0.7	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	0	1	1	1	1	1
303	1.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1
	1.7	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
	1.6	0	0	1	0	1	0	0	0	0	1	0	1	1	1	0	0	1	0	0	1	0	0	1	1
	1.5	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1
	1.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.0	1	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	1	0	0	1	1	1	1	1
	0.7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
	0.6	1	1	0	1	0	1	1	0	1	1	1	1	0	0	0	0	0	1	0	0	1	1	1	1
329	1.6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	1.5	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	1.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	1.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.7	1	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	0.4	0	1	1	1	0	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1	0	0
	0.3	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	0.2	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0
341	2.1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	1	1	1
	1.9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	1.4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.3	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
	1.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.7	0	0	0	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	1	1	0	0	0
	0.6	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
346	2.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.7	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	1	0	1	1
	1.5	0	0	0	1	1	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0
	1.0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	0.8	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	0
	0.6	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
354	1.6	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	1.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
	1.2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.9	1	0	0	1	1	0	0	1	1	0	0	1	0	1	1	1	1	0	0	0	0	1	0	0
	0.8	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	0.7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	0.6	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	0.4	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1

Cultivars: Stellar 1, Westar 2, Hero 3, Reggent 4, Delta 5, Excel 6, Midas 7, Tristar 8, Argentine 9, Bronowski 10, Profit 11, Stallion 12, Horizon 13, Crystal 14, Legend 15, Vanguard 16, Global 17, Touchdown 18, Winfield 19, Bounty 20, Hyola 21, Tower 22, Oscar 23, Yickadee 24

Effect of variable speed source

Analysis of five cultivars collected from trials at four separate sites produced similar profiles within each cultivar (Fig. 2). All cultivars produced band patterns identical to other samples of the same cultivar when tested with primer # 303. The patterns of three cultivars were similar, cv Stellar, Westar and Regent, whilst the other two cultivars, Hero and Delta, were distinct. When analyzed with primer # 329 (Fig. 2), the same three cultivars were similar and Hero and Delta again had unique bands. With primer # 329 one sample of cv

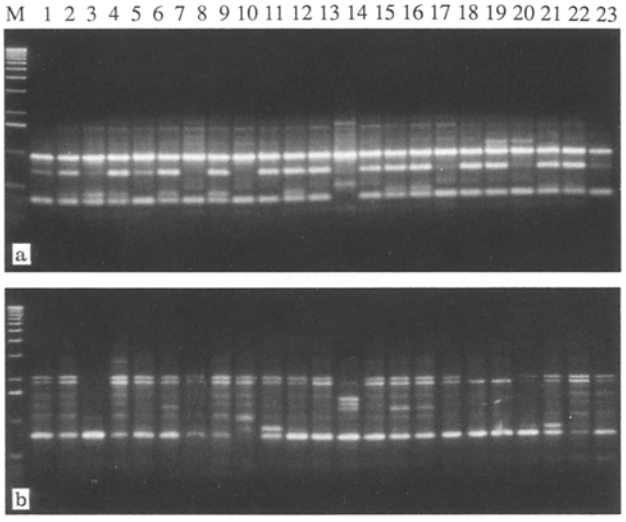

Fig. 1. RAPD profile of DNA from 23 cultivars (numbered as in Table 1) of oilseed rape using primers #329 (**a**) and #341(**b**) (Table 2). Only 23 of the 24 cultivars are shown due to the limitations of the gel box. *M*, DNA marker

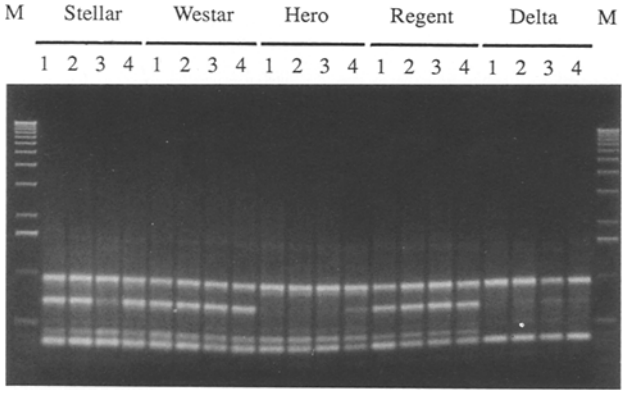

Fig. 2. RAPD profiles of four samples of each of five cultivars of *B. napus* harvested from separate trials at diverse sites in Monitoba and Saskatchewan

Stellar had a less intense band at 750 bp than the other three Stellar samples. One sample of cv Hero had a slightly more intense band then the other Hero samples, also at 750 bp. This variability in bands may represent genetic heterogeneity of the cultivar, rather than variation in experimental conditions. Despite these differences, there was sufficient information using either primer #329, or a combination of primers, to discriminate among the other cultivars.

Instrumental variation

Four cultivars (three *B. napus* and one *B. rapa*) were selected which had distinct polymorphic patterns with primer #329. Identical band patterns were obtained for each of the four cultivars using four different thermocyclers, despite several hours difference in cycling time among the instruments (Fig. 3).

DNA template quality

The DNA extracted from 24 cultivars of oilseed rape, and the five samples of cultivars from the separate trial sites, were extracted over several days. Although concentrations were normalised during dilution, observation of this DNA on agarose gel showed some variation in its fragmentation among samples. Despite this, the RAPD bands were consistent in their intensity, indicating that variation in DNA quality was not a critical factor in the discrimination of the cultivars.

DNA templates were frozen and thawed several times during evaluation of the range of primers and were observed to degrade significantly as indicated by streaking when intact DNA was run on the agarose gel. Although the band patterens were consistent throughout the study, higher levels of DNA template were required to maintain the intensity of the bands after 1–2 months. The problems of DNA degradation were overcome by separating the bulked DNA into several containers prior to freezing and storing one for current use at 4 °C.

Statistical analysis

The number of bands and the individual sizes of the bands recorded are presented as a data matrix in Table 3. Of 43 bands shown, 23 are polymorphic and 20 are monomorphic excluding sample 13 (*Brassica rapa*). Analysis of similarity indices (Nei and Lei 1979), based on the data in the Table 3, produced a similarity matrix. Average linkage cluster analysis (Fig. 4) of the

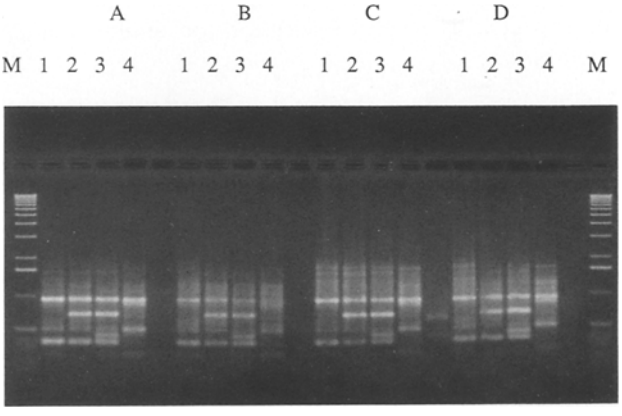

Fig. 3. RAPD profiles generated by four thermocyclers: *A*, Techne MW-2; *B*, Techne PHC-2; *C*, Thermolyne Temp. Tronic; and *D*, Ericomp Ez-Cycler, with primer #329 and four cultivars: *1*, Midas (*B. napus*); *2*, Bronowski (*B. napus*); *3*, Profit (*B. napus*) and *4*, Horizon (*B. rapa*)

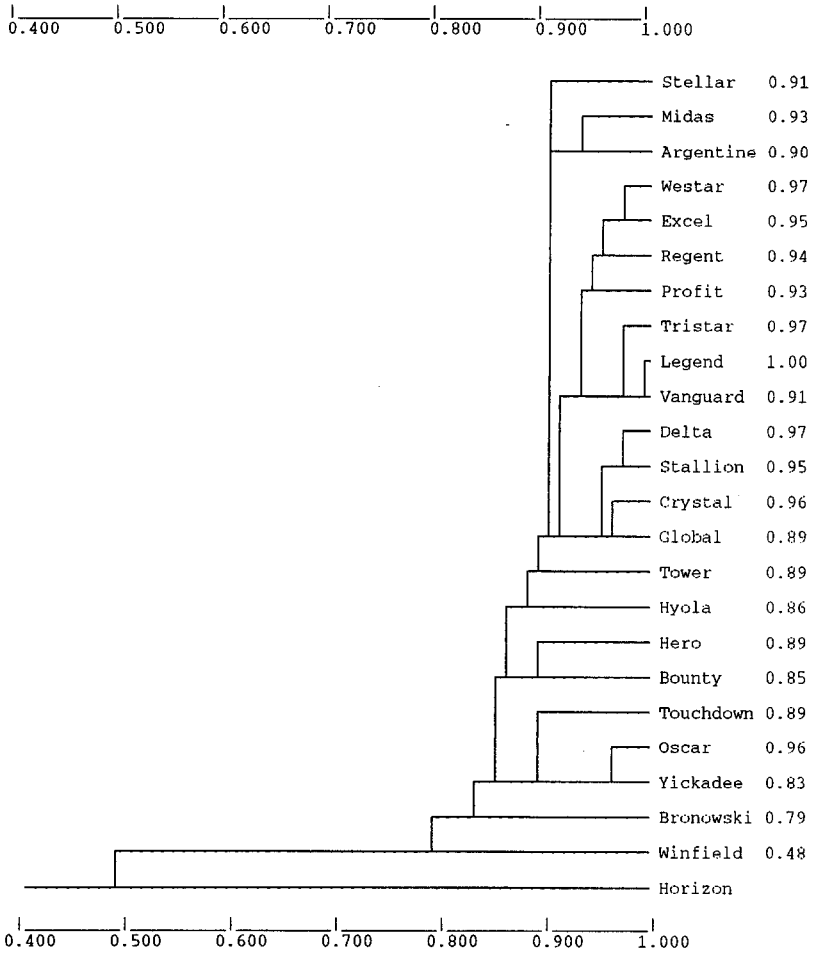

Fig. 4. Average linkage cluster analysis of 24 cultivars of *B. napus* using RAPD data from a 24 × 24 matrix of Nei and Li (1979) similarity indices and NT-SYS software (Rohlf et al. 1971)

similarity indices indicates that the distance separating cv Horizon from *B. napus* types is greater than the distance between all other cultivars. The cluster further indicates that there is a basic similarity among the *B. napus* cultivars used in this study, which is to be expected as result of selection for similar quality factors.

Discussion

The use of Argentine and Bronowski to select primers was based on available pedigree information (Sernyk 1991). These two cultivars had dissimilar backgrounds and were expected to display a high number of polymorphic markers. This deliberate selection may have introduced some bias in that every primer used in subsequent analysis would have at least one band different between the two cultivars. This may have contributed to the distance between these cultivars displayed in the cluster analysis (Fig. 4) although this distance was in agreement with the pedigree information.

Despite the possible bias, some observations could be made regarding the clustering of particular cultivars, based on pedigree information (Sernyk 1991). Oscar and Yickadee, Australian cultivars, clustered together. Crystal, and Stallion (Sweden, Svalöf Seed Ltd.), Global (Denmark, Svalöf Seed Ltd.) and Delta (Sweden, Pioneer Hibred International) clustered closely. Argentine and Midas, which are not canola quality cultivars, had similar patterns. Westar, Excel, Profit (Canada, Agriculture Canada) and Regent (Canada, University of Manitoba) also clustered together. Legend (Andor/SV02215) and Vanguard (Andor/Hermes/Bronowski/Gulle), both Swedish cultivars from Svalöf Seed Ltd., had the same band pattern. The clustering of cultivars of similar seed source adds verification to the RAPD technique.

The RAPD technique discriminated among all of the cultivars tested, with the exception of two, using only six primers. There are several hundred primers available commercially which would ensure the ability of the RAPD technique to differentiate between any two distinct cultivars, as long as the seed is pure and representative of the original breeders' seed.

The technique for DNA extraction is relatively simple, allowing for the extraction of at least 12 samples per day. Higher numbers have been processed in a similar period when a high speed paint shaker (Tai and Tanksley 1990) was used to grind plant material in place of a mortar and pestle. The quality of DNA extracted in this study was high and produced reproducible RAPD products.

The RAPD technique is largely automated requiring little operator input. Most importantly, data generated by the technique is significantly easier to interpret than that of other methods, such as HPLC analysis.

Although the intensity of some of the bands varied for seed from the same cultivar grown at different sites, primers can be selected which produce bands that are consistent among samples from different sites or under different cycling conditions. Since there is a large selection of commercially-available primers, and each primer produces several bands, it is possible to select only those bands which give consistent results, while variable bands are ignored. The patterns produced by the *B. rapa* cultivar examined were distinctly different from *B. napus* cultivars.

There was some variation in band intensity among instruments; however, there were no differences in the results obtained by the different instruments. Therefore the RAPD methodology used in this study was sufficiently robust to be used as a standard technique among laboratories employing different thermocyclers.

Outcrossing species pose particular problems in cultivar discrimination as they are heterogenous populations, rather than a single genotype. There may be selection for a particular subset of the population under different environmental conditions. This may be the reason for the inconsistency in intensity of bands in 2 of the 16 samples compared in Fig. 2. The use of other primers makes it possible to discriminate among cultivars over environments despite these inconsistencies. Only 20 plants of each cultivar were bulked together in an attempt to obtain a characteristic fingerprint of the cultivar. No single plant analysis has been done to determine the degree of heterogeneity within the populations. Sampling DNA from a larger number of plants may overcome the variation observed, particularly in the case of synthetic cultivars. Future studies should include single plant analysis of each cultivar to determine the number of plants required to represent the cultivar for backcrossed cultivars, synthetics and hybrids.

The RAPD technique requires time and effort, considering the period required to grow the seedlings, extract the DNA, and complete the subsequent analysis. With prudent selection of primers, it appears to offer a reliable method for cultivar identification and to have advantages over many of the chemotaxonomic methods used for plant identification, such as glucosinolate or fatty acid concentration, which are susceptible to environmental variation.

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References

Adams H, Vaughan JG, Fenwick GR (1989) The use of glucosinolates for cultivar identification in Swede, *Brassica napus* L. var *napobrassica* (L.) Peterm. J Sci Food Agric 46: 319–324

Barker TA, Vuori TA, Hegedus MR, Myers DG (1992) The use of ray parameters for the discrimination of Australian wheat varieties. Pl Var and Seeds 5:35–45

Buehler RE, McDonald Jr MB, Van Toai TT, St. Martin SK (1989) Soybean cultivar identification using high-performance liquid-chromatography of seed protein. Crop Sci 29: 32–37

Cole RA, Phelps K (1979) Use of canonical variate analysis in the differentiation of swede cultivars by gas-liquid chromatography of volatile hydrolysis products. J Sci Food Agric 30: 669–676

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

Gardiner SE, Forde MB (1988) Identification of cultivars and species of pasture legumes by sodium dodecylsulphate polyacrylamide gel electrophoresis of seed proteins. Pl Var and Seeds 1:13–26

Gilliland TJ (1989) Electrophoresis of sexually and vegetatively propagated cultivars of allogamous species. Pl Var and Seeds 2:15–25

Halligan EA, Forde MB, Warrington IJ (1991) Discrimination of ryegrasses by heading date under various combinations of vernalization and daylength. Pl Var and Seeds 4:115–123

Heaney RK, Fenwick GR (1980) The glucosinolate content of *Brassica* vegetables. A chemotaxonomic approach to cultivar identification. J Sci Food Agri 31:794–801

Huebner FR, Bietz JA, Webb BD, Juliano BO (1990) Rice cultivar identification by high-performance liquid chromatography of endosperm proteins. Cereal Chem 67:129–135

Kidwell KK, Osborn TC (1992) Simple plant DNA isolation procedures. In: Beckman JS, Osborn TC (eds) Plant genomes: methods and physical mapping. Kluwer Academic Publishers, Netherlands, pp 1–14

Marchylo BA, Hatcher DW, Kruger JE (1988) Identification of wheat cultivars by reversed-phase high-performance liquid chromatography of storage proteins. Cereal Chem 65:28–40

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

Plant Varieties Journal (1991) Plant Breeders Rights Office, Ottowa, Ontario, Canada

Rafalski JA, Tingey SV, Willams JGK (1991) RAPD markers – a new technology for genetic mapping and plant breeding. Agbiotech News and Info 3:645–648

Rao TN, Nerkar YS, Patil VD (1990) Identification of cultivars of cotton by sodium dodecylsulphate polyacrylamide-gel electrophoresis (SDS-PAGE) of soluble seed proteins. Pl Var and Seeds 3:7–13

Rohlf FJ, Kishpaugh J, Kirk D (1971) NT-SYS. Numerical taxonomy system of multivariate statistical programs. Tech Rep State University of New York at Stony Brook, New York

Sernyk L (1991) Catalogue of oilseed rape cultivars

Song KM, Osborn TC, Williams PH (1988) Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor Appl Genet 75:784–794

Smith JSC (1988) Identification of pedigrees of hybrid maize (Zea mays L.) cultivars by isozyme electrophoresis and reversed-phase high-performance liquid chromatography. Euphytica 39:199–205

Sneath PH, Sokal RR (1973) Numerical taxonomy. W. H. Freeman and Co., San Francisco, USA

Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. Univ Kansas Sci Bull 38: 1409–1438

Tai TH, Tanksley SD (1990) A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol Biol Rep 8:297–303

Van Der Burg WJ, Van Zwol RA (1991) Rapid identification techniques used in laboratories of the International Seed Testing Assocoation: a survey. Seed Sci Technol 19:687–700

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

White J, Law JR (1991) Differentiation between varieties of oilseed rape (*Brassica napus* L.) on the basis of the fatty acid composition of the oil. Pl Var and Seeds 4:125–132

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

Wrigley CW, Autran JC, Bushuk W (1982) Identification of cereal varieties by gel electrophoresis of the grain proteins. Adv Cereal Sci Technol 5:211–259