African Journal of Biotechnology Vol. 10(16), pp. 3035-3039, 18 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.487
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Characterization of B*rassica napus* Germplasm Based on Molecular Markers

Shahid Ali[1], Iqbal Munir[1], Muhammad Arif[2], Inamullah[2], Farhatullah[3], Ijaz Ali[1], Aqib Iqbal[1], Mushtaq Ahmad[1], M. Wisal Khan[1], S. Jaffar Abbas[1] and Zahoor A. Swati[1]

[1]Institute of Biotechnology and Genetic Engineering, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan.
[2]Department of Agronomy, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan.
[3]Department of Plant Breeding and Genetics, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan.

Accepted 23 February, 2011

Evaluation of genetic relationships is of prime importance in any breeding studies. The present study was carried out to estimate the genetic diversity of *Brassica napus* germplasm using randomly amplified polymorphic DNA (RAPD) primers. A total of 16 *B. napus* genotypes were evaluated using ten RAPD primers. Bivariate data matrix was generated and genetic distances were calculated using unweighted pair group of arithmetic mean (UPGMA) procedure, which amplified 2.31, 2.70, 1.14, 2.46, 1.82, 2.24, 1.78, 3.06, 2.25 and 2.46 alleles per genotype, respectively. All the genotypes showed various levels of genetic polymorphism for the loci detected by using RAPD primers. A total of 217 alleles (bands) were amplified in 16 genotypes using 10 primers giving the average of 13.56 alleles per primer. Among the ten primers used in the present study, primer GLA-09 yielded an average minimum number of bands (1.14), while primer GLD-08 yielded maximum (3.06) number of alleles per genotype on average. Level of genetic polymorphism (estimated as percent genetic distance) observed during the study varied, but on average, and it was in the range of 19 to 93%. Maximum genetic distances were observed among PR-102 and Dunkled, closely followed by PS-1 and PR-111. These findings were further strengthened by dendrogram analyses. Genetically distinct lines pointed out in the present study (PR-102 and Dunkled) could be used in future breeding programs for developing better quality canola inbred lines/varieties.

Key words: Genetic diversity, randomly amplified polymorphic DNA (RAPD), *Brassica napus*, rapeseed.

INTRODUCTION

Oil seed, *Brassica* (rapeseed/mustard), is an important oilseed crop of the world. Rapeseed-mustard accounts for 30.4% of the oilseeds produced in Pakistan, while the remaining 69.6% was imported (Economic survey of Pakistan, 2004-2005). Rapeseed and mustard is cultivated over an area of 257,000 hectares with an annual production of 233,000 tonnes and an average yield of 839 Kg/ha, that contribute about 17% to the total domestic production of edible oil in Pakistan (MINFAL, 2004 - 2005).

Genetic diversity within the genus gives us an important source of variation that can be used to modify *Brassica* crop species by various methods. Estimates of genetic relationships are very important in designing crop improvement programmes, management of germplasm and evolution of conservation strategies. With the advent of recent methods in molecular biology, different molecular markers have been applied to the study of phylogenetic relationships and identification among and within the *Brassica* species. These markers include restriction fragment length polymorphism (RFLP) (Song, 1988a, 1990b; McGrath and Quiros, 1992), randomly amplified polymorphic DNA (RAPD) (Hu and Quiros, 1991; Quiros et al., 1991; Demeke et al., 1992) and simple sequence repeat (SSR) (Kresovich et al., 1995L; Plieske and Struss, 2001) among others.

*Corresponding author. E-mail: iqmunir@hotmail.com. Tel Off: +92-91-9216553, Fax Off: +92-91-9218102.

Abbreviations: RFLPs, Restriction fragment length polymorphisms; **RAPD,** randomly amplified polymorphic DNA; **STS,** sequence-tagged site; **PCR,** polymerase chain reaction.

Table 1. Description of the 16 *B. napus* genotypes used in the present study.

S/N	Genotypes
1	PR-102
2	PS-1
3	PR-111
4	PS-3
5	PM-3
6	BULBUL-98
7	DUNKLED
8	RAINBOW
9	CHALIAR
10	SIREN
11	ABASYN-95
12	HYOLA-42
13	BNWC2AC
14	BNWC3KS
15	BNWC4WA
16	BNWC6SR

DNA-based markers have an advantage over other tests for cultivar identification as DNA is unaffected by environmental factors or the developmental stage of the organism. In recent years, the identification of Brassica cultivars has depended on the application of DNA markers such as restriction fragment length polymorphisms (RFLPs) (Botstein et al., 1980), randomly amplified polymorphic DNA sequences (RAPDs) (Welsh and McClelland, 1990; Williams et al., 1990; Demeke et al., 1992; dos Santos et al., 1994) microsatellite (Charters et al., 1996) and sequence-tagged site (STS) polymerase chain reaction (PCR) markers (Zhang et al., 1997). Despite the development of the newer techniques, RAPD methodologies have retained their advantage in that they are fast, requires no radioactive handling facilities and the costs are relatively minimal. While RAPDs can be used to tag simple, dominant genes as well as identify multiple chromosome intervals controlling a quantitative trait. There are increasing number of reports where RAPDs have been successfully used to estimate genetic variability in *Brassica* (Demeke et al., 1992; Wang et al., 2002; Divaret et al., 1999), common wheat (Liu et al., 1999), maize (Zhang et al., 1997) and barley (Hamza et al., 2004).

The limitation of RAPDs imposed by the lack of molecular polymorphism can be overcome by developing methods to screen RAPD markers and primers in high numbers (Tanhuanpaa et al., 1995). In *Brassica* and its related genera, RAPD markers have been successfully used for identification and polygenetic relationship among and within species (dos Santos et al., 1994; Thromann et al., 1994). The present research was undertaken to estimate the genetic diversity of different lines of *Brassica* using RAPD markers and obtain reliable information for further studies.

MATERIALS AND METHODS

Sixteen rapeseed (*Brassica napus*) genotypes (Table 1) were characterized using RAPD markers. The seed was kindly provided by Pakistan Oilseed Development Board, Agricultural Research Institute, Tarnab Peshawar, Pakistan. The study was conducted in Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University, Peshawar, Pakistan in 2006.

The following protocols were used to study the genetic diversity and to assess the quality parameters of these genotypes.

DNA extraction

For genomic DNA isolation, 10 cm long piece of fresh leaf sample from 3 - 4 weeks old seedlings was collected from the plants in the screen house in the eppendorf tubes and subsequently dropped in a container having liquid nitrogen to freeze the leaf material. The plant material in each eppendorf tube was crushed with a knitting needle to a fine powder. 500 µl DNA extraction buffer (1% SDS, 100 mM NaCl, 100 mM tris base, 100 mM Na_2EDTA, pH 8.5) were added to each eppendorf tube containing the crushed leaf material and mixed well with the knitting needle. 500 µl phenol: chloroform: isoamyl alcohol (in the ratio of 25:24:1) was added and tubes were shaken well until the formation of a homogenous mixture. Samples were then centrifuged at 5000 rpm for 5 min. Aqueous phase was transferred to a fresh tube, to which 500 µl sodium acetate (pH 4.8) and 500 µl isopropanol were also added and mixed gently to precipitate the DNA. Samples were centrifuged at 5000 rpm for 5 min to make DNA pellet. After discarding the supernatant, the pellets were washed with 70% ethyl alcohol, dried at room temperature for an hour, resuspended in 40 - 50 µl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and stored at 4°C. The concentration of DNA was estimated by comparing its intensity with that of the λ DNA of known concentration on a 1.0% agarose Tris Borat ethylenediaminetetraacetic acid (EDTA) (TBE) gel. The DNA was diluted with double distilled, autoclaved and deionized water at the ratio of 1:5 concentrations for use in PCR.

Polymerase chain reaction

The polymerase chain reaction was carried out using protocols of Prasad et al. (2000) with slight modification in thermal profile. For PCR, RAPD primers provided by Genelink were used to assess the genetic diversity. Sequence information of the primers is given in Table 2. PCR reaction was carried out in 25 µl reaction mixture containing 50 - 100 ng of total genomic DNA template, 0.25 µM of each primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase.

Amplification conditions was an initial denaturaton step of 4 min at 94°C, followed by 40 cycles each consisting of a denaturaton step of 1 min at 94°C, annealing step of 1 min at 34°C and an extension step of 2 min at 72°C. The last cycle was followed by 10 min extension at 72°C. All amplification reactions were performed using programmable thermo-cycler. The amplification product was then electrophoresed on 2% agarose/TBE gel and visualized by staining with ethidium bromide and observed under ultraviolet (UV) light using computer programme “uvitec”.

RESULTS AND DISCUSSION

During the present study, ten RAPD primers were used to

Table 2. Sequence information, size Mol. Wt and percentage GC content of 7 RAPD primers used during the present study.

S/N	Name of the primer	Sequence	Size	Mol. Wt	% GC
1	GLA-09	GGGTAACGCC	10	3053.01	70
2	GLA-04	AATCGGGCTG	10	3068.02	60
3	GLD-05	GATGACCGCC	10	3012.99	70
4	GLD-08	GTGTGCCCCA	10	3003.99	70
5	GLD-11	AGCGCCATTG	10	3028	60
6	GLA-02	GGCACTGAGG	10	3093.03	70
7	GLA-03	GAGCCCTCCA	10	2972.97	70
8	GLB-04	GGACTGGAGT	10	3108.04	60
9	GLB-05	TGCGCCCTTC	10	2954.97	70
10	GLB-07	GGTGACGCAG	10	3093.03	70

Mol. Wt = molecular weight of the primer.

Table 3. Average genetic distances among all 16 *B. napus* lines using seven RAPD primers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.19														
3	0.79	0.54													
4	0.42	0.41	0.52												
5	0.44	0.33	0.60	0.22											
6	0.25	0.41	0.38	0.35	0.31										
7	0.93	0.76	0.80	0.74	0.73	0.58									
8	0.24	0.44	0.54	0.33	0.38	0.20	0.55								
9	0.35	0.48	0.58	0.36	0.47	0.25	0.66	0.31							
10	0.40	0.52	0.62	0.42	0.54	0.41	0.75	0.39	0.16						
11	0.61	0.32	0.63	0.45	0.33	0.47	0.84	0.58	0.51	0.51					
12	0.44	0.33	0.54	0.33	0.25	0.35	0.72	0.27	0.54	0.55	0.41				
13	0.49	0.59	0.778	0.51	0.41	0.48	0.76	0.41	0.51	0.66	0.53	0.45			
14	0.80	0.80	0.50	0.63	0.46	0.55	0.91	0.37	0.42	0.50	0.63	0.74	0.66		
15	0.70	0.58	0.73	0.61	0.66	0.59	0.68	0.53	0.59	0.67	0.47	0.40	0.65	0.50	
16	0.58	0.46	0.73	0.51	0.54	0.51	0.78	0.58	0.62	0.66	0.45	0.27	0.40	0.72	0.56

1, PR-102; 2, PS-1; 3, PR-111; 4, PS-3; 5, PM-3; 6, Bulbul-98; 7, Dunkled; 8, Rainbow; 9, BNWC2AC; 10, BNWC3KS; 11, BNWC4WA; 12, BNWC6SR; 13, Hyola-42; 14, Siren; 15, Abasyn-95; 16, Chaliar.

detect level of genetic polymorphism at DNA level among the 16 *B. napus* lines. These RAPD primers were selected arbitrarily and were obtained from Gene Link, Inc. NY 10532, USA. Bivariate data 1-0 were used to estimate genetic distances (Ds). Unweighted pair group of arithamatic means (UPGMA) function (Nie and Li, 1979) was used to estimate genetic distances between the genotypes as follows:

$$GD = 1 - dxy / dx + dy - dxy$$

Where, GD = Genetic distance between two genotypes, dxy = total number of common loci (bands) in two genotypes, dx = number of loci (bands) in genotype 1 and dy = number of loci (bands) in genotype 2.

The ten RAPD primers (GLA-02, GLA-03, GLA-09, GLB-04, GLB-05, GLB-07, GLD-04, GLD-08, GLD-11 and GLD-05) used during the present studies amplified 2.31, 2.70, 1.14, 2.46, 1.82, 2.24, 1.78, 3.06, 2.25 and 2.46 alleles per genotype, respectively. In the present study, all genotypes showed various levels of genetic polymorphism for the loci detected by using RAPD primers. A total of 217 alleles (bands) were amplified in 16 genotypes using 10 primers giving the average of 13.56 alleles per primer. Among the ten primers used in the present study, primer GLA-09 yield on average minimum number of bands (1.14), while primer GLD-08 yielded maximum (3.06) number of alleles per genotype on average. Level of genetic polymorphism (estimated as percent genetic distance) observed during the present study varied, but on average it was in the range of 19 to 93%. Maximum genetic distances were observed among PR-102 and Dunkled, closely followed by PS-1 and PR-111 (Table 3). These findings were further strengthened by dendrogram analyses.

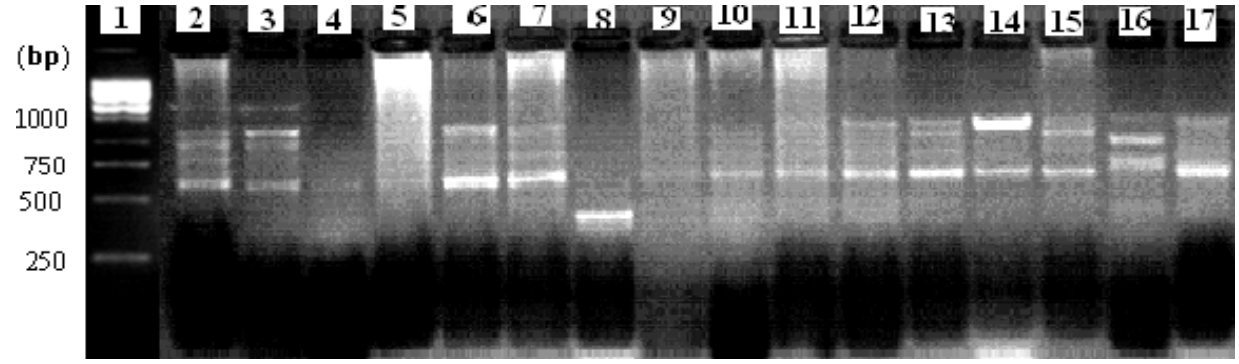

Figure 1. PCR amplification profile for 16 *B. napus* genotypes using RAPD primer GLA-09. 1 = Molecular size marker (1 *K*bp ladder), 2 = PR-102, 3 = PS-1, 4 = PR-111, 5 = PS-3, 6 = PM-3, 7 = Bulbul-98, 8 = Dunkled. 9 = Rainbow, 10 = BNWC2AC, 11 = BNWC3KS, 12 = BNWC4WA, 13 = BNWC6SR, 14 = Hyola-42, 15 = Siren, 16 = Abasyn-95, 17 = Chaliar.

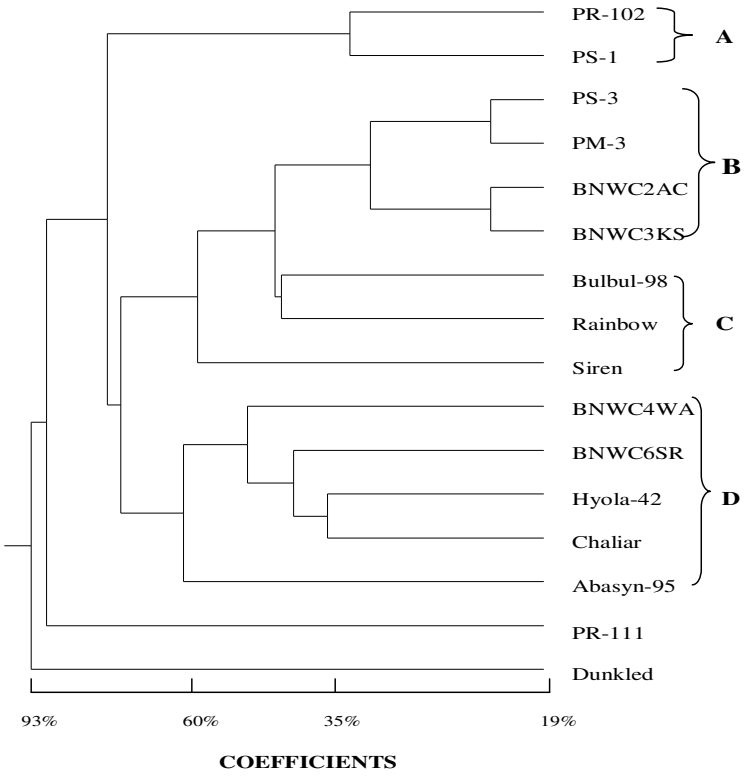

Figure 2. Dendrogram constructed for sixteen *B. napus* lines based on genetic distances using seven RAPD primers.

The genetic dissimilarity coefficient matrix of 16 *B. napus* genotypes based on seven RAPD primers using UPGMA method (Nei and Lie, 1979) was used to construct dendrogram using computer programme "popgene" (Figure 2). The genotypes were grouped into 4 clusters A, B, C and D comprising of 2, 4, 3 and 5 geno-types, respectively. Based on the dendrogram analysis, varieties PR-102 and PS-1 were most distantly related from Dunkled among the group of 16 B*rassica* geno-types. Similar results were reported by Cansian and Echeverrigaray (2000) and Das et al. (1999) who observed more or less similar ranges of genetic dissimi-larities in *Brassica* lines.

Conclusion and recommendations

From the present data, it can be inferred that PCR based assays can be effectively used to analyze the genetic diversity in *B. napus* (Figure 1). Generally, a wide range

of genetic variation exists, ranging from 19 to 93% in various combinations of *B. napus* lines used in the present study. It is recommended that among the 16 *B. napus* genotypes, genetically distinct lines pointed out in the present study should be used in future breeding programs for improving yield and other quality characteristics of *Brassica*.

REFERENCES

Botstein D, White RL, Skolnick MH, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Charters YM, Robertson A, Wilkinson MJ, Ramsay G (1996). PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. Oleifera) using 5'-anchored simple sequence repeat (SSR) primers. Theor. Appl. Genet. 92: 442-447.

Demeke T, Adams RP, Chibbar R (1992). Potential taxonomic use of random amplifed polymorphic DNA (RAPD) a case study in *Brassica*. Theor. Appl. Genet. 92: 990-994.

Divaret I, Margale E, Thomas G (1999). RAPD markers on seed bulks efficiently assess the genetic diversity of *Brassica oleracea* L. collection. Theor. Appl. Genet. 98: 1029-1035.

dos Santos JB, Nienhuis J, Skroch P, Tivang J, Slocum MK (1994). Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. *Theor. Appl. Genet.* 87, 909-915

Economic Survey of Pakistan. (2004-05). Minstry of Finance. Govt. of Pakistan.

Hamza M, Seyis F, Lühs W, Badani AG, Friedt W, Snowdon RJ (2004) Surveying genetic diversity in the *Brassica napus* gene pool using SSR markers. Genome, 37: 342-351.

Kresovich S, Szewc-McFadden AK, Bliek SM, Mc-Ferson JR (1995). Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size- fractionated genomic library of *Brassica napus* L. (rapeseed). Theor. Appl. Genet. 91: 206-211.

Liu R, Qian W, Meng J (2002). Association of RFLP markers and biomass heterosis in trigenomic hybrids of oilseed rape (*Brassica napus x B. campestris*). Theor. Appl. Genet. 105: 1050-1057.

McGrath JM, Quiros CF (1991). Genetic diversity at isozyme and RFLP loci in *Brassica campestris* as related to corp type and geographical origin. Theor. Appl. Genet. 83: 783-790.

MINFAL Agric. Statistics of Pakistan (2004-05). Govt of Pakistan, Ministry of Food, Agric. Livestock Div. (Econ. Wing), Islamabad.

Nei N, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.

Plieske J, Struss D (2001). Microsatellite markers for genome analysis in *Brassica*. *Theor. Appl. Genet.* 102: 689-694.

Son KM, Osborn TC, Williams PH (1988a). *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs) and genome evolution of diploid and amphidiploid species. Theor. Appl. Genet. 75: 784-794.

Song KM, Osborn TC, Williams PH (1990b). *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs) and genome relationship in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). Theor. Appl. Genet. 79: 497-506.

Tanhuanpaa PK, Vilkki JP, Vilkki HJ (1995). Association of a RAPD marker with linolenic acid concentration in the seed oil of rapeseed (*Brassica napus* L.). Genome, 38: 414-416.

Thromann CE, Sharp AG, Schmidt H, Sperling P, Parkin IAP, Luhs W, Lydate DJ, Heinz E (1994). Desturase multigene families of *Brassica napus* arose through genome duplication. Theor. Appl. Genet. 94: 583-591.

Wang JL, Dan B, Hu SY, Tu JX, Luan YF, Meng X, Zhuo G, Nimazhuoma TL (2002). RAPD analysis for the genetic diversity of *Brassica rapa* in Tibet. Yi Chuan Xue Bao. 29: 1021-1027.

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

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

Zhang YX, Brunel D, Delourme R, Fouilloux S (1997). Development of sequence tagged-site PCR markers in oilseed rape (*Brassica napus)*. Plant Anim. Genome, 74: 1763-1769.