
Genetic Diversity Analysis and DNA Fingerprinting of Mungbean (*Vigna radiata* L.) Genotypes Using SSR Markers

Md. Rezwan Molla¹, Iftekhhar Ahmed¹, Md. Motiar Rohman², Md. Amjad Hossain¹,
Md. Aziz Zilani Chowdhury³

¹Molecular Biology Laboratory, Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh

²Molecular Breeding Laboratory, Plant Breeding Division, BARI, Gazipur, Bangladesh

³Crops Division, Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka, Bangladesh

Email address:

rezwanbt@gmail.com (M. R. Molla), ifti.bari@gmail.com (I. Ahmed), motiar_1@yahoo.com (M. M. Rohman),
drmahossain1959@yahoo.com (M. A. Hossain), zilani71@gmail.com (M. A. Z. Chowdhury)

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Abstract: Microsatellite combines several features of an ultimate molecular marker and they are used increasingly in various plant genetic studies and applications. Characterization of mungbean genotypes on the basis of DNA fingerprinting has become an efficient tool to link genotypic variation. This work is reporting the utilization of a small set of five previously developed mungbean microsatellite (SSR) markers for the identification and discrimination of six HYVs and 36 landraces. All five microsatellite markers were found to be polymorphic. Variation was found in number of alleles, allele frequency, observed and expected heterozygosity. Using five primers across 42 genotypes a total of 20 alleles with an average number of 4 alleles per locus were found of which GBssr-MB91 showed highest number of alleles (6) (size ranging from 135 to 152 bp) followed by 4 alleles (from 160 to 176 bp and 175 to 195 bp) and 3 alleles (from 264 to 282 bp and 283 to 304 bp) were detected at the loci LR7322B, LR7323A, LR7323B and GBssr-MB77, respectively. The narrow genetic base could be one of the reasons for the low yield of polymorphic markers in the study. The primer GBssr-MB91 also yielded highest number of PIC value (0.803). Genetic differentiation (F_{st}) values were found in the ranges 0.443 to 0.747 with an average of 0.686 and gene flow (N_m) values ranged from 0.085 to 0.314 with an average of 0.237. Over all Nei's genetic distance value (D) observed from nil to 2.706 among 861 accessions pair resulting as a means of permutation combination of 42 mungbean genotypes. The UPGMA dendrogram based on Nei's genetic distance separated the genotypes, BARI mung-1 and BD6906 from other 40 genotype. Out of 42 genotypes, 36 genotypes were identified with at least one and/or combination of 4 primers.

Keywords: DNA Fingerprinting, Genetic Diversity, Microsatellite (SSR) Marker, Mungbean, Polymorphism

1. Introduction

Mungbean (*Vignaradiata* L. Wilczek) also known as green-gram belongs to subgenus *Ceratotropis* is an important legume food crop in south and Southeast Asia where 80% of the world's mungbean. It is an important crop among the palatable pulses in Bangladesh. This crop provides protein-rich food, restores and maintains the soil fertility by fixing atmospheric nitrogen, and also fits well in different cropping systems. However, the average yield of mungbean has to be as low as 670 kg/ha [1]. There are many reasons for such low

yield [2]. The low productivity of this crop can be attributed to narrow genetic base (resulting in low yield potential land susceptibility to biotic and abiotic stresses) and lack of suitable plant types for different cropping situations [2, 3].

Variability is the touch stone to a breeder to evolve high yielding varieties through selection. The assessment of genetic variation is a major concern of plant breeders and population genetics. Availability of sufficient variation required for the production of new varieties that are aimed

towards the improvement of crop productivity and able to withstand amaze from biotic and abiotic factors. Not quite enough to expose the genetic diversity and do not reflect real genetic relationships. Therefore, molecular markers have several advantages over the traditional phenotypic markers. They are unaffected by environment and detectable in all stages of development. The molecular genetic techniques have been adopted for the management and manipulation of plant genomes DNA markers are the most powerful and widely used because they can portray genome sequence composition [4].

In recent years it has been proved beyond doubt that only identification of crop varieties by quantitative terms is not adequate. Therefore, there is need for documentation with appropriate colour photography and preservation of original seeds. These genotypes/varieties have some identical characters with morphological traits given by the breeders during its release or registration. But those are not adequate and well characterized and documented in the form that can support effective implementation of Plant Variety and Farmers Right Protection Act (PVFRPA) [5]. All these materials have chances of changing its quantitative and qualitative traits due to G x E and outcrossing/mutation renamed as well as re-registered as new.

To overcome these problems, several DNA marker systems are now common use in diversity studies of plants, the most commonly used marker systems are restriction fragment length polymorphism (RFLP) [6], random amplified polymorphic DNA (RAPD) [7], amplified fragment length polymorphism (AFLP) [7], inter simple sequence repeats (ISSRs) [8] and microsatellites or simple sequence repeats (SSRs) [9]. Among them to characterize DNA variation patterns within species and among closely related taxa in *Vigna* species have been RAPD [10], AFLP [11], RFLP [12], ISSR [13], SSRs [10].

Molecular markers have been successfully applied in registration activities like cultivar identification [14], or controls of seed purity of hybrid varieties [15] and also for the variety identification as a part of seed and grain trade [16]. Of all classes of DNA based marker, the microsatellite SSR (Simple Sequence Repeat) is Polymerase Chain Reaction (PCR) based, highly polymorphic, multi-allelic, frequently codominant, highly reproducible, randomly and widely distributed in the genome [17]. Mutations in the motifs and flanking sequences as well as distribution of microsatellites in the genome of a species are exploited to reveal genetic variation and varietal identity. In plants, it has been demonstrated that SSRs are highly informative, locus specific markers in many species [6, 18, 19] identified and distributed throughout the genome. For characterization and documentation, this technique has been recently used in 20 crop species including rice, wheat, maize, barley, rapeseed, soybean, potato and other crops by [5]. In Bangladesh, nine soybean cultivars were identified by microsatellite markers, which have provided identity and might work as protection [20]. Thirteen maize cultivars were also characterized using microsatellite fingerprinting in combination with DUS test

[21] and 94 rice cultivars [22]. Genetic diversity analysis among 13 mungbean cultivars [3] and 10 germplasm (7 exotic and 3 advance line) [23] was performed through polymerase chain reaction (PCR) based random amplification of polymorphic DNA (RAPD).

Based on that experience, the present study has been designed with 42 varieties/land races types of mungbean using the molecular traits i) to analyze genetic diversity and relationship among the genotypes, and ii) to identify unique DNA banding pattern. In this set of study there are materials that are being cultivated, under conservation, being under threat to erode due to ecological imbalance in their habitats and considered to be non-economic in the present context of commercial agriculture with high input-high output production system.

2. Materials and Methods

2.1. Raising of Seedlings and Isolation of Genomic DNA

Seeds selected genotypes were obtained from mungbean collections maintained at Pulses Research Centre (PRC) and Plant Genetic Resources Centre (PGRC) of BARI, Gazipur, Bangladesh which were collected from different location. A total of 42 genotypes including six commercial varieties, one most popular local cultivar and 35 landraces of mungbean representing a wide spectrum of variability were selected for the present study (Table 1). The seedlings were grown in small plastic pots. Bulk DNA was isolated from 2-5 fresh leaves of 10 days old seedlings using following the protocol described by [24] and also used by [25] with some modifications. Excluding usage liquid nitrogen the modified protocol included digestion with homogenization buffer (pH=8.0): [50 mM Tris-HCl, 25 mM EDTA (Ethylenediaminetetraacetic acid), 300 mM NaCl and TEN buffer + 5% SDS (Sodium Dodecyl Sulfate) + 10% PVP (Poly Vinyl Pyrrolidone) + 20% CTAB (Cetyltrimethyl Ammonium Bromide)] at 65°C for 30 min, extraction with phenol: chloroform: isoamyl alcohol (25:24:1), precipitation with ice-cold and extra pure isopropyl alcohol. DNA was purified using two volume of absolute alcohol in presence of 0.3M sodium acetate and pelleted by centrifugation. The pellets were then washed with 70% ethanol, air dried and resuspended in an appropriate volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH=8.0) treated with 2 µl of RNase A for removing of RNA. The quality of extracted DNA was examined under the UV light following agarose gel electrophoresis (1% gel containing 10 mg/ml ethidium bromide).

2.2. Quantification and Optimization of DNA Concentration

The amount of genomic DNA was quantified at 260 nm spectrophotometrically (Spectronic® GENESYS™ 10 Bio). Using the absorbance reading obtained for DNA sample of each mungbean genotypes, the original DNA concentrations were determined and adjusted to 25ng/µl.

Table 1. List of genotypes used in this study.

Sl. no.	Genotypes/Gene bank accession number	Source	Sl. no.	Genotypes/Gene bank accession number	Source
1	BARI mung-1	PRC, BARI	22	BD6889	PGRC, BARI
2	BARI mung-2	PRC, BARI	23	BD6890	PGRC, BARI
3	BARI mung-3	PRC, BARI	24	BD6891	PGRC, BARI
4	BARI mung-4	PRC, BARI	25	BD6892	PGRC, BARI
5	BARI mung-5	PRC, BARI	26	BD6893	PGRC, BARI
6	BARI mung-6	PRC, BARI	27	BD6894	PGRC, BARI
7	Sonamug	PRC, BARI	28	BD6895	PGRC, BARI
8	BD6874	PGRC, BARI	29	BD6896	PGRC, BARI
9	BD6875	PGRC, BARI	30	BD6897	PGRC, BARI
10	BD6876	PGRC, BARI	31	BD6898	PGRC, BARI
11	BD6877	PGRC, BARI	32	BD6899	PGRC, BARI
12	BD6878	PGRC, BARI	33	BD6900	PGRC, BARI
13	BD6879	PGRC, BARI	34	BD6901	PGRC, BARI
14	BD6880	PGRC, BARI	35	BD6902	PGRC, BARI
15	BD6881	PGRC, BARI	36	BD6903	PGRC, BARI
16	BD6882	PGRC, BARI	37	BD6904	PGRC, BARI
17	BD6884	PGRC, BARI	38	BD6905	PGRC, BARI
18	BD6885	PGRC, BARI	39	BD6906	PGRC, BARI
19	BD6886	PGRC, BARI	40	BD6907	PGRC, BARI
20	BD6887	PGRC, BARI	41	BD6908	PGRC, BARI
21	BD6888	PGRC, BARI	42	BD6909	PGRC, BARI

2.3. Identification and Selection of Microsatellite (SSR)

Primers

A set of 14 microsatellite primer pairs (LR7322B, LR7323A, LR7323B, LR7315A, GBssr-MB7, GBssr-MB87, GBssr-MB91, GBssr-MB13, GBssr-MB14, GBssr-MB17, GBssr-MB77, LR733B, LR738A, LR7319B) were identified and characterized for mungbean SSR markers. Preliminarily, the primer pairs were tested for their better responsiveness in amplifying the target genomic region of template DNA and to check the expected PCR product sizes in base pairs. Finally, five primer pairs viz. LR7322 B, LR 7323 A, LR7323 B, GBssr-MB91 and GBssr-MB77 with clear and expected amplified product sizes were selected and used for microsatellite analysis in the present study.

2.4. Polymerase Chain Reaction (PCR)

The Polymerase chain reactions was set up 10 µl volumes containing 1µl 10 x PCR Buffer, 0.25 mM each of the dNTPs, 10 µM of each of primer, 1 unit ampliTaq DNA polymerase (Invitrogen, USA), 75 ng template DNA and a suitable amount of sterile deionized water. The reaction was performed in a oil free Techne, TC 312 thermal cycler. SSRs were amplified under the following “touchdown” PCR conditions: 94°C /5 min denaturation, 45 cycles of 94°C /30 sec, 48-50°C /1 min, decreasing by 0.5°C per cycle, and 72°C /45 sec; 8 cycles of 94°C /30 sec, 45-48-°C/45 sec and 72°C /45 sec; a final extension for 10 min at 72°C. For checking amplification, the PCR products were electrophoretically resolved on 2% agarose gel in 1X TBE. If the primer was shown good band resolution intensity, less smearing, amplifying the target genomic region of template DNA, the PCR protocol considered to be correct.

2.5. Electrophoretic Separation and Visualization of PCR Products

PCR-products were electrophoresed on a 6% denaturing polyacrylamide gel containing 19:1 acrylamide: bis-acrylamide and 8M urea. Electrophoresis was done using the SequiGen GT Sequencing Cell (BIO-RAD Laboratories, Hercules, CA, USA) electrophoresis system. A pre-run of the gel for 30 mins at 120 W was followed by a final run at 60W and 50°C upon loading of denatured PCR products for a specified period of time depending on the size of amplified DNA fragment (usually 1 hour for 100 bp). A molecular weight marker DNA (100 bp DNA ladder, Biobasic, Canada) was loaded on either side of the gel. After completion of electrophoresis, the DNA fragments were visualized following the Promega (Madison, WI) silver-staining protocol.

2.6. Scoring and Analysis of Microsatellite Data

The bands representing particular alleles at the microsatellite loci were scored manually and designated the bands as A, B, C, etc. from the top to the bottom of the gel by three experienced scientists individually. The genotypes of different individuals were hypothetically scored as AA, BB, CC, etc. for homozygous or as AB, AC, BC etc. for heterozygous. A single genotypic data matrix was constructed for all loci. Polymorphism Information Content (PIC) was computed by deducting sum of square values for all the frequencies of different alleles produced by a single marker locus from one using the formula: $PIC=1-\sum X_i^2$, Where, X_i is the frequency of the i -th allele of a particular locus.

PIC provides an estimate of the discriminatory power of a marker by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those

alleles. PIC values range from 0 (monomorphic) to 1 (very high discriminative, with many alleles in equal frequencies). The software DNA FRAG version 3.03 was used to estimate allelic length [26]. Expected (H_e) and observed heterozygosity (H_o) were also calculated as per [27, 18] formula and with the help of POPGENE (version 1.32) [28] computer package program. Estimation of Nei's genetic distance values (D) [27] and construction of UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram was constructed using the software POPGENE (version 1.32) [28].

3. Results and Discussion

All forty two mungbean genotypes were successfully amplified with the five microsatellite primer pairs (LR7322B, LR7323A, LR7323B, GBssr-MB91 and GBssr-MB77). Based on previous results of Kumar *et al.* [29] and Gwag *et al.* [30], primer pairs referred to as loci and DNA bands as alleles. All five microsatellite markers were found to be polymorphic, revealing a total of 20 alleles with an average number of 4 alleles per locus were found in the present study.

The narrow genetic base could be one of the reasons for the low yield of polymorphic markers in the study. At the GBssr-MB91 locus showed highest number of observed alleles (6) among the 42 mungbean genotypes ranging in size from 135 to 152 bp. Likewise, 4 alleles (size ranging from 160 to 176 bp and 175 to 195 bp) and 3 alleles (from 264 to 282 bp and 283 to 304 bp) were detected at the loci LR7322B, LR7323A, LR7323B and GBssr-MB77 respectively in descending order (Table 2) and the effective number of allele was also highest (5.079) for GBssr-MB91 (Table 2). Narrow genetic base has been among the mungbean accessions in this study. Three to five alleles size ranging from 171 to 285 bp were obtained by Kumar *et al.* [29] while conducting isolation of microsatellite markers in mungbean, *Vigna*, although some variation occurred might be due to mutation of dinucleotide repeat units which could also be indicative of varietal differences. Allele frequency ranged from 0.063 to 0.563 observed in the present study. DNA banding patterns were generated by the primer pairs in 42 mungbean genotypes are shown in Figure 1.

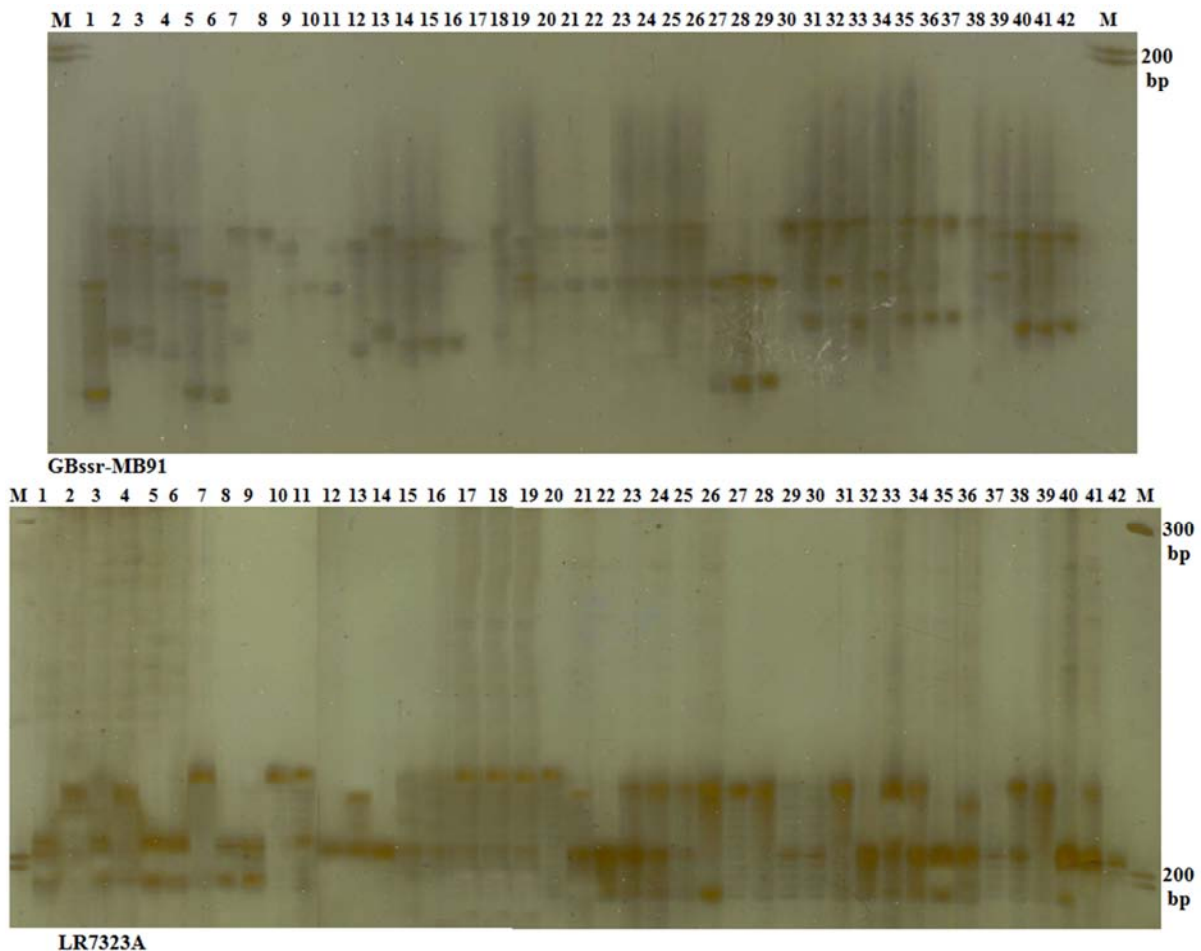


Figure 1. Microsatellite profiles of 42 mungbean genotypes at locus GBssr-MB91 and LR7323A; M: molecular wt. marker (100 bp DNA ladder); Lane 01: BARI mung-1; ; Lane 02: BARI mung-2; Lane 03: BARI mung-3; Lane 04: BARI mung-4; Lane 05: BARI mung-50; Lane 6: BARI mung-6; Lane 07: Sonamug, Lane 08: BD6874; Lane 09: BD6875; Lane 10: BD6876; Lane 11: BD6877; Lane 12: BD6878; Lane 13: BD6879; Lane 14: BD6880; Lane 15: BD6881; Lane 16: BD6882; Lane 17: BD6884; Lane 18: BD6885; Lane 19: BD6886; Lane 20: BD6887; Lane 21: BD6888; Lane 22: BD6889; Lane 23: BD6890; Lane 24: BD6891; Lane 25: BD6892; Lane 26: BD6893; Lane 27: BD6894; Lane 28: BD6895; Lane 29: BD6896; Lane 30: BD6897; Lane 31: BD6898; Lane 32: BD6899; Lane 33: BD6900; Lane 34: BD6901; Lane 35: BD6902; Lane 36: BD6903; Lane 37: BD6904; Lane 38: BD6905; Lane 39: BD6906; Lane 40: BD6907; Lane 41: BD6908; Lane 42: BD6909.

The PIC values, which are reflection of allele diversity, provide an estimate of the discriminating power of a marker by taking into account not only the number of alleles at a locus, but also relative frequencies of these alleles. The PIC values are dependent on the genetic diversity of the cultivars chosen and this investigation had a high proportion of traditional varieties which would have the effect of increasing the PIC values. It is important to indicate that the selection by breeders have increased the frequency of the alleles or allelic combination with favorable effects at the expense of the others, eventually eliminating many of them [31]. The markers and their allele size along with their frequencies and PIC values have been shown in the Table 2. The PIC values for five primers obtained in the present study varied from 0.538 for LR7323B to 0.803 for GBssr-MB91, with an average PIC value of 0.637 (Table 2). Among the markers used in this study GBssr-MB91 and LR7323A

showed higher PIC values than the LR7322B, GBssr-MB77 and LR7323B. Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes. The number of alleles amplified by a primer and its PIC values also depends upon the repeat number and the repeat sequence of the microsatellite sequences [32, 33, 22]. These observations are in agreement with those of Kumar *et al.* [29] and Gwaget *et al.* [30] whose showed that (AG) and (GA) repeats yield higher number of alleles and higher PIC values. GBssr-MB91 and LR7323A having (GA)_n repeat were two most informative microsatellite markers for this set of germplasm, as they yielded 5 alleles. For GBssr-MB91 [(AG)₃₄(GA)₁₄] and LR7323A [(GA)₁₃], 6 and 4 alleles were observed and average PIC values were 0.803 and 0.645, respectively which were not unusual based on repeat number and the repeat motif to that observed in previous study [29, 30].

Table 2. Size and frequency of alleles and diversity index at five SSR loci across 42 mungbean genotypes.

Locus	Repeat Motif	Sequence of primers (5'-3')	Allele sizes (bp)	Allele frequency	PIC
LR7322B	(TC) ₁₀	F: TCAGTCAGTGTTCGATAGCATAGC R: GACACAGAGAGAGAGAGAGAG	176	0.338	0.622
			173	0.095	
			164	0.500	
			160	0.068	
LR7323A	(GA) ₁₃	F: TGACGGAGAGAGAGAGAGAGAG R: TGCTTCCTTTGTCTGAGTTAGAA	195	0.310	0.645
			191	0.083	
			181	0.488	
			175	0.119	
LR7323B	(CT) ₁₀	F: GCTATGCTATCGACACTGACTGA R: GCGCAAAGAGAGAGAGAGAGA	282	0.375	0.538
			273	0.563	
			264	0.063	
			152	0.281	
GBssr-MB91	(AG) ₃₄ (GA) ₁₄	F: GAGGCCAATCCCATAACTTT R: AGCACCACATCAGAGATTCC	150	0.146	0.803
			146	0.244	
			141	0.134	
			139	0.110	
GBssr-MB77	(GTT) ₅ (GA) ₅ A(AG) ₆	F: GGA GAG GAA GGA ACA GGG R: GGC AGA GCA TAA CAT GGC	135	0.085	0.575
			304	0.132	
			293	0.559	
			283	0.309	

Genetic differentiation (*F_{st}*) values were found in the ranges 0.443 to 0.747 with an average of 0.686 and gene flow (*N_m*) values ranged from 0.085 to 0.314 with an average of 0.237 (Table 3). Comparatively least difference was found between the genetic differentiation and gene flow values in 42 mungbean genotypes which are indicative of lower diversity among the genotypes studied were of local cultivars, land races and HYVs. In the present study, variation was found in number of alleles, allele frequency, observed and expected heterozygosity. Across 42 mungbean genotypes, GBssr-MB91 yielded highest average heterozygosity (0.452) followed by LR7323B (0.333), GBssr-MB77 (0.310), LR7323A (0.286)

and LR7322B (0.179) in descending order (Table 4). The observed heterozygosity (*H_o*) showed in similar order where highest *H_o* observed in GBssr-MB91 (0.927) which was not unexpected because maximum exchanges of genetic materials in this particular locus might have occurred across studied mungbean genotypes. Nei's [34] expected heterozygosity for GBssr-MB91 was highest (0.813), which might be due to large number of alleles (6) found in this locus. Kumar *et al.* [29] and Gwaget *et al.* [30] studied different set of primer and observed a higher expected heterozygosity value (0.770) and (0.680) showed in large allele producing locus respectively.

Table 3. Summary of genetic variation statistics for all loci.

Locus	*na	*ne	*I	*Fst	*Nm
LR7322B	4	2.648	1.118	0.747	0.085
LR7323A	4	2.816	1.174	0.557	0.199
LR7323B	3	2.170	0.865	0.545	0.209
GBssr-MB91	6	5.079	1.704	0.443	0.314
GBssr-MB77	3	2.352	0.956	0.571	0.188
Mean	4	3.013	1.163	0.566	0.192

*na = Observed number of alleles, ne = Effective number of alleles, I = Shannon's Information Index and Nm = Gene flow estimated from $F_{st} = 0.25(1 - F_{st})/F_{st}$.

Table 4. Summary of heterozygosity(Het.) statistics for all loci.

Locus	Obs. Hom.	Obs. Het.	Exp. Hom.*	Exp. Het.*	Nei**	Ave. Het.
LR7322B	0.595	0.405	0.369	0.631	0.622	0.179
LR7323A	0.429	0.571	0.347	0.653	0.645	0.286
LR7323B	0.125	0.875	0.452	0.548	0.539	0.333
GBssr-MB91	0.073	0.927	0.187	0.813	0.803	0.452
GBssr-MB77	0.235	0.765	0.417	0.583	0.575	0.310
Mean	0.291	0.355	0.355	0.646	0.637	0.312
St. Dev.	0.218	0.102	0.102	0.102	0.102	0.098

*Expected homozygosity and heterozygosity were computed using Levene [35] ** Nei's [34] expected heterozygosity

Over all Nei's genetic distance value (D) ranged from nil to 2.7058 among 861 pairs resulting as a means of permutation combination of 42 mungbean genotypes (Figure 2). Out of 861 pairs 3.25% showed no genetic distance whereas only one pair BARI mung-2 vs BD6878 showed the highest genetic distance (2.7058) (Table 5). This closeness may be possible in the genetic make up of the locus for which the primers were responsible to distinguish along with low variation also in the morphological traits and geographical sources. The highest genetic distance can be explained by the fact that in one side the local cultivars or land races and on the other side the HYVs have been involved. The distance has been generated during the process of the development of HYVs. The generated distance can further be used for inclusion of gene source from the traditional varieties to more HYVs, which indicates the impact of the genetic fingerprinting and correlating the values with that of the morpho-physiological traits to find out the best performing varieties through appropriate breeding

programmes.

UPGMA dendrogram based on Nei's genetic distance separated the 42 genotypes of mungbean initially into two clusters, "a" and "b" in which 31 genotypes grouped in cluster "a" and other 11 genotypes grouped in Cluster "b". Cluster "a" formed two sub-clusters "c" and "d". Sub-cluster "b" subsequently separated into another two sub-clusters "e", "f" respectively in which "e" contained genotypes BARI mung-2, BD6878, Sonamug and BD6882 and "h" contained mung genotype BARI mung-4, BD6874, BD6876, BD6894, BD6897 and BD6806.

Sub-cluster subsequently formed other sub-clusters namely, "i", "j", "k", "l" and so on (Figure 2). The genotypes have a distinct status in the dendrogram, because there might have effect of morphological traits and geographical sources. The varieties, as for example, BARI mung-5 and BARI mung-6 were grouped together in same sub-cluster which also probably due to similar type of morphological traits and original geographical sources. On the other hand, the variety BARI mung-1, BARI mung-2, BARI mung-3 and BARI mung-4 were scattered in different sub-cluster that might due to distinct breeder's traits like as pigment present at the lowest part of the seedling in the variety BARI mung-2 and it is introduced from same country of origin (AVRDC, Taiwan). Similarly, BARI mung-1 introduced from India (M-7706). On the contrary, BARI mung-2, BARI mung-4 and local cultivar Sonamug were grouped in together in cluster "b" which could be explained BARI mung4 developed from same cross (Sonamug x BARI mung-2).

Allele sizing technologies are well established and can be readily used to size microsatellite alleles from any organism [36]. Utilization of two microsatellite markers in the analysis of mungbeangenotypes revealed a high level of genetic polymorphism which allowed unique genotyping of 44.44% of the studied cultivars and only these three markers were sufficient for unambiguous identification of 15 mungbean genotypes which includes three high yielding, and one most popular local cultivar. In the set of 42 genotypes, 22 alleles were detected which multiplied into a number of observed genotypes at each locus, giving high discrimination value for genotype identification.

These results represent one of the first attempts to find out a small set of microsatellite makers to discriminate mungbean genotypes of Bangladesh providing meaningful data that can be enlarged by additional mungbean genotypes and new microsatellite markers. Among 22 alleles detected, three were specific to three mungbean genotypes. One specific allele was detected in the cultivar BARI mung-2 (GBssrMB-91/152, 141), BD6899 (LR7322B/164,160) and BD6900 (LR7322B/173) (Table 6).

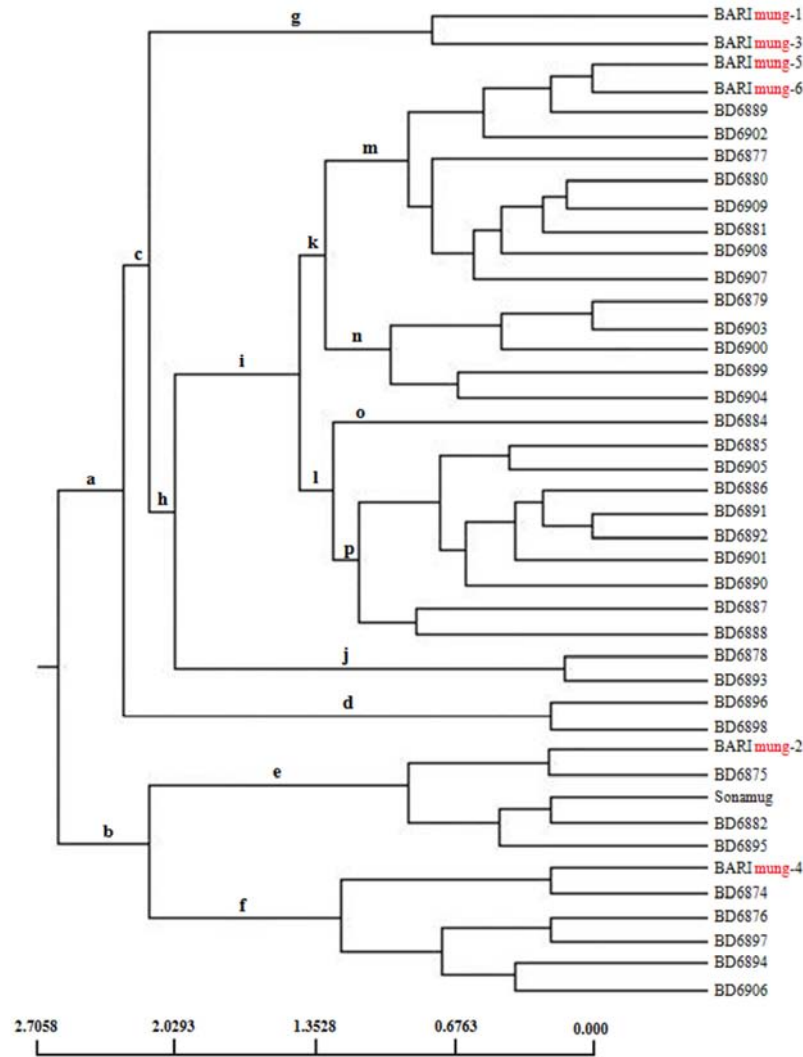


Figure 2. UPGMA dendrogram based on Nei's [27] genetic distance, summarizing the data on differentiation between 42 mungbean genotypes according to microsatellite analysis.

Table 5. Nei's Genetic Distance Values (D) among 42 Mungbean genotypes across 5 SSR markers.

D values	Genotype pairs (Serial no. respective genotypes used)	Number of pairs	%
0.0000	6-5; 8-4; 9-2 and 7; 10-8 and 9; 16-2, 7 and 10; 20-16; 25-24; 26-12 and 16; 27-8, 9 and 16; 28-8, 9 and 16; 30-4, 10, 27 and 28; 31-16 and 29; 36-13; 39-8 and 30	28	3.252
0.0668	42-12 and 14	02	0.232
0.0741	39-27	01	0.116
0.0771	42-15	01	0.116
0.0870	22-5 and 6	02	0.232
0.1054	24-19; 25-19; 34-19, 24 and 25; 38-24 and 25	07	0.813
0.1116	38-18	01	0.116
0.1438	15-12 and 14; 41-14	03	0.348
0.1542	4-2; 28-27	02	0.232
0.1641	35-22; 42-40 and 41	03	0.348
0.1682	24-23; 25-23; 34-27; 39-19	04	0.465
0.1965	24-22; 25-22; 33-13; 34-5 and 6; 36-33; 41-19	07	0.813
0.2027	40-8	01	0.116
0.2209	27-7; 28-7; 35-14; 39-7	04	0.465
0.2231	38-13, 19, 34 and 36	04	0.465
0.2309	40-12 and 14	02	0.232
0.2350	38-7	01	0.116
0.2412	27-23; 39-23 and 28	03	0.348
0.2451	24-18; 25-18	02	0.232
0.2594	3-1; 23-20; 28-10; 35-5 and 6; 37-35; 42-32	07	0.813
0.2736	23-19 and 21; 26-23; 27-19, 24, 25 and 26; 34-23; 35-13; 36-35; 38-23 and 35; 39-24, 25, 26 and 34; 42-11	17	1.974

D values	Genotype pairs (Serial no. respective genotypes used)	Number of pairs	%
0.2798	42-9	01	0.116
0.2877	14-12; 32-15 and 22; 37-32; 40-15; 41-5, 6, 15, 22 and 40	10	1.161
0.3079	23-7	01	0.116
0.3143	19-5 and 6; 21-20; 22-13 and 19; 24-5, 6 and 20; 25-5, 6 and 20; 26-20; 31-20; 32-24 and 25; 33-31; 34-22; 36-22; 37-13 and 36; 38-22, 33 and 37; 40-11; 41-11, 24, 25, 34 and 38	29	3.368
0.3262	14-5 and 6; 22-14; 32-12 and 14; 37-14; 41-12	07	0.813
0.3363	41-9	01	0.116
0.3365	42-35	01	0.116
0.3404	12-11; 14-11; 19-14; 24-7; 25-7; 26-7	06	0.697
0.3466	12-9; 14-9; 16-15; 18-7 and 17	05	0.581
0.3567	19-11; 24-13 and 21; 25-13 and 21; 26-24 and 25; 36-24 and 25; 38-31	10	1.161
0.3648	27-20; 35-32; 37-23; 39-20; 40-35; 41-35; 42-5, 6 and 22	09	1.045
0.3914	34-28; 35-21, 24 and 25; 38-27; 39-38; 42-19	07	0.813
0.3993	11-9; 18-13; 19-18; 34-18; 36-18	05	0.581
0.4032	7-2	01	0.116
0.4055	5-1; 6-1; 32-1,5 and 6; 37-15 and 33; 40-5, 6 and 22	10	1.161
0.4133	23-18, 35-18	02	0.232
0.4236	42-16	01	0.116
0.4315	20-7	01	0.116
0.4418	28-23	01	0.116
0.4478	11-5 and 6; 13-5 and 6; 15-11 and 13; 19-15; 20-11 and 19; 22-11 and 21; 32-11, 13, 19, 21 and 30; 33-24 and 25; 34-1, 20 and 32; 36-5, 6, 15 and 32; 37-21, 24 and 25; 38-5, 6, 20 and 32; 41-13 and 36	34	3.949
0.4581	14-13; 17-11; 19-7 and 17; 21-14; 24-14 and 17; 25-14 and 17; 31-7; 34-7, 14 and 17; 36-14; 38-14 and 17	16	1.858
0.4825	27-10; 32-3 and 23; 35-15; 37-3; 39-10; 42-37	07	0.813
0.4904	9-5 and 6; 15-9; 16-12 and 14; 22-9 and 18; 32-18 and 29; 33-18; 37-18; 41-18	12	1.394
0.5108	19-13; 21-11, 13 and 19; 24-11; 25-11; 26-19 and 21; 30-8 and 13; 31-13, 24 and 35; 34-11, 13, 21 and 26; 36-19, 21, 30, 31 and 34; 38-21 and 26	24	2.787
0.5249	13-3 and 4; 21-2; 26-2; 28-19, 24, 25 and 26; 35-11, 26 and 34; 36-2 and 3; 38-2 and 3; 42-13, 24, 25, 34, 36 and 38	21	2.439
0.5390	15-5 and 6; 22-1 and 15; 33-15; 37-22; 40-32; 41-32	08	0.929
0.5493	10-7; 12-5 and 6; 16-8; 20-17; 22-12; 29-16; 33-7; 41-17	09	1.045
0.5596	23-2; 35-3 and 23; 39-4	04	0.465
0.5675	27-18; 28-18; 35-9; 39-18; 42-18	05	0.581
0.5816	30-18; 31-18; 34-29	03	0.348
0.5917	19-12	01	0.116
0.6020	19-1 and 10; 21-5 and 6; 24-1, 10 and 15; 25-1, 10 and 15; 30-15 and 22; 32-8; 33-19 and 30; 34-10, 15 and 33; 37-19, 30 and 34; 38-15; 40-19; 41-31	24	2.787
0.6161	22-3; 23-22; 28-20; 33-3 and 23; 35-20 and 33; 41-39	08	0.929
0.6264	7-3 and 4; 23-14 and 17; 27-17; 28-17; 35-12; 39-17; 42-14	09	1.045
0.6343	17-9; 18-12 and 14	03	0.348
0.6609	30-16	01	0.116
0.6727	18-5, 6 and 15; 20-18; 29-1, 5, 6 and 15; 37-29	09	1.045
0.6791	19-4; 21-4; 23-13; 24-2 and 3; 25-2 and 3; 26-4; 31-27; 35-30 and 31; 36-23; 38-28; 39-31; 42-21 and 30	16	1.858
0.6931	17-7, 12 and 14; 20-10; 22-20; 23-4; 27-2 and 4; 30-21, 24 and 25; 31-11, 19 and 26; 33-22 and 32; 34-31; 37-1, 5 and 6; 38-11 and 30; 39-2; 41-20, 33 and 37	26	3.020
0.7458	12-8; 13-1 and 12; 21-17; 24-12; 25-12; 26-17; 30-12, 14 and 17; 31-17; 34-12; 36-7 and 12; 38-12	15	1.742
0.7498	18-2 and 3; 42-29	03	0.348
0.7520	32-15; 37-15; 40-15; 41-15	04	0.465
0.7702	5-3; 6-3; 23-1, 5, 6, 10 and 15; 27-1, 5 and 6; 33-27; 35-1; 39-33; 41-23 and 27	15	1.742
0.7843	13-1; 21-15; 26-22; 31-22; 36-1; 38-1; 40-13, 21, 24, 25, 34, 36 and 38; 41-21	14	1.626
0.8047	18-11; 19-9; 21-18; 26-18; 29-19, 24 and 25; 30-9 and 29	09	1.045
0.8166	29-12 and 14	02	0.232
0.8370	14-1; 17-5, 6 and 15; 20-14; 22-17; 32-17; 33-14; 37-7 and 12; 40-17; 41-7	12	1.394
0.8473	3-2; 23-3; 28-2 and 4; 42-23	05	0.581
0.8614	13-4; 19-2 and 3; 23-11; 24-4; 25-4; 27-21; 31-23; 34-2, 3 and 4; 36-4; 38-4; 39-11 and 21	15	1.742
0.8755	15-1; 20-5 and 6; 32-20; 33-1, 5, 6 and 20; 37-20; 40-37; 41-1	11	1.278
0.8959	17-16; 18-10; 29-10 and 22; 32-9; 40-18 and 29	07	0.813
0.9141	14-3; 35-7 and 17	03	0.348
0.9163	13-11; 30-11; 31-21 and 30; 36-11	05	0.581
0.9281	21-7	01	0.116
0.9486	16-11; 19-16	02	0.232
0.9525	15-3; 20-2; 27-22; 33-2; 37-2; 39-1, 5, 6 and 22; 40-3 and 4; 41-3 and 4; 42-1 and 33	15	1.742
0.9730	29-3, 23 and 28	03	0.348
0.9808	18-9; 29-18	02	0.232
1.0075	11-10; 15-8; 20-13; 21-1; 22-8; 26-1, 5, 6 and 10; 30-5 and 6; 31-5, 6 and 10; 33-21 and 26; 36-20; 37-8, 11 and 31; 38-10; 40-8 and 30; 41-30	24	2.787
1.0193	22-7	01	0.116
1.0296	35-2; 42-3	02	0.232

D values	Genotype pairs (Serial no. respective genotypes used)	Number of pairs	%
1.0397	29-17	01	0.116
1.0845	8-3; 21-3; 26-3; 27-11 and 13; 30-3 and 23; 31-2, 3 and 28; 35-8 and 26; 36-27; 39-13 and 36; 42-8	16	1.858
1.0924	13-9; 18-8; 21-9; 24-9; 25-9; 29-11, 13 and 21; 34-9; 36-9 and 29; 39-9 and 29	13	1.510
1.0964	14-4; 39-14	02	0.232
1.0986	10-1, 5 and 6; 22-10; 32-10; 33-10; 40-1 and 20; 41-10	09	1.045
1.1513	17-13; 21-12; 31-14; 36-17	04	0.465
1.1757	10-3; 15-4; 20-4; 22-2; 28-1, 5 and 6; 32-27; 33-28; 37-27; 39-15, 32 and 37; 40-23; 41-28; 42-20	16	1.858
1.1836	18-1; 33-29; 41-29	03	0.348
1.2040	11-8; 13-8; 21-8; 24-8; 25-8; 26-8, 11 and 13; 30-19; 34-30; 36-8 and 26; 38-8	13	1.510
1.2425	7-1, 5 and 6; 12-1; 17-10; 18-16; 32-7; 33-12 and 17; 37-17	10	1.161
1.2528	4-3; 27-3; 28-3; 35-27; 39-3 and 35; 42-4 and 39	08	0.929
1.2606	18-4; 29-27; 35-29	03	0.348
1.2951	11-1; 30-20; 31-15; 32-26 and 31; 33-8 and 11; 37-26; 40-31; 41-26	10	1.161
1.3195	12-3; 17-2 and 4; 23-12; 27-14	05	0.581
1.3540	16-7 and 13; 21-16; 24-16; 25-16; 34-16; 36-16; 38-16	08	0.929
1.3722	23-8; 28-11 and 21; 42-31	04	0.465
1.3863	14-7; 20-1 and 15; 29-3; 40-33	05	0.581
1.4390	11-7	01	0.116
1.4452	16-1, 5 and 6; 22-16; 33-16	05	0.581
1.4634	2-1; 4-1; 5-2 and 3; 6-2 and 4; 20-3; 22-4; 27-15; 28-22, 32-2 and 28; 33-4; 37-4; 40-2 and 39; 41-2	17	1.974
1.4979	9-8; 29-8; 31-9	03	0.348
1.5223	16-3 and 4; 23-16; 35-16	04	0.465
1.5301	15-7; 40-7	02	0.232
1.5404	35-4; 42-27	02	0.232
1.5890	9-1; 20-9; 37-9	03	0.348
1.6072	12-4; 14-2; 39-12; 42-7	04	0.465
1.6094	30-26; 31-8	02	0.232
1.6661	9-3 and 4; 29-2 and 4; 39-29	05	0.581
1.7006	8-1, 5 and 6; 13-10; 20-8; 21-10; 30-1; 31-1; 36-10; 40-26	10	1.161
1.7329	29-7	01	0.116
1.7777	8-2; 11-3 and 4; 28-13; 30-2; 31-4; 36-28	07	0.813
1.7918	15-10; 37-10; 40-10	03	0.348
1.8444	8-7; 14-8; 17-8; 26-14; 30-7; 31-12	06	0.697
1.8688	10-2 and 4; 15-2; 28-15; 32-4; 35-10; 37-28; 40-27 and 38; 42-10	10	1.161
1.9356	12-10; 14-10; 17-1; 20-12	04	0.465
1.9459	35-28; 42-2 and 8	03	0.348
2.0127	17-3; 27-12; 28-12 and 14	04	0.465
2.0794	12-7	01	0.116
2.1910	26-17; 29-26	02	0.232
2.2154	39-16	01	0.116
2.2822	29-20; 33-9	02	0.232
2.3026	19-8; 34-8	02	0.232
2.3592	23-9; 39-9	02	0.232
2.3937	26-15; 41-8	02	0.232
2.4708	11-2; 42-26	02	0.232
2.7058	12-2	01	0.116
	Total	861	100.00

Table 6. Fingerprinting key showing distinguishing characteristics of 42 mungbean genotypes as generated using SSR marker profiles (“_” mention distinguishing base pair).

Sl. no.	Genotypes	Band position due to primers (bp)					Distinguishing primer
		LR7322B	LR7323A	LR7323B	GBssrMB-91	GBssrMB-77	
1	BARI Mung 1	160	181, 175	282, 273	146, 135	293, 283	LR7322B+LR7323B
2	BARI Mung 2	176	191	273, 264	152, 141	293, 283	GBSSRMB91
3	BARI Mung 3	160	181, 175	273	152, 141	293, 283	LR7322B+LR7323B
4	BARI Mung 4	176	191	273, 264	150, 139	293, 283	LR7322B+LR7323A
5	BARI Mung 5	164	181, 175	282, 273	146, 135	293, 283	Not identified
6	BARI Mung 6	164	181, 175	282, 273	146, 135	293, 283	Not identified
7	Sonamug	176	195	273	152, 141	293, 283	LR7323A+LR7323B+GBSSRMB91
8	BD6874	..	181, 175	..	152	304	LR7323A+GBSSRMB91
9	BD6875	164	181, 175	..	150, 139	..	LR7323A+GBSSRMB91+GBSSRMB77
10	BD6876	..	195	273	146	..	LR7323A+LR7323B+GBSSRMB91
11	BD6877	164	195, 181	..	150, 146	304, 293	LR7322B+LR7323A+GBSSRMB91
12	BD6878	164	181	282, 273	150, 139	304	LR7323B+GBSSRMB91

Sl. no.	Genotypes	Band position due to primers (bp)					Distinguishing primer
		LR7322B	LR7323A	LR7323B	GBssrMB-91	GBssrMB-77	
13	BD6879	173, 164	191, 181	282, 273	150, 146	293, 283	Not identified
14	BD6880	164	181	282, 273	150, 139	304, 293	LR7323B+GBSSRMB91+GBSSRMB77
15	BD6881	173, 164	181	282, 273	150, 139	304, 293	LR7322B+LR7323A
16	BD6882	..	181	..	150, 139	..	LR7323A+GBSSRMB91
17	BD6884	176, 164	195, 181	LR7322B+LR7323A
18	BD6885	176, 164	195, 181	282, 273	152, 141	..	LR7323B+GBSSRMB91
19	BD6886	176, 164	195, 181	282, 273	150, 146	293, 283	LR7323A+GBSSRMB91
20	BD6887	176, 164	195	..	152, 146	293	LR7322B+LR7323A
21	BD6888	176, 164	191, 181	..	152, 146	293	LR7322B+LR7323A
22	BD6889	164	191, 181	282, 273	152, 146	293, 283	LR7323A+LR7323B+GBSSRMB91
23	BD6890	176	195, 181	282, 273	152, 146	293	LR7322B+LR7323A
24	BD6891	176, 164	195, 181	282, 273	152, 146	293, 283	Not identified
25	BD6892	176, 164	195, 181	282, 273	152, 146	293, 283	Not identified
26	BD6893	176	195, 175	..	152, 146	293, 283	LR7322B+LR7323A
27	BD6894	176	195	282, 273	146, 135	293, 283	LR7323A+LR7323B+GBSSRMB91
28	BD6895	176	195	273	146, 135	..	LR7323A+LR7323B+GBSSRMB91
29	BD6896	..	181	273, 264	146, 135	..	LR7323A+LR7323B
30	BD6897	173, 164	181	..	152	..	LR7322B+LR7323A+GBSSRMB91
31	BD6898	173, 164	195	..	152, 141	293, 283	LR7322B+LR7323A
32	BD6899	164, 160	181	282, 273	152, 146	304, 293	LR7322B
33	BD6900	173	195, 181	282, 273	152, 141	293, 283	LR7322B
34	BD6901	176, 164	195, 181	282, 273	146, 135	293, 283	LR7323A+LR7323B+GBSSRMB91
35	BD6902	164	181, 175	282, 273	152, 141	293	LR7323A+LR7323B+GBSSRMB91
36	BD6903	173, 164	191, 181	282, 273	152, 141	293, 283	Not identified
37	BD6904	..	181	282, 273	152, 141	293	LR7323A+GBSSRMB91+GBSSRMB77
38	BD6905	176, 164	195, 181	282, 273	152, 141	293, 283	LR7323A+LR7323B+GBSSRMB91+GBSSRMB77
39	BD6906	176	195	282, 273	150, 146	293, 283	LR7323A+LR7323B+GBSSRMB91
40	BD6907	164	181, 175	273, 264	150, 139	304, 293	LR7322B+LR7323A+LR7323B
41	BD6908	164	195, 181	282, 273	150, 139	293, 283	LR7323A+LR7323B+GBSSRMB91
42	BD6909	164	181	282, 273	150, 139	304, 293	LR7322B+LR7323A+LR7323B+GBSSRMB91

Except BARI mung-5, BARI mung-6, BD6879, BD6891, BD6892 and BD6903 other 36 genotypes showed unique and differential DNA banding patterns across one and/or combination of six primers. Since these cultivars also differ for several morphological traits like as plant height, plant growth habit, seed coat colour, smoothness and seed size it seems unlikely that the observed level of similar banding pattern is correct and more likely explanation is that the original DNA samples were mislabeled or duplicated. Amplification of a 152/141 bp band with GBssrMB-91 primer pair could distinguish BARI mung-2 while the absence of this band and presence of a 164/160 bp band with LR7322B could identify BD6899, besides presence of the same band along with a 195 bp fragment amplified with LR7323A and 273 bp with LR7323B, 282/273 bp with LR7323B, 181/175 bp with LR7323A and 282/273 bp with LR7323B, 181bp with LR7323A and 195/181bp with LR7323A could distinguish Sonamug, BD6885, BD8902, BD6904 and BD6905 respectively. BD6900 showed a unique DNA band of 173 bp for the primer LR7322B. Rest of the genotypes were identified with the combination of more than one locus where two primer combination for 14 genotypes, three primer for 12 genotypes and four primer combination were used to discriminate only two genotypes.

Discriminating locus along with their band size against each genotype was mentioned in Table 6.

4. Conclusion

The set of microsatellite markers used here provides a positive assessment to the ability of SSR marker to produce unique DNA profiles of mungbean genotypes. The results of the present study could be applied as baseline information to maintain the appropriate identity and the construction of a database of all mungbean cultivars and their wild relatives grown in Bangladesh and in broad sense, to protect the plant varieties of Bangladesh. Inter-mating cultivars from the major distinct gene pools could provide new genetic recombination to exploit in cultivar development programme.

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