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MOLECULAR CHARACTERIZATION OF HYACINTH BEAN GERMPLASM COLLECTED FROM BANGLADESH AND EXOTIC SOURCES

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Abstract

Hyacinth bean (*Lablab purpureus* (L.) Sweet) has versatile use as vegetable, pulse, fodder, hay, silage, green manure and cover crop. The experiment was conducted with 65 accessions of hyacinth bean from 17 countries of Asia, Africa and Europe for molecular characterization and phylogenetic relationship. Simple sequence repeat marker (SSR) derived from Medicago and cowpea was used. A total of 47 polymorphic alleles were counted. Two to five alleles per marker were detected with an average of 3.62. PIC values ranged from 0.21 to 0.72 with a mean of 0.53. Average gene diversity was exhibited 0.59 ranging from of 0.24 to 0.76. The maximum genetic distance (0.86) was observed between accessions of Bangladesh and India. Phylogenetic analysis classified the 65 accessions into 8 groups ranging from 1 to 22 accessions per group. Small values of genetic dissimilarities (0 to 0.29) showed without relating geographic distribution. Comparatively greater diversity was exhibited among the accessions in Bangladesh than the remaining 16 countries. The analysis of molecular variance indicated a fixation index of 0.0399 suggesting a low differentiation among the hyacinth bean accessions in Bangladesh and free gene flow among the populations. Genetically diverse accessions were identified that could be potentially source of germplasm for further crop improvement in the country.

Keywords: *Lablab purpureus*, SSR markers, genetic diversity, phylogenetic relationship, Bangladesh.

Introduction

Hyacinth bean is known as country bean or shim, mainly grown for its young pods and immature seeds for vegetable purpose, dry seed is used as pulse and often as soup and in many food preparations in Bangladesh, South and South-East Asia (Yawalkar and Ram, 2004). Lablab has two botanical types, the garden types and the field types. The garden type produces a twining stem and branches and is used as a green vegetable. Field type is erect and bushy, and is used for seed production, forage, green manure, as well as being a cover crop to prevent soil erosion and control weeds. Garden types are cultivated more than the field types all over in

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Bangladesh. Verdcourt (1970) recognized three sub-species: uncinatus, purpureus and bengalensis It was supposed that ssp. *purpureus* and ssp. *bengalensis* were genetically very similar and most of the domesticated hyacinth bean in Bangladesh belongs either to ssp. *purpureus* or ssp. *bengalensis*. BARI have released 10 and Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh released five varieties of hyacinth bean. Hyacinth bean grown on approximately 17,126 ha of land across the country during the winter season, yield an average of 5.50 t of fresh pods per ha for a total yield of about 94,356 t (BBS, 2014).

Hyacinth bean has advantages in terms of environmental tolerances and unique nutritional profiles when compared to the widely cultivated crops. Its unique alleles that could be applied in future agriculture and crop breeding (Varshney *et al.*, 2010). Diversity and relationship among 103 accessions of *Lablab purpureus* from 26 countries of diverse origin were measured through AFLP marker (Maass *et al.* 2005). AFLP and a number of EST-derived markers from a range of legumes were used to test 78 Lablab accessions from India, CPI (Australia) and ILRI (Ethiopia) for genetic diversity (Venkatesha *et al.* 2007). AFLP diversity analysis clearly showed limited genetic variation within the accessions from India compared to a more diverse set of 15 accessions from CPI and ILRI. The biggest challenge for molecular characterization and evaluation of hyacinth bean is the lack of SSR markers. There are many different legume genera and species at PGRC collection and it is expensive and time consuming to develop SSR markers for each species. Many accessions need to be processed with a large set of markers in order to access genetic diversity. It is necessary therefore that DNA marker be abundant in the genome, highly variable among accessions, easy to use and potentially high-throughput. Microsatellites or simple sequence repeats generally meet these requirements for assessing genetic diversity. SSRs markers are generally considered more effective than other techniques because they are co-dominant and highly reproducible (Mondini *et al.*, 2009).

Polymorphic bands in RAPD, AFLP and SAMPL, can also be converted into locus specific SCARs, which is then prove to be as effective as SSR markers. Transferred SSR markers of Medicago, soyabean, cowpea and peanut have been used for characterization and evaluation across the legume family including *Lablab purpureus* (Wang *et al.*, 2004; Wang *et al.*, 2006a, b). Research has previously focused on morphological variation in order to understand the origin and diversity of a crop, however in the past 20 years, studies of molecular markers have taken over as they are not affected by the environment in the same way of morphology which are typically highly variable (Robotham and Chapman, 2017; Rai *et al.*, 2011; Tertivanidis *et al.*, 2008). Bangladesh is rich in hyacinth bean diversity. Systematic research such as collection, conservation, characterization and evaluation and utilization of hyacinth bean has been done at Plant Genetic

Resources Centre (PGRC) of BARI (Islam *et al.*, 2002; Islam 2008; Islam and Haque, 2009). Few studies at molecular level have been already done in different countries but a few or none reports included germplasm from Bangladesh. Therefore, extensive research in this area is needed. PGRC of BARI conserved 751 accessions in the gene bank. The purpose of this study was to characterize and evaluate the accessions of hyacinth bean andto know the genetic diversity of the accessions and to establish a phylogenetic relationship of the accessions from Bangladesh with the other countries.

Materials and methods

Plant materials

The experiment was carried out at the Biotechnology Laboratory of the Bangladesh Institute of Nuclear Agriculture (BINA) Mymensingh, Bangladesh. Sixty five accessions of hyacinth bean of which 38 accessions from 37 districts, 4 varieties of BARI and 23 accessions from 16 countries of Asia, Africa and Europe were used in this study (Table 1). The accessions were selected on the basis of different qualitative and quantitative characters, different geographical locations (district or country) and diversity analysis. The Bangladeshi 38 accessions were collected from 20º35' to 26º75' N latitude and 88º03' to 92º75' E longitude of 37 districts. The varieties, BARI Hyacinth Bean-1, BARI Hyacinth Bean-2 and BARI Hyacinth Bean-4 were grown all over Bangladesh during winter (photo sensitive) but BARI Hyacinth Bean-3 was grown during summer and winter (photo insensitive) (Table 1). The 23 exotic accessions from 16 countries were collected from AVRDC, Taiwan (TOT prefixed before the number). There were eight accessions namely CPI 34777, CPI 35894, CPI 52508, CPI 76996, CPI 81626, CPI 100602, CPI 106548 and ILRI 14437 selected from Core Collection (Pengelly and Maass, 2001 and Maass *et al.*, 2005). The accessions were obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Tropical Agriculture, Brisbane, Australia (CPI prefixed before the number) and the International Livestock Research Institute (ILRI) Addis Ababa, Ethiopia (ILRI-prefixed before the number). The germplasm were accessioned at PGRC BARI by prefixing BD before the number (Table 1). Nine seeds per accession were planted into plastic pots having 15 cm diameter and 20 cm height containing a mixture of loamy soil and decomposed cowdung (5:1). Three seeds were planted in each plastic pot. Watering was done just after seed sowing for proper germination of seeds. Young, actively growing fresh leaves of hyacinth bean were collected from 25-days old seedlings for extracting genomic DNA. Leaves were washed in distilled water and ethanol and were kept in ice and then preserved in -80^0C freezer for subsequent use.

Table 1. List of hyacinth bean accessions collected from different countries for SSR analysis

BARI Acc. No.	Collector's number	Name of district	Origin	BARI Acc. number	Collector's number	Name of district	Origin
BD-6	NH-13	Comilla	Bangladesh	BD-8785	MHML-173	Feni	Bangladesh
BD-31	MOI-46	Pabna	Bangladesh	BD-8802	MHQ-98	Jamalpur	Bangladesh
BD-82	MOI-99	Rangpur	Bangladesh	BD-8808	MHQ-164	Sherpur	Bangladesh
BD-86	MOI-104	Gaibandha	Bangladesh	BD-8815	MRA-29	Cox's Bazar	Bangladesh
BD-101	MOI-121	Naogaon	Bangladesh	BD-8835	BARI Hya-1	Variety	Bangladesh
BD-111	SMH-137	Magura	Bangladesh	BD-8867	AEMM-371/1	Chittagong	Bangladesh
BD-114	AM-140	Hobiganj	Bangladesh	BARI Hya-3	-	Variety	Bangladesh
BD-122	AM-148	Hobiganj	Bangladesh	BARI Hya-4	-	Variety	Bangladesh
BD-128	MOI-154	Dinajpur	Bangladesh	**Bangladesh: Total Accession-42, District-37**			
BD-130	MOI-156	Panchagarh	Bangladesh	**BARI Acc. No.**	**AVRDC no.**	**Other no.**	**Origin**
BD-1785	ODMH-19	Netrakona	Bangladesh	BD-8529	36950172	Etho-1	Ethiopia
BD-1799	MMH-162	Barguna	Bangladesh	BD-9441	TOT 7255	CPI 34777*	India
BD-1805	MMH-140	Patuakhali	Bangladesh	BD-9442	TOT 7256A	CPI 81626*	India
BD-1809	MMH-71	Jhalakati	Bangladesh	BD-9444	TOT 7260	CPI 100602*	Kenya
BD-1816	MMH-211	Gopalganj	Bangladesh	BD-9446	TOT 7261B	CPI 106548*	India
BD-1818	MMH-38	Barisal	Bangladesh	BD-9447	TOT 7262A	CPI 35894*	Denmark
BD-1830	MHML-56	Luxmipur	Bangladesh	BD-9449	TOT 7263	CPI 52508*	Mozambique
BD-1839	MHML-111	Noakhali	Bangladesh	BD-9451	TOT 7264B	CPI 76996*	Zambia
BD-2884	AEMM-115	Khagrachhari	Bangladesh	BD-9452	TOT 7396A	CAM 177	Cambodia

BARI Acc. No.	Collector's number	Name of district	Origin	BARI Acc. number	Collector's number	Name of district	Origin
BD-7775	BARI Hya 2	Variety	Bangladesh	BD-9455	TOT 7777	CAM 417	Cambodia
BD-7978	M-226	Sirajganj	Bangladesh	BD-9457	TOT 7265	ILRI 14437*	Zimbabwe
BD-7985	MH-250	Mymensingh	Bangladesh	BD-9459	TOT 6752	PD 13	India
BD-7988	MT-143	Faridpur	Bangladesh	BD-9461	TOT 2029	LV 4018	Indonesia
BD-7993	MT-222	Rajbari	Bangladesh	BD-9462	TOT 4008A	HPL 98-13	Laos
BD-8001	OD-325	Rangamati	Bangladesh	BD-9463	TOT 4008B	HPL 98-13	Laos
BD-8005	OS-32	Jessore	Bangladesh	BD-9469	TOT 7118	LHL 03-0025	Malaysia
BD-8006	OS-164	Narail	Bangladesh	BD-9472	TOT 2713	PHL 2631	Philippines
BD-8018	T-137	Kushtia	Bangladesh	BD-9473	TOT 2716	PHL 2635	Philippines
BD-8027	T-240	Meherpur	Bangladesh	BD-9477	TOT 4881		Taiwan
BD-8039	T-495	Chuadanga	Bangladesh	BD-9478	TOT 0583		Thailand
BD-8737	MA-74	Natore	Bangladesh	BD-9481	TOT 2465		Thailand
BD-8752	MA-225	Nawbabgonj	Bangladesh	BD-9483	TOT7905		Uzbekistan
BD-8757	MA-254	Rajshahi	Bangladesh	BD-9484	TOT3932	TB 90	Viet Nam
BD-8770	MHA-67	Gazipur	Bangladesh	**Exotic: Total accession-23, Country-16**			

Where, '*'Selected the accessions from core collection (Pengelly and Maass 2001 and Maass *et al*. 2005).

DNA preparation

Approximately 0.1 g leaf samples were ground by pestle and mortar using 660 µl extraction buffer and 40 µl 20% SDS. DNA extraction was done by CTAB mini-preparation method (IRRI, 1997). DNA was dissolved and diluted in 0.1 X TE (1mM Tris, 0.1mM EDTA, P^{H}8.0) to make the concentration of 25ng/µl as PCR templates. DNA preparation was confirmed with 1% agarose gel electrophoresis at 120 V for 1 hour 20 minutes. DNA concentration was measured at A260 nm absorbance through Double bean Spectrophotometer (SQ-4802 USA).

PCR primer selection and amplification

Ten pairs of primers from cowpea (*Vigna unguiculata* (L) Walp genomic SSRs and 15 pairs of primers from Medicago (*Medicago truncatula*) expressed sequence tag (EST)-derived SSRs were selected for polymorphism survey from the hyacinth bean germplasm evaluation by Wang *et al.,* (2007 and 2004). Among them, 13 primers were selected which showed clear polymorphism (Table 3). The PCR was performed in a SIGMA PCR System with a 48-PCR tube format. The PCR tubes (0.2 ml) were set on the wells of the thermocycler plate. Two µl of genomic DNA were used in a 15 µl total volume with a final concentration of 10X PCR buffer, 1.0 µl dNTPs, 1.0 µl each primer, 0.5 µl Tag DNA polymerase and 8.25 µl ddH_2O. The PCR was programmed as initial denaturing at $94P^{oP}C$ for 4 minutes, denaturing at $94P^{oP}C$ for 1 minute, annealing at $50P^{oP}C$ for 30 seconds, polymerizing at $72P^{oP}C$ for 40 seconds. Step 2 to 4 for 10 cycles. Again denaturing at $94P^{oP}C$ for 1 minute, annealing at $45P^{oP}C$ for 30 seconds, polymerizing at $72P^{oP}C$ for 40 seconds. Step 6 to 8 for 35 cycles. Extension at $72P^{oP}C$ for 10 minutes. Completion of cycling programme, reactions were held at 4°C (Wang *et al.,* 2007). Each of the amplifications was repeated at least twice for ensuring the results.

PCR fragment separation and microsatellite data analysis

Four µl loading dye was added with the PCR products. Ten µl PCR products were run in 3.5% agarose gel. DNA marker (100 bp) was loaded on either side of the gel. The electrophoresis was run for 2.30 hours at 90 V. The gel was stained for 20 minutes in ethidium bromide. DNA bands were visualized under UV light using a Gel DOC. The bands representing particular alleles at the microsatellite loci were scored by AlphaEase FC 4.0 (Software). Power Marker 3.23 (Liu and Muse, 2005) package software has been used in microsatellite data analysis (Table 3). The population was divided through F-statistics (Wright, 1965). The genetic structure of the accessions was investigated by the analysis of molecular variances (AMOVA). The fixation index was used for analyzing the genetic structure (Kiambi *et al.,* 2005). Genetic distance measures used for frequency data were calculated following Nei (1973). The unweighted pair-group method with

arithmetic mean (UPGMA) dendrogram was drawn, and Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura *et al*., 2007).

Results and discussion

Allelic variation

Thirteen SSR markers generated clear polymorphic amplicons (DNA fragments or bands in agarose gels) among the 65 accessions of hyacinth bean (Table 3, Fig.1). A total of 47 bands were scored from 13 SSR markers. The maximum 5 alleles were exhibited in primers VM71, AQ842128 and MtSSRNFBF46 while the minimum, 2 alleles from VM22, AW127626 and MtSSRNFBG31. The primers exhibited an average of 3.62 alleles among the 65 accessions of hyacinth bean (Table 3). On the other hand, 42 accessions from Bangladesh and the 23 accessions from other 16 countries showed an average of 3.23 and 3.00 alleles, respectively (Table 4). The informativeness of SSR markers was measured by PIC values. The highest PIC value 0.72 was found in AQ842128 followed by 0.70 in MtSSRNFBF46 while the lowest 0.21 was found in VM22 with an average of 0.53. More PIC value was found in accessions from Bangladesh (0.50) than other countries (0.41). The maximum heterozygocity was observed in MtSSRNFBF46 (0.86) followed by VM71 (0.72) and AW127626 (0.57) with an average of 0.23 among the 65 accessions. The lowest heterozygosity was exhibited in VM70 (0.03), while 6 markers (VM31, VM22, AQ842128, AW584539, BF647899 and AW688861) did not detect any heterozygosity. Major allele frequency ranged from 0.29 (AW584539) to 0.86 (VM22) with an average of 0.52. However, the lower major allele frequency was obtained from Bangladesh (0.55) than other countries (65). The highest size of allele 213 bp was found in AW584539 and their range was 200 to 218 bp. The second highest size of allele 183 bp was reported in VM31.The lowest size of allele 52 bp was exhibited in VM71 ranging from 52 to 85 bp among the 65 accessions. The banding pattern of AW584539 primer is shown in Fig. 1.

Gene diversity and genetic distance

The maximum gene diversity was exhibited in AQ842128 (0.76) and the minimum was observed in VM22 (0.24). The average gene diversity for all microsatellite loci in 65 accessions was 0.59 (Table 3). Gene diversity of 0.56 and 0.47 was exhibited in accessions from Bangladesh and exotic countries, respectively. It is noted that a marker detecting a lower number of alleles also showed the lower gene diversity, compared to markers detecting a higher number of alleles that showed the higher gene diversity. There were significant deviations from Hardy-Weinberg Equilibrium for all microsatellite loci from 65 accessions (Table 5). The values of pair-wise comparison of Nei's (1973) genetic distance (D) between

Table 2. List of the selected primer which were used for molecular characterization

Name	Origin	Repeats	Forward primer	Reverse primer	Amplication size
VM14	Cowpea	(AG)24	5'-.AATTCGTGGCATAGTCACAAGAGA	5'-TAAAGGAGGGCATAGGGAGGTAT	144
VM22	Cowpea	(AG)12	5'- GCG GGT AGT GTA TAC AAT TTG	5'- GTACTGTTCCATGGAAGATCT	217
VM31	Cowpea	(CT)16	5'- CGCTCTTCGTTGATGGTTATG	5'-GTGTTCTAGAGGGTGTGATGGTA	200
VM70	Cowpea	(AG)20	5'- AAA ATC GGG GAA GGA AAC C	5'-GAAGGCAAAATACATGGAGTCAC	186
VM71	Cowpea	(AG)12	5'-TCGTGGCAGAGAATCAAAGACAC	5'-TGGGTGGAGGCAAAAACAAAAC	225
AQ842128	*Medicago*	(TA)23	5'-TCAATGCTGATGCCATTTTC	5'-TCGCGTATTATAGCACAACACC	209
AW584539	*Medicago*	(ACA)8	5'-TTGATGGGCAATACATGTCG	5'- GTTGAAGGAAGGTGGTGGTG	204
AW127626	*Medicago*	(GTTT)7	5'- CATTTTGAAGGAAGGAAGAAGG	5'-ATTTGGAAGCGGAATGTGAA	191
AW688861	*Medicago*	(CAACT)7	5'-TTGTTGTGTGGCTTCTTTGG	5'-AAACCAACCACCTGTGTTGAC	195
BF647899	*Medicago*	(AT)34	5'- CTGTCAACAAGGGGTTAGGTG	5'-TGCATCTACACCCAAAACAAA	266
MtSSRNFAW16	*Medicago*	(TTC)17	5'- ATCGTCCCCACTGTGTCTTC	5'- GTGGGGTTGGTGAGAGTGTT	142-175
MtSSRNFBF46	*Medicago*	(GTAT)6	5'- ACCCCTGCTGAAACAGCATA	5'- CTCTCCCCTAGCCTCAAAGC	174-196
MtSSRNFBG31	*Medicago*	(GGTTT)5	5'- GAGCAAAGGGGTTTGTCTCA	5'-GCAACTCCAGCTGCATCTTT	176-200

Table 3. Allelic variation of the microsatellite loci in 65 accessions of hyacinth bean from Bangladesh and Exotic countries

Locus	Repeats	No. of genotype	No. of allele	Amplicon size (bp)	Allele with highest size	Major allele frequency	Availability	Gene diversity	Hetero zygosity	PIC
VM14	(AG)24	5	3	61-110	61	0.55	0.98	0.59	0.22	0.53
VM22	(AG)12	2	2	54-58	58	0.86	1.00	0.24	0.00	0.21
VM31	(CT)16	4	4	177-190	183	0.39	0.88	0.72	0.00	0.67
VM70	(AG)20	4	4	58-90	64	0.60	1.00	0.56	0.03	0.50
VM71	(AG)12	5	5	52-85	52	0.35	1.00	0.73	0.72	0.68
AQ842128	(TA)23	5	5	90-119	98	0.36	0.91	0.76	0.00	0.72
AW584539	(ACA)8	4	4	200-218	206, 213	0.29	0.89	0.74	0.000	0.69
AW127626	(GTTT)7	3	2	56-76	76	0.67	1.00	0.44	0.57	0.34
AW688861	(CAACT)7	3	3	50-62	56	0.58	1.00	0.53	0.00	0.44
BF647899	(AT)34	4	4	100-117	107	0.40	0.97	0.68	0.00	0.62
MtSSRNFAW16	(TTC)17	4	4	51-75	57	0.60	1.00	0.58	0.09	0.53
MtSSRNFBF46	(GTAT)6	6	5	50-86	86	0.38	1.00	0.74	0.86	0.70
MtSSRNFBG31	(GGTTT)5	2	2	150-190	150	0.77	0.88	0.35	0.46	0.29
Average		**3.92**	**3.62**			**0.52**	**0.96**	**0.59**	**0.23**	**0.53**

Where, PIC-Polymorphism Information Content

Table 4. Allelic variation of the microsatellite loci in 42 accessions of hyacinth bean from Bangladesh and 23 accessions from exotic countries separately

Locus	No. of genotype		No. of allele		Amplicon size (bp)	Major allele frequency		Gene diversity		Hetero zygosity		PIC	
	BGD	EXO	BGD	EXO	BD/OBD	BGD	EXO	BGD	EXO	BGD	EXO	BGD	EXO
VM14	5	4	3	3	61-110	0.43	0.76	0.65	0.39	0.17	0.30	0.57	0.35
VM22	2	1	2	1	54-58	0.79	1.00	0.34	0.00	0.00	0.00	0.28	0.00
VM31	4	3	4	3	177-190	0.38	0.61	0.71	0.55	0.00	0.00	0.66	0.49
VM70	3	3	3	3	58-70	0.57	0.65	0.53	0.48	0.00	0.09	0.43	0.40
VM71	4	3	4	3	52-85	0.44	0.67	0.65	0.46	0.76	0.65	0.59	0.39
AQ842128	5	4	5	4	90-126	0.27	0.59	0.78	0.60	0.00	0.00	0.75	0.55
AW584539	4	4	4	4	200-218	0.38	0.39	0.73	0.67	0.00	0.00	0.68	0.61
AW127626	3	3	2	2	56-76	0.75	0.52	0.38	0.50	0.40	0.87	0.30	0.37
AW688861	3	2	3	2	50-62	0.79	0.78	0.36	0.34	0.00	0.00	0.33	0.28
BF647899	4	3	4	3	100-117	0.53	0.61	0.63	0.55	0.00	0.00	0.57	0.49
MtSSRNFAW16	3	4	3	4	51-65	0.55	0.70	0.60	0.48	0.00	0.26	0.53	0.44
MtSSRNFBF46	4	4	3	5	55-86	0.48	0.39	0.59	0.72	0.81	0.96	0.50	0.67
MtSSRNFBG31	2	2	2	2	150-190	0.78	0.75	0.34	0.38	0.44	0.50	0.28	0.30
Average	**3.54**	**3.08**	**3.23**	**3.00**		**0.55**	**0.65**	**0.56**	**0.47**	**0.20**	**0.28**	**0.50**	**0.41**

Where, ‘BGD’ and ‘EXO’, indicate accessions from Bangladesh and Exotic country, respectively.

accessions were computed from combined data for 13 primers and 65 accessions (Table 6). The higher genetic distance (0.86) was observed between accessions of Bangladesh (BD-8006, Narail) and India (BD-9459). However the lowest genetic distance (0.0) was obtained between accessions BD-7978 (Sirajganj) and BD-2884 (Khagrachhari), BD-8770 (Gazipur) and BD-8785 (Feni) from Bangladesh. Considering the 42 accessions from Bangladesh, the higher genetic distance (0.79) was exhibited between accessions BD-82 (Rangpur) and BD-114 (Hobiganj), and between accessions BD-101 (Naogoan) and BD-1799 (Barguna). However, the lowest (0.0) was obtained from the same accessions as earlier from Bangladesh. Considering the 23 accessions from Asia, Africa and Europe (exotic accessions), the higher genetic distance (0.62) was exhibited between accessions BD-9461 (Indonesia) and BD-9462 (Laos), and the lower genetic distance (0.02) was found between accessions BD-9441 (CPI 34777, India) and BD-9457 (ILRI 14437, Zimbabwe) (Table 7).

Germplasm characterization is an important component of breeding programme for an effective and efficient utilization of plant genetic resources. Microsatellite markers have shown high levels of polymorphism in many crops including rice, wheat, barley, maize, sorghum, soyabean, beans, Brassica species, alfalfa, sunflower and tomato. The lowest heterozygosity was exhibited in VM70 (0.03), while 6 markers did not detect any heterozygosity. Lack of heterozygocity could be attributed by the inbreeding nature of hyacinth bean. The proportion of heterozygocity is likely to be low due to self-pollination. Similar findings were observed in cowpea by Padulosi (1993). The number of allele amplified per primer pair was from 3 to 25 for rice (Yang *et al.*, 1994), 11 to 26 for soybean (Rongwen *et al.*,1995), 3 to 16 for wheat (Plaschke *et al.*, 1995), 2 to 23 for maize (Senior *et al.*, 1998) and 2 to 7 for cowpea (Li *et al.*, 2001). In this study, two to five alleles with an average of 3.62 per primer pair were amplified from the 65 accessions of hyacinth bean. This result is agreed with the results of Wang *et al.*, (2007). Wang *et al.* (2007) studied on 47 accessions of hyacinth bean and found a total of 90 bands with a mean of 3.6 alleles per SSR marker and the markers were derived from *Medicago*, cowpea and soybean. Thus the level of microsatellite polymorphism in hyacinth bean, although relatively high, is much lower than other crops. The possible reason for the low level of microsatellite polymorphism is that the cultivated hyacinth bean is relatively narrow genetic base compared with other crops.

Genetic diversity of cultivated hyacinth bean and its wild species have been extensively investigated by means of RAPD (Liu, 1996; Sultana *et al.* 2000; Rai *et al.* 2010), AFLP (Maass *et al.*, 2005) and Transferred SSR (Wang *et al.*, 2007, 2004, 2006a, b). The cultivated hyacinth bean had lower genetic diversity than many other crops including legume crops (Pasquet, 1993; Pasquet, 1999). The low genetic diversity in cultivated hyacinth bean may be a result of narrow genetic base. The low level of genetic diversity at DNA level among hyacinth bean

accessions could be increased by using its wild relatives to broaden the genetic base. A high level of genetic variation was observed between the accessions of cultivated and wild form (Liu, 1996); However the lower or moderate level of variations were exhibited from the accessions of cultivated form Sultana *et al.,* 2000; Maass *et al.,* 2005; Wang *et al.,* 2007).

Table 5. Hardy-Weinberg Equilibrium of different accessions of hyacinth bean

Name of marker	Degrees of freedom	χ^2 value	χ^2 P-value
VM14	3	57.70	0.000
VM22	1	65.00	0.000
VM31	6	171.00	0.000
VM70	6	130.80	0.000
VM71	10	114.88	0.000
AQ842128	10	236.00	0.000
AW584539	6	174.00	0.000
AW127626	1	5.31	0.021
AW688861	3	130.00	0.000
BF647899	6	189.00	0.000
MtSSRNFAW16	6	130.38	0.000
MtSSRNFBF46	10	119.69	0.000
MtSSRNFBG31	1	4.98	0.026
Total	**69**		

Table 6. Analysis of molecular variance of hyacinth bean accessions

Sources of variation	Sum of squares	% of variation	F statistics (Fst)
Among populations	100.90	11	0.0399
Within population (BGD)	483.20	51	
Within population (EXO)	182.75	19	
Within accessions (BGD)	108.00	11	
Within accessions (EXO)	80.00	8	
Total	954.85	100	

Where, 'BGD' and 'EXO' indicate accessions from Bangladesh and Exotic countries

Population structure and phylogenetic tree analysis

The analysis of molecular variances (AMOVA) for the total accessions among the populations (Bangladesh and exotic countries) revealed 11% genetic variation while the variation within population from Bangladesh and exotic countries were 51% and 19%, respectively (Table 6). Variation within accessions from

Bangladesh and exotic countries exhibited 11% and 8%, respectively (Table 6). The fixation index (Fst) was low 0.0399, indicating low differentiation among hyacinth bean accessions in Bangladesh. Sixty-five accessions from 17 countries of Asia, Africa and Europe were used to make dendrogram based on Nei's (1973) genetic distance using UPGMA (Fig.2). There were 42 accessions from 37 districts of Bangladesh and 23 accessions from 16 countries. The dissimilarity value ranged from 0.00 to 0.29. The 65 accessions were classified into eight groups by selecting a cut off point at 0.23 on dissimilarity within the dendrogram. Number of accession in each group was ranged from 1 (Group-II) to 22 (cluster-VI). Group-I formed with 3 accessions from Bangladesh. Only one accession, BD-1839 from Noakhali in Bangladesh formed Group II.

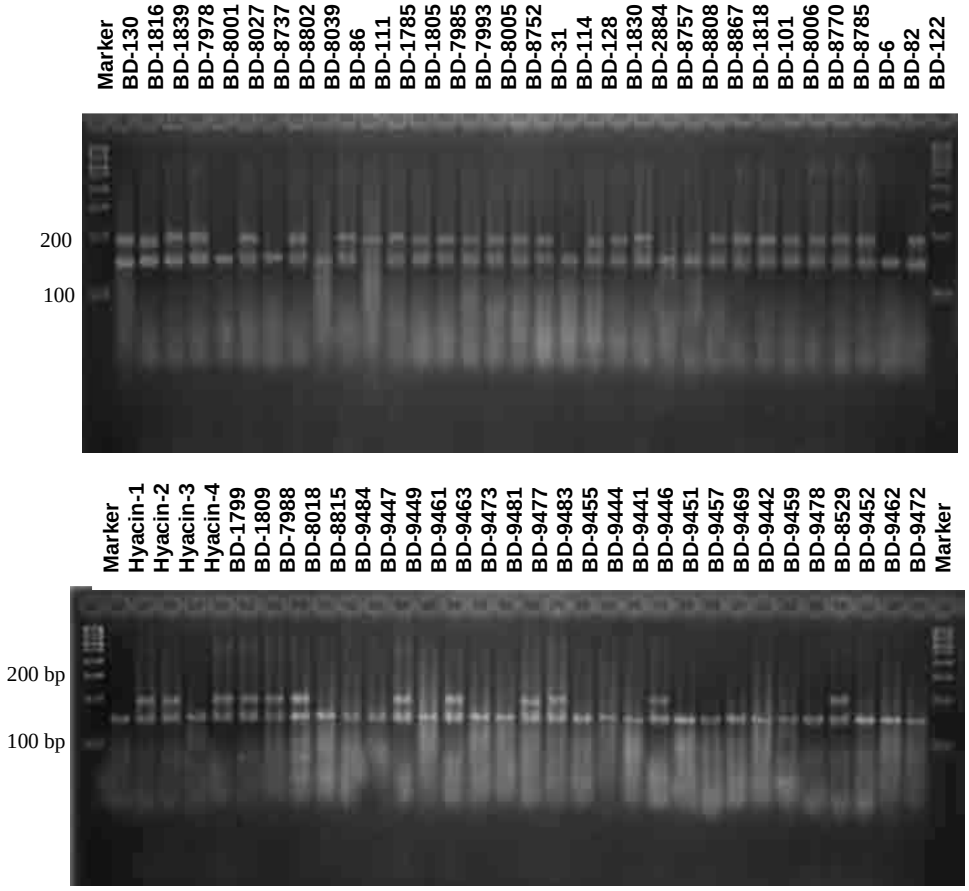

Fig. 1. Amplicons generated by PCR and separated by agarose gel electrophoresis. Each lane contained either 5 µl molecular marker (100 bp ladder) or 15µl PCR products in reaction solution from one of the 65 accessions. Fragments were separated by electrophoresis in a 3.5% agarose gel. PCR products were generated with the primer pair AW 584539.

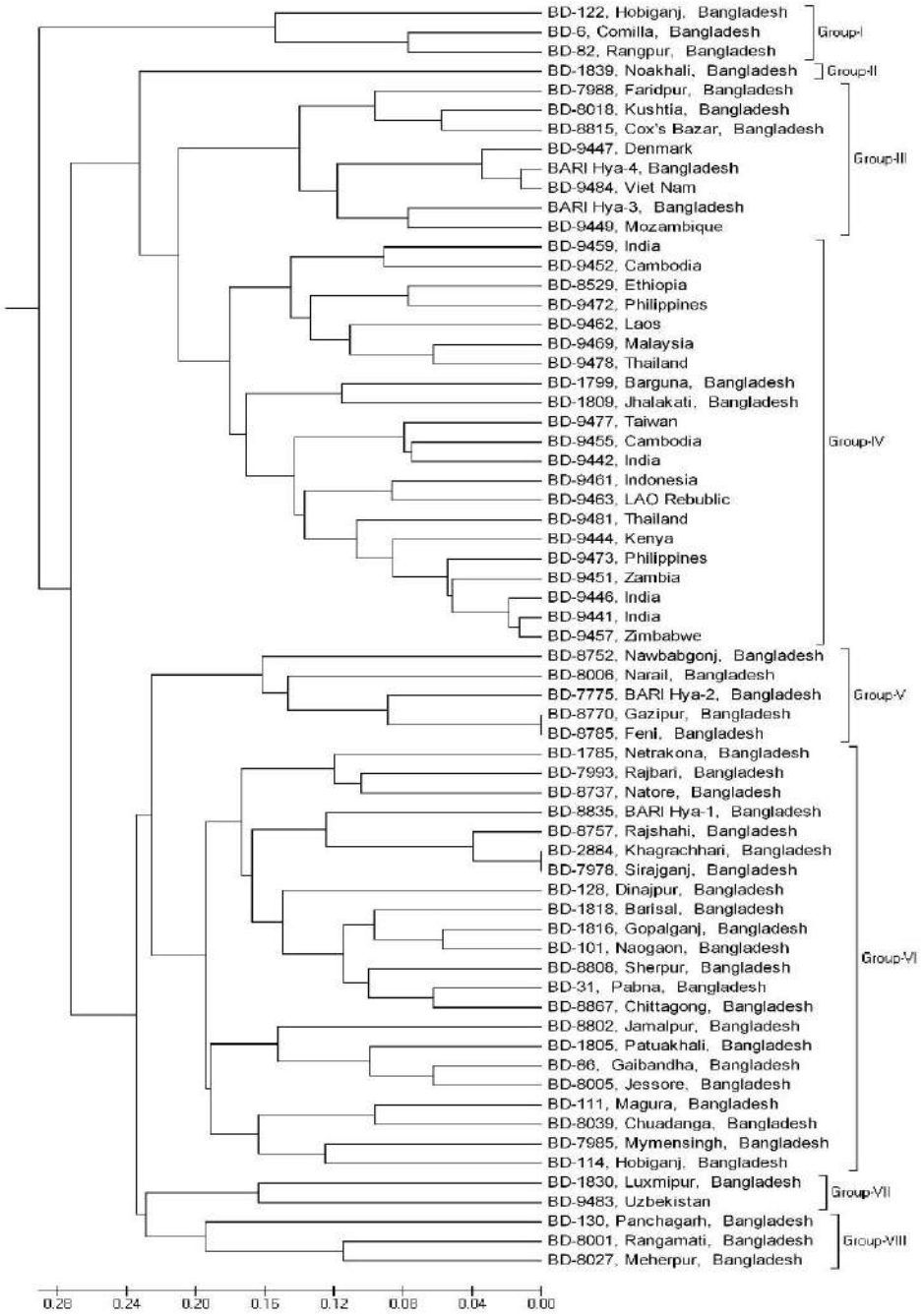

Fig. 2. Dendrogram of genetic relationship (UPGMA) among 65 *Lablab purpureus* accessions from Bangladesh and 16 countries of Asia, Africa and Europe.

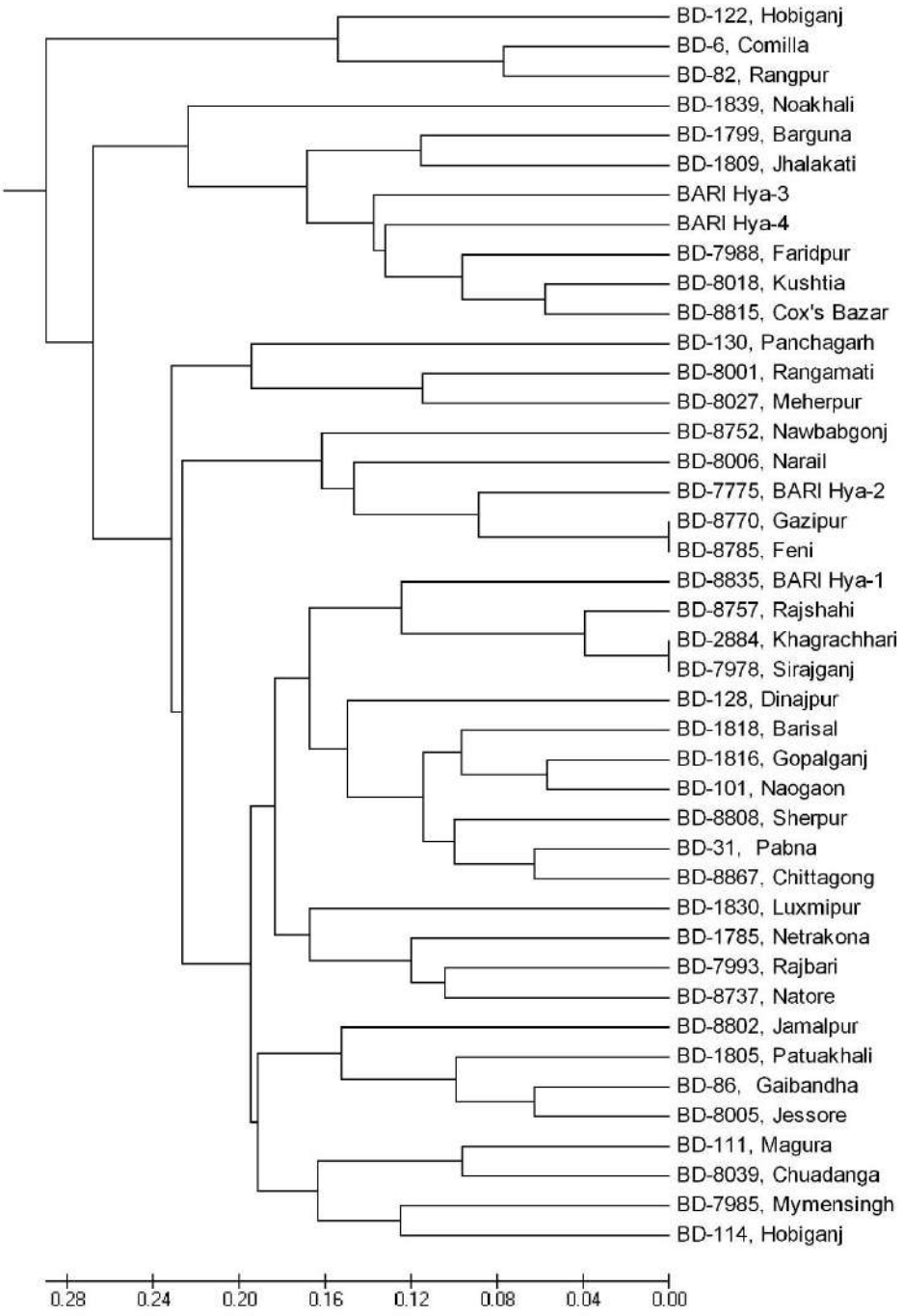

Fig. 3. Dendrogram of genetic relationship (UPGMA) among 42 *Lablab purpureus* accessions from Bangladesh.

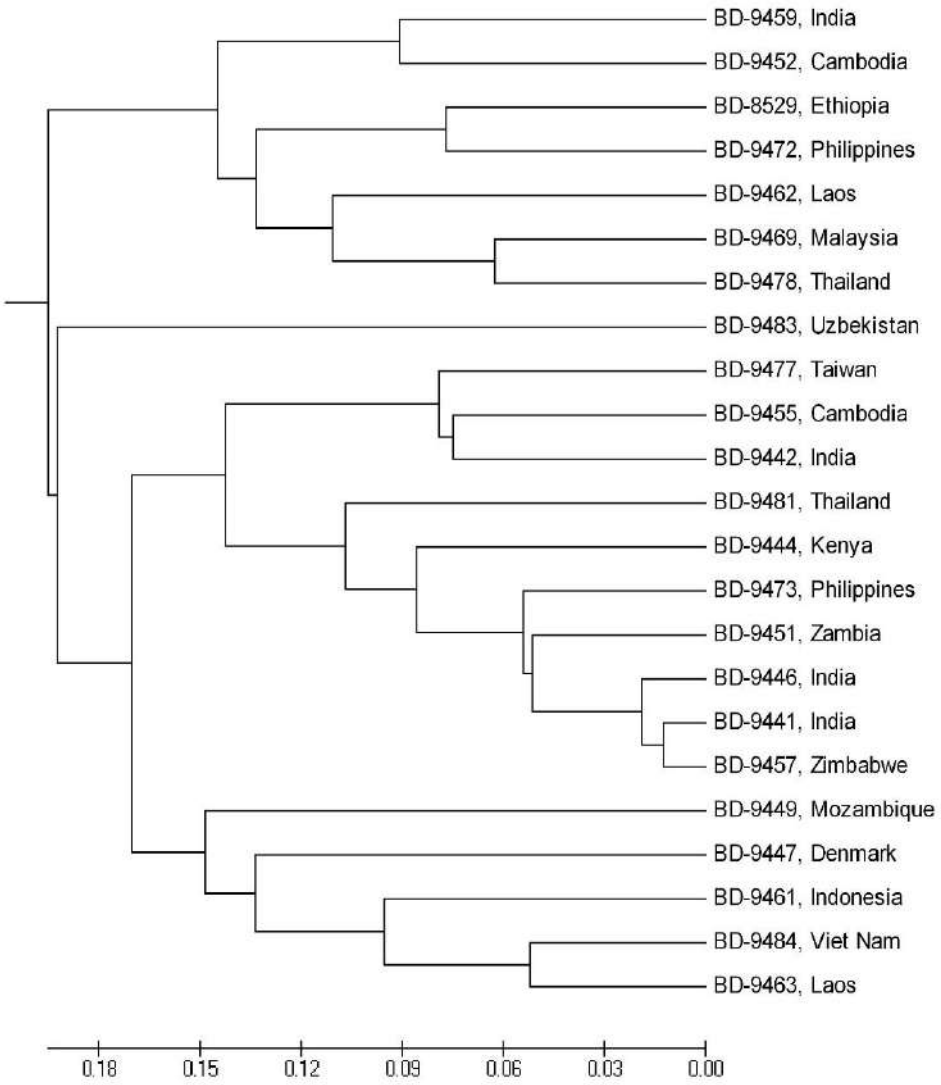

Fig. 4. Dendrogram of genetic relationship (UPGMA) among 23 *Lablab purpureus* accessions from 16 countries of Asia, Africa and Europe (except Bangladesh).

The accession from Denmark (CPI 35894A/BD-9447), Mozambique (CPI 52508/BD-9449), two released variety of BARI (BARI Hyacinth bean-3 and BARI Hyacinth bean-4) and other three accessions from Bangladesh formed Group-III. Group IV composed of 21 accessions from 13 countries of Asia and Africa. Among them, four accessions from India, two accessions each from Bangladesh, Laos, Philippines, Thailand and Cambodia, one accession each from Malaysia, Indonesia, Taiwan, Ethiopia, Zambia, Kenya and Zimbabwe. This is an important

group having six accessions, CPI 34777 (India), CPI 106548 (India), CPI 81626 (India), CPI 100602 (Kenya), CPI 76996 (Zambia) and ILRI 14437 (Zimbabwe) from core collection of Pengelly and Maass (2001). Group-V formed with five accessions from Bangladesh. Group VI is the largest group and it contains 22 accessions from 20 districts in Bangladesh indicate the close relationship. BARI hyacinth bean-1 and BARI hyacinth bean-2 were present in groups VI and V, respectively. However, these two varieties were also accommodated into two separate groups in morphological study (Islam, 2012). Similarly, BD-1830 (Bangladesh) and BD-9483 (TOT 7905, Uzbekistan) were aggregated into Group-VII (Fig.2) and they were distributed in separate clusters in morphological study. Three accessions from Bangladesh formed Group-VIII. The 63 accessions were distributed among the 10 clusters with different combinations in D^2 analysis (Islam, 2012). Forty two accessions from Bangladesh and 23 accessions from 16 countries of Asia, Africa and Europe (except Bangladesh) were again used to make dendrogram based on Nei's (1973) genetic distance using UPGMA. The dissimilarity value ranged from 0.00 to 0.285 (Fig.3) and 0.0 to 0.185 (Fig. 4) from the accessions of Bangladesh and exotic countries, respectively. The results showed distinct differentiation in variation among many accessions while less distinct differentiation in others. The accessions from Bangladesh have distinctness and more diverse than the accessions of exotic countries.

Wright (1978) and Kiambi *et al.* (2005) suggested that the fixation index (Fst) in a range of 0- 0.05 indicated little differentiation, 0.05-0.15 moderate differentiation, 0.15–0.25 large differentiation and above 0.25 indicates a very large differentiation. The lack of differentiation among regions and hyacinth bean accessions is an indication of both high level of gene flow between regions as well as lack of sufficient time for significant genetic differentiation among geographic regions. Gene flow can be considered as a function of seed exchange in different ways is influenced by natural and human selection pressures. The low differentiation is in agreement with a random amplified polymorphic DNA (RAPD) study on cowpea germplasm (Tosti and Negri, 2002).

Groupings of the 65 accessions based on microsatellite polymorphisms generally agreed with the morphological studied (Table 7). Several discrepancies were present, such as the CPI 35894 and CPI 52508 in group III, CPI 100602 and CPI 81626 in group IV while they fall in group IX and VIII, respectively in morphological study (Islam, 2012). Such incongruities were also observed in the other studies (Plaschke *et al.* 1995, Senior *et al.* 1998). Comparison of the grouping produced by the present study with that constructed by morphological study showed consistency only in the large group. This lack of consistency between different marker techniques was also observed in soyabean (Powell *et al.* 1996). This may be due to the fact that different markers systems detected different components of DNA variation, subject to different evolutionary mechanisms. In this study, there is not any wild accession to compare easily between morphological and molecular

Table 7. Genetic distance of hyacinth bean accessions based on 13 microsatellite alleles

Accession number	BD 114	BD 2884	BD 101	BD 8006	BD 8770	BD 8785	BD 82	BD 1799	BD 7978	BD-9484	BD-9447	BD-9449	BD-9461	BD-9481	BD-9477	BD-9483	BD-9455	BD-9455	BD-9441	BD-9450	BD-9457	BD-9469	BD-9459	BD 8529	BD-9462	BD-9472
BD 114	**0**																									
BD 2884	0.33	**0**																								
BD 101	0.56	0.38	**0**																							
BD 8006	0.48	0.54	0.38	**0**																						
BD 8770	0.45	0.53	0.43	0.23	**0**																					
BD 8785	0.58	0.56	0.4	0.33	**0**	**0**																				
BD 82	0.79	0.73	0.58	0.5	0.33	0.48	**0**																			
BD 1799	0.65	0.63	0.79	0.56	0.5	0.54	0.48	**0**																		
BD 7978	0.27	**0**	0.33	0.58	0.47	0.52	0.71	0.69	**0**																	
BD-9484	0.46	0.44	0.6	0.52	0.5	0.5	0.69	0.25	0.48	**0**																
BD-9447	0.35	0.46	0.54	0.54	0.47	0.6	0.58	0.44	0.46	0.11	**0**															
BD-9449	0.58	0.48	0.48	0.56	0.5	0.38	0.71	0.38	0.44	0.17	0.27	**0**														
BD-9461	0.48	0.38	0.62	0.62	0.58	0.6	0.75	0.42	0.42	0.21	0.4	0.42	**0**													
BD-9481	0.4	0.52	0.77	0.73	0.56	0.58	0.65	0.48	0.48	0.39	0.39	0.52	0.35	**0**												
BD-9477	0.6	0.5	0.73	0.73	0.73	0.71	0.6	0.42	0.54	0.29	0.48	0.5	0.19	0.31	**0**											
BD-9483	0.48	0.46	0.62	0.46	0.58	0.6	0.6	0.35	0.5	0.46	0.48	0.5	0.38	0.44	0.42	**0**										
BD-9455	0.56	0.58	0.63	0.71	0.56	0.6	0.4	0.5	0.55	0.36	0.34	0.5	0.33	0.27	0.17	0.46	**0**									
BD-9455	0.63	0.46	0.71	0.71	0.69	0.6	0.71	0.29	0.5	0.32	0.5	0.38	0.25	0.31	0.25	0.21	0.43	**0**								
BD-9441	0.53	0.68	0.78	0.73	0.57	0.6	0.63	0.23	0.68	0.19	0.38	0.38	0.23	0.15	0.23	0.33	0.3	0.13	**0**							
BD-9450	0.4	0.56	0.65	0.73	0.67	0.67	0.73	0.4	0.52	0.34	0.43	0.48	0.27	0.21	0.31	0.35	0.32	0.23	0.1	**0**						
BD-9457	0.45	0.6	0.75	0.7	0.57	0.63	0.6	0.25	0.6	0.22	0.35	0.4	0.25	0.18	0.25	0.3	0.3	0.15	0.02	0.13	**0**					
BD-9469	0.6	0.63	0.71	0.63	0.58	0.6	0.56	0.25	0.59	0.36	0.43	0.33	0.33	0.32	0.38	0.38	0.38	0.2	0.15	0.23	0.18	**0**				
BD-9459	0.69	0.77	0.77	0.86	0.67	0.67	0.65	0.4	0.75	0.52	0.61	0.56	0.38	0.44	0.35	0.54	0.34	0.44	0.2	0.4	0.23	0.3	**0**			
BD 8529	0.6	0.54	0.65	0.62	0.65	0.67	0.52	0.35	0.5	0.5	0.52	0.46	0.27	0.35	0.31	0.31	0.33	0.29	0.23	0.31	0.23	0.13	0.29	**0**		
BD-9462	0.67	0.77	0.69	0.62	0.53	0.48	0.56	0.35	0.75	0.54	0.6	0.42	0.62	0.54	0.58	0.56	0.52	0.52	0.4	0.56	0.43	0.25	0.35	0.38	**0**	
BD-9472	0.58	0.52	0.63	0.63	0.73	0.65	0.58	0.48	0.48	0.52	0.54	0.4	0.37	0.4	0.29	0.48	0.29	0.38	0.38	0.4	0.35	0.19	0.38	0.15	0.33	**0**

diversity. Another difficulty is that out of 150 accessions from morphological study, 63 accessions were present in molecular analysis. Two released varieties namely, BARI Hyacinth bean-1 and BARI Hyacinth bean-2; and other 8 accessions from core collection (Pengelly and Maass, 2001) have been compared with respect to morphological and molecular diversity which supports this finding. The transferred SSR classification was consistent with previous reports from SSR, RAPD and AFLP studies. Transferred SSRs markers have been used to assess genetic diversity and to examine phylogenetic relationships among plant germplasm (Wang *et al.* 2004; 2006a, b). Sultana *et al.* (2000) found the dissimilarity value ranged from 0.006 to 0.134 in cultivated accessions and in wild accessions it was from 0.070 to 0.250 through RAPD analysis on 102 accessions. Liu (1996) reported a high level of genetic variation was detected through RAPD analysis between cultivated and wild accessions of hyacinth bean and genetic variation among Asian collections was significantly higher than that among African collections from cultivated form.

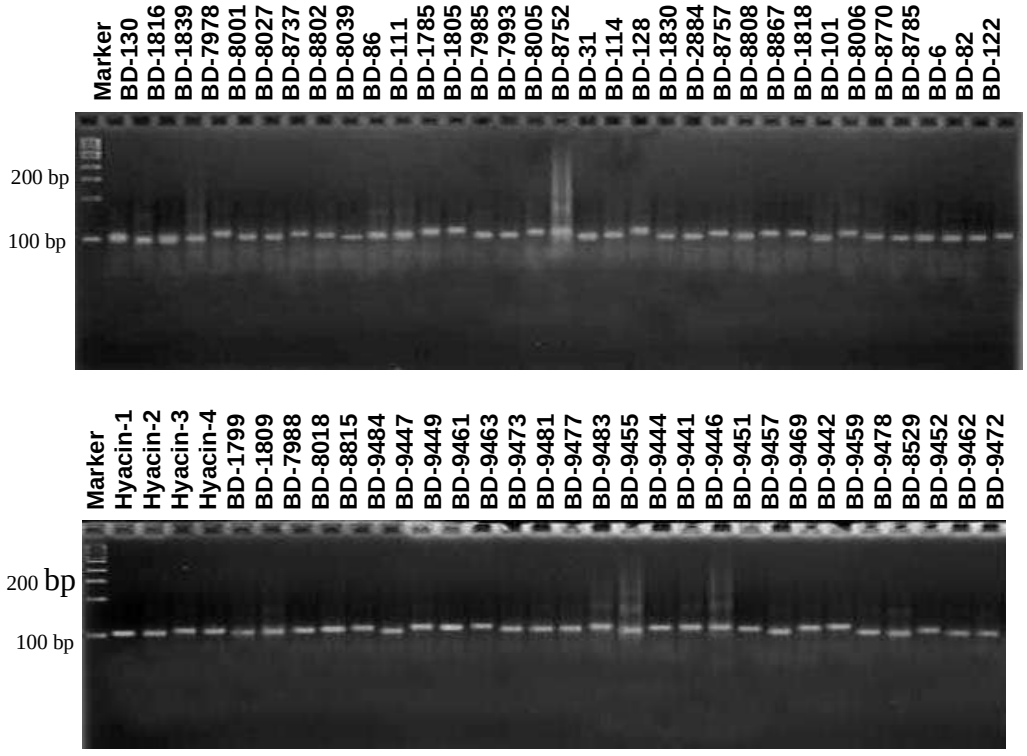

Fig. 5. Amplicons generated by PCR and separated by agarose gel electrophoresis. Each lane contained either 5 µl molecular marker (100 bp ladder) or 15µl PCR products in reaction solution from one of the 65 accessions. Fragments were separated by electrophoresis in a 3.5% agarose gel. PCR products were generated with the primer pair BF647899

Phylogenetic relationships observed in this study were partially agreed with morphological and molecular characters. This relationship observed in molecular markers was not in full agreement with that observed by morphological characters (Sultana. *et al.* 2000; Rai *et al.* 2010; Maass *et al.* 2005). In general, classification based on morphological characters has often showed confusion for taxonomists but the discriminatory power of molecular markers can provide information that will complete taxonomic studies (Carolin, 1992).

The success of cross-species amplification rate could be improved by using SSRs based on express sequence tags rather than using genomic SSR, since express sequence tags come from transcribed regions of the genome, and are likely to be conserved across a broader taxonomic range. In conclusion, microsatellite markers are polymorphic in hyacinth bean. They can be used to distinguish accessions of hyacinth bean. The degree of the polymorphism is relatively low in hyacinth bean compared with the other crops. No significant relation was detected between geographic region and genetic diversity. The polymorphism needs to be expanded. There are 751 accessions of hyacinth bean in the PGRC collection. Only 65 accessions were used in this experiment. Thus the collection should be expended to include more of the genetic diversity available including wild species. This study would aid selection and utilization of germplasm in crop improvement programmes in hyacinth bean.

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