ISSN: 2518-2021 (Print), 2415-4474 (Online) Fundam Appl Agric 2016, 1(1): 17-22

Fundamental and Applied Agriculture

Journal homepage: www.f2ffoundation.org/faa

Genetics and Breeding
ORIGINAL ARTICLE

RAPD analysis of *Brassica napus* with introgressed cross and reciprocal of 6th generation lines

Umme Rosemila[1]*, Lutfur Rahman[1], Lutful Hasan[1] and Mohammad Mahbub-Ur-Rahman[2]

[1]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Mujibnagar Integrated Agricultural Development Project, Department of Agriculture Extension, Kushtia, Bangladesh

A R T I C L E I N F O

Article history:

Received: 01 March 2016
Received in revised form: 12 April 2016
Accepted: 07 May 2016
Available online: 25 May 2016

Academic Editor: Md. Parvez Anwar

Keywords:

Brassica napus
RAPD
Genetic relationship
Gene diversity
Polymorphic band
Reciprocal effect

A B S T R A C T

Assessment of the genetic variation and relatedness among the genotypes of *Brassica napus* is of great importance for variety selection, breeding purpose, hybridization, evaluation and conservation of the diverse gene pool. Random Amplified Polymorphic DNA (RAPD) assay was conducted to characterize two different varieties of *Brassica napus* viz. BINA Sarisha-4 (D), BINA Sarisha-5 (E) and their sixth generation cross and reciprocal cross selection progeny lines. Total genomic DNA was isolated from *Brassica* leaves following SDS extraction, Phenol: Choloroform: Isoamyl alchohol purification and ethanol precipitation method followed by using four decamer random primers to amplify genomic DNA. A dendrogram was constructed using unweighed pair group method of arithmetic mean (UPGMEA). The primers produced 26 RAPD bands of which 13 were considered as polymorphic. The percentage of polymorphic loci is 50%. The proportion of polymorphic loci and gene diversity estimates were 11.54% and 0.049 for the cross progeny BINA Sharisha-4 (D) × BINA Sarisha-5 (E); 26.92% and 0.1241 for the reciprocal cross selection progeny BINA Sarisha-5 (E) × BINA Sarisha-4 (D); 23.08% and 0.1054 for BINA Sarisha-5 (E); 3.85% and 0.0115 for BINA Sarisha-4 (D). High level of differentiation between the varieties (D) & (E) (Gst = 0.6553) and low level of gene flow (Nm = 0.2630) estimates across all loci reflected the existence of high level of genetic variation between two parents and cross and reciprocal cross progeny lines. The UPGMA dendrogram based on Nei's (1972) genetic distance indicated segregation of 4 accessions into two major clusters. Cluster one consists of cross and reciprocal cross selection progeny lines of (D) and (E) where they are closely related with the least genetic distance (0.1697). While the two parents (E) and (D) were grouped in cluster two. The highest genetic distance (0.3043) was observed between the reciprocal cross progeny lines (E) × (D) and the parent (D), which was the widest genetic diversity. The RAPD is a simple and fast technique to compare the genetic diversity and the patterns of variations between the varieties and progeny of *Brassica spp*.

Copyright © 2016 Rosemila et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

The genus *Brassica* belongs to the order Rhoedales and family Brassicaceae (Formerly Cruciferae) is featured with extensive diversity and includes several important agricultural and horticultural crops. The oilseed, *Brassica* include five major species viz. *B. nigra* (L.) Koch, *B. oleracea* L., *B. rapa* L., *B. carinata* A. Braun, *B. juncea* Czern & Coss and *B. napus* L. Among the oleiferous *Brassica* species, *B. rapa* and *B. napus* are commonly known as rapeseed, while those of *B. nigra* and *B. carinata* as mustard (Yarnell 1956) are the principle source of edible oil in Bangladesh. *B. napus* L. (rapeseed; 2n = 4x = 38), a non-pungent allotetraploid species, has been introduced in the country by Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA). In *Brassica,* a considerable degree of genetic variation exists among different species (Malik et al. 1995; Nanda et al. 1995). Desirable gene(s) may not be available in a single species or varieties and some of these do not express phenotypically. Such condition may require incorporation of the desired characters to commercial varieties/lines through intra and inter specific hybridization. In respect of combining

*Corresponding author: rosemilabau@gmail.com

ability, many workers have found different reciprocal effects in parents and hybrids (Rawat 1992; Pszezola 1993) for different characters. Thus genetic variation is an important issue to choose a good parent, good combination and to know the reciprocal effects.

For an effective breeding program, the extent and nature of genetic diversity within crop species is essential. There are various techniques for studying the genetic variability of crop germplasm including morphological traits, total seed protein, isozymes and various types of molecular markers. Molecular markers based on the Deoxyribonucleic Acid (DNA) sequence are more varied and reliable in this regard (Raghunathachari et al. 2000). Recent advances in the field of molecular marker opens up a new horizon for the plant breeder to hasten the transfer of desirable genes among varieties and to introgress novel genes from related species. These include Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR), Random Amplification of Polymorphic DNA (RAPD) (Williams et al. 1993; Karp et al. 1997) and Amplified Fragment Length Polymorphism (AFLP) (Vos et al. 1995). Among them, RAPD markers generated by the Polymerase Chain Reaction (PCR) have widely been using since 1990's to assess intra specific genetic variation at molecular level. RAPD analysis can be used extensively for various purposes which include to identify genetic variation (Bered et al. 2001; Tahian et al. 2003), genetic relatedness (Monte et al. 1999; Shan et al. 2001), genetic diversity analysis (Zheng et al. 2001; Maric et al. 2004) and phylogenetic relationship (Tsuji et al. 2000; Goriunova et al. 2004). The advantages of this technique are its ability to detect extensive polymorphisms, simplicity, rapidity and very small amounts of genomic DNA which allows the analysis of single seeds and young seedlings. Several studies of RAPD in different crops like soybean (William et al. 1990), Rice (Welsh and McClelland 1990) and tomato (Klein-Lankhorst et al. 1991) have concluded that RAPD markers are appropriate for mapping as well as DNA fingerprinting and population genetic diversity analysis.

The general objective of this study was therefore to assess the genetic variation and relatedness among the genotypes of two *Brassica napus* varieties viz. BINA Sarisha-4(D), BINA Sarisha-5(E) and their two colchiploid-C_6 cross and reciprocal cross lines by PCR based RAPD technique. It is important particularly for variety selection, for breeding purpose, hybridization, evaluation and conservation of the diverse gene pool. Considering these viewpoints the present research work was carried out to determine the genetic variation of the parents with their intra and inter-varietal cross and reciprocal progenies.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the BAU-SID/MoA Genetic Fingerprinting Laboratory of the Department of Genetics and Plant Breeding, Biotechnology laboratory, Department of Biotechnology and Professor Mohamod Hossain central laboratory of Bangladesh Agricultural University, Mymensingh.

Study Material

Two parental genotypes of *Brassica napus* viz. BINA Sarisha-4 (D) and BINA Sarisha-5 (E) and their two cross and reciprocal selection of sixth generation hybrids (from C_6 populations) were used in this study. The source of seeds was BAU-SID/MoA Genetic Fingerprinting Laboratory of the Department of Genetics and Plant Breeding. Seeds were sown and germinated in the pot placed in the green house of the Department of Genetics and Plant Breeding. Fresh and young leaf samples were collected from 12-days old seedlings and used as the source of genomic DNA.

Experimental Procedure

Total genomic DNA was isolated from *Brassica* leaves following Sodium Dodecyl Sulphate (SDS) extraction, Phenol: Chloroform: Isoamyl alcohol (25:24:1; v:v:v) purification and ethanol precipitation method (Ghosh et al. 2009; Amani et al. 2011). Initially, healthy portion of the youngest leaves were cut apart with sterile scissors and forceps. The leaf materials were then washed in sterile distilled water and ethanol and dried on fresh tissue paper to remove spore of microorganism and any other sources of foreign DNA. Approximately 0.1 – 0.2 g of leaf tissue was cut into small pieces and taken into eppendorf tubes (1.5 mL). After adding 400 µL extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 300 mM NaCl, 1% SDS, pH=8.0) (Edwards et al. 1991), the samples were ground with tissue homogenizer followed by further addition of 400 µL extraction buffer making the total volume 800 µL. Then the ground samples were vortexed for 20 seconds and incubated at 65°C for 5 min in a hot water bath. Following further mixing with a vortex mixture for about 20 seconds, the samples were again incubated at the same temperature for approximately 10 minutes. The extract was then centrifuged for 10 minutes at 13,000 rpm to precipitate the cell debris and the upper aqueous phase of about 600 µL was taken into another microcentrifuge tube. For purification, equal volume (600 µL) of Phenol: Chloroform: Isoamyl alcohol (25:24:1, v/v/v) was added to the tube and vortexed for few seconds. Then the solution was centrifuged for 10 min at 13,000 rpm. The upper aqueous phase was removed carefully without upsetting the lower portion and placed in a new eppendorf tube, to which an equal volume of Isopropanol was added, mixed and incubated for 20 minutes at -20^0C. DNA was precipitated first and visualized as white strands with 600 µL of absolute (100%) ethanol and pelleted by centrifugation for 10 minutes at 13,000 rpm. After discarding the liquid completely, re-precipitation of the DNA solution was done with 400 µL of 70% ethanol. Then 20 µL of 3 M sodium acetate was added to the DNA solution and pelleted by centrifugation for 3 minutes at 13000 rpm. After removing the liquid completely, the pellets were then air dried and dissolved in an appropriate volume of (20 - 50 µL) TE buffer. Finally, the DNA samples were stored at -20°C. The DNA samples were evaluated both quantitatively and qualitatively using spectrophotometer and agarose gel electrophoresis, respectively. DNA quality was checked by electrophoresis in 1% agarose gel and quantified (Table 1) using a spectrophotometer at 260 nm wave length (Spectronic® GENESIS™).

Table 1. Absorbance readings and concentrations of different DNA samples

Brassica variety	Serial No.	Absorbance reading (at 260 nm)	DNA concentration (ng/µl)
BINA Sarisha-4 (D) × BINA Sarisha-5(E)	$B_{1\text{-}1}$	0.007	350
	$B_{1\text{-}2}$	0.009	450
	$B_{1\text{-}3}$	0.010	500
BINA Sarisha-5(E) × BINA Sarisha-4(D)	$B_{2\text{-}1}$	0.009	450
	$B_{2\text{-}2}$	0.006	300
	$B_{2\text{-}3}$	0.009	450
BINA Sarisha-5(E)	$B_{3\text{-}1}$	0.009	450
	$B_{3\text{-}2}$	0.015	750
	$B_{3\text{-}3}$	0.012	600
BINA Sarisha-4(D)	$B_{4\text{-}1}$	0.012	600
	$B_{4\text{-}2}$	0.016	800
	$B_{4\text{-}3}$	0.012	600

Eleven primers of random sequence were screened for four different varieties to evaluate their suitability for amplification

of the DNA sequences, which could be scored accurately. The details of the primers are given in Table 2. A final subset of four primers (69AB10C9, 70AB10C10, 61AB10G1 and 66AB10G6) exhibiting good quality banding patterns and sufficient variability were selected for further analysis. The selected four primers produced comparative maximum number of high intensity band with minimal smearing. The number of fragments amplified per primer varied (Table 3).

Table 2. Parameters of the random primers used in the present study for screening

Primer code	Sequence	GC content (%)
63AB10A3	GTCGCCGTCA	70
68AB10A8	GTGTGCCCCA	70
62AB10C2	GGACCCAACC	70
69AB10C9*	CTCTGGAGAC	60
70AB10C10*	GGTCTACACC	60
61AB10G1*	ACCGCGAAGG	70
64AB10G4	TCTGGTGAGG	60
66AB10G6*	ACCTGAACGG	60
71AB10G11	AGCGCCATTG	60
72AB10G12	AGGGCGTAAG	60
73AB10T13	CTGGGGACTT	60

*selected for RAPD analysis of all samples of *Brassica*

Table 3. RAPD primers with corresponding bands scored and their size range together with polymorphic bands observed in *Brassica* genotypes

Primer codes	Sequences (5´ - 3´)	Total number of bands scored	Size range (bp)	Number of polymorphic bands
69AB10C9	CTCTGGAGAC	5	428-2029	2
70AB10C10	GGTCTACACC	7	92-2348	3
61AB10G1	ACCGCGAAGG	7	234-2083	4
66AB10G6	ACCTGAACGG	7	380-1988	4
Total		26		13

PCR Amplification and Electrophoresis

The amplification conditions were based on Williams et al. (1990) with some modification. PCR reactions were performed on each DNA sample in a 10 µL reaction mixtures containing 1 µL of 10X Ampli *Taq* polymerase buffer, 2.5 µL of primer, 1.0 µL of dNTPs, 2.50 µL of Ampli *Taq* DNA polymerase (Genei Pvt. Ltd., Bangalore, India) and 4.0 µL of genomic DNA and 1.30 µL of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mixture was denatured at 94°C for 3 minutes followed 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C and elongation or extension at 72°C for 2 minutes. After final extension step of 7 minutes at 72°C PCR products from each sample were confirmed by running 1.4% agarose gel containing 6.0 µL Ethidium bromide (10 mg/ml) in 1X TBE buffer at 120V for 1hr. 2.5 µL Loading dye (1/10th volume of stock) was added to the PCR products and loaded in the wells. Molecular marker DNA (λ DNA *Hind* III and 100bp DNA ladder) was also loaded on either side of the gel. RAPD bands were observed under ultra violet light on a trans illuminator and documented by taking photograph using a Gel documentation system. Using primers, 69AB10C9, 70AB10C10, 61AB10G1 and 66AB10G6 the banding patterns of two *Brassica* varieties and their 6th generation cross and reciprocal selection progeny lines have been shown in Figures 1 through 4.

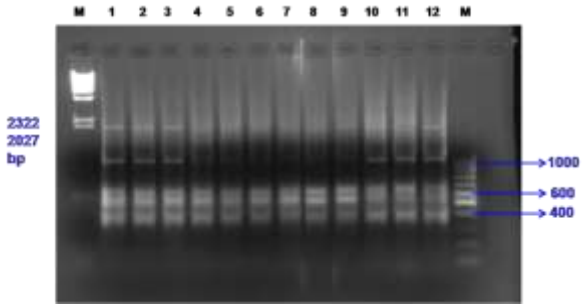

Figure 1. RAPD profiles of two different varieties of *Brassica napus*, viz. BINA Sarisha-4 (D) and BINA Sarisha-5 (E) along with their 6th generation cross and reciprocal selection progeny lines using 69AB10C9 primer. Lane 1-3: BINA Sarisha-4 (D) × BINA Sarisha-5 (E), Lane 4-6: BINA Sarisha-5 (E) × BINA Sarisha-4 (D), Lane 7-9: BINA Sarisha-5 (E) and Lane 10-12: BINA Sarisha-4 (D). Ms: Molecular weight markers (λ DNA *Hind* III digest/100 bp DNA ladder)

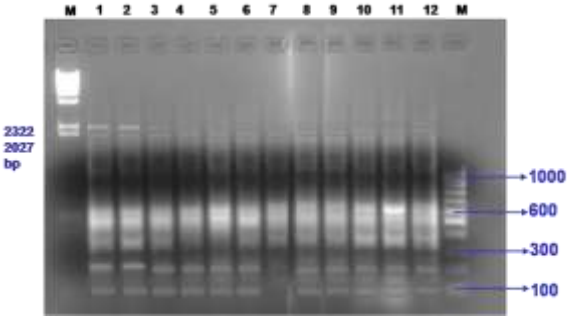

Figure 2. RAPD profiles of four different varieties (one cross, one reciprocal cross and two parental variety of 6th generation) of *Brassica napus*, using 70AB10C10 primer. Lane 1-3: BINA Sarisha-4 (D) × BINA Sarisha-5 (E), lane 4-6: BINA Sarisha-5 (E) × BINA Sarisha-4 (D), lane 7-9: BINA Sarisha-5 (E), and lane 10-12: BINA Sarisha-4 (D). Ms: Molecular weight markers (λ DNA *Hind* III digest/100 bp DNA ladder)

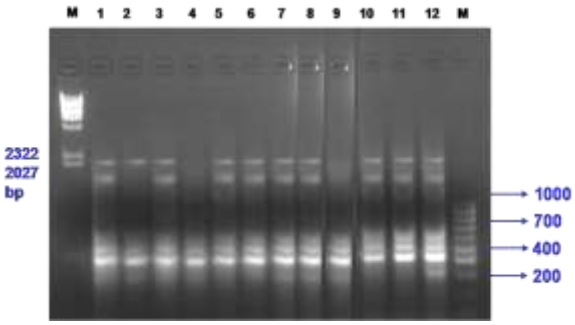

Figure 3. RAPD profiles of four different varieties (one cross, one reciprocal cross and two parental variety of 6th generation) of *Brassica napus*, using 61AB10G1 primer. Lane 1-3: BINA Sarisha-4 (D) × BINA Sarisha-5 (E), lane 4-6: BINA Sarisha-5 (E) × BINA Sarisha-4 (D), lane 7-9: BINA Sarisha-5 (E) and lane 10-12: BINA Sarisha-4 (D). Ms: Molecular weight markers (λ DNA *Hind* III digest/100 bp DNA ladder)

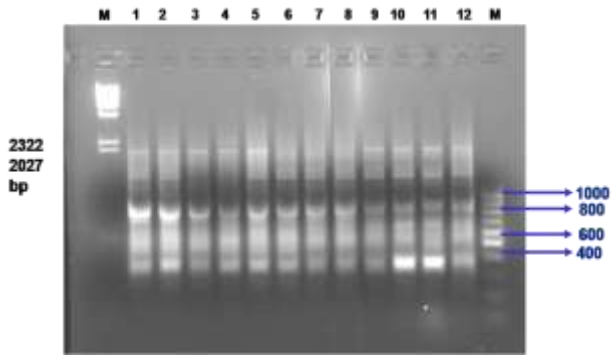

Figure 4. RAPD profiles of four different varieties (one cross, one reciprocal cross and two parental variety of 6th generation) of *Brassica napus*, using 66AB10G6 primer. Lane 1-3: BINA Sarisha-4 (D) × BINA Sarisha-5 (E), lane 4-6: BINA Sarisha-5 (E) × BINA Sarisha-4 (D), lane 7-9: BINA Sarisha-5 (E), and lane 10-12: BINA Sarisha-4 (D). Ms: Molecular weight markers (λ DNA *Hind* III digest/100 bp DNA ladder)

RAPD Data Analysis and Dendrogram Construction

All distinct bands or fragments (RAPD markers) were threreby given identification numbers according to their position on the gel and were scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. This was used for estimating polymorphic loci, Nei's (1973) gene diversity, population differentiation (G_{st}), gene flow (N_m), genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the populations using computer program POPGENE (Version 1.31). The same program was also used to perform homogeneity test in different loci between variety pairs.

RESULTS AND DISCUSSION

Polymorphic Loci and Gene Diversity

The highest number and proportion of polymorphic loci was found in the reciprocal progeny line BINA Sarisha-5 (E) × BINA Sarisha-4 (D) which were 7 and 26.92 respectively, whereas the lowest values of these traits were recorded in parent variety BINA Sharisha-4 (Table 4). However, the highest level of Nei's (1973) gene diversity value (0.1241) was found in C_6 reciprocal cross progeny line BINA Sarisha-5 (E) × BINA Sarisha-4 (D) (98.72%) and the lowest value (0.0115) was found in parent variety BINA Sarisha-4 (D).

Table 4. Number and proportion of polymorphic bands, gene diversity obtained in different *Brassica* genotypes

Variety/line	Number of polymorphic loci	Proportion of polymorphic loci (%)	Gene diversity
BINA Sharisha-4 (D) × BINA Sharisha-5 (E)	3	11.54	0.0491
BINA Sharisha-5 (E) × BINA Sharisha-4 (D)	7	26.92	0.1241
BINA Sharisha-5 (E)	6	23.08	0.1054
BINA Sharisha-4 (D)	1	3.85	0.0115

Intra and Inter Variety Similarity Indices

Table 5 (A and B) showed that intra-variety similarity indices (Si) were higher (ranged from 92.62 to 98.72%) than inter variety similarity indices (Sij) (ranging from 88.44 to 97.63) indicating low genetic variation between two individuals of same variety than between two individuals of different varieties using RAPD technique. Among the parents and their cross and reciprocal cross progeny lines intra-varietal similarities indices (Si) was found the highest in BINA Sarisha-4 (98.72%). The highest inter-variety similarity indices of 97.63% was found between BINA Sharisha-4 (D) × BINA Sharisha-5 (E) vs. BINA Sharisha-4 (D) while BINA Sharisha-5 (E) × BINA Sharisha-4 (D) vs BINA Sharisha-4 (D) showed least inter-variety similarity indices (88.44%).

Table 5. Summary of band sharing based similarity indices within and between individuals of four different *Brassica* genotypes

A. Intra-varietal similarity indices (Si)

Variety and progeny line	Band sharing values (%)				
	69AB 10C9	70AB 10C10	61AB 10G1	66AB 10G6	Average
BINA Sharisha-4 (D) × BINA Sharisha-5 (E)	100	100	88.85	93.94	95.69
BINA Sharisha-5 (E) × BINA Sharisha-4 (D)	100	94.87	81.68	93.94	92.62
BINA Sharisha-5 (E)	100	88.88	88.88	100	94.44
BINA Sharisha-4 (D)	100	100	94.87	100	98.72

B. Inter-varietal similarity indices (Sij)

Varietal pair	Band sharing values (%)				
	69AB 10C9	70AB 10C10	61AB 10G1	66AB 10G6	Average
BINA Sharisha-4 (D) × BINA Sharisha-5 (E)- BINA Sharisha-5 (E) × BINA Sharisha-4 (D)	78.086	94.86	87.14	94.94	88.76
BINA Sharisha-4 (D) × BINA Sharisha-5 (E)- BINA Sharisha-5 (E)	75	94.44	91.72	96.96	89.53
BINA Sharisha-4 (D) × BINA Sharisha-5 (E)- BINA Sharisha-4 (D)	100	100	93.56	96.96	97.63
BINA Sharisha-5 (E) × BINA Sharisha-4 (D)- BINA Sharisha-5 (E)	100	92.70	88.13	93.93	93.69
BINA Sharisha-5 (E) × BINA Sharisha-4 (D)- BINA Sharisha-4 (D)	75	94.86	89.95	93.93	88.44
BINA Sharisha-5 (E)- BINA Sharisha-4 (D)	75	94.44	92.70	100	90.54
Average	83.85	95.22	90.53	96.12	91.43

Genetic Distance

Original measures of Nei's genetic distance (Nei, 1972) between varieties were computed from combined data sets for the 4 primers ranging from 0.1697 to 0.3043 (Table 6). The highest genetic distance (0.3043) was observed between BINA Sharisha-5 (E) × BINA Sharisha-4 (D) vs. BINA Sharisha-4 (D) pair and the lowest genetic distance (0.1697) was found in BINA Sharisha-4 (D) × BINA Sharisha-5 (E) vs BINA Sharisha-5 (E) × BINA Sharisha-4 (D) progeny lines pair.

Genetic Differentiation and Estimation of Gene Flow

Nei's (1973) analysis of gene diversity in subdivided populations presented the gene flow (Nm) value of 0.2630 and the proportion of total genetic diversity (Gst) was 0.6553 (Table 7). Hardy-Weinberg expectation of average heterozygosity in sub- population (Ht) was 0.2104, whereas; the heterozygosity (Hs) was 0.0725 (Table 7).

Table 6. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values among studied *Brassica* varieties and their progeny lines

Variety	BINA Sharisha-4 (D)×BINA Sharisha-5 (E))	BINA Sharisha-5 (E)×BINA Sharisha-4 (D)	BINA Sharisha-5 (E)	BINA Sharisha-4 (D)
BINA Sharisha-4 (D) × BINA Sharisha-5 (E)	****	0.8439	0.7503	0.8415
BINA Sharisha-5 (E) × BINA Sharisha-4 (D)	0.1697	****	0.8383	0.7377
BINA Sharisha-5 (E)	0.2873	0.1764	****	0.8031
BINA Sharisha-4 (D)	0.1726	0.3043	0.2193	****

Table 7. Summary of genetic variation statistics across all loci

Locus	Sample size	Ht	Hs	Gst	Nm*
69AB10C9-1	12	0.5000	0.0000	1.0000	0.0000
69AB10C9-2	12	0.5000	0.0000	1.0000	0.0000
69AB10C9-3	12	0.0000	0.0000	****	****
69AB10C9-4	12	0.0000	0.0000	****	****
69AB10C9-5	12	0.0000	0.0000	****	****
70AB10C10-1	12	0.0000	0.0000	****	****
70AB10C10-2	12	0.0000	0.0000	****	****
70AB10C10-3	12	0.0000	0.0000	****	****
70AB10C10-4	12	0.0000	0.0000	****	****
70AB10C10-5	12	0.3249	0.0749	0.7694	0.1498
70AB10C10-6	12	0.2470	0.1220	0.5061	0.4880
70AB10C10-7	12	0.2470	0. 1220	0.5061	0.4880
61AB10G1-1	12	0.4107	0.2440	0.4058	0.7321
61AB10G1-2	12	0.4910	0.3660	0.2546	1.4621
61AB10G1-3	12	0.0000	0.0000	****	****
61AB10G1-4	12	0.0000	0.0000	****	****
61AB10G1-5	12	0.0000	0.0000	****	****
61AB10G1-6	12	0.4541	0.1969	0.5663	0.3829
61AB10G1-7	12	0.4541	0.1969	0.5663	0.3829
66AB10G6-1	12	0.4541	0.1969	0.5663	0.3829
66AB10G6-2	12	0.4107	0.2440	0.4058	0.7321
66AB10G6-3	12	0.0000	0.0000	****	****
66AB10G6-4	12	0.4777	0.1220	0.7446	0.1715
66AB10G6-5	12	0.5000	0.0000	1.0000	0.0000
66AB10G6-6	12	0.0000	0.0000	****	****
66AB10G6-7	12	0.0000	0.0000	****	****
Mean	12	0.2104	0.0725	0.6553	0.2630
St. DEV		0.0501	0.0113		

H_t = Hardy-Weinberg average heterozygosity expected in sub-population

H_s = Hardy-Weinberg average heterozygosity obtained in sub-population

G_{st} = Co-efficient of gene differentiation

Nm = Estimate of gene flow from Gst or Gcs. e.g.,$N_m=0.5(1- G_{st})/G_{st}$

Dendrogram

Based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithemetic Mean (UPGMA), the 4 accessions were segregrated into two major clusters (Fig. 5). Cross and reciprocal cross selection of the parents BINA Sharisha-4 (D) × BINA Sharisha-5 (E) and BINA Sharisha-5 (E) × BINA Sharisha-4 (D) were grouped into cluster I and two parent varieties BINA Sharisha-5 (E) and BINA Sharisha-4 (D) were clustered into another group (cluster II). In cluster I, BINA Sharisha-4 (D) × BINA Sharisha-5 (E) and BINA Sharisha-5 (E) × BINA Sharisha-4 (D) formed sub-cluster I and sub-cluster II respectively where they are closely related with the least genetic distance (0.1697). In cluster II, BINA Sharisha-5 (E) and BINA Sharisha-4 (D) formed sub cluster I and sub cluster II respectively where they showed closer relationship with relatively higher genetic distance (0.2193). The highest genetic distance (0.3043) was observed between BINA Sharisha-5 (E) × BINA Sharisha-4 (D) and BINA Sharisha-4 (D).

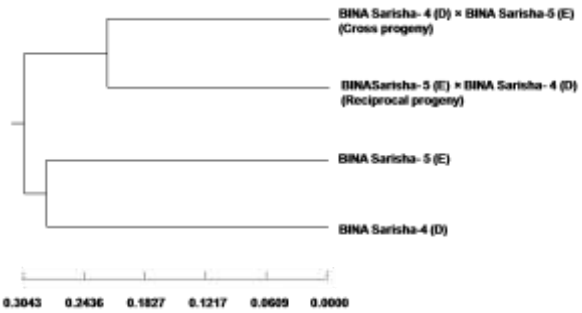

Figure 5. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between two *Brassica napus* varieties and their 6th generation cross and reciprocal selection progeny lines according to RAPD analysis

In this study, the RAPD technique was found in discriminating the four *Brassica* genotypes. Varieties and progeny lines showed higher intra-varietal similarity and lower frequency of polymorphic loci are likely to have low heterozygosity as compared to those exhibiting lower intra-varietal similarity. The present study showed that out of 4 RAPD markers, 13 scorable bands (50%) were considered as polymorphic. This proportion of polymorphism is lower compared to some previous RAPD analysis in *Brassica*, e.g. 81.72% in 9 Chinese mustard crops accessions (Fu et al. 2006), 76% in *B. napus* germplasm from Spain and Great Britain (Cartea et al. 2005). This difference can be attributed to the primers used and the genotypes evaluated.

The results are in consistent with the fact that the low or high level of genetic distance exists between cultivars with their same/ different origins. As they were originated from the same species, genetic variation among them was generally low. However, the highest genetic distance was found between the reciprocal cross progeny line and BINA Sarisha-4 (D). On the other hand the cross and reciprocal cross progeny lines showed the least genetic distance. As well as this study indicates that the reciprocal cross progeny and BINA Sarisha-4 (D) variety contain the highest genetic variation while the cross and reciprocal cross population contain least genetic variation.

CONCLUSION

The genetic variations among the studied varieties and progeny indicate that the RAPD method could be used as an effective tool for molecular genetic analysis among the cross and reciprocal cross progeny lines and their parents. However, large number of samples and higher number of primers would be necessary to generate and construct an appropriate genetic relationship, for reliable identification of samples and analysis of genetic diversity among different varieties and cultivars. In conclusion, RAPD markers have been proved to be powerful tools for molecular genetic analysis of *Brassica spp.* varieties for plant breeding program to assess genetic diversity available to allow for the development of new varieties. The results of this study can be used for future diversity assessment and genetic analysis among the cross and reciprocal cross for selection of *Brassica* materials important for cultivation purposes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

Amini J, Kazemi R, Abbasi AR, Salmanian AH. 2011. A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis. Iranian J Biotech, 9(1): 69-71.

Bered F, Barbosa NJF, Rocha BM, Carvalho FIF, da-Rocha BM, de-Carvalho FIF. 2001. Genetic variability in common wheat based on morphological traits, coefficients of parentage and RAPDs. J New Seeds, 3(2): 73-87.

Cartea ME, Soengas P, Picoaga A, Ordas A. 2005. Relationships among *Brassica napus* (L). germplasm from Spain and great Britain as determined by RAPD markers. Genet Resour Crop Evol, 52(6): 655-662.

Edwards K, Johnstone C, Thompson C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res, 19: 1349.

Fu J, Zhang MF, Qi XH. 2006. Genetic diversity of traditional Chinese mustard crops *Brassia juncea* as revealed by phenotypic differences and RAPD markers. Genet Resour Crop Evol, 53(7): 1513-1519. doi: 10.1007/s10722-005-7763-3.

Ghosh KK, Haque ME, Parvin MSS, Akhter F, Rahim MM. 2009. Genetic diversity analysis in *Brassica* varieties through RAPD markers. Bangladesh J Agril Res, 34 (3): 493-503.

Goriunova SV, Kochieva EZ, Chikida NN, Pukhalskii VA. 2004. Phylogenetic relationships and intraspecific variation of D-genome *Aegilops* L. as revealed by RAPD analysis. Genetika, 40(5): 642-51.

Karp A, Kresovich S, Bhat KV, Ayad WG, Hodgkin T. 1997. Molecular tools in plant genetic resources conservation: a guide to the technologies. in IPGRI (International Plant Genetic Resources Institute). Technical Bulletin, Rome, Italy.

Klein-Lankhorst RM, Vermunt A, Weide R, Liharska T, Zabel P. 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplied polmyorphic DNA (RAPD). Theor Appl Genet, 83: 83-108.

Malik V, Singh H, Singh D. 1995. Gene action of seed yield and other desirable characters in rapeseed. Ann Biol (Ludhiana), 11(1/2): 94-97.

Maric S, Boloric S, Mortinic J, Pejic I, Kozumplik V. 2004. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers. Plant-Breeding, 123(4): 366-369.

Monte JV, Casanova C, Soler C. 1999. Genetic variation in Spanish populations of the genus *Aegilops* revealed by RAPDs. Agronomie, 19(5): 419-427.

Nanda R, Bhargava SC, Tonar DPS. 1995. Rate and duration of siliqua and seed filling and their relation to seed yield in *Brassica* sp. Indian J Agric Sci, 64(4): 227-231.

Nei M. 1972. Genetic distance between populations. Amer Naturalists, 106:283-292.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proc Natl Aca Sci, 70: 3321-3323.

Pszczola J. 1993. Estimation of combining ability for selected traits in winter sweden rape in two sets of diallel crosses. Postepy Nauk Rolniczych, 40/45(5): 33-40

Raghunathachari P, Khanna VK, Singh US, Singh NK. 2000. RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). Current Sci, 79(7): 994-998.

Rawat DS. 1992. Analysis of reciprocal differences in Indian mustard. Acta Agronomica Hungarica, 41(3-4): 227-233.

Shan WX, Li YX, Song SJ, Guo PZ, Wu XS, Li YX. 2001. The RAPD marker analysis of wheat outbreeding population due to selection differentiation. Herditas-Beijing, 23(1): 33-37.

Tahian AS, Abo A, Elafa I. 2003. Multivariate and RAPD analysis of spring wheat cultivars. Anut J Agril Sci, 34(5): 1-25.

Tsuji K, Ohnishi O, Xin P. 2000. Origin of cultivated Tatary buckwheat (*Fagopyrum tataricum* Gaertn.) revealed by RAPD analysis. Genet Resour Crop Evol, 47(4): 431-438.

Vos P, Hogers R, Bleeker M, Reijans M, Van DLT, Hornes M, Freijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP a new technique for DNA fingerprinting. Nucleic Acids Res, 23: 4407–4414.

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

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primer are useful as genetic markers. Nucl Acids Res, 18(22): 6531-6535.

Williams JGK, Hanafey MK, Rafalski JA, Tingey SV. 1993. Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol, 218: 705-740.

Yarnell SH. 1956. Cytogenetics of Vegetable Crops. Bot Rev, 22: 81-166.

Zheng YL, Chen YO, Wei YM, Zhou YH, Zhang ZO. 2001. Esterase, gliadins and RAPD variations among Sichuan wheat cultivars. Wheat Info Ser, 92: 5-8.