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Assessment of Genetic Diversity of Local and Exotic *Brassica napus* Germplasm

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Abstract: Estimation of genetic diversity of *Brassica* germplasm provides the basis for rapeseed/mustard genetic improvement. Studies were undertaken to estimate the genetic diversity of 30 lines of *Brassica napus* using Randomly Amplified Polymorphic DNA (RAPD) primers. A total of 30 *B. napus* genotypes of local and exotic origin were characterized using molecular markers. Four RAPD primers were used to estimate the genetic distances among the genotypes in all the possible combinations. The genetic diversity study revealed different levels of genetic polymorphism for RAPD primers GLA05, GLA07, GLA09 and GLA10, resulting in amplification of 5.7, 3.5, 3.1 and 5.4 scorable bands (loci) per genotype, respectively. Individual genetic distances observed among *B. napus* genotypes ranged from 6.5 to 51%. Bivariate data matrix was generated and genetic distances were calculated using Unweighted Pair Group of Arithmetic Mean (UPGMA) procedure. The UPGMA cluster analyses revealed maximum genetic dissimilarity for 8966-1 and 8969-1 genotypes, closely followed by Ganyou-5, 89127-1, 89111-2 and Mlep-048. It is recommended that among the thirty *B. napus* genotypes, genetically distinct lines pointed out in the present study, should be used in future breeding programs for improvement of *Brassica napus*.

Key words: Genetic diversity, RAPD, *brassica napus*, rapeseed

INTRODUCTION

Pakistan is facing crucial shortage of edible oil and a lot of its foreign exchange is being used on the import of oil. Presently, the domestic production of edible oil meets only 34% of country's requirements and Pakistan continues to import large quantities of edible oil to fulfill the local demand. According to the Economic Survey of Pakistan, oil import costed US $ 372.88 millions in the form of foreign exchange during 2004-2005. The shortage of edible oil is increasing day by day as a result of rapidly growing population of the country. Rapeseed contributes about 16-18% towards the total domestic production of edible oil. Recent figures published in the Economic Survey of Pakistan (2004-2005) indicate that rapeseed is being cultivated over an area of 244 thousand hectares with an annual production of 227 thousand tons of seed.

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 relationship are very important in designing crop improvement programs, management of germplasm and evolution of conservation strategies. The identification of genotypic variation is a requirement of seed industry, including variety registration, seed certification and improvement of breeding programs. The use of morphological characteristics for genotype identification and discrimination is central to the breeding programs. However, the robustness of morphological descriptors is questionable since they are based on both the genetic makeup of the variety and the interaction of the genotype with the environment. Furthermore, the increased number of varieties with similar genetic origin and the recent introduction of transgenic varieties raise concerns that morphological data may not have sufficient discriminating power to clearly distinguish among such genotypes.

For genotype identification, the use of DNA profiling techniques represents several advantages over the morphological characterization. DNA sequences are not affected by environmental conditions and identification can be determined at any stage of plant growth (Lakshmikumaran, 2000; Smith and Smith, 1992). Finally, the use of the Polymerase Chain Reaction (PCR) has enabled the development of simple and rapid DNA profiling methods (Justo de *et al*., 2004; Morell *et al*., 1995).

Random Amplified Polymorphic DNA (RAPD) markers offer quick screening of different regions of the genome for genetic polymorphisms. The standard RAPD procedure uses a single 10-base-long random

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oligonucleotide as a primer to amplify short stretches of the genome by PCR (Osborn and Lukens, 2003; Hu *et al*., 1995). The use of RAPD markers for fingerprinting varieties has been investigated in many crops, including canola (Zhu and Wu, 1998; Mailer *et al*., 1994). The RAPD markers are easier and quicker to use and are preferred in applications where the relationships between closely-related breeding lines are of interest (Halldén *et al*., 1994). The present study was undertaken to estimate the genetic diversity of different lines of *B. napus* using RAPD markers and obtain reliable information that can be an asset to any *Brassica* breeding program.

MATERIALS AND METHODS

The presented study was conducted at the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University Peshawar during the year 2004-2005. During this study the thirty *Brassica napus* lines (Table 1) were characterized molecularly.

A small-scale total genomic DNA isolation procedure (Weining and Langridge, 1991) was used with modification. DNA samples were then treated with 40 µg RNAase-A at 37°C for 1 hour. For Polymerase Chain Reaction (PCR) 1: 5 dilution of DNA was made in double distilled, deionized autoclaved water. Quality of DNA samples was checked on a 0.8% Agarose/TBE gel. Four Randomly Amplified Polymorphic DNA primers (GLA05, GLA07, GLA09 and GLA10) purchased from GeneLink, Inc. NY 10532, USA, were used during the present analysis. Detailed description and sequence information of the primers is given in the Table 2.

PCR reactions were carried out in 25 µL reaction using standard protocols. Amplification conditions for RAPDs involved an initial denaturation step of 4 min at 94°C, followed by 40 cycles each consisting of a denaturation step of 1 min at 94°C, an annealing step of 1 min at 36°C and an extension step of 2 min at 72°C. The last cycle was followed by 7 min extension at 72°C. The amplification products were electrophorased on 2.0% agarose/TBE gels and visualized by staining with ethidium bromide and viewed under UV light (Sambrook *et al*., 1989). For genetic diversity analysis, all scorable bands (alleles) were scored as present (1) or absent (0). The bivariate 1-0 data were used to estimate genetic dissimilarity following Nei and Li (1979). The bivariate 1-0 data were also used to construct a dendrogram using computer program PopGene32 version 1.31 (http://www.ualberta.ca./~fyeh/fyeh).

RESULTS AND DISCUSSION

During the present study four RAPD primers GLA05, GLA07, GLA09 and GLA10 were used to detect level of genetic polymorphism at DNA level among the 30 genotypes used during the present studies. Only the scorable bands were included in the analyses. Every single band was considered as a single locus/allele for all the genetic analyses. The loci were scored as present / absent. Bivariate data 1-0 were used to estimate genetic distances (Ds). Unweighted Pair Group of Arithamatic Means (UPGMA) function (Nei and Li, 1979) was used to estimate genetic distances between the genotypes as follows:

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

The 1-0 bivariate data matrix for thirty *Brassica napus* lines based on the data of four RAPD primers using UPGMA method was used to construct a dendrogram which is presented in Fig. 1. In general the dendrograms agreed with the average dissimilarity matrix presented in Table 1. Based on the dendrogram analysis the thirty Brassica napus lines can be categorized in 3 major groups i.e., A, B and C. *Brassica* lines 8966-1 and 8969-1, were most distantly related among the 30 lines used in the study. The other lines which showed more genetic dissimilarities were Ganyou-5, 89127-1, 89111-2 and Mlep-048. These results were in close agreement with the results of genetic distances where similar relationships

Fig. 1: *PCR amplification products of 30 *B. napus* lines using RAPD primer GLA10, {1) Printol, 2) con – 1, 3) con – 2, 4) con – 3, 5) Cyclone, 6) Norseman, 7) Bullet 8)sponser, 9) westar, 10) vanguard, 11) Ganyou – 5, 12) Crusher, 13) Oscar, 14) Rain Bow, 15) ks – 75, 16) 89111 – 2, 17) 8948 – 2, 18) 8966 – 1, 19) 8969 – 1, 20) Bln -877, 21) 89206 – 2, 22) 89127 – 1, 23) 89111 – 1, 24) 9214 – 10, 25) Mlep – 048, 26) DGL, 27) dunkled, 28) rain bow, 29) con 1, 30) con 2}

between the varieties were found. The lines that showed maximum genetic distances in dendrogram analyses also showed higher Genetic Distances (GD) in average dissimilarity matrix values (Table 1).

During present study, RAPD markers were found to be useful, allowing better evaluation of genetic diversity at DNA level. Level of genetic polymorphism (estimated as percent genetic distance) observed during present study varied (Table 3), but in general it was in the range of 16 to 37.7 %. Similar results were reported by Das *et al.* (1999) who observed more or less similar ranges of genetic dissimilarities in *Brassica* lines. For individual RAPD primers, higher level of genetic polymorphism was found in case of RAPD primer GLA10 where higher levels of genetic variability were observed among different comparisons, indicating its power for the identification of individual genotypes (Table 4). The Dendrogram constructed by Unweighted Pair Group of Arithamatic Means (UPGMA) was generally in agreement with the genetic distances calculated, indicating that the RAPD technique can be used reliably for estimation of genetic variability in rapeseed. Generally polymorphism in amphidiploids is less than that observed in diploid species. The level of polymorphism found in the present study in *B. napus* lines was less then 38%. Similar findings were reported by Uzunova *et al.* (1995) who reported less then 45% level of polymorphism in *B. napus*. In *B. oleracea*, a diploid specie, higher level of more than 80% polymorphism and in *B. juncea* approximately 60% polymorphism was reported by Cheung *et al.* (1997). Lower levels of polymorphism in amphidiploids may be attributed to the lower level of out-crossing due to a weak and often non-existing self-incompatibility system (Rakow

Table 1: Description of the 30 *Brassica napus* lines used in the current study

Sr. No.	Genotypes	Sr. No.	Genotypes
1	Printol	16	89111 – 2
2	Con – 1	17	8948 – 2
3	Con – 2	18	8966 – 1
4	Con – 3	19	8969 – 1
5	Cyclone	20	Bln -877
6	Norseman	21	89206 – 2
7	Bullet	22	89127 – 1
8	Sponser	23	89111 – 1
9	Westar	24	9214 – 10
10	Vanguard	25	Mlep – 048
11	Ganyou – 5	26	DGL
12	Crusher	27	Dunkled
13	Oscar	28	Rain bow-1
14	Rain Bow	29	Con 1
15	Ks – 75	30	Con 2

Table 2: Detailed description and sequence information of the primers used

Name of primer	Sequence	Size	TM (°C)	Mol. Wt (Da)
A05 primer	(AGGGGTCTTG)	10bp	29.5	2987.9
A07 primer	(GAAACGGGTG)	10bp	29.5	3037.0
A09 primer	GGGTAACGCC)	10bp	33.6	3012.9
A10 primer	(GTGATCGCAG)	10bp	33.6	3012.9

TM = Melting temperature of primer; Mol. Wt = Molecular weight of the primer

Table 3: Description of dissimilarity among 30 *Brassica napus* lines in Percentage for primer genelink A-10 primer

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	42																												
3	42	0																											
4	25	42	60																										
5	50	20	50	50																									
6	14	33	55	14	42																								
7	28	20	50	28	33	16																							
8	62	40	62	62	20	57	50																						
9	25	42	60	0	50	14	28	62																					
10	50	20	50	28	33	16	0	50	28																				
11	50	20	50	28	33	16	0	50	28	0																			
12	28	20	50	50	33	42	33	50	50	33	33																		
13	37	33	55	37	42	28	16	57	37	16	16	16																	
14	14	33	55	37	42	28	42	57	37	42	42	16	28																
15	28	50	66	50	57	42	57	50	50	57	57	33	42	16															
16	37	57	70	37	62	50	42	57	37	42	42	42	28	50	42														
17	28	50	66	50	57	42	57	50	50	57	57	33	42	16	0	16													
18	50	50	66	66	33	62	57	20	66	57	57	33	42	42	33	42	33												
19	57	25	57	57	40	50	40	25	57	40	40	40	50	50	40	50	40	40											
20	0	42	60	25	50	37	50	62	25	50	50	28	37	14	28	37	28	50	57										
21	0	42	60	25	50	37	50	62	25	50	50	28	37	14	28	37	28	50	57	0									
22	57	25	33	57	40	50	40	25	57	40	40	40	50	50	40	50	40	40	0	57	57								
23	28	20	66	28	33	16	33	50	28	33	33	33	42	16	33	62	33	57	40	28	28	40							
24	14	33	55	37	42	50	42	57	37	42	42	16	28	28	42	28	42	42	50	14	14	50	42						
25	25	42	60	25	50	37	28	62	25	28	28	28	14	37	50	14	50	50	57	25	25	57	50	14					
26	14	33	55	37	42	28	42	57	37	42	42	16	28	0	16	50	16	42	50	14	14	50	16	0	37				
27	57	20	57	57	40	50	40	25	57	40	40	40	50	50	40	50	40	40	0	57	57	0	40	50	57	50			
28	25	42	60	44	28	37	50	42	44	50	50	28	37	14	28	55	28	28	57	25	25	57	28	37	44	14	57		
29	28	20	50	50	33	42	33	50	50	33	33	0	16	16	33	42	33	33	40	28	28	40	33	16	28	16	40	28	
30	25	42	60	25	50	14	28	62	25	28	28	28	14	14	28	37	28	50	57	25	25	57	28	37	25	14	57	25	28

{1) Printol, 2) con – 1, 3) con – 2, 4) con – 3, 5) Cyclone, 6) Norseman, 7) Bullet 8) Sponser, 9) Westar, 10) Vanguard, 11) Ganyou – 5, 12) Crusher, 13) Oscar, 14) Rain Bow, 15) ks – 75, 16) 89111 – 2, 17) 8948 – 2, 18) 8966 – 1, 19) 8969 – 1, 20) Bln -877, 21) 89206 – 2, 22) 89127 – 1, 23) 89111 – 1, 24) 9214 – 10, 25) Mlep – 048, 26) DGL, 27) dunkled, 28) rain bow, 29) con 1, 30) con 2}

Table 4: Description of average dissimilarity among 30 *Brassica napus* lines in Percentage for all four genelink primers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
2	25																												
3	19	13																											
4	16	31	23																										
5	27	18	20	21																									
6	14	18	17	14	14																								
7	24	9	22	24	24	10																							
8	30	16	23	24	12	24	28																						
9	6	25	23	10	27	14	24	30																					
10	21	18	13	15	15	8	10	20	15																				
11	19	26	27	23	29	21	23	33	13	15																			
12	20	30	31	25	27	31	35	31	25	27	27																		
13	24	28	20	24	24	17	20	28	24	10	25	17																	
14	20	30	24	26	26	20	29	24	26	20	34	18	20																
15	28	38	30	27	28	26	36	19	33	28	41	21	14	8															
16	24	34	31	30	34	28	32	28	24	24	19	36	27	29	30														
17	25	34	32	30	30	28	38	22	25	30	38	30	29	22	15	19													
18	42	42	40	46	32	36	41	29	38	38	50	46	40	38	38	40	37												
19	45	41	37	39	33	38	41	23	45	33	47	45	42	38	33	42	33	19											
20	22	32	31	23	22	22	31	25	34	28	41	34	32	22	23	31	20	40	40										
21	13	36	34	19	31	30	39	34	25	31	31	7	19	18	20	34	23	38	50	27									
22	27	31	27	37	29	33	37	25	21	29	29	23	38	39	35	38	33	35	35	41									
23	20	22	23	20	19	23	23	19	26	15	27	30	29	13	21	28	18	32	32	15	32	32							
24	16	26	20	22	22	21	25	21	22	17	29	26	20	16	32	20	25	24	34	18	29	34	11						
25	33	42	35	27	32	31	35	28	33	27	30	28	18	21	28	19	34	49	43	29	32	46	31	22					
26	29	38	37	34	34	32	42	31	28	34	29	33	33	26	28	23	23	51	47	23	39	35	26	22	28				
27	50	36	34	35	30	34	38	19	41	30	31	41	42	35	29	26	30	47	30	30	51	19	29	31	29	17			
28	25	34	28	30	24	24	33	23	36	25	25	35	29	19	26	20	23	27	42	27	38	36	13	16	26	19	27		
29	34	36	32	33	28	32	36	25	33	28	29	31	29	26	34	24	31	44	39	23	43	35	27	23	22	8	16	23	
30	27	34	34	27	31	24	33	29	27	26	34	32	23	25	27	29	21	48	44	16	36	27	24	27	32	14	21	30	17

{1) Printol, 2) con – 1, 3) con – 2, 4) con – 3, 5) Cyclone, 6) Norseman, 7) Bullet 8) Sponser, 9) Westar, 10) Vanguard, 11) Ganyou – 5, 12) Crusher, 13) Oscar, 14) Rain Bow, 15) ks – 75, 16) 89111 – 2, 17) 8948 – 2, 18) 8966 – 1, 19) 8969 – 1, 20) Bln -877, 21) 89206 – 2, 22) 89127 – 1, 23) 89111 – 1, 24) 9214 – 10, 25) Mlep – 048, 26) DGL, 27) dunkled, 28) rain bow, 29) con 1, 30) con 2}

and Woods, 1987). Level of genetic polymorphism (estimated as percent genetic distance) observed during present study varied in the range of 16 to 37.7%.

CONCLUSIONS AND RECOMMENDATIONS

It is evident from present data that PCR based assays like RAPDs can be used effectively to estimate genetic variability in *B. napus* (Fig. 2). Easy handling of the technique make it especially suitable for breeding programs where large numbers of lines have to be analyzed.

Generally a lot of genetic variation exists, ranging from a minimum average of 0.6% to the maximum average of 51% in various combinations of the *B. napus* germplasm investigated in the present study. Maximum genetic distances were observed among the lines 8966-1, 89206-2, 89127-1, DGL and Dunkled. The maximum value of 51% was observed for combinations DGL+89661 and Dunkled+89206-2 (equally in both cases), while the minimum value of 6.3 % for combination Printol+Bullet. It is recommended that among the 30 *B. napus* genotypes, genetically distinct lines, pointed out in the present study, should be used in future breeding programs for improving yield and quality characteristics of *Brassica*.

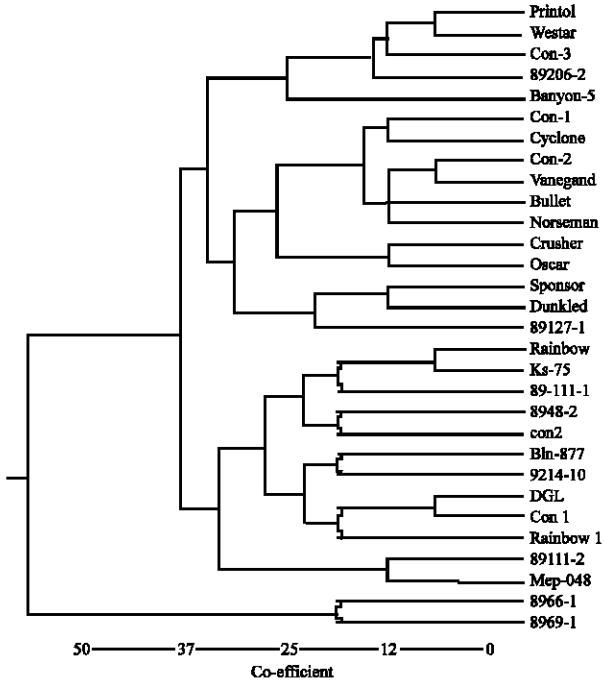

Fig. 2: Dendrogram of all the 30 *Brassica napus* lines based on genetic distances estimated from data of four RAPD primers

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