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Genetic diversity analysis of mustard (*Brassica* spp.) germplasm using molecular marker for selection of short duration genotypes

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Molecular characterization of 16 mustard (*Brassica* spp.) genotypes by using 12 RAPD markers revealed that three primers GLA-11, OPB-04 and OPD-02 showed good technical resolution and sufficient variations among different genotypes. A total of 40 RAPD bands were scored of which 38 (94.87%) polymorphic amplification products were obtained. Besides, the primer OPD-02 amplified maximum number of polymorphic bands (100.00%) while the primer GLA-11 and OPB-04 generated the least (92.31%) polymorphic bands, which were minimal in number. The present study produced 13.33 scorable bands per primer and 12.67 polymorphic bands per primer. Frequencies of maximum number of polymorphic loci were found to be high with the exception of GLA-11(0.750), OPB-04 (0.875) and OPD-02 (0.750). The estimate of Nei's genetic diversity for the entire genotypes of mustard was 0.3596 and Shannon's information index was 0.535. There was a high level of genetic variation among the mustard genotypes studied from the proportion of polymorphic loci point of view. The values of pair-wise comparison of Nei's genetic distance between genotypes were computed from combined data for the three primers; ranged from 0.1054 to 0.9862. BINA Sarisha-3 and BINA Sarisha-4 showed the lowest genetic distance of 0.1054 where Tori-7 and NAP-0758-2 showed highest genetic distance of 0.9862. The 16 mustard genotypes were differentiated into three main clusters: BARI Sarisha-14, BARI Sarisha-9, BARI Sarisha-15, BINA Sarisha-4, BINA Sarisha-3, BARI Sarisha-8, Sampad and Tori-7 in cluster A, NAP-0763, NAP-0721-1, BARI Sarisha-4, BARI Sarisha-6, NAP-0762-2 and NAP-0848-2 in cluster B and NAP-0838 and NAP-0758-2 were grouped into cluster C by making dendrogram based on Nei's genetic distance using unweighted pair group method of arithmetic means (UPGMA).

Key words: Mustard, diversity analysis, RAPD marker, genetic distance, cluster analysis.

INTRODUCTION

Oilseed *Brassicas* occupy an important position in the rainfed agriculture throughout the world. In most of the regions of the world, its cultivation has increased drastically during the last decades and, by now; it is the third largest contributor of the world supply of vegetable oil. Rapeseed is one of the most important oil and protein

rich annual crops in the world. Seed provides oil both for industrial and culinary purposes. The oils extracted from mustard contain high protein (37%), and feed concentration which is highly palatable to livestock. In Bangladesh, the seed yield of mustard/rapeseed is about 1000 kg/ha, which is very low in comparison to other developed countries. On the other hand, the area of cultivation of mustard in Bangladesh is lower due to rice based cropping system which is difficult to change. Most of the released mustard cultivars are long day duration (105 to 115 days) and thus, did not fit well for cultivation between *Aman* and *Boro* rice cropping system. Where a long duration mustard crop is grown after *Aman* and prior to the *Boro*, however, the transplanting of *Boro* may be pushed into February, resulting in a later harvest and greater exposure to early flood risk. So, if we can develop the short duration (75 to 80 days) lines, which would be successfully cultivated between *Aman* and *Boro* rice rotation without affecting this popular cropping pattern. So, it is urgent to analyze the genetic diversity and its response for the selection of short duration mustard genotypes for increasing our cropping intensity.

Diversity at marker loci is currently the most feasible strategy for characterizing diversity in mustard. Molecular markers provide the best estimate of genetic diversity, since these are independent of the confounding effects of environmental factors. In mustard, various marker systems have been used for assessing the genetic diversity. There is increasing number of reports where molecular markers like restriction fragment length polymorphism (RFLP) (Diers and Osborn, 1994; Hallden et al., 1994), random amplified polymorphic DNAs, (RAPDs) (Ghosh et al., 2009; Yildirim et al., 2010; Khan et al., 2011), amplified fragment length polymorphism (AFLP) (Sun et al., 2001; Zhao et al., 2005) and microsatellites or simple sequence repeats (SSRs) (Abbas et al., 2009; Wang et al., 2009) have been used to study genome organization, varietal differences and diversity analysis in *Brassicas.* Among molecular marker RAPDs are increasingly being employed in genetic research owing to their speed and simplicity (Williams et al*.,* 1990; Welsh and McClelland, 1990). In mustard, RAPD method can be used as fast and effective approaches for genetic variation and relationship (Ananga et al*.,* 2008), detecting polymorphism at the DNA level, genetic diversity analysis (Chen et al*.,* 2000), measurement of genetic distance. It is important particularly for variety selection for breeding purpose, hybridization evaluation and conservation of their diverse gene pool.

Iqbal et al. (2014) conducted an experiment entitled genetic diversity analysis of mustard germplasm based on phenotypic traits for selection of short duration genotypes in the same laboratory with same genotypes. In the present experiment, a comparative discussion was done between the diversity among the genotypes based on phenotypic traits and diversity obtained from molecular level. Keeping in mind the present study was undertaken to determine the genetic variability and diversity among different mustard genotypes.

MATERIALS AND METHODS

The experiment was conducted at the Molecular and Biotechnology Laboratory, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, to characterize 16 genotypes of mustard (Table 1) at molecular level with markers. DNA from 16 mustard genotypes were isolated from actively growing fresh leaves of 20 to 25 days old seedlings using Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Murray and Thompson (1980), and later modified by Doyle and Doyle (1990). 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 re-precipitation 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 (10 mM Tris. HC1, 1 mM EDTA, pH = 8.0). DNA quality was checked by electrophoresis in 0.8% agarose gel and quantified 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. The primers used in RAPD analysis were found to discriminate different genotypes (two randomly chosen individuals from two different genotypes to evaluate their suitability for amplification of the DNA sequences) effectively. Among the 12 primers (Table 2) initially tested, 3 primers (GLA-11, OPB-04 and OPD-02) yielded comparatively maximum number of amplification products with high intensity and minimal smearing, good resolution and also clear bands which were selected for further analysis.

DNA amplification by PCR and electrophoresis

The amplification conditions originally recommended by Williams et al. (1990) were applied with slight modifications. The following components were used to prepare PCR cocktail (Table 3). The total volume of PCR cocktail was 8 µl per sample. 2 µl genomic DNA was added with 8 µl PCR cocktail and finally, total volume was 10 µl. DNA amplification was performed in an oil-free thermal cycler. The PCR tubes were set on the wells of the thermocycler plate. Then, the machine was run according to the following setup: Initial denaturation at 94°C for 3 min; denaturation at 94°C for 1 min;

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Abbreviations: UPGMA, Unweighted pair group method of arithmetic means; **RFLP,** restriction fragment length polymorphism; **RAPDs,** random amplified polymorphic DNAs; **AFLP,** amplified fragment length polymorphism; **SSRs,** simple sequence repeats; **CTAB,** cetyl trimethyl ammonium bromide.

Table 1. List of the mustard genotypes used in the experiment.

Name of the genotypes	Name of the species	Sources of the genotypes
BARI Sarisha-4	*Brassica campestris* L.	BARI
BARI Sarisha-6	*Brassica campestris* L.	BARI
BARI Sarisha-8	*Brassica napus* L.	BARI
BARI Sarisha-9	*Brassica campestris* L.	BARI
BARI Sarisha-14	*Brassica campestris* L.	BARI
BARI Sarisha-15	*Brassica campestris* L.	BARI
Tori-7	*Brassica campestris* L.	BARI
Sampad	*Brassica campestris* L.	BAU
BINA Sarisha-3	*Brassica campestris* L.	BINA
BINA Sarisha-4	*Brassica campestris* L.	BINA
NAP-0763	*Brassica napus* L.	RARS, Jamalpur
NAP-0762-2	*Brassica napus* L.	RARS, Jamalpur
NAP-0838	*Brassica napus* L.	RARS, Jamalpur
NAP-0721-1	*Brassica napus* L.	RARS, Jamalpur
NAP-0758-2	*Brassica napus* L.	RARS, Jamalpur
NAP-0842-2	*Brassica napus* L.	RARS, Jamalpur

The genotypes of BARI and BINA are the varieties and the genotypes of RARS, Jamalpur are the advanced lines.

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

Primer code	Sequence (5'- 3')
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
GLA-5	AGGGGTCTTG
GLA-9	GGGTAACGCC
GLA-11*	CAATCGCCGT
OPB-02	TGATCCCTGG
OPB-04*	GGACTGGAGT
OPC-01	TTCGAGCCAG
OPC-02	GTGAGGCGTC
OPG-5	GTGATCGCAG
OPD-02*	GGACCCAACC

*Selected for RAPD analysis of all samples of the 16 mustard genotypes.

Table 3. Components of PCR cocktail (for 8 reactions).

Reagent	Amount per sample (µl)	Total (µl)
Ampli *Taq* polymerase buffer	1.0	8
Primer	2.5	20
dNTPs	1.0	8
Ampli *Taq* DNA polymerase	0.2	1.6
Sterile deionized water	3.3	26.4

annealing at 35°C for 1 min; elongation or extension at 72°C for 2 min; Cycle to step 2 for 40 more time; incubation at 72°C for 7 min; completion of cycling program (45 cycles), reactions were held at 4°C. The amplified products were separated electrophoretically on

Table 4. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in 16 mustard genotypes.

Primer code	Sequences (5´-3´)	Total number of bands scored	Band size ranges (bp)	Number of polymorphic bands	Proportion of polymorphic loci (%)
GLA-11	CAATCGCCGT	13	220-950	12	92.31
OPB-04	GGACTGGAGT	13	250-980	12	92.31
OPD-02	GGACCCAACC	14	150-1000	14	100.00
Total		40		38	284.62
Average		13.33		12.67	94.87

1.5% agarose gel containing ethidium bromide. Molecular weight marker of 100 bp DNA ladder was electrophoresed alongside with the PCR products. DNA bands were observed on UV-trans-illuminator 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.

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, Nei's (1973), gene diversity, population differentiation, (G_{st}), gene flow (N_m), genetic distance (D) and to construct a UPGMA dendrogram among populations using a computer program, POPGENE (Version 1.31) (Yeh et al., 1999).

RESULTS AND DISCUSSION

Twelve (12) primers were initially employed on 16 mustard genotypes for their ability to produce polymorphic bands and 3 primers (GLA-11, OPB-04 and OPD-02) which gave reproducible and distinct polymerphic amplified products were selected. DNA amplification from all the primers tested in this study was not consistently reproducible and is a very common feature of RAPD technique. The present findings agree with those of Hadrys et al. (1992) and Williams et al. (1993). Technical problems from amplification of the RAPD technique in the field of genetic population research have also been reported by many authors Schierwater and Ender, 1993; Lynch and Milligan, 1994). A total of 40 RAPD bands were scored of which 38 (94.87%) polymorphic amplification products were obtained by using these arbitrary primers (Table 4). The size of the amplification products ranged from 100 to 1000 bp. Ghosh et al. (2009) reported 59 reproducible DNA bands generated by four arbitrary selected primers of which 58 (98.03%) bands were proved to be polymorphic and these bands ranged from 212 to 30686 bp in size. The selected 3 primers produced comparatively maximum number of high intensity band with minimal smearing, good technical resolution and sufficient variation among different variants. The dissimilar numbers of bands were generated by primer GLA-11, OPB-04 and OPD-02 (Table 4). Besides, the primer OPD-02 amplified maximum number of polymorphic bands (100.00%) while the primer GLA-11 and OPB-04 generated the least (92.31%) polymorphic bands which were minimal in number. The banding patterns of 16 mustard genotypes using primers GLA-11, OPB-04 and OPD-02 are shown in Figures 1, 2 and 3 respectively. Number of RAPD markers scored for each individual 16 mustard genotypes for each primer are presented in Table 4.

A total of 254 clear and repeatable bands were amplified from three RAPD primers (Table 5). The primers GLA-11, OPB-04 and OPD-02 produced 74, 85 and 95, respectively polymorphic bands in 16 mustard genotypes. This proportion of polymorphism is higher compared to some previous molecular analysis in mustard genotypes in primers are confirmed in higher percentage of polymorphism. The present experiment produced 13.33 scorable bands per primer and 12.67 polymorphic bands per primer. The reasons of the considerable number of average scorable and polymorphic bands consist of 60 to 70% GC content. Fukuoka et al. (1992) observed an increase in the number of bands with increasing GC content of the primer. The explanation for this correlation between the GC content of the primer and the number of bands is that the stability of base complementation is higher when G is pairing with C by three hydrogen bonds than that of the complementation of A with T by two hydrogen bonds. The DNA polymorphisms were detected according to the presence and absence of band. Absence of band may be caused by failure of primer to anneal a site in some individuals due to nucleotide, sequences difference or by insertions or deletions between primer sites Clark and Lanigan, 1993. Frequencies of maximum number of polymorphic loci were found to be high with the exception of GLA-11(0.750), OPB-04(0.875) and OPD -02 (0.750) (Table 6). The values of Nei's (1973) genetic diversity and Shannon's information index for different accessions of 16 mustard genotypes across all loci are shown in Table 7. The estimate of Nei's genetic diversity for entire genotypes of mustard was 0.3596 and Shannon's information index was 0.535. There was a high level of genetic variation among the studied mustard genotypes

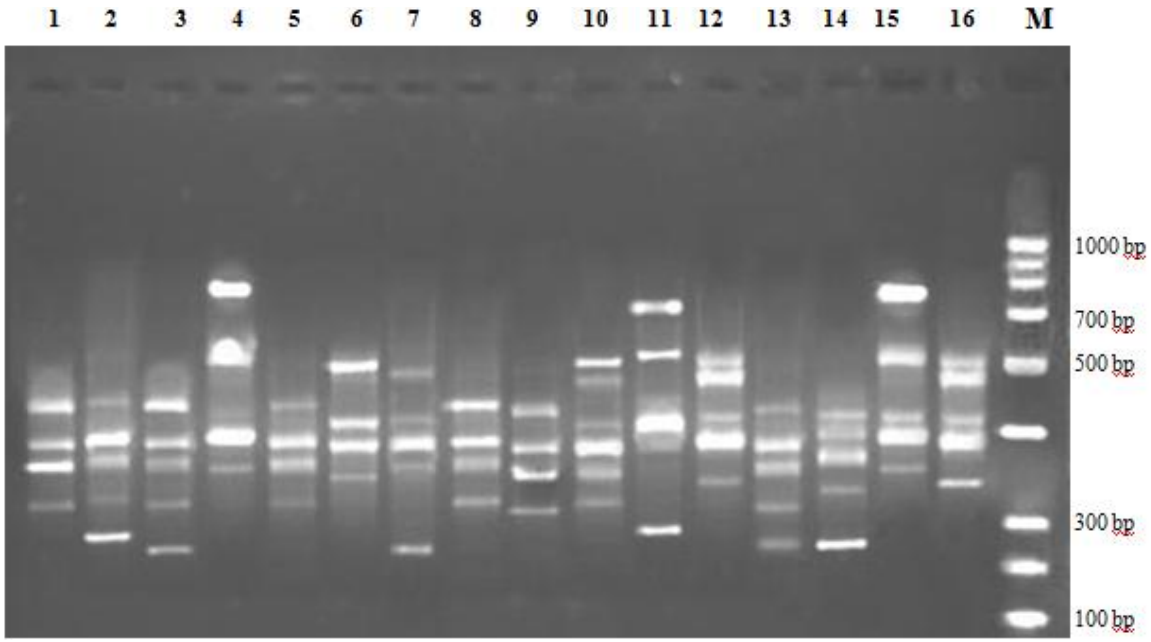

Figure 1. RAPD profile of 16 mustard genotypes using primer GLA-11. M, Molecular weight marker (100 bp DNA ladder in size). 1, BARI Sarisha-14; 2, Tori-7; 3, BARI Sarisha-8; 4, NAP-0763; 5, BINA Sarisha-4; 6, NAP-0721-1; 7, BARI Sarisha-4; 8, BINA Sarisha-3; 9, BARI Sarisha-15; 10, NAP-0838; 11, NAP-0762-2; 12, NAP-0848-2; 13, BARI Sarisha-9; 14, Sampad; 15, BARI Sarisha-6; 16, NAP-0758-2.

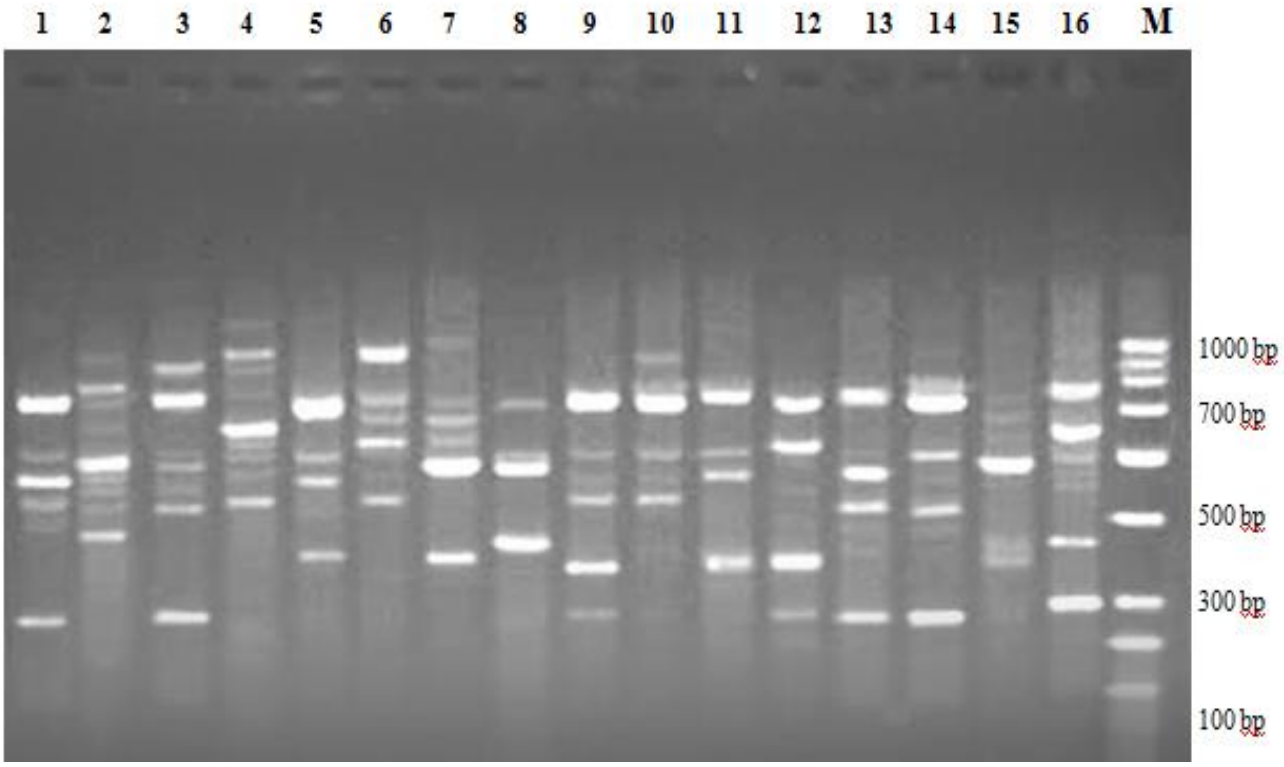

Figure 2. RAPD profiles of 16 mustard genotypes using primer OPB-04. M, Molecular weight marker (100 bp DNA ladder in size). 1, BARI Sarisha-14; 2, Tori-7; 3, BARI Sarisha-8; 4, NAP-0763; 5, BINA Sarisha-4; 6, NAP-0721-1; 7, BARI Sarisha-4; 8, BINA Sarisha-3; 9, BARI Sarisha-15; 10, NAP-0838; 11, NAP-0762-2; 12, NAP-0848-2; 13, BARI Sarisha-9; 14, Sampad; 15, BARI Sarisha-6; 16, NAP-0758-2.

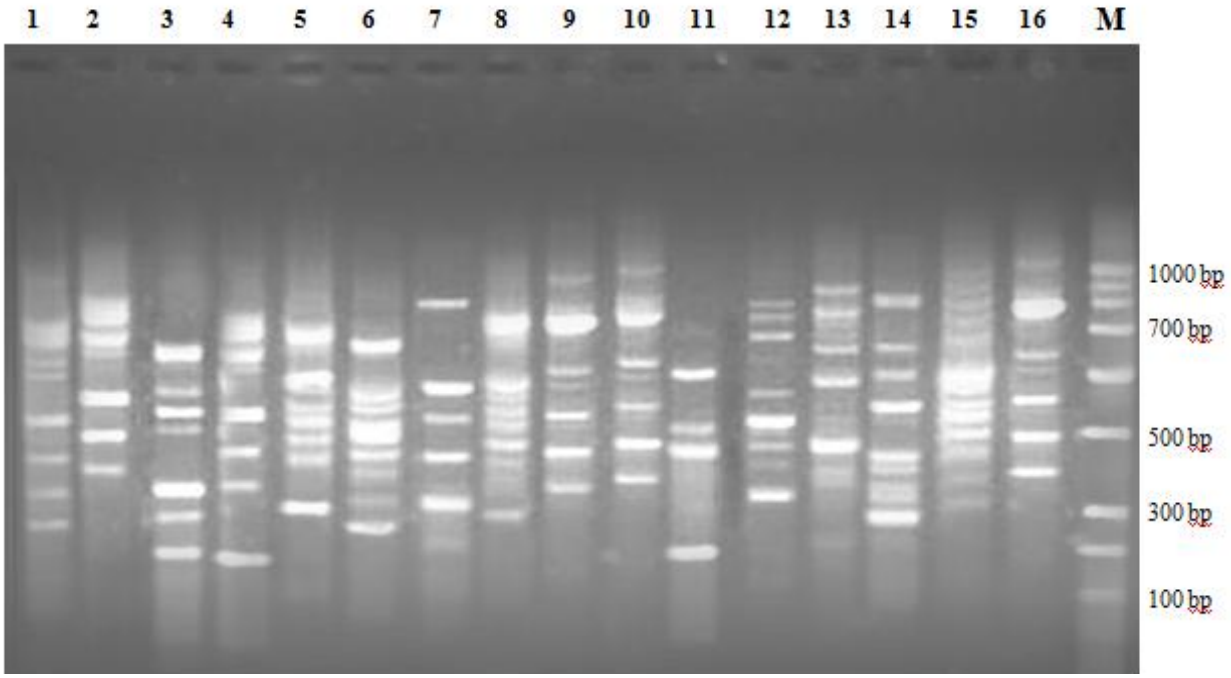

Figure 3 RAPD profiles of 16 mustard genotypes using primer OPD-02. M, Molecular weight marker (100 bp DNA ladder in size). 1, BARI Sarisha-14; 2, Tori-7; 3, BARI Sarisha-8; 4, NAP-0763; 5, BINA Sarisha-4; 6, NAP-0721-1; 7, BARI Sarisha-4; 8, BINA Sarisha-3; 9, BARI Sarisha-15; 10, NAP-0838; 11, NAP-0762-2; 12, NAP-0848-2; 13, BARI Sarisha-9; 14, Sampad; 15, BARI Sarisha-6; 16, NAP-0758-2.

Table 5. Number of polymorphic bands observed in 16 mustard genotypes after PCR amplification on with RAPD primers GLA-11, OPB-04 and OPD-02.

Genotype	GLA-11	OPB-04	OPD-02	Total bands
BARI Sarisha-14	4	6	6	16
Tori-7	4	8	5	17
BARI Sarisha-8	5	7	7	19
NAP-0763	4	8	6	18
BINA Sarisha-4	3	4	7	14
NAP-0721-1	4	5	6	15
BARI Sarisha-4	5	6	5	16
BINA Sarisha-3	4	4	7	15
BARI Sarisha-15	4	6	7	17
NAP-0838	6	6	5	17
NAP-0762-2	6	4	4	14
NAP-0848-2	5	4	7	16
BARI Sarisha-9	5	5	6	16
Sampad	5	5	8	18
BARI Sarisha-6	5	2	4	11
NAP-0758-2	5	5	5	15
Total bands	74	85	95	254

from the proportion of polymorphic loci point of view.

Considering the genetic distance values, the result indicate that some mustard genotypes were genetically different from each other and some were tend to be

Table 6. Frequencies of polymorphic RAPD markers in mustard genotypes.

Loci	Gene frequency	Loci	Gene frequency
GLA11-1	0.1250	OPB04-8	0.8750
GLA11-2	0.0625	OPB04-9	0.3125
GLA11-3	0.5000	OPB04-10	0.5625
GLA11-4	0.2500	OPB04-11	0.2500
GLA11-5	0.4375	OPB04-12	0.4375
GLA11-6	0.5000	OPB04-13	0.4375
GLA11-7	0.0000	OPD02-1	0.1875
GLA11-8	0.7500	OPD02-2	0.1875
GLA11-9	0.2500	OPD02-3	0.4375
GLA11-10	0.1875	OPD02-4	0.5000
GLA11-11	0.5000	OPD02-5	0.1875
GLA11-12	0.1250	OPD02-6	0.5000
GLA11-13	0.2500	OPD02-7	0.1250
OPB04-1	0.0625	OPD02-8	0.7500
OPB04-2	0.1875	OPD02-9	0.4375
OPB04-3	0.2500	OPD02-10	0.7500
OPB04-4	0.1250	OPD02-11	0.5000
OPB04-5	0.8125	OPD02-12	0.5000
OPB04-6	0.1875	OPD02-13	0.1875
OPB04-7	0.3125	OPD02-14	0.1875

Table 7. Summary of genetic diversity and Shanon information index statistics for all loci.

Loci	Observed number of alleles	Effective number of alleles	Gene diversity (h)	Shanon information index (i)
GLA11-1	2.0000	1.2800	0.2188	0.3768
GLA11-2	2.0000	1.1327	0.1172	0.2338
GLA11-3	2.0000	2.0000	0.5000	0.6931
GLA11-4	2.0000	1.6000	0.3750	0.5623
GLA11-5	2.0000	1.9692	0.4922	0.6853
GLA11-6	2.0000	2.0000	0.5000	0.6931
GLA11-7	1.0000	1.0000	0.0000	0.0000
GLA11-8	2.0000	1.6000	0.3750	0.5623
GLA11-9	2.0000	1.6000	0.3750	0.5623
GLA11-10	2.0000	1.4382	0.3047	0.4826
GLA11-11	2.0000	2.0000	0.5000	0.6931
GLA11-12	2.0000	1.2800	0.2188	0.3768
GLA11-13	2.0000	1.6000	0.3750	0.5623
OPB04-1	2.0000	1.1327	0.1172	0.2338
OPB04-2	2.0000	1.4382	0.3047	0.4826
OPB04-3	2.0000	1.6000	0.3750	0.5623
OPB04-4	2.0000	1.2800	0.2188	0.3768
OPB04-5	2.0000	1.4382	0.3047	0.4826
OPB04-6	2.0000	1.4382	0.3047	0.4826
OPB04-7	2.0000	1.7534	0.4297	0.6211
OPB04-8	2.0000	1.2800	0.2188	0.3768
OPB04-9	2.0000	1.7534	0.4297	0.6211
OPB04-10	2.0000	1.9692	0.4922	0.6853
OPB04-11	2.0000	1.6000	0.3750	0.5623

Table 7. Contd.

Loci	Observed number of alleles	Effective number of alleles	Gene diversity (h)	Shanon information index (i)
OPB04-12	2.0000	1.9692	0.4922	0.6853
OPB04-13	2.0000	1.9692	0.4922	0.6853
OPD02-1	2.0000	1.4382	0.3047	0.4826
OPD02-2	2.0000	1.4382	0.3047	0.4826
OPD02-3	2.0000	1.9692	0.4922	0.6853
OPD02-4	2.0000	2.0000	0.5000	0.6931
OPD02-5	2.0000	1.4382	0.3047	0.4826
OPD02-6	2.0000	2.0000	0.5000	0.6931
OPD02-7	2.0000	1.2800	0.2188	0.3768
OPD02-8	2.0000	1.6000	0.3750	0.5623
OPD02-9	2.0000	1.9692	0.4922	0.6853
OPD02-10	2.0000	1.6000	0.3750	0.5623
OPD02-11	2.0000	2.0000	0.5000	0.6931
OPD02-12	2.0000	2.0000	0.5000	0.6931
OPD02-13	2.0000	1.4382	0.3047	0.4826
OPD02-14	2.0000	1.4382	0.3047	0.4826
Mean	1.9750	1.6183	0.3596	0.5350
Standard deviation	0.1581	0.3003	0.1269	0.1560

similar. The values of pair-wise comparison of Nei's genetic distance between genotypes were computed from combined data for the three primers, ranged from 0.1054 to 0.9862 which was near similar to previous study. The smaller number of pair-wise differences (high genetic similarity) among some genotypes was likely due to their genetical relatedness. On the other hand, large number of pair-wise differences (low genetic similarity) was observed among those genotypes developed from genetically distant parental lines. Comparatively higher genetic distance (0.9862) was found among Tori-7 vs. NAP-0758-2 genotypes pair than other genotypes combination. The lowest genetic distance (0.1054) was revealed among BINA Sarisha-3 vs. BINA Sarisha-4 as the genotypes were morphologically very similar. Considering the genetic distance values, the results indicated that the genotypes were genetically different from each other which could be used in breeding programme to have potential genetic gains.

Sixteen (16) mustard genotypes of the experiment were used to make dendrogram based on Nei's genetic distance using UPGMA. In this study, 16 mustard genotypes had been differentiated into three main clusters: BARI Sarisha-14, BARI Sarisha-9, BARI Sarisha-15, BINA Sarisha-4, BINA Sarisha-3, BARI Sarisha-8, Sampad and Tori-7 in Cluster A, NAP-0763, NAP-0721-1, BARI Sarisha-4, BARI Sarisha-6, NAP-0762-2 and NAP-0848-2 in cluster B and NAP-0838 and NAP-0758-2 were grouped in cluster C (Figure 4). Genotypes included in cluster A were totally different from the genotypes included in cluster B and cluster C. So, genetic relationship was not present between cluster A genotypes with cluster B and cluster C. The cluster A was divided into two sub-clusters. In sub-cluster II of cluster A, there was Tori-7 alone whereas, sub-cluster I was divided in two sub-sub clusters. In sub-sub cluster II, there was BARI Sarisha-8 and Sampad whereas sub-sub cluster I was divided in two sub-sub-sub clusters. In sub-sub-sub cluster II, there was BINA Sarisha-3 and BINA Sarisha-4 whereas sub-sub-sub cluster I was divided in two sub-sub-sub-sub clusters. Sub-sub-sub-sub cluster I included BARI Sarisha-14 and BARI Sarisha-14 and Sub-sub-sub-sub cluster II included only BARI Sarisha-15. The cluster B was divided into two sub-clusters. In sub-cluster I of cluster B, there was NAP-0763 and NAP-0721-1 whereas, sub-cluster II was divided in two sub-sub clusters. Sub-sub cluster I included BARI Sarisha-4 and BARI Sarisha-6 and Sub-sub cluster II included NAP-0762-2 and NAP-0838-2. Finally, the cluster C included NAP-0838 and NAP-0758-2. According to Ghosh et al. (2009), 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 and another group contained BINA Sharisha-4, BINA Sharisha-5, and BARI Sharisha-13. Genotypic variations based on molecular characterization indicated that genotypes belonging to different clusters depend on their genetic components itself, but not at geographical origin at all. Therefore, it could be concluded that for further research program, especially for hybridization, genotype could be selected from different clusters, to achieve maximum heterosis regarding yield.

Iqbal et al. (2014) conducted an experiment entitled genetic diversity analysis of mustard germplasm based on phenotypic traits for selection of short duration genotypes in the same laboratory with same genotypes.

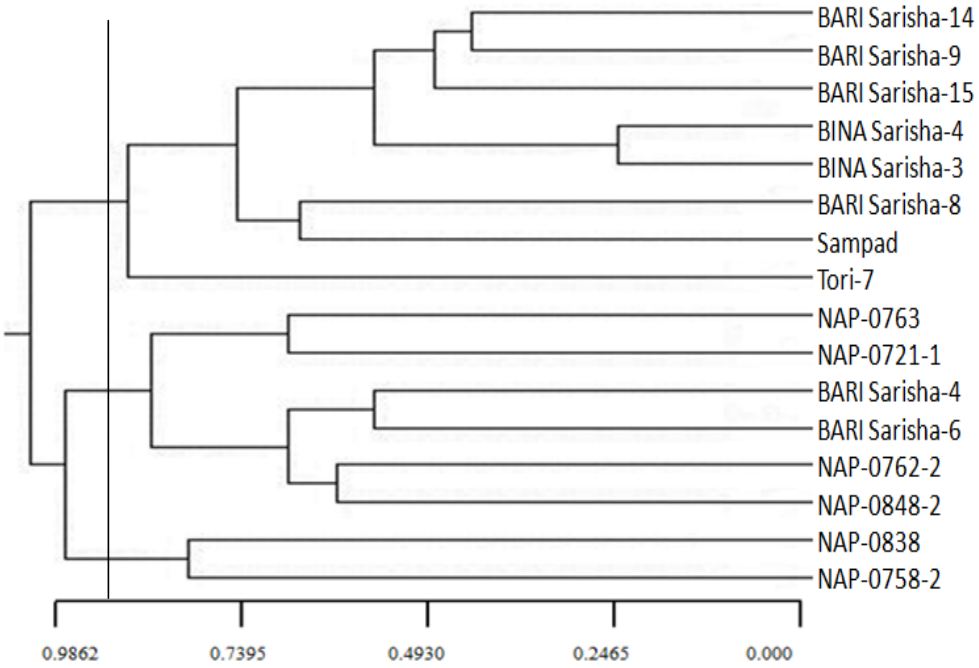

Figure 4. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's genetic distance; summarizing data on differentiation in 16 mustard genotypes according to RAPD analysis.

They suggested that, on basis of lowest days to mature groups included Tori-7, BARI Sarisha-14, BARI Sarisha-9, BARI Sarisha-15 can be crossed with higher seed yield group which included NAP-0838, NAP-0758-2, NAP-0762-2, NAP-0848-2 and BARI Sarisha-4 for developing short durated high yielding varieties.

Now after the comparison between the results of phenotypic traits and molecular markers, it can be revealed that genotypes that belongs to lowest days to mature groups represents the genotypes of cluster I of molecular markers and genotypes that belongs to clusters III and II of molecular markers represents most of the genotypes of higher seed yield group. Therefore, it could be concluded that the next crossing combination might be between the genotypes: Tori-7, BARI Sarisha-14, BARI Sarisha-9, BARI Sarisha-15 and Sampad with the genotypes: NAP-0838, NAP-0758-2, NAP-0762-2, and NAP-0848-2 for developing short duration high yielded variety which are genotypically and phenotypically significantly differentiated.

Conflict of interests

The authors did not declare any conflict of interest.

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