

## Molecular characterization of oil seed *Brassica* using RAPD markers

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### Abstract

Twelve varieties of oil seed *Brassica* spp. were characterized at molecular level using Random Amplified Polymorphic DNA (RAPD) primers to explore genetic diversity and to find out relationship among them. Twelve random primers used in the study generated 94 RAPD fragments and 53 (56.38%) of them were considered as polymorphic indicating high level of polymorphism within the materials. The size of amplified fragments ranged between 300-3000 bp. The values of pair-wise genetic distance ranged from 0.1613 to 0.5543. To find out phylogenetic relationships among the varieties, dendrogram based on Nei's genetic distance was constructed using Unweighted Pair Group Method of Arithmetic Means (UPGMA) separating the 12 *Brassica* spp into two major clusters C<sub>1</sub> and C<sub>2</sub>. This result will be useful for designing future breeding programmes for improvement of *Brassica* varieties.

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### Introduction

Economically, *Brassica* (Fam.: *Brassicaceae*) is categorized into oilseed, vegetable and condiment crops (Ghosh *et al.*, 2009). Oilseed *Brassica* ranks third after soybean and palm oil in the global production (McVetty and Duncan, 2015). In this sub-continent three species of *Brassica*, namely, rapeseed, (*Brassica rapa*, Syn. *Brassica campestris* L.); mustard (*Brassica juncea* L. and *Brassica napus* L.) are cultivated for the production of oil. The varieties of *B. nigra* and *B. carinata* are also known as mustard but it is not commercially cultivated in Bangladesh. *Brassica* represented by rapeseed and mustard plays a vital role in vegetable oil production in Bangladesh. It is the most important edible oil crop, which covers the highest acreage of 66% of the total oil seed acreage of Bangladesh (BBS, 2010). This crop is mostly grown under residual soil moisture in winter season and due to the lack of using improved varieties as well as poor cultural practices, the average yield is quite lower than that in the developed countries (Hasanuzzaman *et al.*, 2008).

The probable origin of *Brassica* was in the Mediterranean-middle eastern area and a secondary centre of origin and differentiation of the species *B. rapa* and *B. juncea* was appears in China (Nishi, 1980). Since their introduction into China thousands of years ago, these two species have remarkably been changed in forms, structure and productivity by domestication. As a result of the allogamous breeding system in *Brassica*, there is a large amount of morphological variability in the many sub-species, botanical varieties and cultivar groups of *B. rapa* and *B. juncea* (Li, 1981; Lee, 1982; Opena *et al.*, 1988).

Therefore, assessment of genetic variability is a first step for the future breeding program specially like these naturally synthesized complex crop species *Brassica*. Improvements through genetical manner have largely been contributed to the high productivity of many crops. However, breeders tend to concentrate on specific genotypes, which combine traits of

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interest and could be used as progenitors in several breeding programs. With the development of a wide range of molecular techniques, marker assisted breeding is now used to enhance flexibility, selection of the breeding materials of conventional breeding programs in crop improvement (Frey *et al.*, 2004; Lu *et al.*, 2004). Among them, RAPD markers, generated by the polymerase chain reaction (PCR) has widely been used to assess intra-specific genetic variation at molecular level (Williams *et al.*, 1990). In some recent studies on *Brassica* crops, RAPD technique has successfully been used to determine the genetic relationships with different related species (Ren *et al.*, 1995); to identify cultivars (Hu and Quiros, 1991) and genetic diversity analysis among the crop germplasm (Teklewold and Becker, 2006; Ahmad *et al.*, 2009). Moreover, the main advantages of RAPD over other molecular methods are the low sample DNA requirements, high frequency of detectable polymorphic DNA bands and independent from the effects of environmental factors (Kuras *et al.*, 2004).

Although there are many varieties of *Brassica* spp. available in Bangladesh, but the present varieties are mostly characterized on the basis of their morphological features. Therefore, an authentic characterization and streamlining of these varieties are needed. RAPD is an efficient method for varietal identification, study of polymorphism, gene mapping, biodiversity, genetic map construction, hybridization and phylogenetic relationship in mustard varieties (Sharma and Sharma, 1999). The aims of the present study were to characterize the varieties of *Brassica* spp. which are commonly grown in Bangladesh using RAPD primers. The main goal was to elucidate the phylogenetic relationship among *Brassica* spp on the basis of

their molecular data by PCR based RAPD technique.

### Materials and methods

A total of 12 varieties of *Brassica* spp. namely *Brassica campestris* var. BARI Sarisha-1 (Tori-7), *Brassica juncea* var. BARI Sarisha-2 (Rai-5), *Brassica campestris* var. BARI Sarisha-6 (Dhali), *Brassica campestris* var. BARI Sarisha-9 *Brassica juncea* var., BARI sarisha-10, *Brassica juncea* var. BARI Sarisha-11, *Brassica campestris* var. BARI Sarisha-12, *Brassica napus* var. BARI Sarisha-13, *Brassica campestris* var. BARI Sarisha-14, *Brassica campestris* var. BARI Sarisha-15, *Brassica juncea* var. BARI Sarisha-16 and *Brassica campestris* var. BARI Sarisha-17 were investigated in this study. These 12 varieties of *Brassica* were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. The experiment was performed in the Tissue Culture Laboratory of Bangladesh Council of Scientific and Industrial Research, (BCSIR), Dhaka-1205. To extract genomic DNA, young and actively growing fresh leaves were collected from each of the 12 varieties of *Brassica* spp. seedlings. Total genomic DNA was extracted by using a modified CTAB method (Doyle and Doyle, 1987). DNA quality was checked by electrophoresis in 1% agarose gel and quantified through a spectrophotometer (Analytikjena, Specord 50, Germany) at 260 nm wave length.

To perform RAPD analysis, seventeen decamer primers (Operon Technology, USA and Sigma, USA) were applied of which twelve primers exhibited good quality banding patterns and sufficient variability. These twelve primers were selected for further analysis. The details of the twelve primers were given in Table I.

**Table I. Twelve arbitrary primers (RAPD primers) used in the present study**

Primer code	Sequence (5'—3')	Anneal. temp.(°C)	G+C content (%)
OPA-1	CAG GCC CTT C	36.4	70
OPA-2	TGC CGA GCT G	40.7	70
OPA-4	AAT CGG GCT G	35.1	60
OPA-5	AGG GGT CTT G	32.6	60
OPA-6	GGT CCC TGA C	35.2	70
OPA-7	GAA ACG GGT G	34.1	60
OPA-10	GTG ATC GCA G	33.1	60
OPG-02	GGC ACT GAGG	33.6	70
OPG-19	GTC AGG GCA A	34.7	60
UBC-3	CCT GGG CTT A	35.2	60
UBC-336	GCC ACG GAG A	39.8	70
UBC-355	GTA TGG GGC T	32.7	60

The following components were used to prepare 25 µl PCR cocktail-Taq buffer A 10x (10 mM Tris-HCl with 1.5 mM MgCl<sub>2</sub>) - 2.5 µl; primer (10 µM) - 1.0 µl; dNTPs mix (10 mM) - 0.5 µl; Taq DNA polymerase (5 U/µl) - 0.2 µl; Template DNA (25 ng/µl) - 2 µl and sterile de-ionized distilled water- 18.8 µl. DNA amplification is carried out in Thermal cycler (Applied Biosystems, USA) for 35 cycles after initial denature at 95°C for 5 min, denature at 95°C for 1 min, annealing at 30-36°C for 30 sec, extension at 72°C for 3 min and final extension at 72°C for 5 min. The amplified products were separated by electrophoresis in 1% agarose gel. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system (MS major science UVDA). The photographs were critically examined on the basis of presence (1) or absence (0), size of bands and overall polymorphism of bands. The scores obtained using all

## Results and discussion

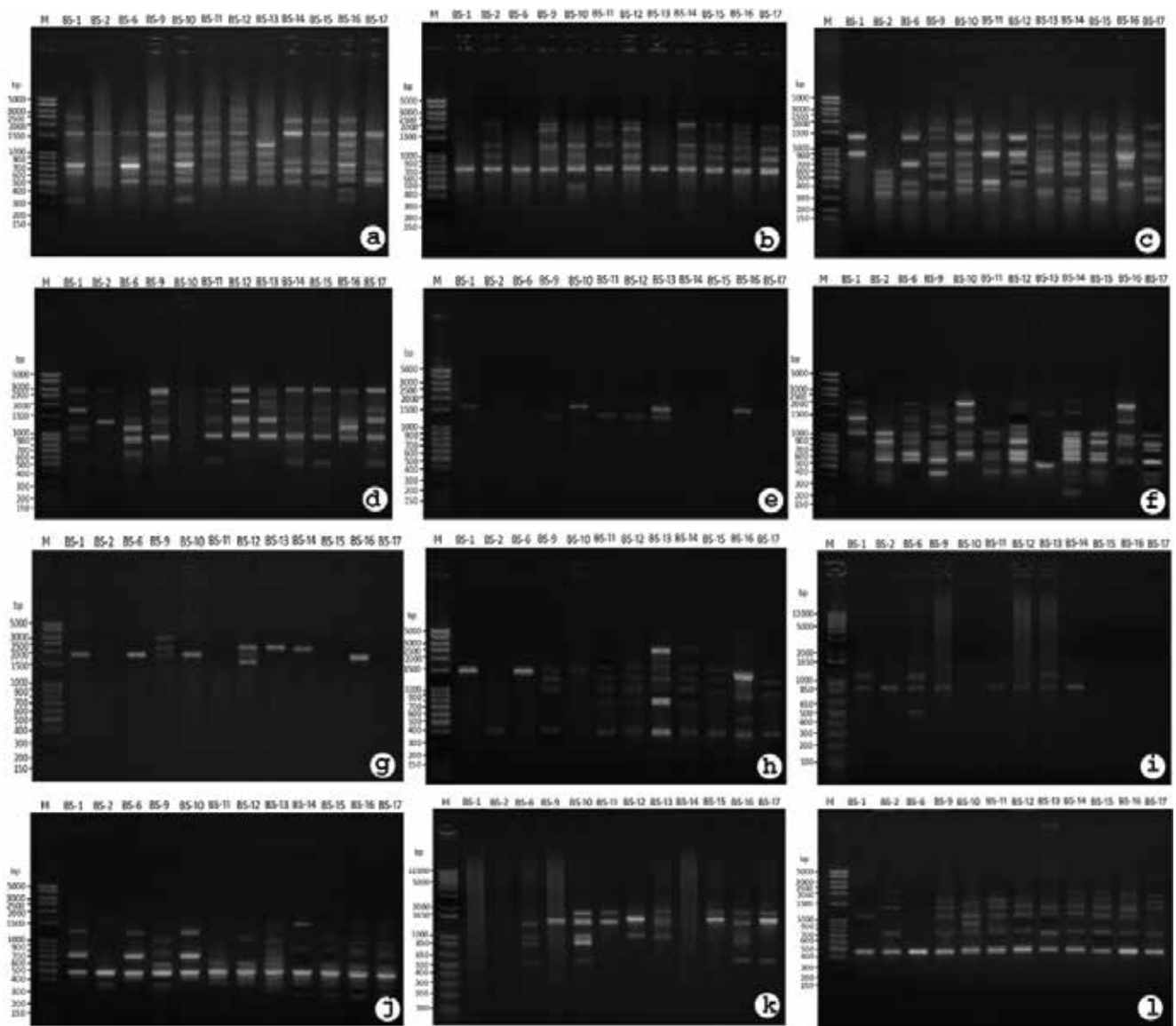
Twelve oligo-nucleotide primer combinations, namely, OPA-1, OPA-2, OPA-4, OPA-5, OPA-6, OPA-7, OPA-10, OPG-2, OPG-19, UBC-3, UBC-336 and UBC-355 were used for RAPD analysis of 12 *Brassica* varieties. Each primer combination showed different banding patterns. The 12 *Brassica* varieties selected for the present study represent a broad spectrum of variation for several phenotypic traits. The primer sequence, band size and banding pattern of 12 *Brassica* spp. varieties were shown in Table II. Fig. 1 showed the amplification profiles generated with the 12 primers across the 12 *Brassica* varieties.

**Table. II. Compilation of RAPD analysis in 12 varieties of *Brassica* spp**

Primer Sequences (5'—3')	Total fragment or loci	Size ranges (bp)	Number of Polymorphic loci	Polymorphisms (%)
CAG GCC CTT C	12	300-3000	8	66.66
TGC CGA GCT C	7	500-2250	6	85.71
AAT CGG GCT G	12	300-2000	10	83.33
AGG GGT CTT G	9	500-3000	7	77.78
GGT CCC TGA C	2	1300-1600	1	50
GAA ACG GGT G	4	1500-3000	3	75
GTG ATC GCA G	13	250-2000	3	23.07
GGC ACT GAG G	6	500-1700	4	66.67
GTC AGG GCA A	7	500-2000	5	71.43
CCT GGG CTT A	9	300-2500	3	33.33
GCC ACG GAG A	3	500-1200	1	33.33
GTA TGG GGC T	10	300-1500	2	20
Total	94		53	56.38

primers in the RAPD markers analysis were pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei's (1972) gene diversity, genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the germplasm using computer program "POPGENE 32" (Version 1.32).

Only 3 common bands of two sizes (500 and 800 bp) were observed in three primer combinations namely OPA-2, primer-UBC-3 and primer-UBC-355 (Fig. 1: b, j and l.). The different sized common band indicated the sharing of similar DNA fragments among 12 varieties. Although these varieties had some common RAPD bands, sufficient polymorphisms regarding RAPD fragments were observed.



**Fig. 1.** RAPD analyses of 12 *Brassica* spp. Varieties.(a) RAPD analysis with primer OPA-1, (b) RAPD analysis with primer OPA-2, (c) RAPD analysis with primer OPA-4, (d) RAPD analysis with primer OPA-5, (e) RAPD analysis with primer OPA-6, (f) RAPD analysis with primer OPA-7, (g) RAPD analysis with primer OPA-10, (h) RAPD analysis with primer OPG-2, (i) RAPD analysis with primer OPG-19, (j) RAPD analysis with primer UBC-3 (k) RAPD analysis with primer UBC-336, (l) RAPD analysis with primer UBC-355

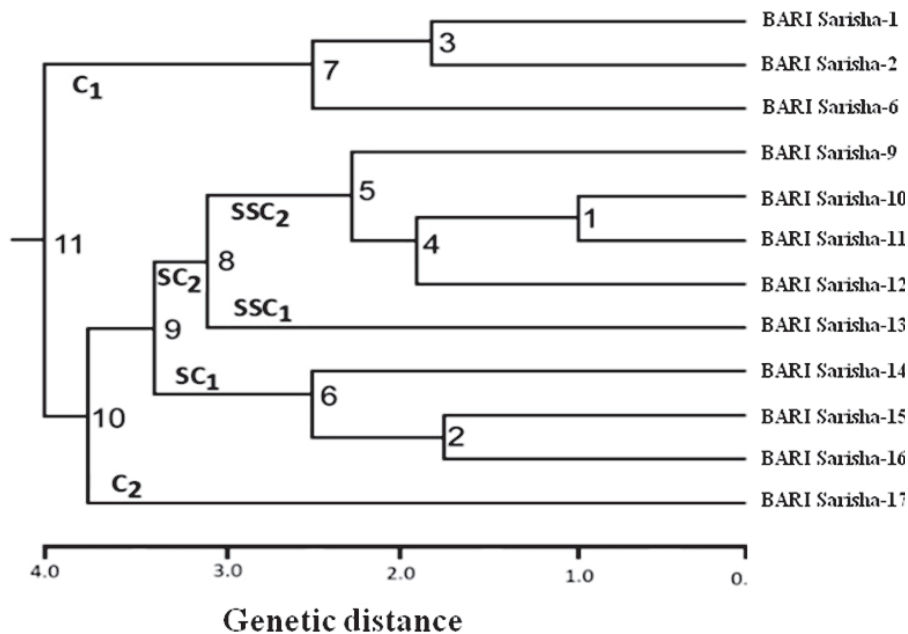
The twelve random primers generated 94 RAPD fragments or loci of which 53 (56.38%) were considered as polymorphic and this indicated significant level of polymorphism existed within population. Ali *et al.* (2007) scored a significant degree of polymorphism in 15 mustard (*Brassica juncea* L.) germplasms using RAPD markers. The molecular weight of bands analyzed by them ranged from 100 to 7000 bp of which 59.36% were polymorphic. Ahmad *et al.* (2007) reported

59% and 60.09% polymorphism in *B. napus* L. and *Brassica campestris* L. lines, respectively. Usunova *et al.* (1995) observed less than 45% level of polymorphism in different varieties of *Brassica napus*.

The RAPD fragments size ranges were 300-3000 bp. The number of amplification products generated by each primer varied from 2 (OPA-6) to 13 (OPA-10) with an average of

7.83 bands per primer. Primer OPA-2 showed the highest percentage of polymorphism (85.71%) while lowest percentage of polymorphism was observed using primer UBC-355 (20%) (Table-II). Light and bright bands were produced in the RAPD reactions. Light bands produced from low homology between the primer and the pairing site on the DNA strand (Thormann *et al.*, 1994). Saha *et al.* (2008) reported the presence of polymorphic bands in nine *Brassica* varieties have had range from 212 to 2272 bp in size and got highest proportion of polymorphic loci (37.29 %). Ahmad *et al* (2009) reported 97.70% polymorphism 20 genotypes of *Brassica juncea* and the amplified amplicons were ranged

Based on Nei's (1972) genetic distance, a dendrogram was prepared using UPGMA where the 12 *Brassica* varieties were segregated into two cluster or groups C<sub>1</sub> and C<sub>2</sub> (Fig. 2). The major cluster could be divided into two sub-cluster in which first sub-cluster included three *Brassica* varieties of *B. campestris* namely BARI Sarisha-1, BARI Sarisha-2 and *B. juncea* BARI Sarisha-6 while the second sub-group included rest of the varieties (Fig. 2). The second subgroup was further divided into sub-subclusters. The values of pair-wise comparison Nei's (1972) genetic distance among 12 *Brassica* varieties computed from combined data from the twelve RAPD primers ranged from 0.1613 to 0.5543 (Table III). The



**Fig. 2.** UPGMA dendrogram constructed based on Nei's (1972) genetic distance summarizing data on differentiation among 12 varieties of *Brassica* Spp. In which, *Brassica campestris* var. BARI Sarisha-1 (Tori-7), *Brassica juncea* var. BARI Sarisha-2 (Rai-5), *Brassica campestris* var. BARI Sarisha-6 (Dhali), *Brassica campestris* var. BARI Sarisha-9, *Brassica juncea* var., BARI sarisha-10, *Brassica juncea* var. BARI Sarisha-11, *Brassica campestris* var. BARI Sarisha-12, *Brassica napus* var. BARI Sarisha-13, *Brassica campestris* var. BARI Sarisha-14, *Brassica campestris* var. BARI Sarisha-15, *Brassica juncea* var. BARI Sarisha-16 and *Brassica campestris* var. BARI Sarisha-17

from 0.1 kb to 5.1 kb. A narrower range of genetic diversity has also been formerly documented by Yuan *et al.* (2004), Astarini *et al.* (2004) in *B. napus* and *B. oleracea* respectively. A diverse level of polymorphism in different crops has been reported such as chickpea 98.14% (Rasool, 2013), peanut 96% (Lang and Hang, 2007), eggplant 57.89% (Biswas *et al.*, 2009) and chilli 90% (Paran *et al.*, 1998).

highest genetic distance (0.5543) was found between *B. campestris* var. BARI Sarisha-17 vs *B. juncea* BARI Sarisha-10. The lowest (0.1613) genetic distance was observed between *B. campestris* var. BARI Sarisha-15 vs BARI Sarisha-14. The difference between the highest and the lowest value of genetic distance revealed the wide range of variability persisting among the 12 mustard varieties. High

**Table III. Summary of Nei's (1972) genetic distances of 12 varieties of *Brassica* spp**

Varieties	BS-1	BS-2	BS-6	BS-9	BS-10	BS-11	BS-12	BS-13	BS-14	BS-15	BS-16	BS-17
BS-1	****											
BS-2	0.3386	****										
BS-6	0.2126	0.3386	****									
BS-9	0.4490	0.2806	0.5179	****								
BS-10	0.3238	0.4002	0.3238	0.4490	****							
BS-11	0.3689	0.1866	0.3689	0.2528	0.3689	****						
BS-12	0.4658	0.2948	0.5002	0.1995	0.3092	0.1866	****					
BS-13	0.3536	0.4324	0.4490	0.3536	0.4162	0.3386	0.3092	****				
BS-14	0.5002	0.2392	0.5002	0.3092	0.5002	0.2666	0.3536	0.4658	****			
BS-15	0.4002	0.1866	0.4324	0.2806	0.4658	0.2126	0.3238	0.4002	<b>0.1613</b>	****		
BS-16	0.4490	0.4002	0.3844	0.5179	0.3844	0.3689	0.4324	0.5179	0.4658	0.3689	****	
BS-17	0.5179	0.2258	0.5179	0.3844	<b>0.5543</b>	0.3092	0.4002	0.4829	0.3092	0.1995	0.3536	****

Here,

0.5543 indicates the highest genetic distance and 0.1613 showed the lowest genetic distance between two different varieties of *Brassica* spp.

BS-1= *Brassica campestris* var. BARI Sarisha-1 (Tori-7), BS-2= *Brassica juncea* var. BARI Sarisha-2 (Rai-5), BS-6= *Brassica campestris* var. BARI Sarisha-6 (Dhali), BS-9= *Brassica campestris* var. BARI Sarisha-9, BS-10= *Brassica juncea* var., BARI Sarisha-10, BS-11= *Brassica juncea* var. BARI Sarisha-11, BS-12= *Brassica campestris* var. BARI Sarisha-12, BS-13= *Brassica napus* var. BARI Sarisha-13, BS-14= *Brassica campestris* var. BARI Sarisha-14, BS-15= *Brassica campestris* var. BARI Sarisha-15, BS-16= *Brassica juncea* var. BARI Sarisha-16, BS-17= *Brassica campestris* var. BARI Sarisha-17.

genetic distance values between varieties pair were found due to difference in genetic constituent. The varieties of lowest genetic distance can be used as parental source for breeding line to improve mustard varieties. Ghosh *et al.* (2009) reported wide genetic diversity ranging from 0.049 to 0.768 among 8 genus of *Brassica* varieties in Bangladesh. Iqbal *et al.* (2015) reported genetic distance ranged from 0.1054 to 0.9862 among the 16 mustards varieties of Bangladesh according to the values of pair-wise comparison of Nei's.

### Conclusion

The results of the present investigation showed high level of polymorphisms (56.38%) among the twelve varieties of *Brassica*. Broad range of polymorphism revealed wide

variability in *Brassica* spp. variety. This observed variability would be helpful for future breeding program to improve *Brassica* varieties in Bangladesh.

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