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RAPD MARKERS BASED GENETIC DIVERSITY ANALYSIS OF ONION (ALLIUM CEPA) GERMPLASM

Agriculture

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ABSTRACT

Onion is one of the most popular spice crop, which is used for seasoning wide range of dishes as well as folk medicine. Assessment of genetic diversity helps to use the onion germplasm as breeding materials for its improvement. Molecular marker based diversity assessment is more powerful, rapid and reliable compared to morphology based genetic diversity assessment. Thirty eight onion germplasm were characterized by using 10 random amplified polymorphic DNA (RAPD) markers for estimating genetic diversity within the germplasm. The germplasm exhibited wide range of variability which could be used in cross breeding for getting superior F1 generation. Out of 10 primers, four gave polymorphism among onion germplasm. Genetic diversity of 38 germplasm was analyzed using variation at 34 RAPD loci, of which 91.89% were polymorphic. The highest pair wise genetic distance was 1.046 and the lowest was 0.056. The mean gene diversity among all the germplasm was 1.54 and Shannon's information index was 0.3188. The UPGMA dendrogram, based on Nei`s genetic diversity showed 6 clusters. The frequency of polymorphic loci ranged from 0.0263 to 1.0000, indicates that there exists a wide genetic diversity among the germplasm and corroborated the usefulness of RAPD markers for estimating genetic diversity in onion.

KEYWORDS

Divergence, onion, polymorphism, RAPD

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most economically important spice crops. It is used in every day for seasoning wide range of dishes. Onion can be eaten as raw and/or cooked. It is an indispensable item in every kitchen as vegetables and condiment to enhancing flavor of the food stuffs. Therefore, onion is popularly referred as "queen of kitchen". Among the spice crops grown in Bangladesh, onion ranks top regarding area and production (AIS, 2020). At present, total production of onion in Bangladesh is about 1.95 million metric tons of bulbs from 0.185 million hectares of land (BBS, 2021) but we get only 1.7 million metric tons for consumption due to 30 - 40% losses in storage (Anon, 2020). Whereas, the annual national requirements about 2.5 - 2.7 million metric tons. Presently, Bangladesh has about 0.8 - 1.0 million metric tons/year shortage of onion. Therefore, we are imported onion every year to meet up the demand by spending foreign currency. In Bangladesh, onion yield is lower (12.32 t/ha) than major onion growing countries (19.10 t/ha) (NHRDF, 2018).

The assessment of genetic diversity of plant populations may be carried out by characterizing and evaluating the morphological traits or by using molecular markers. However, the field evaluation of morphological characteristics is not solely a complete reliable method due to variable environmental factors. Currently, many molecular markers are routinely used to evaluate genetic diversity in plants. Improvement in yield and quality is normally elevated by selecting desirable characters of the genome which could be achieved by using molecular markers (Rashid *et al*., 2013). RAPD markers are used for quickly and easily determine the genetic diversity of plant material within the population, breeding lines, as well as general collection germplasm. They are also useful in genetic analysis of the resistance to the particular diseases of vegetable crops (Cvikic *et al*., 2009 and Zdravkovic *et al*., 2011). Considering the above facts, the aim of present research was designed to assess the genetic diversity of collected onion germplasm, which could have contributed to designing cross breeding and germplasm enhancement.

MATERIALAND METHODS

The study was conducted at the Department of Genetics and Plant Breeding laboratory and central laboratory of Bangladesh Agricultural University, Mymensingh, Bangladesh. Gnomic DNA sample was extracted from young actively grown leaf tissue of 35 days old seedling of 38 onion germplasm (Table 1) following modified CTAAB method Doyle and Doyle (1990). Approximately 100 mg of leaf tissue was grinded with liquid nitrogen. The grinded tissues were mixed with 400 µl extraction buffer. After adding additional 400 µl extraction buffer, the samples were vortex for 30 second and incubated at 65 °C for 15 minutes in hot water bath. The extract was centrifuged for 10 minutes at 14000 rpm to allow precipitation of the cell debris. 500 µl upper clear liquid was transferred to another tube. 600 µl of phenol: Chloroform: Isoamyl alcohol (v: v: v= 25: 24: 1) was added and mixed gently for purification. The solution was centrifuged for 10 minutes at 14000 rpm. DNA was precipitated using 800 µl of absolute alcohol and pelleted by centrifugation for 3 minutes at 14000 rpm. After discarding the liquid completely, the DNA solution was precipitated by adding 400 µl of 70% ethanol with 20 µl 3 M sodium acetate and pelleted by centrifugation for 3 minutes at 14000 rpm. When the liquid removed completely, the pellets were air dried and re-suspended in 25 µl of TE buffer. DNA samples were stored at -20 ˚C for future use.

Table 1. Accessions number, local name and place of collection of 38 germplasm are analyzed in the present study

No. of accessions	Local name of germplasm	Place of collection	No. of accessions	Local name of germplasm	Place of collection
AC0143	Taherpuri	Rajshahi	AC0267	N-53	Myanmar
AC0191	East Bengal	Jessore	AC0268	Nasik red	Jessore
AC0210	N-53 dark red	India	AC0275	N-53	Joypurhat
AC0214	N-53 red	India	AC0276	Gota	Bogra,
AC0222	Raja red	Natore	AC0277	N-53	Egypt
AC0223	Belari red	Rajshahi,	AC0278	B.S.S 441	Dinajpur
AC0242	Nasik	Gaibandha	AC0279	orient	Jhenaidah
AC0245	Shuk sagar	Meherpur	AC0281	White bulb	Nilphamari,
AC0247	BSS 442	Joypurhat	AC0282	Lucifera	Dhaka
AC0250	IR 0N-1	Faridpur	AC0283	Unknown	Turkey
AC0251	IR 0N-2	Bogra	AC0284	Unknown	Naogaon
AC0252	N-53	Magura	AC0285	Unknown	Pakistan
AC0259	Bombai Lal	Pabna	AC0286	Unknown	Chuadanga
AC0260	Kerala	India	AC0287	Zittka	Manikgonj
AC0261	IR-8	Natore	AC0288	Bomby	Kushtia
AC0262	Lalsagar	Joypurhat	AC0291	Unknown	Local market
AC0264	UP line	Faridpur	AC0293	Unknown	Faridpur
AC0265	IR-ON-18	Rajbari	AC0295	Rangilla-7	Joypurhat
AC0266	Nasik red	Dinajpur	AC0296	Ranga-3	Barma

A total of 10 decamer random primers (Operon Technologies, Inc, Alameda, California, USA) (Table 2) were screened on a sub-sample of two randomly chosen individuals from each genotype, to test their

suitability for amplifying onion with accurate score. On the basis of intensity or resolution of bands, repeatability of markers and consistency of the primers were evaluated.

Table 2. Primer code and sequences are used for the detection of polymorphism in onion

Primer codes	Sequence (5′ to 3′)
OPAD-05	ACCGCATGGG
OPA-09	GGGTAACGCC
OPD-03	GTCGCCGTCA
OPC-05	GATGACCGCC
OPA-11	CAATCGCCGT
OPA-12	TCGGCGATAG
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC

A final subset of 4 primers (OPAD-05, OPA-09, OPD-03 and OPC-05) out of 10 was selected for the analysis of 38 germplasm of onion. For more confirmation of the reproducibility of RAPD markers, the PCR was repeated three times.

PCR amplification for RAPD

The amplification of PCR was done based on Willaams *et al*. (1990) with some modifications. PCR reactions were performed on each DNA sample in a 10 µl reaction mix contained 1 µl of 10× Taq polymerase buffer, 0.25 µl of 10 mM primer, 1 µl of 250 mM dNTPs, 0.5 µl of $MgCl_2$, 0.25 µl of 1 unit Taq DNA polymerase (Banglore Genei, India), 1 µl of 100 ng of gnomic DNA and 5 µl sterile deionized water. The PCR was performed in an oil free thermal cycler (Master Cycler Gradient, Eppendorf, USA). The reaction mix was pre-heated at 94 °C for 3 minutes followed by 40 cycles of 1 min denaturation at 94 °C, 1 min annealing at 34 °C and elongation or extension at 72 °C for 2 minutes. After the last cycle, a final step of 7 minutes at 72 °C was added to allow complete extension of all amplified fragments of DNA.

Agarose gel electrophoresis

The PCR product from each sample was separated electro-phoretically on 1.5% agarose gel (Fisher Biotech, USA) containing ethidium bromide in 1× TBE buffer at 120 v for 80 min and 100 bp ladder was electrophoreses alongside the RAPD reactions. DNA bands were observed on computerized UV light chamber and photographed.

Data analysis

The RAPD bands were scored visually on the basis of their presence (1) or absence (0), separately for each genotype of onion and each primer. The scores obtained using all primers in the RAPD analysis were pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei`s (1973) gene diversity, population differentiation (GST), gene flow (Nm,) genetic distance (D) and constructing a UPGMA (Unweighted pair Group Method of Arithmetic Means) dendrogram among populations using POPGENE (version 1.31) (Yeh *et al*., 1999) computer programme. For estimating gene frequencies of RAPD loci was done based on the assumption of a two alleles system. From the two alleles, only one was capable of amplify action of a RAPD band by primer annealing at an unknown genomic position (locus). The other was the "null" allele incapable of amplification, because of the primer-annealing site by mutation. Only a null homozygote was detectable as negative for the RAPD band of interest. Under the assumption of Hardy-Weinberg equilibrium, the null allele frequency (q) may be $(N/n)^{½}$, where N and n are the number of band of negative individuals observed and the sample size, respectively. The frequency of the other allele (p) was 1-q. The assumption of the two alleles system enables us to calculate the Nei`s, (1972) from the RAPD pattern. The similarity index values (SI) between the RAPD profiles of any two individuals on the same gel were calculated from RAPD markers according to the following formula.

Similarity Index (SI) = $2N_{AB}/(N_A+N_B)$

Where, N_{AB} is the total number of RAPD bands shared by individuals A, N_A and N_B are the numbers of fragments scored for each individual, respectively (Lynch, 1990). Within cultivars, similarity (S_i) was calculated as the average of SI across all possible comparisons between individuals within a cultivar. Between cultivars, similarity (Sij) was calculated as the average similarity between randomly paired individuals from cultivars I and J (Lynch, 1991).

RESULTS

The RAPD technology provides a quick and efficient screen for DNA sequence based polymorphism at a very large number of loci. Primarily 10 primers were used; however only four primers OPAD-05, OPA-09, OPD-03 and OPC-05 were produced clear band and scored as DNA fragments with patterns for 38 onion germplasm.

Polymorphism in onion germplasm

Selected four RAPD primers were used for analyzing 38 onion germplasm, which gave 37 consistent and differential amplification products (Table 3). The number of bands per primer varied from 7 and 14 fragments, and was amplified with an average of 9.25 bands/primer. Among them, 34 bands (91.89%) were polymorphic (either occurring in or absent in < 95% of all germplasm) and the remaining bands were found as monomorphic, i.e. they were present in all of the tested germplasm (Figure 1 to 4).

The primer OPA-09 was generated the maximum number of bands (14) followed by OPAD-05 and OPD-03 bands (8 bands) and the minimum number were obtained from OPC-05 (07 bands). The primer of OPA-09 was amplified the highest number of polymorphic bands (13); OPAD-05 showed 8 bands that were polymorphic in nature. The primers of OPD-03 produced 7 polymorphic bands. Whereas, the lowest of polymorphism was observed in the primer OPC-05 (6). On an average, 8.5 polymorphic bands were counted per primer.

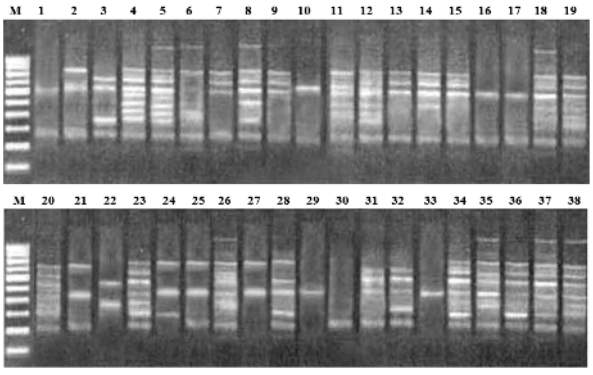

Figure 1. RAPD profile of 38 varieties using primer OPAD-05. M: 100 bp DNA ladder.

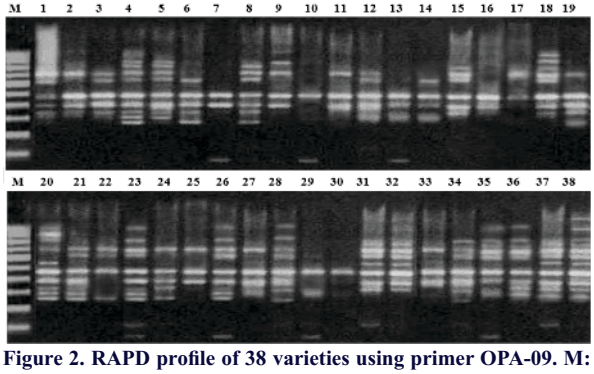

Figure 2. RAPD profile of 38 varieties using primer OPA-09. M: 100 bp DNA ladder.

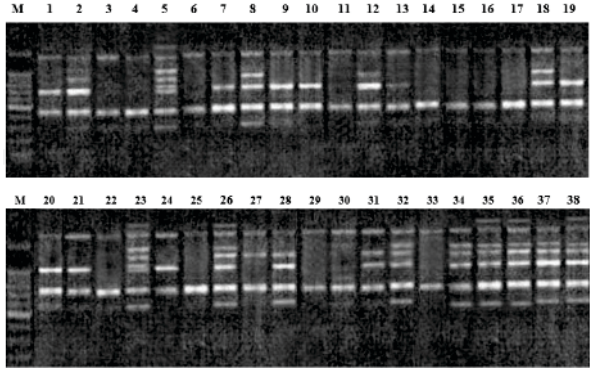

Figure 3. RAPD profile of 38 varieties using primer OPD-03. M: 100 bp DNA ladder.

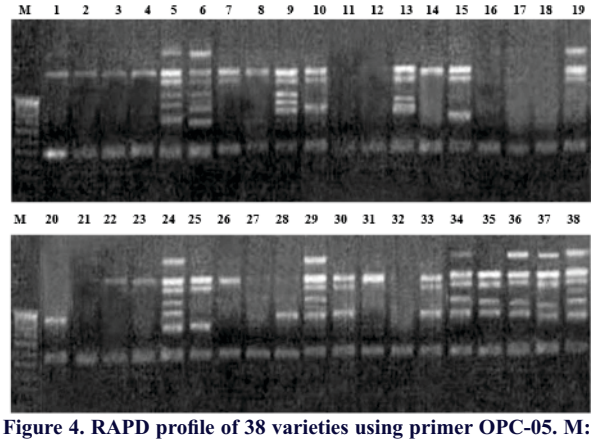

Figure 4. RAPD profile of 38 varieties using primer OPC-05. M: 100 bp DNA ladder.

Where; 1 = AC0288, 2 = AC0281, 3 = AC0275, 4 = AC0252, 5 = AC0265, 6 = AC0291, 7 = AC0260, 8 = AC0282, 9 = AC0266 , 10 = AC0276, 11 = AC0264, 12 = AC0192, 13 = AC0250, 14 = AC0251, 15 = AC0261, 16 = AC0284, 17 = AC0262, 18 = AC0279, 19 = AC0214, 20 = AC0286, 21 = AC0287, 22 = AC0278, 23 = AC0268, 24 = AC0267, 25 = AC0285, 26 = AC0247, 27 = AC0293, 28 = AC0259, 29 = AC0283, 30 = AC0242, 31 = AC0295, 32 = AC0296, 33 = AC0277, 34 = AC0143, 35 = AC0222, 36 = AC0223, 37 = AC0245, 38 = AC0210.

Table 3. RAPD primers with corresponding bands score and their size range together with polymorphic bands observed in 38 onion germplasm

Primer code	Sequences (5´- 3´)	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPAD-05	ACCGCATGGG	8	8	100.00
OPA-09	GGGTAACGCC	14	13	92.86
OPD-03	GTCGCCGTCA	8	7	87.50
OPC-05	GATGACCGCC	7	6	85.71
Total		37	34	91.89
Average		9.25	8.5	91.89

The frequencies of maximum polymorphic loci were found in OPA-09 as 8 (1.000), OPD-03 as 2 (1.000), OPC-05 as 7 (1.000), OPAD-05 as 8 (0.8684), OPA-09- as 9 (0.8684) and OPA-09 as 5 (0.7895) (Table 4). The minimum frequency was found in OPAD-05 as 5 (0.0263), OPD-03 as 5 (0.0526), OPA-09 as 13 (0.0789), OPA-09 as 1 (0.1053), OPA-09 as 7 (0.1316) and OPC-05 as 6 (0.1316).

The frequency of polymorphic loci ranged from 0.0263 to 1.0000, indicated wide genetic diversity among the onion germplasm and showed by RAPD markers.

Table 4. Frequencies of polymorphic RAPD markers in 38 onion germplasm

RAPD Markers	Gene frequency	RAPD Markers	Gene frequency	RAPD Markers	Gene frequency	RAPD Markers	Gene frequency
OPAD05-1	0.2105	OPA09-3	0.2105	OPA09-12	0.3158	OPD03-7	1.0000
OPAD05-2	0.7105	OPA09-4	0.3158	OPA09-13	0.0789	OPD03-8	0.3421
OPAD05-3	0.6316	OPA09-5	0.7895	OPA09-14	0.1842	OPC05-1	0.2368
OPAD05-4	0.7632	OPA09-6	0.5789	OPD03-1	0.1579	OPC05-2	0.7368
OPAD05-5	0.0263	OPA09-7	0.1316	OPD03-2	1.0000	OPC05-3	0.4737
OPAD05-6	0.5789	OPA09-8	1.0000	OPD03-3	0.2632	OPC05-4	0.2632
OPAD05-7	0.4211	OPA09-9	0.8684	OPD03-4	0.4211	OPC05-5	0.3684
OPAD05-8	0.8684	OPA09-10	0.5789	OPD03-5	0.0526	OPC05-6	0.1316
OPA09-1	0.1053	OPA09-11	0.3947	OPD03-6	0.6316	OPC05-7	1.0000
OPA09-2	0.2895						

A dendrogram was constructed based on Nei`s (1972) genetic distance using Unweighted pair Group Method of Arithmetic Means (UPGMA), which revealed segregation of the 38 onion germplasm into six clusters (Figure 5). The cluster VI was included the highest number of germplasm (11), which were collected from different geographical locations. The cluster II contained 9 germplasm, cluster IV contained 7 germplasm, cluster I contained 6 germplasm, cluster III contained 3 germplasm and cluster V contained only 2 germplasm. Germplasm in the same cluster may represent members of one heterotic group. The maximum variability for selection from segregation populations may be achieved by utilizing germplasm from different clusters as parents of crosses (Gwanama *et al*., 2000).

The highest Nei`s (1972) genetic distance (1.046) was estimated between the germplasm. The highest pair wise genetic distance of germplasm was 1.046 and the lowest pair wise genetic distance was 0.056 (Figure 5). In addition, high level of average genetic distance (0.972) was also observed. The Nei`s gene diversity varied from 1.0000 to 1.9945, with an average of 1.5429, and Shannon`s information index range was 0.0000 to 0.4986, with an average of 0.3188 (Table 5). Result revealed wide variability among the 38 onion germplasm.

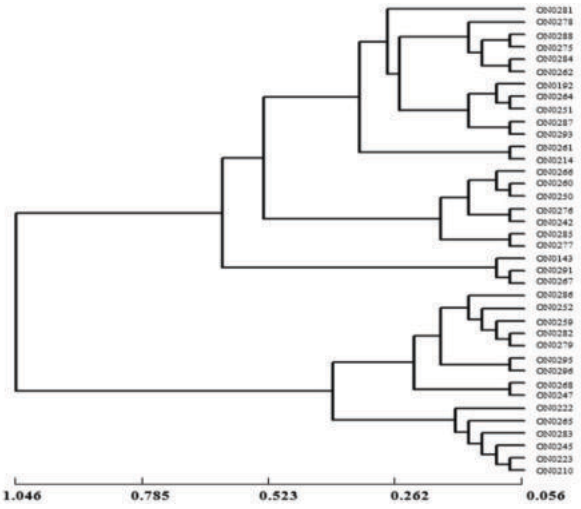

Figure5. Dendrogram was constructed based on Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation in 38 onion germplasm according to RAPD analysis.

Table 5. Estimation of genetic variability using RAPD marker's in 38 onion germplasm.

Loci	Sample Size	Effective number of alleles	Nei'(s) Gene diversity (h)	Shanon information index (i)
OPAD05-1	38	2.0000	1.4979	0.3324
OPAD05-2	38	2.0000	1.6988	0.4114
OPAD05-3	38	2.0000	1.8705	0.4654
OPAD05-4	38	2.0000	1.5662	0.3615
OPAD05-5	38	2.0000	1.0540	0.0512
OPAD05-6	38	2.0000	1.9514	0.4875
OPAD05-7	38	2.0000	1.9514	0.4875
OPAD05-8	38	2.0000	1.2962	0.2285
OPA09-1	38	2.0000	1.2321	0.1884
OPA09-2	38	2.0000	1.6988	0.4114
OPA09-3	38	2.0000	1.4979	0.3324
OPA09-4	38	2.0000	1.7610	0.4321
OPA09-5	38	2.0000	1.4979	0.3324
OPA09-6	38	2.0000	1.9514	0.4875
OPA09-7	38	2.0000	1.2962	0.2285
OPA09-8	38	1.0000	1.0000	0.0000
OPA09-9	38	2.0000	1.2962	0.2285

OPA09-10	38	2.0000	1.9514	0.4875
OPA09-11	38	2.0000	1.9151	0.4778
OPA09-12	38	2.0000	1.7610	0.4321
OPA09-13	38	2.0000	1.1702	0.1454
OPA09-14	38	2.0000	1.4297	0.3006
OPD03 -1	38	2.0000	1.3623	0.2659
OPD03-2	38	1.0000	1.0000	0.0000
OPD03-3	38	2.0000	1.6335	0.3878
OPD03-4	38	2.0000	1.9514	0.4875
OPD03-5	38	2.0000	1.1108	0.0997
OPD03-6	38	2.0000	1.8705	0.4654
OPD03-7	38	1.0000	1.0000	0.0000
OPD03-8	38	2.0000	1.8186	0.4501
OPC05-1	38	2.0000	1.5662	0.3615
OPC05-2	38	2.0000	1.6335	0.3878
OPC05-3	38	2.0000	1.9945	0.4986
OPC05-4	38	2.0000	1.6335	0.3878
OPC05-5	38	2.0000	1.8705	0.4654
OPC05-6	38	2.0000	1.2962	0.2285
OPC05-7	38	1.0000	1.0000	0.0000
Mean	38	1.8919	1.5429	0.3188
St. Dev		0.3148	0.3283	0.1626

DISCUSSION

This research assessed genetic diversity among onion cultivars collected from different locations by using RAPD markers. Dennequin et al. (1997) and Tanikawa et al. (2002) reported that RAPD markers have been used only in limited studies of *A. cepa* diversity. The result of their study confirmed high genetic similarity among the cultivars studied. Moreover, the Jaccard's similarity coefficient (0.73) between On16 (*A. cepa*) and On17 (*A. ascalonicum*) is in agreement with USDA (2011) that *A. ascalonicum* is a taxon of *A. cepa*. The genetic distance among the germplasm was 0.056 to 1.046 and the gene diversity was 1.54. The clustering pattern based on morphological traits of the germplasm did not match in some cases with the RAPD based clustering, but majority of the cases matched between clustering pattern of morphological on RAPD data. This variation might be the influence of environments fact on phenotypic expression of characters of a crop. This agrees with Dhortre et al. (2010) who opined that differences between genotypes of same origin can be attributed to genetic buildup, heterogeneity in trait development and previous selection activity. Furthermore, the distribution of genotypes into different clusters irrespective of their location supports the works of Lee et al. (1996), Mohanty (2001) and Khar et al. (2006) which ruled out any association between geographical distribution and genetic divergence in rain-fed onions in different parts of India. Pramanick et al. (1992) also stated that the lack of correspondence between geographical distribution and genetic diversity in *A. cepa* indicates the role of drift, natural and artificial selections and differential gene expression due to genotype × environment interactions.

Most of the farmers in Bangladesh obtain their onion seeds from other farmers as well as from the neighboring countries by legal or illegal ways. It is probable that a greater diversity in onion would present in a wider collection from more distant geographical regions was included in this study. Therefore, we found genetic distance (1.046) among the germplasm. We also estimated the highest pair wise genetic distance of germplasm 1.046 and the lowest as 0.056 with high level of average genetic distance (0.972). Gwanama *et al*. (2000) obtained a range of genetic distance of 0.13 - 0.41 with a mean gene distance of 0.31 among 31 accessions of *Cucumis moschata*. In this study, we found 7 to 14 bands/primer which was amplified with an average of 9.25 bands per primer. Among them, 34 bands (91.89%) were polymorphic. Lee *et al.* (1996) found a mean genetic distance of 0.30, although individual distance among pairs of germplasm was 0.91. Their study involved inbred lines, showed closer genetic relationships between individual germplasm within distantly related clusters. Adsul (2009) obtained the similar results, where 31 primers yielded 77.2% polymorphic bands in 14 onion germplasm and separated into four clusters. Adesoye et al. (2012) found 54 RAPD bands across the 15 onion germplasm by using six RAPD primers and the number of bands per primer ranged from 6 to 13 with a mean of 9 bands per primer.

Dennequin et al. (1997) and Tanikawa et al. (2002) showed the relative high genetic diversity between the white and light brown cultivars (75%) and the purple and light brown cultivars (54.72%), the overall genetic diversity was low between the phenotypic classes. The alleles effective in polymorphism ranged from 1.19 ± 0.32 to 1.46 ± 0.35 in the light brown and purple phenotypes, giving gene diversity of 0.11 ± 0.19 and 0.27 ± 0.18, respectively. This low diversity may be attributed to selection by farmers with *A. cepa*. This proposition is supported by Wilkie et al. (1993) and Tanikawa et al. (2002) who reported a relatively small number of polymorphisms in cultivars of *A. cepa* and *A. ascalonicum*, respectively, stating that the low RAPD-type polymorphism observed was reasonably high when compared with the intensive selection of modern day cultivars. The clustering of the genotypes together may be as a result of adaptation to the environment since they are from the same location. A dendrogram of 38 genotypes with six clusters was constructed based on Nei`s (1972) genetic distance using UPGMA (Figure 5). However, the high genetic distance between some phenotypic classes provides a broad genetic base for further character selection in cultivars of *A. cepa*. From the cluster analysis, although most of the small sized cultivars belonged to the same cluster, but similarity between all the clusters based on size was still relatively high. It is therefore suggested that the differences in size may be due to the environment and genotype × environment interactions rather than genetic factors. Sultan (1995) and Schwaegerle and Bazzaz (1987) suggested that mere observation of plasticity in a phenotypic trait does not necessarily imply that the response is adaptive, Pigliucci (2001) opined that phenotypic plasticity is an important means by which individual plants in natural populations may cope with environmental heterogeneity.

CONCLUSION

Random amplified polymorphic DNA (RAPD) analysis was done to study the genetic diversity of 38 onion germplasm. With the help of polymerase chain reaction (PCR) amplifications, 4 primers were generated 37 DNA bands, of which 34 (91.89%) was polymorphic. The results of RAPD banding pattern indicated that high level of genetic diversity was present in the germplasm. It was revealed that Nei`s (1973) genetic diversity (1.54) and Shannon`s information index (0.319) across all loci also support high level genetic diversity in 38 onion germplasm. The UPGMA based dendrogram on Nei`s (1972) genetic distances segregated the germplasm into six clusters. Cluster VI was consisted 11 germplasm followed by cluster II (9), cluster IV (7) cluster I (6), cluster III (3) and Cluster V represented only 2 genotypes. On the basis of morphological data and RAPD markers clustering pattern synchronized 54.00% of cluster membership among the germplasm. These diverse germplasm from different cluster will help in parent selection for future breeding program to develop hybrid onion varieties.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Adesoye AI, Obi OC, Fasola TR, Ayodele AE. 2012. Assessment of genetic diversity in two *Allium* spp. using random amplified polymorphic DNA markers. J. Med. Plants Res. 6**:** 4741-4747.
2. Adsul GG. 2009. Genetic diversity analysis of some onion germplasm lines (*Allium cepa* L.). (Doctoral dissertation), North Maharashtra University, Jalgaon, India.
3. AIS (Agricultural Information Service). 2020. Krishi Dairy. Khamarbari, Farmgate, Dhaka-1215, Bangladesh.
4. Anonymous 2020. Annual Research Report. Spices Research Centre, Bangladesh Agricultural Research Institute, Shibgonj, Bogra, Bangladesh.
5. BBS (Bangladesh Bureau of Statistics). 2021. Statistics Division, Ministry of Planning, Government of the People's Republic of Bangladesh.
6. Cvikic D, Pavlovic N, Zdravkovic M, Prodanovic S. 2009. RAPD marker related to elongated fruit shape in pepper (*Capsicum annuum* L.). Acta Hort. 830: 97-100.
7. Dennequin ML, Panaud O, Robert T, Ricroch A. 1997. Assessment of genetic relationships among sexual and asexual formsof *A ilium cepa* using morphological traits and RAPD markers. Heredity 78:403-409.
8. Dhortre M, Allolli TB, Hulihalli UK, Athani SI. 2010. Genetic diversity study in Kharif Onion (*Allium cepa* Var. *cepa* L.). Karnataka J. Agric. Sci. 23(5):811-812.
9. Doyle AJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissues. Focus. 12**:** 13-15.
10. Elo KS, Ivanoff JA, Vuorinen, Piironen J. 1997. Inheritance of RAPD markers and detection of inter-specific hybridization with brown trout and Atlantic salmon. Aquaculture. 152: 55-65.
11. Gwanama C, Labuschange MT, Botha AM. 2000. Analysis of genetic variation in *Cucurbita moschata* by RAPD markers. Euphytica. 113: 19-24.
12. Khar A, Asha DA, Mahajan V, Lawande KE. 2006.Genetic Diversity analysis in Elite lines of late Kharif (Rangda) Onion. J. Maharashtra Agric. University. 31(1):49-52.
13. Lee SJ, Shin JS, Park KW, Hong YP. 1996. Detection of genetic diversity using RAPD-

PCR and sugar analysis in watermelon [*Citrulus lanatus* (Thumb.) Mansf.] germplsm. Theor. Appl. Genet. 92: 719-725.
14. Mohanty BK. 2001. Genetic variability, inter-relationship and path analysis in onion. J. Trop. Agric. 39:17-20.
15. Nei M. 1972. Genetic distance between populations. The American Naturalist. 196: 283-292.
16. Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceeding of National Academy for Science, USA. 70: 3321-3323.
17. NHRDF (National Horticultural Research and Development Foundation). 2018. Annual Report. New Delhi-10058, India.
18. Pigliucci M. 2001. Phenotypic Plasticity: Beyond Nature and Nurture. Johns Hopkins University Press, Baltimore, USA.
19. Pramanick KK, Singh N, Kalda TS. 1992. Genetic divergence in eggplant (*Solanum melongena* L.). Indian J. Hort. 49:371-375.
20. Rashid M, Islam A, M. Mian M, Hossain T, Kabir M. 2013. Multivariate analysis in onion (*Allium cepa* L.). Bangladesh J. of Agric. Res. 37: 573-582.
21. Schwaegerle KE, Bazzaz FA. 1987. Differentiation among nine populations of Phlox: response to environmental gradients. Ecology 68:54-64.
22. Sultan SE. 1995. Phenotypic plasticity and plant adaptation. Acta Bot. Neerl. 44: 363-383.
23. Tanikawa T, Takagi M, Ichii M. 2002. Cultivar identification and genetic diversity in onion (*Allium cepa* L.) as evaluated by random amplified polymorphic DNA (RAPD) analysis. Jpn. Soc. Horti. Sci. 71(2):249-251.
24. USDA (United States Department of Agriculture) 2011. National Genetic Resources Program. Germplasm Resources Information Network - (GRIN). National Germplasm Resources Laboratory, Beltsville, Maryland, USA. http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl
25. Wilkie S, Isaac PG, Slater RJ. 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theor. Appl. Genet. 86:497-504.
26. Yeh FC, Yang RC, Boyle T. 1999. POPGENE VERSION 1.32 Microsoft Window-based Free Ware for population Genetic Analysis.
27. https://sites.ualberta.ca/~fyeh/popgene_download.html
28. Zdravkovic J, Pavlovic N, Mijatovic M, Zdravkovic M, Adzic S, Pavlovic R, Boskovicrakocevic LJ. 2011. The level of resistance to late blight Phytophthora infestans (Mont.) de Bary in tomato breeding genotypes in Serbia. African J. of Agril. Res. 6: 5475-5480.