GENETIC DIVERSITY ANALYSIS IN *BRASSICA* VARIETIES THROUGH RAPD MARKERS

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ABSTRACT

This investigation was aimed at exploring the genetic diversity and relationship among nine *Brassica* varieties, namely BARI Sharisha-12, Agrani, Sampad, BINA Sharisha-4, BINA Sharisha-5, BARI Sharisha-13, Daulot, Rai-5, Alboglabra using Random Amplified Polymorphic DNA (RAPD) markers. In total, 59 reproducible DNA bands were generated by four arbitrary selected primers of which 58 (98.03%) bands were proved to be polymorphic. These bands ranged from 212 to 30686 bp in size. The highest proportion of polymorphic loci and gene diversity values were 37.29% and 0.1373, respectively, for BARI Sharisha-12 and the lowest proportion of polymorphic loci and gene diversity values were 8.47% and 0.0318, 8.47% and 0.0382 for BINA Sharisha-4 and Rai-5, respectively. A dendrogram was constructed using unweighted pair group method of arithmetic mean (UPGMA). The result of cluster analysis indicated that the 9 accessions were capable of being classified into 2 major groups. One group consists of BARI Sharisha-12, Agrani, Sampad, Daulot, Rai-5, Alboglabra. where Daulot and Rai-5 showed the lowest genetic distance of 0.049. And another group contains BINA Sharisha-4, BINA Sharisha-5, and BARI Sharisha-1 3, where BINA Sharisha-5 and BARI sharisha-13 showed genetic distance of 0.071.

Key Words: RAPD, Brassica, genetic distance, polymorphic band.

Introduction

Crops of the genus *Brassica* are widely used in the cuisine of many cultures to the many choices of edible forms in the genus. Economically, *Brassica* is loosely categorized into oilseed, vegetable, and condiment crops. Among the *Brassica* species, the varieties of *B. nigra, B. carinata,* and *B. juncea* are commonly known as mustard, while those of *B. campestris* and *B. napus* as rapeseed (Yarnell, 1956). *B. oleracea* is known as cole crop which includes two major groups, the vegetable and the oilseed group. Assessment of genetic variability is the first step in any crop improvement programme. Molecular techniques viz., Restriction fragment length

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polymorphism (RFLP), Simple sequence repeats (SSR), Random amplification of polymorphic DNA (RAPD) (Williams *et al.,* 1990) and the recently developed Amplified fragment length polymorphism (AFLP) (Vos *et al.,* 1995) are the modern tools for precise estimation of genetic variation in crop species. Among them, RAPD markers, generated by the polymerase chain reaction (PCR) has widely been used since 1990s to assess intra specific genetic variation at molecular level (William *et al.*, 1990). The technique of RAPD gained importance due to its simplicity, efficiency, relative ease to perform and non-requirement of sequence information. It is, therefore, the aim of this work is to provide genetic variation and relatedness of some *Brassica* varieties by PCR based RAPD technique, as it is important, particularly for selection of diverse parents to be used in hybridization programme.

Materials and Method

Nine varieties of *Brassica* spp. viz., BARI Sharisha-12, Agrany, Sampad, BINA Sharisha-4, BINA Sharisha-5, BARI Sharisha-13, Daulot, Rai-5, Alboglabra were used in the study. Out of the nine varieties, three were BINA varieties (Agrany, BINA Sharisha-4, and BINA Sharisha-5), four BARI varieties (BARI Sharisha-12, BARI Sharisha-13, Daulot, and Rai-5), one was BAU variety (Sampad) and Alboglabra was exotic line from Germany. Seed germination was performed in the laboratory and the germinated seeds were sown in the pot placed in the green house of the Department of Genetics and Plant Breeding of Bangladesh Agricultural University. Fresh and young leaf samples were collected from 12-day old seedlings. Genomic DNA was extracted from young leaf following Phenol: Chloroform: Isoamyl alcohol (25:24:1; v:v:v) purification and ethanol precipitation method. DNA was precipitated with 800 µl of absolute ethanol where DNA became visible as white strands by flicking the tube several times with fingers. DNA was pelleted by centrifugation and reprecipitation of the DNA solution was done by adding 400 µl of 70% ethanol. The pellets were then air dried and dissolved in 50 µl of TE buffer (10mM Tris. HC1, 1 mM EDTA, pH=8.0). DNA quality was checked by electrophoresis in 2.5% agarose gel and quantified (Table 1) using a spectrophotometer at 260 nm wave length (Spectronic® GENESIS™). The DNA samples were evaluated both quantitatively and qualitatively using spectrophotometer and agarose gel electrophoresis, respectively.

Table 1. Absorbance readings and concentrations of different DNA samples.

Brassica variety	Serial No.	Absorbance reading (at 260 mm)	DNA concentration (ng/μl)
BARI Sharisha-12	B_{1-1}	0.038	1900
	B_{1-2}	0.047	2350
	B_{1-3}	0.034	1700
Agrani	B_{2-1}	0.013	650
	B_{2-2}	0.018	900
	B_{2-3}	0.016	800
Sampad	B_{3-1}	0.012	600
	B_{3-2}	0.009	450
	B_{3-3}	0.014	700
BINA Sharisha-4	B_{4-1}	0.025	1250
	B_{4-2}	0.020	1000
	B_{4-3}	0.018	900
BINA Sharisha-5	B_{5-1}	0.010	500
	B_{5-2}	0.009	450
	B_{5-3}	0.036	1800
BARI Sharisha-13	B_{6-1}	0.083	4150
	B_{6-2}	0.036	1800
	B_{6-3}	0.003	150
Daulot	B_{7-1}	0.057	2850
	B_{7-2}	0.038	1900
	B_{7-3}	0.024	1200
Rai-5	B_{8-1}	0.042	2100
	B_{8-2}	0.021	1050
	B_{8-3}	0.028	1400
Alboglabra	B_{9-1}	0.021	1050
	B_{9-2}	0.028	1400
	B_{9-3}	0.022	1100

Table 2. RAPD primers with corresponding bands scored polymorphic bands observed in nine *Brassica* varieties.

Primer codes	Sequences (5´-3´)	Total no. of bands scored	Size range (bp)	No. of polymorphic bands
OPB-04	GGACTGGAGT	11	300-1929	10
OPC-05	GATGACCGCC	19	376-3068	19
OPC-09	CTCACCGTCC	14	212-2034	14
OPD-02	GGACCCAACC	15	396-2272	15
Total		59		58

The primers used in RAPD analysis were found to discriminate different varieties and single exotic line (Two randomly chosen individuals from two different varieties to evaluate their suitability for amplification of the DNA

sequences) effectively. Among the 14 primers (Table 3) initially tested, 4 primers (OPB-04, OPC-05, OPC-09, and OPD-02) yielded comparatively maximum number of amplification products with high intensity and minimal smearing, good resolution and also clear bands. The number of fragments amplified per primer varied (Table 2).

Table 3. Parameters of the Operon random primers used in the present study for screening.

Primer Code	Sequences (5´-3´)	GC content (%)	Reference
OPA-02	TGCCGAGCTG	70	Jain *et al.* (1994) and Cartea *et al.* (2005)
OPA-05	AGGGGTCTTG	60	Jain *et al.* (1994) and Cartea *et al.* (2005)
OPA-07	GAAACGGGTG	60	Jain *et al.* (1994) and Cartea *et al.* (2005)
OPA-09	GGGTAACGCC	70	Jain *et al.* (1994) and Cartea *et al.* (2005)
OPB-03	CATCCCCFTG	60	Cartea *et al.* (2005)
OPB-04*	GGACTGGAGT	60	Cartea *et al.* (2005)
OPB-05	TGCGCCCTTC	70	Cartea *et al.* (2005)
OPB-10	CTGCTGGGAC	70	Cartea *et al.* (2005)
OPC-05*	GATGACCGCC	70	Jain *et al.* (1994) and Cartea *et al.* (2005)
OPC-09*	CTCACCGTCC	70	Jain *et al.* (1994) and Cartea *et al.* (2005)
OPD-02*	GGACCCAACC	70	Cartea *et al.* (2005)
OPK-10	GTGCAACGTG	60	Rabbani *et al.* (1998)
OPL-07	AGGCGGGAAC	70	Rabbani *et al.* (1998)
OPL-14	GTGACAGGCT	60	Rabbani *et al.* (1998)

* Selected for RAPD analysis for all samples of the nine varieties

DNA amplification by PCR and electrophoresis

PCR reactions were performed on each DNA sample in a 10 µl reaction mix containing 1.0 µl of l0x Ampli Taq polymerase buffer, 2.5 µl primer, 1.0 µl dNTPs, 0.2 µ1 Ampli Taq DNA polymerase (Genei Pvt. Ltd., Bangalore, India) and 4.0 µ1 of genomic DNA and 1.3 µ1 sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf, Germany). The reaction mix was preheated at 95°C for 3 mm followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 36°C and extension at 72°C for 2 min. After the last cycle, a final step of 7 mm at 72°C was added to allow complete extension of all amplified fragments.
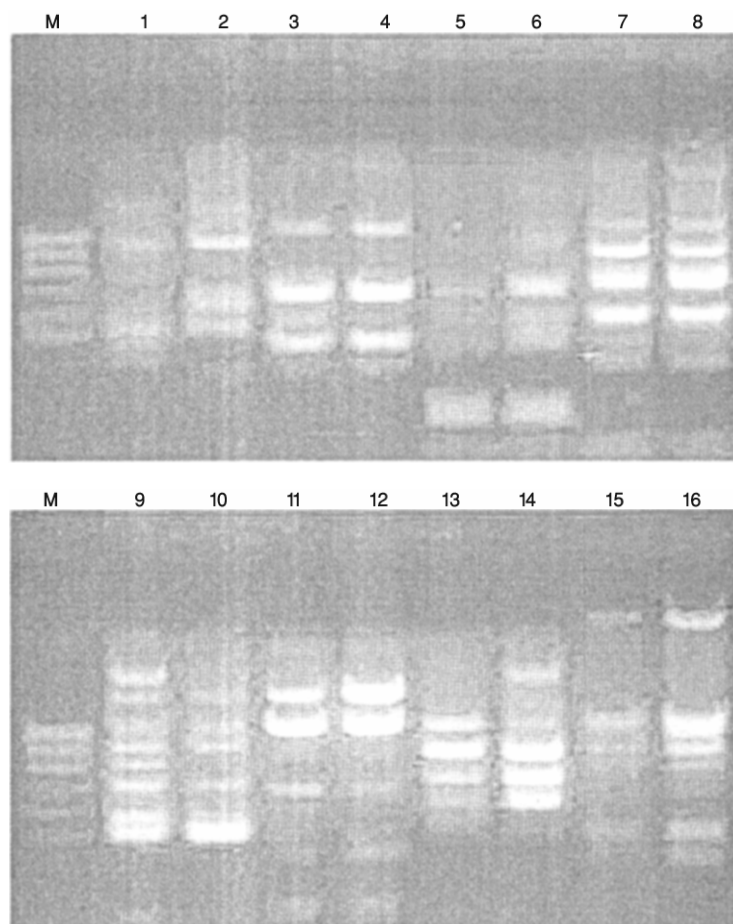
Fig. 1. Primer section PCR amplification products directed by 14 different decamer random primers using DNA from two randomly chosen individuals from different *Brassica* varieties Lane 1-2; OPA-02 Lane 3-4 OPA-07, Lane 5-6; OPA-09 Lane 7-8 OPB-04 Lane 9-10 OPB-05, Lane 11-12 OPC-05, Lane 13-14; OPC-09, Lane 15-16, OPD -02, M. Molecular weight marker (100 bp DNA ladder).

The amplified product from each sample was separated electrophoretically on 2.5% agarose gel containing ethidium bromide (SRL, India) in lx TBE buffer. A molecular weight DNA marker (100 bp DNA ladder) was loaded in the first lane of the gel. Electophoresis was carried out at 83v for 50 min to get the dye 3/4 of the gel length. DNA bands were observed on UV-transilluminator in the dark chamber of the Image Documentation System (uvitec, UK) and the image was viewed on the monitor, focused, acquired, saved and printed on thermal paper.
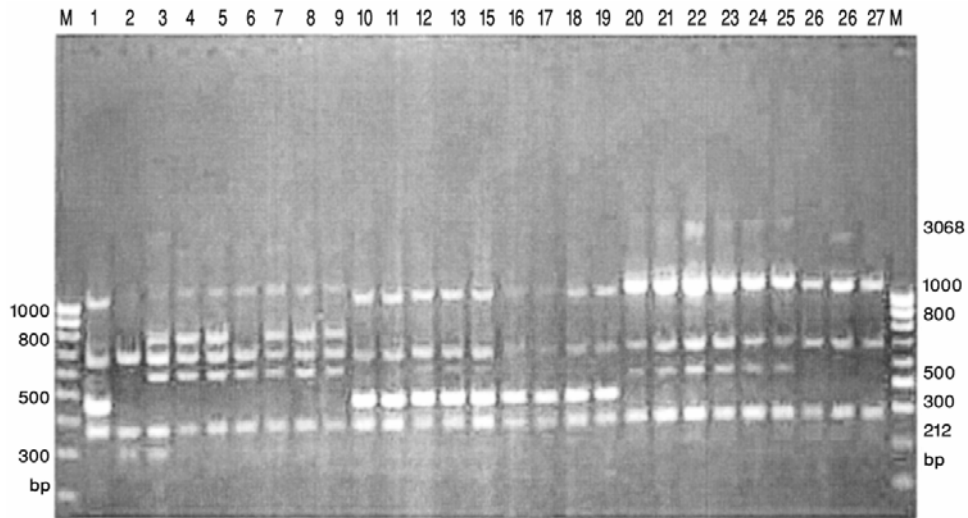
Fig. 2. RAPD profiles of nine *Brassica* varieties using primer OPB-04 Lane 1-2 BARI
Sharisha-12, Lane 4-6 Agrani Lane 7-9, Sampad Lane 12-12 BINA Sharisha-4,
13-16 BINA Sharisha-5, Lane 16-18, BINA Sharisha-13 Lane 19-21, Daulot Lane
22-24, Rai-5, Lane 25-27, Alboglabra M Molecular weight marker (100 bp DNA
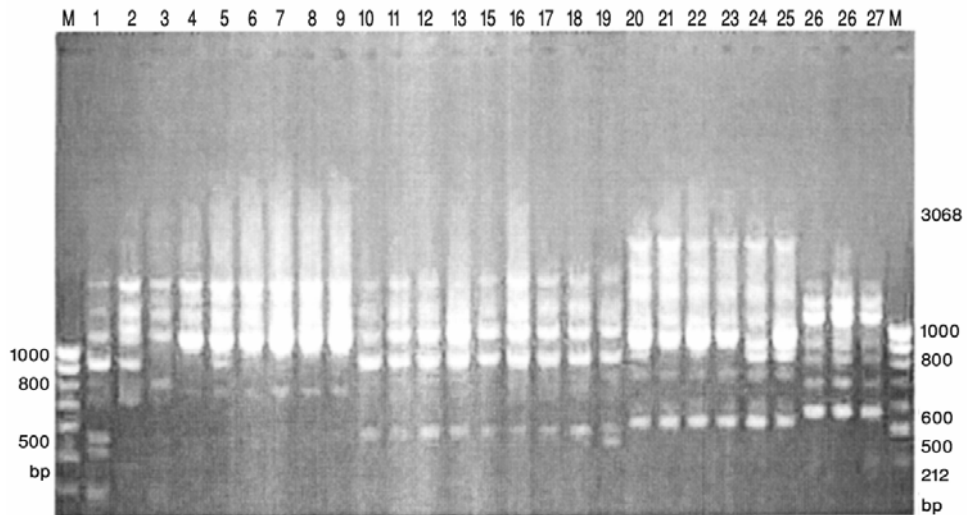ladder in both sides).

Fig. 3. RAPD profiles of nine *Brassica* varieties using primer OPC-05 Lane 1-2 BARI
sharisha-12, Lane 4-6 Agrani Lane 7-9, Sampad Lane 10-12 BINA Sharisha-4 13-
15 BINA Sharisha-5, Lane 16-18, BINA Sharisha-13 Lane 19-21 Daulot Lane 22-
24 Rai-5, Lane 25-27, Alboglabra, M Molecular weight marker (100 bp DNA
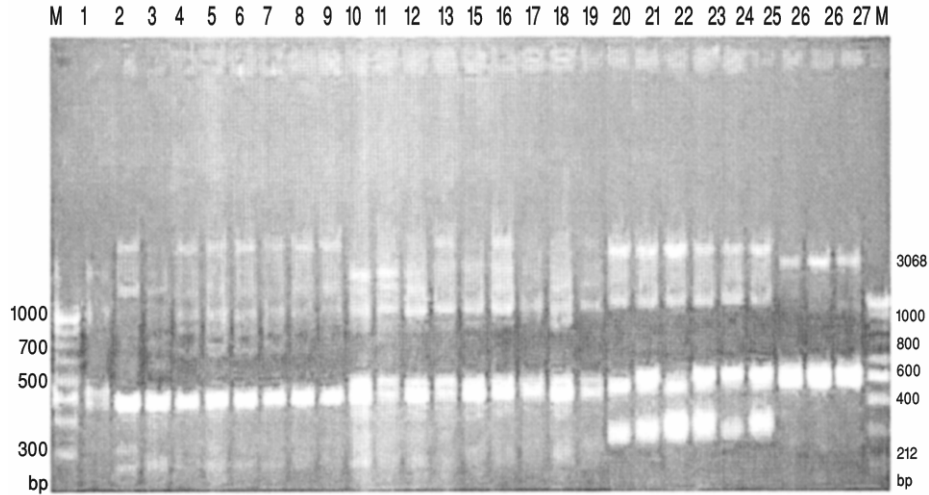ladder in both sides).

Fig. 4. RAPD profiles of nine *Brassica* varieties using primer OPC-09 Lane 1-2 BARI sharisha-12, Lane 4-6 Agrani Lane 7-9, Sampad Lane 10-12 BINA Sharisha-4 13-15 BINA Sharisha-5, Lane 16-18, BINA Sharisha-13 Lane 19-21 Daulot Lane 22-24 Rai-5, Lane 25-27, Alboglabra, M Molecular weight marker (100 bp DNA ladder in both sides).
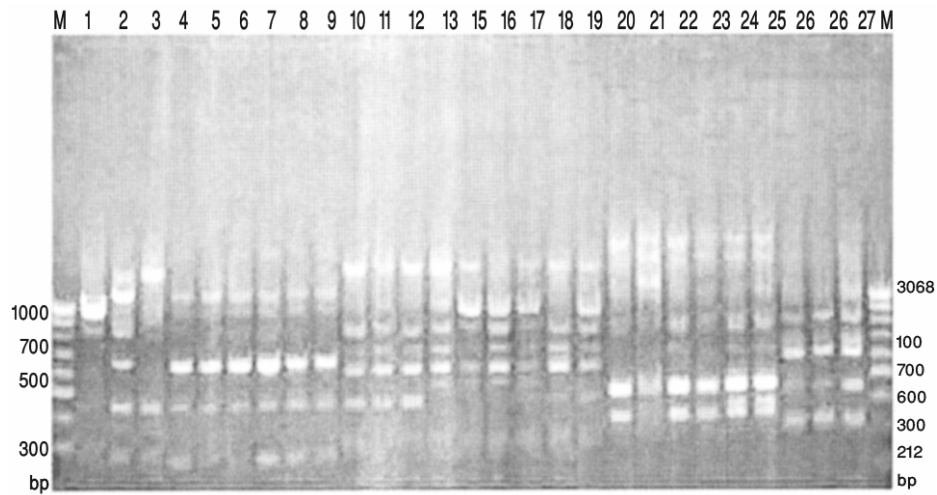

Fig. 5. RAPD profiles of nine *Brassica* varieties using primer OPD-02 Lane 1-2 BARI sharisha-12, Lane 4-6 Agrani Lane 7-9, Sampad Lane 10-12 BINA Sharisha-4 13-15 BINA Sharisha-5, Lane 16-18, BINA Sharisha-13 Lane 19-21 Daulot Lane 22-24 Rai-5, Lane 25-27, Alboglabra, M Molecular weight marker (100 bp DNA ladder in both sides).

RAPD data analysis

All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and 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 pooled to create a single data matrix. This was used to estimate polymorphic loci (Neis, 1973), gene diversity, population differentiation (G_{st}), gene flow (N_m), genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer programme, POPGENE (Version 1.31) (Yeh *et al.,* 1999).

Results and Discussion

The highest number and proportion of polymorphic loci was found in the variety of BARI Sharisha-12, which were 22 and 37.29%, respectively, whereas the lowest values of these traits was recorded in both the varieties of BINA Sharisha-4 and Rai-5 (Table 4). However, the highest and lowest Nei's (1973) gene diversity values were found in BARI Sharisha-12 and BINA Sharisha-4, respectively.

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

Variety	No. of polymorphic loci	Proportion of polymorphic loci (%)	Gene diversity
1. BARI Sharisha-12	22	37.29	0.1373
2. Agrani	12	20.34	0.0865
3. Sampad	7	11.86	0.0547
4. BINA Sharisha-4	5	8.47	0.0318
5. BINA Sharisha-5	12	20.34	0.0833
6. BARI Sharisha-13	14	23.73	0.0966
7. Daulot	9	15.25	0.0617
8. Rai-5	5	8.47	0.0382
9. Alboglabra	14	23.73	0.0871

Table *5.* presented the pair-wise gene flow (N_m) values. The highest gene flow (N_m) values was found in BINA Sharisha-5 - BARI Sharisha-13 varietals pair which was 1.45. On the other hand, the lowest gene flow value (0.07) was observed in BINA Sharisha-4 — Rai-5 (Table *5).*

Table 5. Gene flow value (N_m) in different varietal pair (below diagonal.

Variety	BARI Sharishs-12	Agrani	Sampad	BINA Sharishs-4	BINA Sharishs-5	BARI Sharishs-13	Daulot	Rai-5
Agrani	0.59							
Sampad	0.42	1.09						
BINA Sharishs-4	0.31	0.13	0.09					
BINA Sharishs-5	0.54	0.22	0.13	0.51				
BARI Sharishs-13	0.76	0.28	.21	0.49	1.45			
Daulot	0.33	0.22	0.17	0.09	0.17	0.22		
Rai-5	0.26	0.17	0.12	0.07	0.14	0.18	1.10	
Alboglabra	0.48	0.25	0.19	0.14	0.28	0.34	0.25	0.24

The highest Nei's (1972) genetic distance (0.768) was observed in BINA Sharisha-4 — Rai-5 varietals pair whereas, the lowest genetic distance (0.049) was estimated in Daulot — Rai-5 varietals pair (Table 6). Furthermore, high level of genetic distance was found in BINA Sharisha-4–Daulot (0.764); Sampad--BINA Sharisha-4 (0.722) varietals pair and low level of genetic distance was observed in Agrani-Sampad (0.071), BINA Sharisha-5–BARI Sharisha-13 (0.071) varietals pair (Table 6).

Table 6. Summary of Nei's (1972) genetic distance (below diagonal) values between nine *Brassica* varieties.

Variety	BARI Sharishs-12	Agrani	Sampad	BINA Sharishs-4	BINA Sharishs-5	BARI Sharishs-13	Daulot	Rai-5
Agrani	0.23							
Sampad	0.293	0.071						
BINA Sharishs-4	0.356	0.672	0.722					
BINA Sharishs-5	0.261	0.556	0.613	0.126				
BARI Sharishs-13	0.189	0.447	0.483	0.150	0.071			
Daulot	0.411	0.450	0.438	0.764	0.614	0.506		
Rai-5	0.461	0.495	0.490	0.768	0.598	0.528	0.049	
Alboglabra	0.307	0.473	0.487	0.579	0.403	0.354	0.394	0.332

Based on Nei's (1972) genetic distance using Unweighted Pair Group Method of Arithmetic Mean (UPGMA), the 9 accessions were segregated into two major clusters (Fig. 6). As a result, BARI Sharisha-12, Agrani, Sampad, Daulot, Rai-5, Alboglabra clustered into one group that had two sub-clusters.BARI Sharisha-12,

Agrani, and Sampad were grouped in sub-cluster I and Daulot, Rai-5, Alboglabra were grouped in sub-cluster II. In sub-cluster I, Agrani and Sampad showed closer relationship with minimal genetic distance (0.071) and in sub-cluster II. Daulot and Rai-5 showed closer relationship with lowest genetic distance (0.049). BINA Sharisha-4, BINA Sharisha-5 and BARI Sharisha-13 clustered into another group where BINA Sharisha-5, and BARI Sharisha-13 showed closer relationship with minimal genetic distance (0.071).
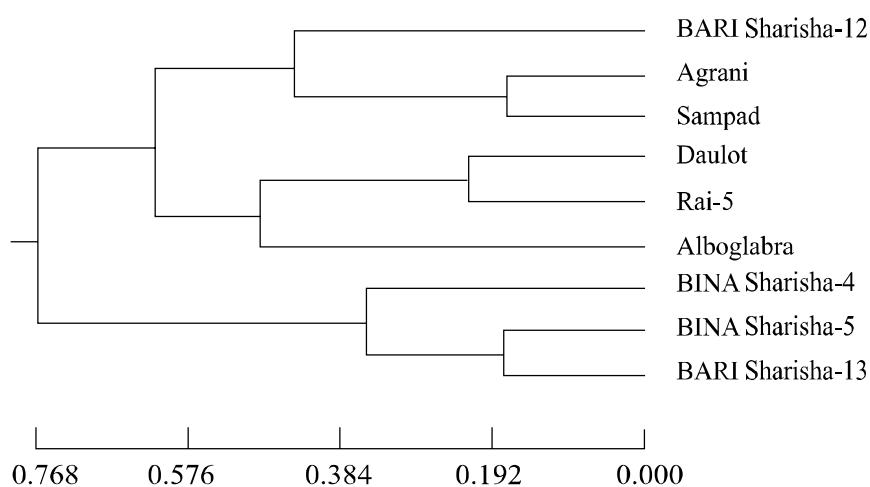

Fig. 6. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between nine *Brassica* varieties according to RAPD analysis.

The information can be used in breeding programmes. It has been proved that RAPD can be suitable and efficient tool for genetic characterization of many plant species including oilseed rape (Hu *el al.,* 1999).

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