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Research Article
Analysis of Yardlong Bean (*Vigna unguiculata* Subsp. *sesquipedalis*) Genetic Diversity using RAPD Markers

[1]Muhammad Shahidul Haque, [1]Afifa Azad, [1]Nihar Ranjan Saha and [2]Muhammad Monirul Islam

[1]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh 2202, Bangladesh

Abstract

Background and Objective: Yardlong bean is a well-known vegetable because of its long, slender, succulent pods and its protein content. In the present study genetic variation and relationships among yardlong bean genotypes were investigated thorough RAPD marker technique. **Materials and Methods:** Thirty six genotypes of yardlong bean were planted in the field to characterize their genetic relatedness and identify variation at molecular level. Sixteen decamer RAPD oligonucleotide primers were screened and out of these, 10 primers were chosen for further evaluation. **Results:** Altogether 94 RAPD bands were scored of which 82 were polymorphic. The amplified product size ranged from 150-900 bp. The primer OPB-08 amplified maximum number of polymorphic bands. The average number of polymorphic bands/primer was 8.2. The frequency of polymorphism ranged from 0.0278-0.9722. Nei's pair-wise genetic distance ranged from 0.055-0.83. A UPGMA dendrogram of genetic similarity was constructed based on 82 polymorphic bands obtained from 10 primers. The UPGMA dendrogram divided the studied individuals into four main clusters. BD-1528 and BD-1524 accession formed distinct cluster (I and II, respectively). Most of the genotypes were observed in cluster IV (29 genotypes) which was further sub-divided into two sub-clusters which were subdivided into sub-sub-clusters making the genotypes diverse. Accessions collected from Bangladesh Agricultural Research Institute, showed significant diversification as they were widely distributed in 4 clusters. **Conclusion:** The variability among the genotypes has the potential to be used in future plant breeding program as well as for commercial production.

Key words: Genetic diversity, genotype, molecular marker, RAPD, yardlong bean

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Corresponding Author: Muhammad Shahidul Haque, Department of Biotechnology, Bangladesh Agricultural University, 2202 Mymensingh, Bangladesh

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yardlong bean (*Vigna unguiculata* sub. sp. *sesquipedalis*) is a climbing annual of Fabaceae or Leguminosae family. Its synonyms are bora, long-podded cowpea, asparagus bean, pea bean, snake bean, garter bean and Chinese long bean. It has the ability to fix considerable atmospheric nitrogen with *Rhizobium* bacterium in the symbiosis process and thus it improves soil productivity[1].

Yardlong bean is widely spread throughout the tropics as a minor vegetable crop. But it is mostly cultivated in India, Bangladesh as well as Southeast Asia and Oceania[1]. Though it is botanically more closely related to cowpea, it is much more a trailing and climbing plant than the cowpea, often reaching 9-12 feet in height[2] and it is characterized by its very long pods (30-90 cm in length).

As a vegetable yardlong bean is very nutritive. The tender green pods are rich in crude protein (3.5-5%) as well as they are a good source of vitamin A (941 IU) and C (13 mg), iron (2.5 mg), calcium (80 mg), phosphorus (74 mg) and dietary fibre (2 g)[3]. Fiber is very useful for health, in particular eases the digestive system and gives a sense of long lasting satiety. It can be used as medicine to reduce cholesterol in the blood and to help kidney's and spleen's function[4].

Yardlong bean is one of the economically important vegetable crops in Bangladesh. The area occupied by this crop was 16284 acres and the production was 25651 million t during[5] the year 2017-2018. It is one of the vegetables having exporting potential in Bangladesh. But the production rate is much lower than other Asian countries. Here the breeding of yardlong bean is usually practiced without any genomic background. The genetic diversity of yardlong bean should be explored for long term success in its breeding programs.

Genetic diversity plays an important role in the success of any breeding program. Knowledge of genetic diversity is very useful for plant improvement as it promotes the efficient use of genetic variations in breeding programs through supporting proper selection of cross combination among large sets of parental genotypes. A clear characterization of accessions is a necessary first step to facilitate breeding efforts and will benefit plant breeder in choosing the proper parental materials. Morphological attributes are generally limited and have environmental influence. DNA markers, on the contrary, provide a larger number of characters which are unaffected by environmental influence and have therefore been used extensively to study relationships within and between crop species[6,7]. Several molecular marker techniques have been used to measure genetic diversity of a range of plant species. Williams *et al.*[8] first developed random amplified polymorphic DNA (RAPD) and is still among the most widely used because of its simplicity and cost effectiveness. RAPD analysis has proven effective for diversity studies in several legume species, including cowpea[9,10], yardlong bean[11,12], mung bean and blackgram[13]. To construct genetic linkage map RAPD was also further applied together with other markers[14]. The objective of this present study was to analyze the genetic diversity of yardlong bean genotypes by utilizing RAPD techniques.

MATERIALS AND METHODS

Plant materials: A total of 36 genotypes (25 accessions and 11 commercial cultivars) of yardlong bean (Table 1) were collected and grown at the experimental Farm (24°75' North Latitude and 90°50' East longitude), Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh, during the period of April-June, 2019.

Table 1: List with source of 36 yardlong bean genotypes

Name of the genotypes	Source
Kagornatki	Lal Teer seed company
Saba	Lal Teer seed company
Lal Benny	Lal Teer seed company
Toki	Lal Teer seed company
Banalata	Lal Teer seed company
1070	Lal Teer seed company
Bari Borboti-1	Lal Teer seed company
BD-10292	BARI
BD-1529	BARI
BD-1528	BARI
BD-1527	BARI
BD-1524	BARI
BD-1519	BARI
BD-1517	BARI
BD-1516	BARI
BD-1511	BARI
BD-9830	BARI
BD-9831	BARI
BD-9833	BARI
BD-9852	BARI
BD-10069	BARI
BD-10070	BARI
BD-10071	BARI
BD-10073	BARI
BD-10290	BARI
BD-10085	BARI
BD-10082	BARI
BD-10079	BARI
BD-10077	BARI
BD-10076	BARI
BD-10075	BARI
BD-10074	BARI
Khagrachari local	Khagrachhari
Kashikanchon	Satkhira
Felon mirershorai local	Hathazari, Chattogram
Thai variety	Local market

DNA extraction: After 3 weeks of sowing, newly opened fresh young leaves were picked from three plants/genotype for DNA extraction. 0.3 g fresh leaf sample was ground into fine powder and DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega Corporation, U.S.A), according to the manufacturer's instructions without any additional modifications.

Primer screening: Initially sixteen RAPD primers (Macrogen, Korea) were screened on a sub-sample of two randomly chosen individuals from 2 different genotypes to evaluate their suitability for amplifying yardlong bean RAPDs that could be scored accurately. Primers were selected from those chosen for previous studies of yardlong bean genetic diversity[15]. Primers were evaluated based on intensity of bands, consistency within individual, presence of smearing and potential for population discrimination. A final subset of ten primers out of sixteen exhibited good quality amplified band and were selected for analysis of the whole sample set of the 36 genotypes of yardlong bean.

PCR amplification: A total of 20 µL PCR reaction volume containing 10 µL Go-Taq green master mix (Promega Corporation, U.S.A), 7 µL nuclease free H_2O, 2 µL primer and 1 µL of template DNA, loaded in PCR machine (Biometra Tone) for DNA amplification. The PCR amplification was carried out with a thermal profile described by Sarutayophat *et al.*[15] with a little modification in annealing temperature of following cycling parameters: initial denaturation step at 94°C for 3 min, followed by 35 amplification cycles of denaturation at 94°C for 30 sec, annealing at 30°C for 30 sec, extension at 72°C for 1 min with a final extension at 72°C for 5 min. Afterwards the PCR products are separated by electrophoresis on a separation gel containing 6% polyacrylamide gel runs in TBE buffer for about 1.5-2.0 h at 60 volts. After completion of electrophoresis the gel was soaked in ethidium bromide (0.5 µg mL^{-1}) solution for 30 min and photographed under UV light. Molecular weights were estimated with a 50 bp DNA Ladder Plus (Promega Corporation, U.S.A).

Statistical analysis: RAPD markers were scored visually of their presence (1) or absence (0) of bands, separately for each genotypes of yardlong bean and each primer. For more accuracy, 2 independent persons performed band scoring. Bands not identified by the two readers were considered as non-scorable. For each primer, the number of different bands and the frequency of polymorphic bands were calculated. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, expected heterozygosity, Nei[16] gene diversity (h), Shannon's information index (I), pair-wise Nei[17] genetic distance. A dendrogram based on Nei[17] genetic distance constructed using UPGMA (Unweighted Pair Group Method of Arithmetic Mean) among the genotypes using POPGENE[18] computer program.

RESULTS

Level of polymorphism: Sixteen primers were evaluated among 36 genotypes of yardlong bean to start with and utterly 10 primers (OPA-09, OPB-04, OPB-07, OPB-08, OPC-05, OPC-06, OPR-12, OPZ-08, OPZ-12, OPZ-13) produced comparatively higher number of high intensity bands with minimal smearing and good technical resolution, showed clear polymorphism and were selected to use for genetic diversity analysis in this study. The RAPD profiles of the amplified products of 4 representative primers are shown in Fig. 1a-d. A total of 94 RAPD bands were scored of which 82 were polymorphic amplification products obtained by using these arbitrary primers which varied significantly among the primers. The size of the amplified products ranged from 150-900 bp (Table 2). OPB-08 generated the highest number of 14 bands and 13 of which were polymorphic (92.86%), followed by OPZ-13 (12 bands). Primer OPZ-08 produced the lowest number of scorable bands (4). Primers OPB-08, OPZ-13, OPC-06 and OPB-04 gave the highest polymorphism percentage in a range of 90-93%. The primer OPA-09 showed least polymorphism (72.73%) compared to others. The average number of bands/primer was 9.4 whereas the polymorphic bands scored 8.2/primer. The number of polymorphic loci was 86.28% in average. Thoroughly 1857 clear bands were amplified from ten RAPD primers in 36 yardlong bean genotypes. It revealed that the RAPD profiles showed a high level of genetic variability among the studied individuals in this study.

The overall observed and effective numbers of alleles were 1.8723 and 1.4690, respectively (Table 3). Average values of Nei[16] gene diversity (h) and Shannon's information index (I) across all primer against genotypes for all loci were found 0.2782 and 0.4229, respectively. The overall mean expected heterozygosity was 0.2782. Minimum frequency of polymorphism ranged in between 0.0278-0.1111 and maximum frequency ranged from 0.6111-0.9722 (Table 4).

Table 2: RAPD primers with corresponding bands score and their size range together with polymorphic bands score

Primers	Sequences (5'-3')	Band size	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)
OPA-09	GGGTAACGCC	180-900	11	8	72.73
OPB-04	GGACTGGAGT	200-900	10	9	90.00
OPB-07	GGTGACGCAG	280-900	8	7	87.50
OPB-08	GTCCACACGC	140-900	14	13	92.86
OPC-05	GATGACCGCC	150-900	9	8	88.89
OPC-06	GAACGGACTC	140-900	11	10	90.91
OPR-12	ACAGGTGCGT	160-900	7	6	85.71
OPZ-08	GGGTGGGTAA	300-900	4	3	75.00
OPZ-12	TCAACGGGAC	180-900	8	7	87.50
OPZ-13	GACTAAGCCC	250-900	12	11	91.67
Total			94	82	862.76
Average			9.4	8.2	86.28

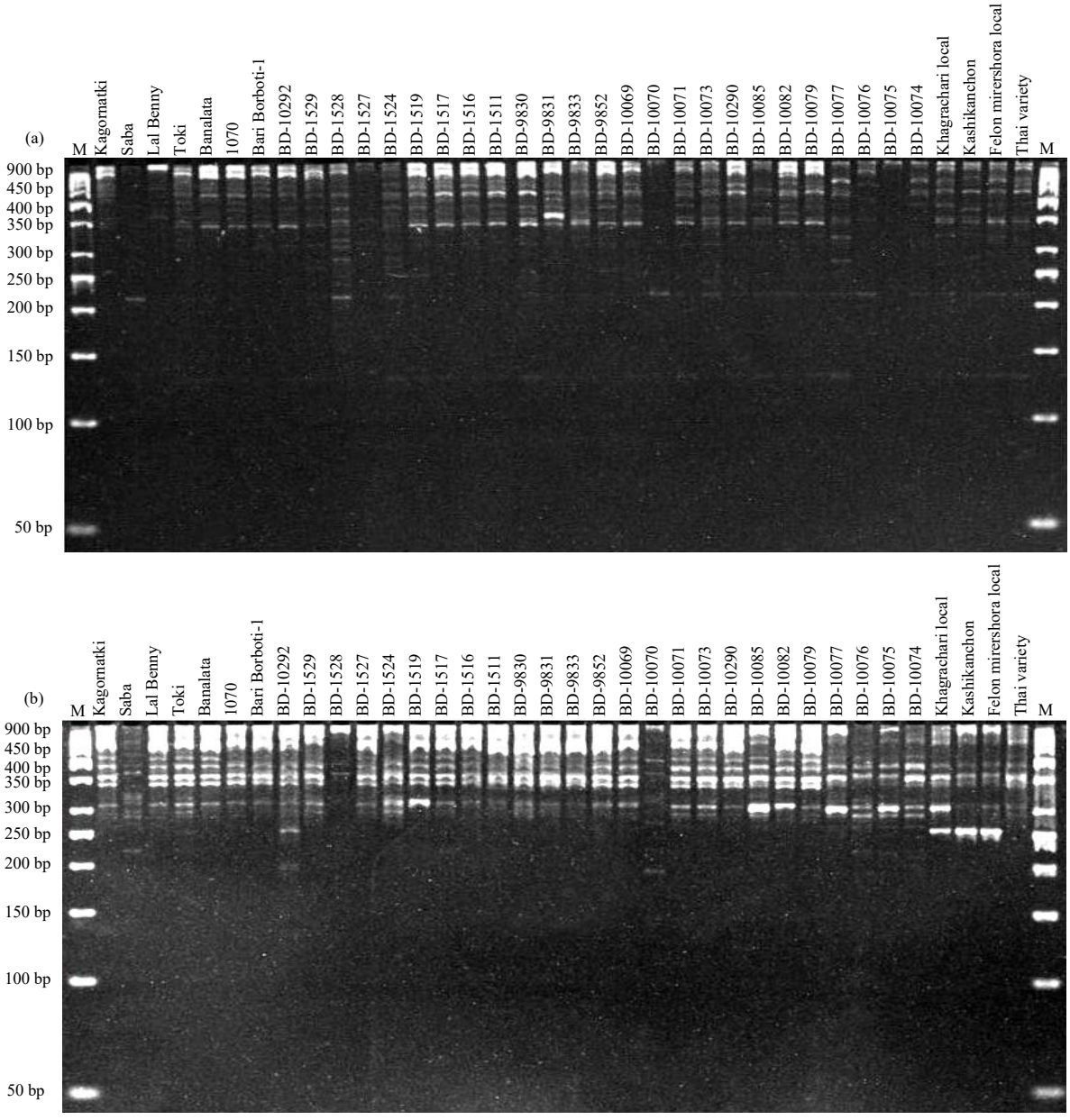

Fig. 1(a-d): Continue

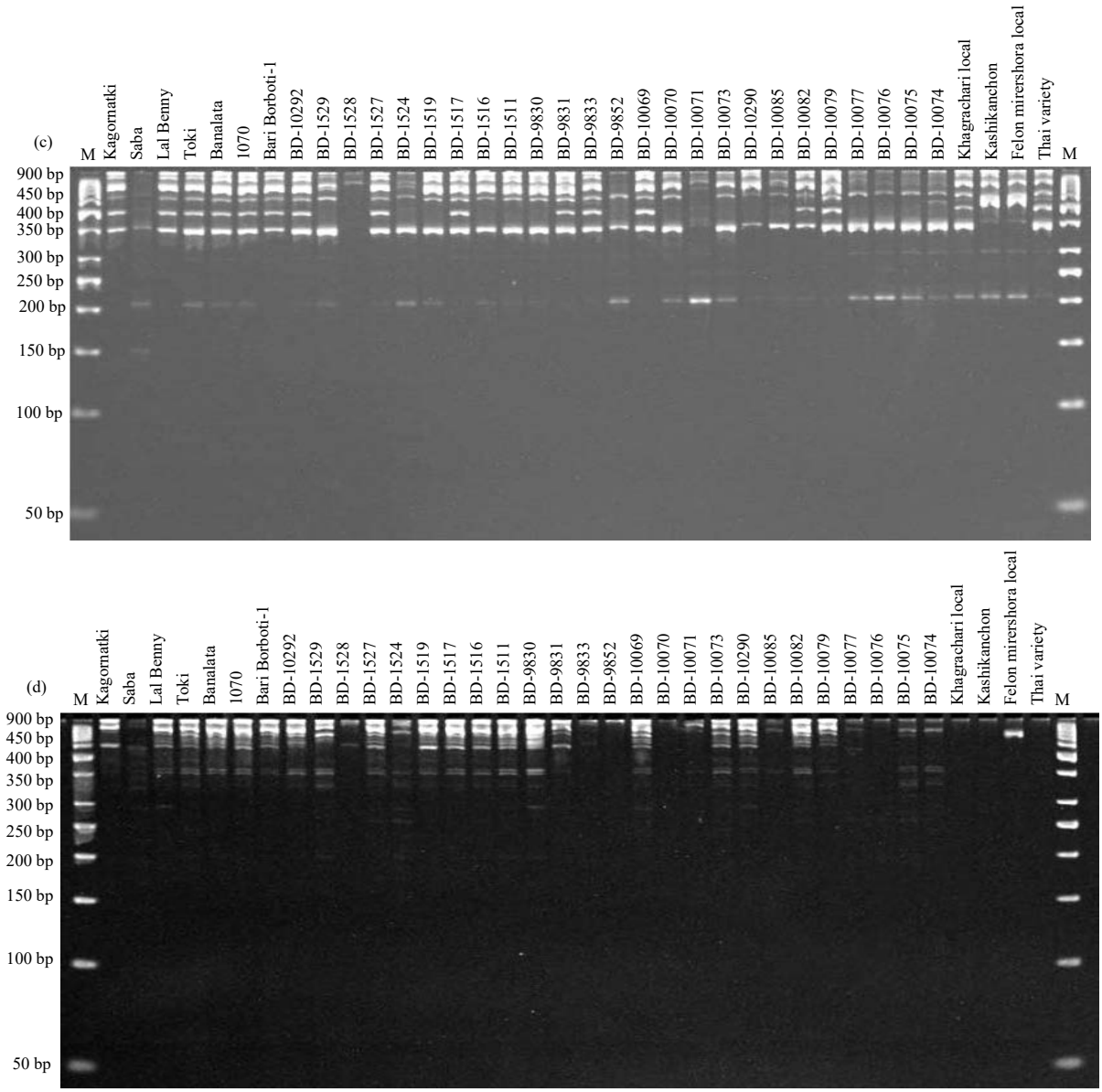

Fig. 1(a-d): Banding pattern of (a) OPB-08, (b) OPZ-13, (c) OPC-05 and (d) OPB-04 primer in 36 yardlong bean genotypes
M: DNA ladder

Table 3: Mean value and standard deviation of several allelic information

Allelic information	Mean over all loci	Standard deviation
Observed number of alleles (na)	1.8723	0.3355
Effective number of alleles (ne)	1.4690	0.3550
Gene diversity (h)	0.2782	0.1747
Shannon information index (i)	0.4229	0.2341
Heterozygosity	0.2782	0.0305

Genetic relatedness: The pair-wise genetic distance in this study ranged from 0.055-0.83. The maximum genetic distance was 0.83 and observed between accessions of BD-1528 and BD-10075. BD-1528 also had large genetic distance value of 0.737 with BD-10074, 0.652 with BD-1524 and 0.612 with Kashikanchon. Besides, Saba had large genetic distance of 0.806 with Kashikanchon. The Bari borboti-1 had minimum genetic distance value of 0.055 with 1070. Other accessions that had minimum pair-wise genetic distance (0.089) in between themselves were Banalata and 1070, Bari borboti-1 and Kagornatki, BD-1519 and BD-1516. The difference between the highest and the lowest value of genetic distance revealed the wide range of variability persisting among the yardlong bean genotypes.

Table 4: Minimum and maximum frequencies of polymorphic RAPD markers in 36 yardlong bean genotypes

Primers	Locus numbers	Minimum frequency	Maximum frequency
OPA-09	11	0.0833	0.6111
OPB-04	10	0.0833	0.7778
OPB-07	8	0.1111	0.9722
OPB-08	14	0.0833	0.8889
OPC-05	9	0.0278	0.9722
OPC-06	11	0.0833	0.9167
OPR-12	7	0.0833	0.8611
OPZ-08	4	0.0556	0.9722
OPZ-12	8	0.0556	0.8333
OPZ-13	12	0.0833	0.9167

Among the cultivated variety Saba had shown the largest genetic distance of 0.806 with Kashikanchon and Bari borboti-1 had the least genetic distance value of 0.089 with Kagornatki. Accessions collected from Plant Genetic Resource Centre (PGRC) of BARI (Bangladesh Agricultural Research Institute) demonstrated the highest genetic distance of 0.83 between BD-1528 and BD-10075 while the lower genetic distance of 0.089 found between BD-1519 and BD-1516.

Cluster analysis was performed using the unweighted pair group method of arithmetic means (UPGMA) to study the genotypes based on genetic distance level. The UPGMA dendrogram segregated the studied individuals into four main clusters and a few rather sub-clusters and sub-sub-clusters. The clustering of 36 yardlong bean genotypes is shown in Fig. 2. Cluster I and III consists of a single genotype BD-1528 and BD-1524, respectively. Accessions BD-10070, BD-10074, BD-10075, BD-10076 and Saba formed cluster II. Cluster IV was the largest consisting of the rest of the genotypes (29) and subdivided into 2 sub-clusters which were further subdivided into sub-sub-clusters. The PGRC genotypes had a great diversity. They were distributed into 4 clusters.

DISCUSSION

This study showed extensive level of polymorphism as 10 primers (OPA-09, OPB-04, OPB-07, OPB-08, OPC-05, OPC-06, OPR-12, OPZ-08, OPZ-12, OPZ-13) out of 16 primers, showed clear variation at the DNA level (polymorphisms) which indicates the presence of genetic diversity. Genetic diversity is a prerequisite for genetic improvement of agricultural crops. Presence of genetic diversity in crop populations is not simply detected by morphological characteristics. Molecular markers such as RAPD markers provide a more reliable method for identification of varieties/species than morphological characters. By using these arbitrary primers, a total of 94 RAPD bands were scored of which 82 were polymorphic amplification products (proportion of polymorphic loci 86.28% in average) and the fragment size ranged from 150-900 bp. Pooprompan *et al.*[11] analyzed various varieties of yardlong bean by RAPD and reported fragment size variation from 500-2200 bp, while Phansak *et al.*[12] reported size of 940-1100 bp of amplified products generated by RAPD primers. Primers used by the two research groups were different from primers used in this experiment. But four primers were common with the present study, in the experiment of Sarutayophat *et al.*[15] they reported amplified fragments ranged from 225-1650 bp.

In this study, 86.28% genetic polymorphism was estimated among 36 yardlong bean genotypes. Tantasawat *et al.*[19] reported 91.03% polymorphism investigating 23 yardlong bean and 7 cowpea genotypes. RAPD has been widely used to study genetic polymorphism in cowpea[9,10,20]. In contrast, other studies also described lowers levels of polymorphism[21], e.g., 12 and 18.5%[22] with RAPD markers. The average number of bands/locus was 9.4 whereas the polymorphic bands scored 8.2/locus. This result is in agreement with Sarutayophat *et al.*[15], they analysed 37 yardlong bean/cowpea accessions with five RAPD primers (OPC-06, OPR-12, OPZ-03, OPZ-08 and OPZ-13) and reported 7.6 bands and 4.6 polymorphic bands/locus in average. The observed and effective numbers of alleles were obtained 1.8723 and 1.4690, respectively in this study. Similar results were obtained for several other plant species in previous studies by De Britto *et al.*[23].

There was a high level of genetic variation among the studied genotypes of yardlong bean from the proportion of polymorphic loci point of view. Nei[16] gene diversity (h) was 0.2782 on average. Mafakheri *et al.*[24] reported high genetic diversity at molecular level using SSR markers (0.146 on average). The overall mean expected heterozygosity was found 0.2782. The expected heterozygosity was accounted for the frequency of the different types of alleles or loci in the population[25]. In most cultivated plant species higher mean heterozygosity have been reported for 0.30 in soyabean[26], 0.32 in common bean[27], 0.361 in green beans[28], 0.444 in mung bean[29]. The frequency of polymorphic RAPD markers in this

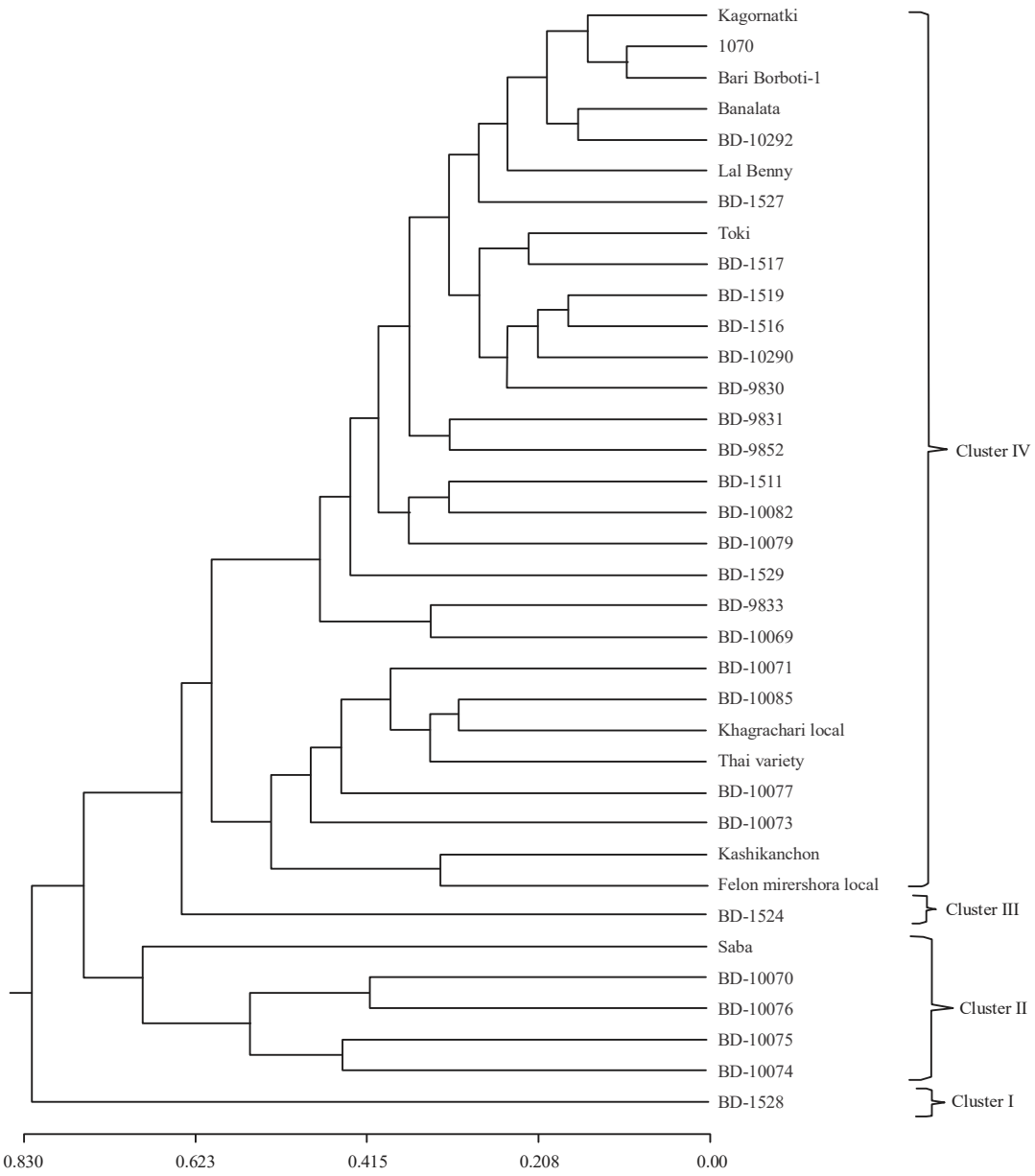

Fig. 2: Dendrogram for 36 Yardlong bean genotypes showing the genetic similarity and relatedness

study ranged in between 0.0278 and 0.9722. The ability to resolve genetic variation may be more directly related to the number of polymorphism detected by the marker techniques and the percentage of polymorphic RAPDs. However, it does not correlate with the influence of rare and common alleles on the genetic highest frequency across the genome[30]. The Nei[17] genetic distance ranged from 0.055-0.83. The maximum genetic distance was 0.83 and observed between accessions BD-1528 and BD-10075 and the minimum genetic distance was 0.055 and observed in between Bari borboti-1 and 1070. Lakhanpaul *et al.*[31] reported that pair-wise genetic distances ranged from 0.06-0.30 using RAPD markers for black gram, another *Vigna* species.

Genetic relationships will help plant breeders to prevent gene erosion within varieties by selecting a large number of different clones of each variety[32]. The knowledge of genetic relationships among genotypes provides useful information to address breeding programs and germplasm resource management[33]. The UPGMA dendrogram based on Nei[17] genetic distance constructed four distinct clusters. Cluster IV was the largest cluster consisting of most of the genotypes (29). Among the commonly cultivated varieties, Kagornatki, Lal

benny, Banalata and Toki clustered in the same group and high yielding variety, Saba formed a separate cluster. Similar result was reported by Alam *et al.*[34] using different primers. They reported Lal benny and Kegornatki in the same cluster whereas Saba formed a distinct cluster. Phansak *et al.*[35] separated the yardlong bean accessions into three groups by STMS analysis and suggested that the clustering of accessions were not correlated to geographical origin. These results correlated with taxonomic relationships in *Vigna* established using RAPD and RFLP analysis[13,36]. The results of the present experiment exposed that the selected yardlong bean genotypes were widely distributed in four clusters as they were highly variable regarding RAPD based molecular characterization which indicated a wide polymorphism as well as prolonged diversification among the genotypes.

CONCLUSION

The present piece of study was undertaken to analyze the molecular diversity and genetic relatedness among the collected germplasm of yardlong bean. Genetic variation based on molecular characterization indicated that the genotypes belonging to different clusters have characteristic distinction at the DNA level and extended diversification which is a prerequisite for genetic improvement of agricultural crops. It could be concluded that this variability may also be used for further research program, especially for hybridization and also for selection of superior genotypes for commercial cultivation at farmer's level as well as for breeding new genotypes of yardlong bean in Bangladesh.

SIGNIFICANCE STATEMENT

This study discovers the molecular diversification among yardlong bean genotypes available in Bangladesh. The information can be potentially used for selection of parents for breeding for genetic improvement and genotypes for commercial production. Exploration of molecular level diversity can help development of new variety by hybridization and breeding. This study will help the researcher to uncover the critical areas such as genetic mapping of yardlong bean as well as to know the specific functioning of genes which will help to develop super varieties with favorable characteristics.

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REFERENCES

1. Pandey, R.K. and E. Westphal, 1989. *Vigna unguiculata* (L.) Walp. In: PROSEA (Plant Resources of Southeast Asia) No. 1: Pulses, Van der Maesen, L.J.G. and S. Somaatmadja (Eds.). Pudoc Scientific Publishers, Wageningen, The Netherlands, ISBN-13: 978-9022009840, pp: 77-81.
2. USDA., 2012. Plant guide: Yardlong bean *Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* (L.) Verdc. United States Department of Agriculture, Natural Resources Conservation Service, Washington, DC., USA.
3. Singh, J., G. Kalloo and K.P. Singh, 2001. Vegetable crops: Nutritional security. Technical Bulletin No. 6, Indian Institute of Vegetable Research, Varanasi, India, pp: 1-56.
4. Rubatzky, V.E. and M. Yamaguchi, 1997. World Vegetables: Principles, Production and Nutritive Values. 2nd Edn., Chapman and Hall New York, USA., ISBN-13: 9780834216877, Pages: 844.
5. BBS., 2019. Statistical Year Book Bangladesh 2018. 38th Edn., Bangladesh Bureau of Statistics (BBS), Dhaka, Bangladesh, ISBN-978-984-475-020-3, Pages: 569.
6. Asare, A.T., B.S. Gowda, I.K.A. Galyuon, L.M. Aboagye, J.F. Takrama and M.P. Timko, 2010. Assessment of the genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) germplasm from Ghana using simple sequence repeat markers. Plant Genet. Resour., 8: 142-150.
7. Basha, A.I., S. Padulosi, K. Chabane, A. Hadj-Hassan, E. Dulloo, M.A. Pagnotta and E. Porceddu, 2007. Genetic diversity of Syrian pistachio (*Pistacia vera* L.) varieties evaluated by AFLP markers. Genet. Resour. Crop Evol., 54: 1807-1816.
8. Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.
9. Ba, F.S., R.S. Pasquet and P. Gepts, 2004. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. Genet. Resour. Crop Evol., 51: 539-550.
10. Fall, L., D. Diouf, M.A. Fall-Ndiaye, F.A. Badiane and M. Gueye, 2003. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] varieties determined by ARA and RAPD techniques. Afr. J. Biotechnol., 2: 48-50.
11. Pooprompan, P., P. Tamiesak and K. Hosaki, 1996. Use of Random Amplified Polymorphic DNA (RAPD) for identification of yardlong bean cultivars. Proceedings of the 22nd Congress on Science and Technology of Thailand, October 16-18, 1996, Bangkok, Thailand.
12. Phansak, P., P.W.J. Taylor, P. Srinives and O. Mongkolporn, 2001. Level of polymorphisms in five accessions of yardlong bean revealed by RAPDs and microsatellites. Agric. Sci. J., 32: 185-189.

13. Kaga, A., N. Tomooka, Y. Egawa, K. Hosaka and O. Kamijima, 1996. Species relationships in the subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. Euphytica, 88: 17-24.
14. Ouedraogo, J.T., B.S. Gowda, M. Jean, T.J. Close and J.D. Ehlers *et al*., 2002. An improved genetic linkage map for cowpea (*Vigna unguiculata* L.) combining AFLP, RFLP, RAPD, biochemical markers and biological resistance traits. Genome, 45: 175-188.
15. Sarutayophat, T., C. Nualsri, Q. Santipracha and V. Saereeprasert, 2007. Characterization and genetic relatedness among 37 yardlong bean and cowpea accessions based on morphological characters and RAPD analysis. Songklanakarin J. Sci. Technol., 29: 591-600.
16. Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA., 70: 3321-3323.
17. Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.
18. Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao, 1999. POPGENE version 1.31, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada.
19. Tantasawat, P., J. Trongchuen, T. Prajongjai, W. Seehalak and Y. Jittayasothorn, 2010. Variety identification and comparative analysis of genetic diversity in yardlong bean (*Vigna unguiculata* spp. *sesquipedalis*) using morphological characters, SSR and ISSR analysis. Sci. Hortic., 124: 204-216.
20. Badiane, F.A., D. Diouf, D. Sane, O. Diouf, V. Goudiaby and N. Diallo, 2004. Screening cowpea [*Vigna unguiculata* (L.) Walp.] varieties by inducing water deficit and RAPD analyses. Afr. J. Biotechnol., 3: 174-178.
21. Menendez, C.M., A.E. Hall and P. Gepts, 1997. A genetic linkage map of cowpea (*Vigna unguiculata*) developed from a cross between two inbred, domesticated lines. Theor. Applied Genet., 95: 1210-1217.
22. Tosti, N. and V. Negri, 2002. Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* subsp. *unguiculata*) landraces. Genome, 45: 268-275.
23. De Britto, A.J., G. Prabha, T.L.S. Raj and P.B.J.R. Kumar, 2010. Genetic diversity analysis in five *Cassia* species using RAPD markers. J. Basic Applied Biol., 4: 162-167.
24. Mafakheri, K., M.R. Bihamta and A.R. Abbasi, 2017. Assessment of genetic diversity in cowpea (*Vigna unguiculata* L.) germplasm using morphological and molecular characterisation. Cogent Food Agric., Vol. 3, No. 1. 10.1080/23311932.2017.1327092.
25. Mohammadi, S.A. and B.M. Prasanna, 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci., 43: 1235-1248.
26. Ude, G.N., W.J. Kenworthy, J.M. Costa, P.B. Cregan and J. Alvernaz, 2003. Genetic diversity of soybean cultivars from China, Japan, North America and North American ancestral lines determined by amplified fragment length polymorphism. Crop Sci., 43: 1858-1867.
27. Maras, M., J. Sustar-Vozlic, B. Javornik and V. Meglic, 2008. The efficiency of AFLP and SSR markers in genetic diversity estimation and gene pool classification of common bean (*Phaseolus vulgaris* L.). Acta Agric. Slov., 91: 87-96.
28. Sarikamis, G., F. Yasar, M. Bakir, K. Kazan and A. Ergul, 2009. Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from Eastern Turkey. Genet. Mol. Res., 8: 880-887.
29. Gwag, J.G., J.W. Chung, H.K. Chung, J.H. Lee and K.H. Ma *et al*., 2006. Characterization of new microsatellite markers in mung bean, *Vigna radiata* (L.). Mol. Ecol. Notes, 6: 1132-1134.
30. Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.
31. Lakhanpaul, S., S. Chadha and K.V. Bhat, 2000. Random Amplified Polymorphic DNA (RAPD) analysis in Indian mung bean (*Vigna radiata* (L.) Wilczek) cultivars. Genetica, 109: 227-234.
32. Ruhl, E., H. Konrad, B. Lindner and E. Bleser, 2004. Quality criteria and targets for clonal selection in grapevine. Acta Hortic., 652: 29-33.
33. Roldan-Ruiz, I., F.A. van Eeuwijk, T.J. Gilliland, P. Dubreuil and C. Dillmann *et al*., 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. Theor. Applied Genet., 103: 1138-1150.
34. Alam, S.S., R. Tasmin, I. Jahan, M.A. Habib and S.S. Sultana, 2013. Fluorescent banding and RAPD analysis of five cultivars in *Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc. Cytologia, 78: 73-79.
35. Phansak, P., P.W.J. Taylor and O. Mongkolporn, 2005. Genetic diversity in yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) and related *Vigna* species using sequence tagged microsatellite site analysis. Sci. Hortic., 106: 137-146.
36. Fatokun, C.A., D. Danesh, N.D. Young and E.L. Stewart, 1993. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theor. Applied Gene., 86: 97-104.