Journal of Agricultural Science; Vol. 13, No. 2; 2021
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Genetic Diversity Analysis of Soybean [*Glycine max* (L.) Merrill.] Germplasms in Bangladesh Using SSR Markers

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Received: October 31, 2020 Accepted: December 8, 2020 Online Published: January 15, 2021

doi:10.5539/jas.v13n2p1 URL: https://doi.org/10.5539/jas.v13n2p1

Abstract

The purpose of the present study was to investigate the genetic diversity and molecular characterization of 50 soybean genotypes in Bangladesh with 20 SSR markers. Genomic DNA was isolated by modified standard cetyl tri-methyl ammonium bromide (CTAB) extraction protocol and alleles were amplified by polymerase chain reaction (PCR). Allele sizes were estimated in comparison with 50 bp DNA ladder. The software NTSYSpc version 2.2 and POPGENE version 1.31 were utilized for molecular data analysis and preparation of dendrogram. Polymorphic Information Content (PIC) values varied from 0.53 (Satt664) to 0.98 (Satt009, Satt330 and Satt522) with the mean value 0.897 and expected heterozygosity varied from 0.4059 (Satt685) to 0.1246 (Satt664) with the mean value 0.244. The dendrogram analysis depicted that the 50 genotypes were grouped in four (4) major clusters. The most diverse genotypes were SBG-1, PM-78-6-3-13, BS-3 and AGS-31, which suggest that the simple sequence repeat (SSR) markers are very efficient for genetic diversity analysis. The similarity matrix revealed the diversity among genotypes. The diverse genetic materials obtained from the present study on genetic diversity of soybean genotypes in Bangladesh may be utilized in the future breeding programme.

Keywords: genetic diversity, soybean, SSR primers, varietal identification

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is the most vital economic legume crops in the world. It supplies vegetable oil which is a crucial source of protein for human and animal nutrition. It is also an important source of bio-diesel (Pimentel & Patzek, 2008).

Genetic diversity is the evaluation of genetic variability within species which is important for the survival and adaptiveness of the species. The genetic diversity analysis is important to broaden the genetic base of soybean germplasm which can be further used in breeding programs.

DNA markers based molecular characterization of soybean germplasms was more informative, stable, and reliable, as compared to traditional methods like agronomic traits, pedigree analysis and morphological diversity analysis. Molecular markers were used for the identification of genetically diverse genotypes which were further used in a breeding program (Maughan et al., 1996; Thompson & Nelson, 1998). Molecular markers were used to evaluate genetic variation within the collected germplasms of soybean genotypes which was reported by Mohammadi and Prasanna (2003).

There are various molecular markers, like restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms (SNPs), and microsatellites or simple sequence repeats (SSRs) are often used in the evaluation of soybean. Molecular profiling is preferable in breeding programs because it is reliable, authentic and less influenced by environmental fluctuations (Vinu et al., 2013). Molecular markers can separate the specific genotypes by identifying the presence of unique bands.

For genetic diversity analysis and molecular characterization in soybean, SSR markers become very important because of their abundance, codominance, high reproducibility (Kujane et al., 2019; Koutu et al., 2019), high polymorphism compared to RFLPs, AFLPs, and RAPDs (Kumawat et al., 2015; Chakraborty et al., 2018; Moniruzzaman et al., 2019) and have a much greater ability to identify unique alleles in parental and elite soybean germplasm than any other markers (Tantasawat et al., 2011).

Russel et al. (2004) found that SSR markers are useful for the determination of genetic relationship and dissimilarity within a population. Microsatellites or SSR markers have high resolving power for evaluation of population (Li et al., 2010). Simko et al. (2012) reported that SSR markers have a high success rate in diversity analysis. Microsatellites or SSR markers are efficiently used for genetic and genomic analysis, genotypic characterization, varietal protection, seed purity estimation, germplasm conservation, diversity analysis, paternity and pedigree analysis, quantitative trait locus (QTL) mapping and marker-assisted breeding (Munir et al., 2013). Wang et al. (2010) and Guan et al. (2010) stated that SSRs marker can be efficiently wont for evaluation of genetic diversity and the relationship among soybean genotypes.

Although there are some studies on soybean diversity analysis but there are very few studies administered in Bangladesh to assess the molecular markers based genetic diversity analysis of soybean. So, the present study was conducted for the identification and genetic diversity analysis of fifty (50) soybean genotypes using 20 SSR markers.

2. Material and Methods

The study was carried out at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh during the period of December 2015 to June 2016, located at 24°43′23″N-90°25′53″E with an altitude of 518 m above sea level. Fifty (50) soybean genotypes having high yield potential were used as experimental materials for this study. All these genotypes with their sources of collection are given in Table 1.

Table 1. Experimental materials with their sources of collection

Sl No.	Genotypes	Sources of collection	Sl No.	Genotypes	Sources of collection
1	G-2120	BAU, Mymensingh	26	BS-3	BAU, Mymensingh
2	Gc-84040-27-1	BAU, Mymensingh	27	KADSING	BAU, Mymensingh
3	MTD-16	BAU, Mymensingh	28	AGS-302	BAU, Mymensingh
4	AGS-79	BAU, Mymensingh	29	GAURAB	BAU, Mymensingh
5	ASSET-93-19-1	BAU, Mymensingh	30	MTD-176	BAU, Mymensingh
6	PM-78-6-3-13	BAU, Mymensingh	31	JOYAWIYAJA	BAU, Mymensingh
7	G-2261	BAU, Mymensingh	32	AGS-314	BAU, Mymensingh
8	Pb-1/SOHAG	BAU, Mymensingh	33	BS-13	BAU, Mymensingh
9	HIHS-WIHS	BAU, Mymensingh	34	LG-92P-1139	BAU, Mymensingh
10	MTD-451	BAU, Mymensingh	35	CH-1	BAU, Mymensingh
11	BRAGG	BAU, Mymensingh	36	ASSET-93-19-2	BAU, Mymensingh
12	GC-83005-9	BAU, Mymensingh	37	TAINANS	BAU, Mymensingh
13	SBG-1	BAU, Mymensingh	38	Lokon	BAU, Mymensingh
14	SY-35	BAU, Mymensingh	39	16-81021-16-1	BAU, Mymensingh
15	NO-205	BAU, Mymensingh	40	SBM-15	BINA, Mymensingh
16	MTD-6	BAU, Mymensingh	41	SBM-18	BINA, Mymensingh
17	AGS-66	BAU, Mymensingh	42	SBM-22	BINA, Mymensingh
18	ACC-1222	BAU, Mymensingh	43	SBM-09	BINA, Mymensingh
19	BARI-6	BARI, Gazipur	44	SANTAR-05A	BAU, Mymensingh
20	BINA-2	BINA, Mymensingh	45	AGS-278	BAU, Mymensingh
21	BINA-3	BINA, Mymensingh	46	ASSET-93-19-5	BAU, Mymensingh
22	BINA-1	BINA, Mymensingh	47	G-10180	BAU, Mymensingh
23	BINA-4	BINA, Mymensingh	48	PK-416	BAU, Mymensingh
24	BARI-5	BARI, Gazipur	49	DAVIS	BAU, Mymensingh
25	MINA HAI	BAU, Mymensingh	50	YESOY-4	BAU, Mymensingh

2.1 Plant Growth

The plants are grown at the glasshouse of Bangladesh institute of nuclear agriculture (BINA), Mymensingh, Bangladesh during the period of December 2015 to March 2016. The plastic pot was prepared with the mixture of 12 kg air-dried sandy loam soil and cowdung at a ratio of 4:1 holding 28% moisture at field capacity (FC). Fertilizer was applied in the soil of the pot uniformly with 0.15, 0.18, 0.36 and 0.1 g urea, triple superphosphate, muriate of potash, and gypsum corresponding to 24-30-60-15 kg NPKS per hectare, respectively. Six healthy seeds were sown in a pot. The seedlings were emerged within 6-7 days after sowing. After two weeks of Seedling emergence, only three healthy seedlings of uniform size were keep in a pot and the rest were thinned out.

2.2 DNA Isolation

The molecular work was carried out at the molecular laboratory in Plant Breeding Division and Biotechnology Divisions of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh during the period of February 2016 to June 2016.

The fresh young leaves were collected from 21 days old soybean plants for DNA extraction. The total genomic DNA were extracted following the modified standard CTAB DNA extraction protocol (J. J. Doyle & J. L. Doyle 1990). The quality of DNA was estimated with 1% agarose gel and quantification of DNA concentration was done by a Thermo Scientific, NanoDrop 2000c spectrophotometer. Dilution of DNA samples were made by using Tris-EDTA (TE) buffer to get a final concentration 100 ng/µl. Then, the polymerase chain reaction (PCR) amplification of SSRs marker was done by using the diluted DNA sample. The SSR markers developed by Cregan et al. (1999) were used in the present study. Twenty (20) SSR markers were chosen from 18 chromosomes/linkage groups (out of 20 chromosomes) of soybean for genetic diversity analysis of 50 accessions of soybean germplasm. The supplementary Table 2 represents the name, position, and sequences of the SSR markers.

Table 2. Sequence of SSR Marker for DNA fingerprinting

Sl. No.	SSR locus	Chromosome number	Linkage Group (LG)	Forward primer sequence (5'→3')	Reverse primer sequence (5'→3')
1.	Satt165	5	A1	CACGAATAACTTGACACATT	TAAAAACAAAGCAAACATAAA
2.	Sat_406	8	A2	GCGCGTGTGGTGGTTACATTA	GCGTTTGCAGCCATTTCCATTTAC
3.	BE806308	11	B1	GCGATTTGACCCCGTTCATACAT	GCGGCAGAAATCCGCTCTCTTTA
4.	Satt509	11	B1	GCGCTACCGTGTGGTGGTGTGCTACCT	GCGCAAGTGGCCAGCTCATCTATT
5.	Satt194	4	C1	GGGCCCAACTGATATTTAATTGTAA	GCGCTTTGTGTTCCGATTTTGAT
6.	Satt184	1	D1a	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT
7.	Satt163	18	G	AATAGCACGAGAAAAGGAGAGA	GTGTATGTGAAGGGGAAAAACTA
8.	Sat_210	18	G	GCGCCAGCAACAAAGTTCCTGACAAA	GCGCATGCAAATGAAATAATAA
9.	Sat_339	16	J	GCGAAAAAGGATTATGTAAACGGTATAAAAA	GCGGTCGAAAATTTAGGATGTAAGATGTTA
10.	Satt009	3	N	CCAACTTGAAATTACTAGAGAAA	CTTACTAGCGTATTAACCCTT
11.	Satt157	2	Dlb	GGGCTCACTCTCGATAGTAGGTATAAAG	GGGATACCAAAAGGAATAATTGTCTT
12.	Satt434	12	H	GCGTTCCGATATACTATATAATCCTAAT	GCGGGGTTAGTCTTTTTATTTAACTTAA
13.	Satt545	5	A1	CAATGCCATTCCATATTTGTT	CAATTGCCCTAGTTTTGATAG
14.	Satt664	19	L	GCGTAGATGCTCAACATCAACACTAATCTG	GCGGACGATGAAGAAATATACTATTACGAA
15.	Satt685	15	E	ATCGTGGCATGTCTCACTAC	GAGGCGGAAGGAAATCTAAT
16.	Satt260	9	K	GCGCCAAATGTATACTTTAAATTCTT	GCGGGTTTAGCTAAAATAGTTCGTGC
17.	Satt330	20	I	GCGCCTCCATTCCACAACAAATA	GCGGCATCCGTTTCTAAGATAGTTA
18.	Satt331	10	O	GCAGAGTCCCCCCTAAATATAG	CGGGAACAACCACACTCTCCATT
19	Satt460	6	C2	GCGCGATGGGCTGTTGGTTTTTAT	GCGCATACGATTTGGCATTTTTCTATTG
20.	Satt522	13	F	GCGAAACTGCCTAGGTTAAAA	TTAGGCGAAATCAACAAT

Source: Cregan et al. (1999).

2.3 Simple Sequence Repeats (SSR) Analysis

The volume of 20 µL reaction mixture was used for each PCR reaction. The PCR reaction mixture was prepared with 8 µL nuclease-free water, 10 µL 1× GoTaq green master mix (Promega Corporation, Madison, Wisconsin, USA), 10 pmol of each primer and 100 ng of genomic DNA. The T3 thermocycler (Biometra, Germany) was used to perform the PCR reactions. Thermal profile was slightly modified from Cregan et al. (1999) which was set up with initial denaturation step 95 °C for 02 min followed by the 35 cycle of denaturation 94 °C for 60 s,

annealing 55-58 °C (as recommended for each SSR primer) for 02 min, extension 72 °C for 60 s and final extension 72 °C for 10 min). The 6% polyacrylamide gels were used for size separation of amplified SSR fragments. The gels were staining with ethidium bromide (0.5 µg/ml) in 1X TBE buffer and photographs were taken on AlphaImager HP (USA). The fragment sizes were calculated with a compared of 50 bp ladder (Promega Corporation, Madison, Wisconsin, USA).

2.4 SSR Allele Scoring and Data Analysis

The PCR products from all the polymorphic SSR markers were analyzed by scoring qualitatively for presence or absence. The SSR markers produced clean bands were scored as 1 (present) and 0 (absent) for each primer. The polymorphism information content (PIC) was calculated by using the following equation according to Botstein et al. (1980):

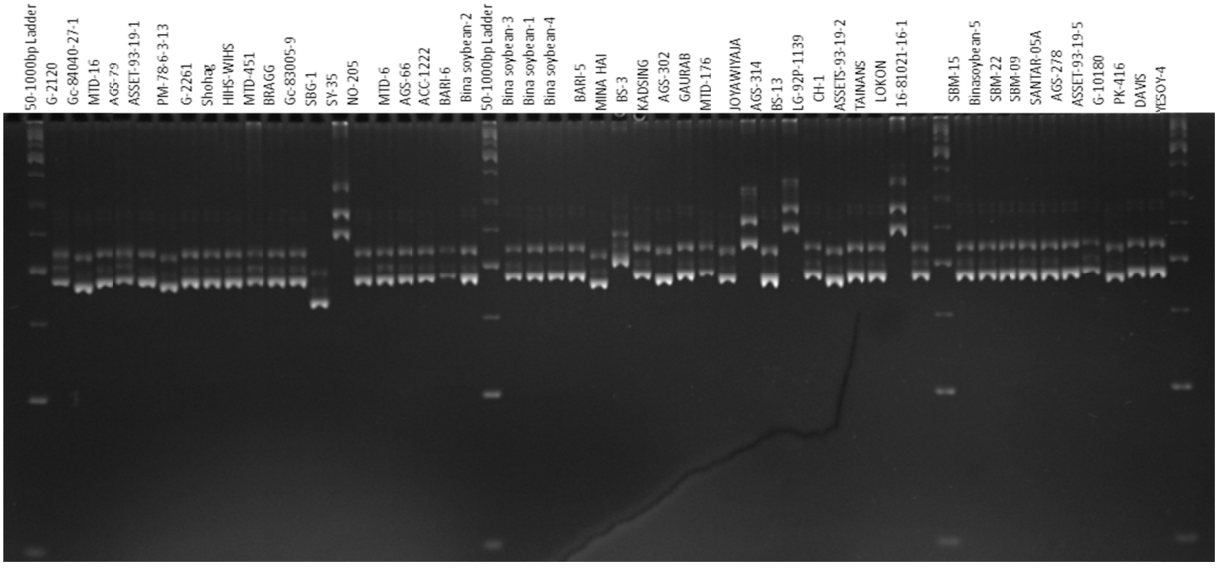
$$\text{PIC} = 1 - \Sigma P_i^2 \quad (1)$$

Where, P_i is the frequency of the i^{th} allele in the set of genotypes analyzed, calculated for each SSR locus.

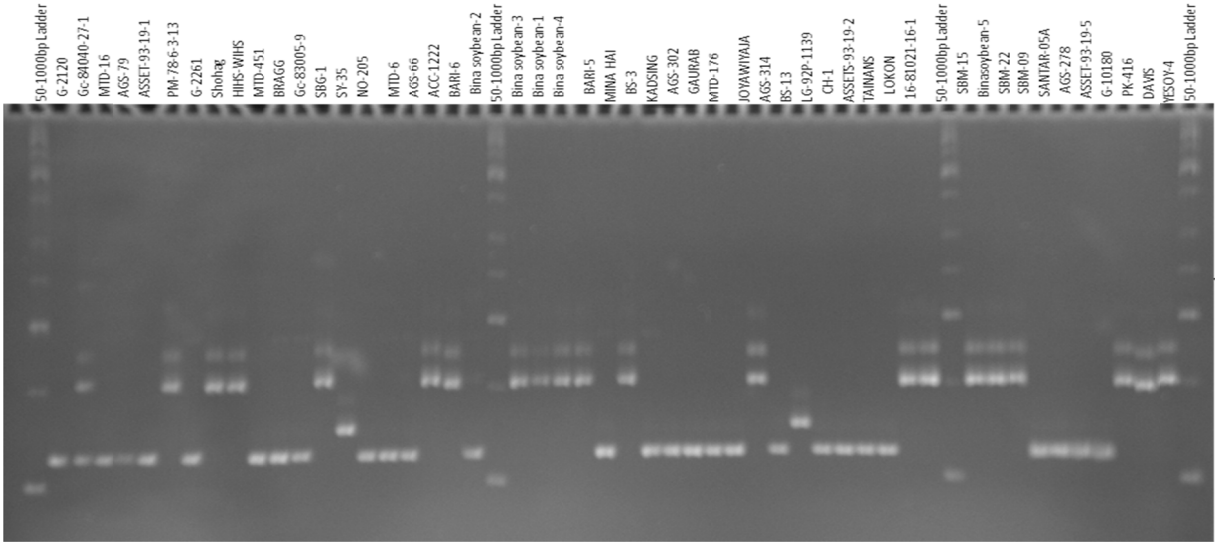
The genetic similarity matrix was measured by Jaccard's similarity coefficient. The resulting similarity matrix was further analyzed in the program NTSYSpc version 2.2 (Rohlf, 2000) for the construction of dendrogram by using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm.

3. Results and Discussion

The results of genetic diversity parameters obtained at each locus among 50 soybean genotypes are presented in Table 3. The genetic variability or similarity analysis is very important for the protection of soybean germplasm, their improvement and efficient management. Microsatellite markers based genetic distance information are suitable for the creation of genetic variation by using genetically dissimilar genotypes (Vieira et al., 2007; Vinu et al., 2013). DNA fingerprinting approaches can identify huge amount of loci for extensive variability. The PCR based fingerprinting assay, *i.e.*, SSR or microsatellite markers were used for the evaluation of 50 soybean genotypes. The SSR markers were exhibited a higher number of DNA polymorphism than other molecular markers. In the present study, out of total 20 SSR markers, 18 markers produced polymorphism, which produced 90% amplification of primer in the 50 soybean genotypes. The amplification of 50 soybean genotypes at primer Satt165 and Satt545 were presented in Figure 1.



(A) Satt165



(B) Satt545

Figure 1. The gel electrophoresis DNA bands amplified by (A) Satt165 and (B) Satt545 marker in 50 soybean genotypes

3.1 Gene Diversity

The gene diversity of a locus is a basic measurement which estimated the expected proportion of heterozygous genotypes within a population under Hardy-Weinberg equilibrium (Nei, 1972). In the present study, the gene diversity was higher in loci Satt685 (0.406) and lower in loci Satt664 (0.125) with a mean diversity of 0.245 (Table 3). The marker having the highest number of alleles indicated higher gene diversity and marker having the lowest number of alleles indicated lower gene diversity.

Genetic diversity for specific locus was assessed by polymorphic information content (PIC). Allelic diversity and gene frequency among the genotypes were estimated with PIC value. The PIC values are greater than 0.5 for all markers used in the present study. The PIC values varied from 0.53 (Satt664) to 0.98 (Satt009, Satt330, and Satt522) with an average of 0.897 (Table 3) which indicates the informativeness of SSR markers. High PIC values are suggested for the identification of diverse soybean genotypes. The PIC values varied from 0.55 to 0.66 with an average of 0.36 for the marker Sat554, Sat180, Sat600 and Sat478 having 4 alleles per locus were reported by Hipparagi et al. (2017). Kumawat et al. (2015) reported that the PIC value was 0.50 for SSRs primer Satt411 and 0.41 for primer BE806308 on genetic diversity analysis of 82 soybean accessions in India. Previous works were reported the PIC values ranged from 0.199 to 0.87 (Wang et al., 2006, Hisano et al., 2007; Zhang et al., 2013; Kim et al., 2014; Bisen et al., 2015).

The expected Heterozygosity was higher in Satt685 (0.4059) and lower in Satt664 (0.1246) with an average of 0.244 (Table 3). The study revealed that the group gene frequency varied from 0.12 (Satt522) to 0.69 (Satt664) and the average of 0.29. The range of Effective number of alleles were 1.172 (Satt664) to 1.761 (Satt685) with a mean of 1.402. The Shannon's Information index ranges from 0.223 (Satt664) to 0.584 (Satt685). The Shannon's information index of 0.383 (mean) in the present study was lower than the results obtained by Zhao et al. (2018) who reported Shannon's information index of 2.528. The results of Shannon's information index indicating the presence of moderate genetic variation among the genotypes (Table 3).

Table 3. Group gene frequency, effective number of alleles, genetic diversity/heterozygosity, Shannon's Information index and PIC value

Sl. No.	Name of SSR marker	Group gene frequency	Effective number of alleles (ne*)	Gene diversity/ heterozygosity (h*)	Shannon's information index (I*)	PIC value
1	Satt009	0.15	1.298	0.189	0.311	0.98
2	Satt163	0.41	1.400	0.255	0.406	0.84
3	Satt165	0.43	1.280	0.201	0.342	0.81
4	Satt184	0.27	1.459	0.269	0.414	0.92
5	Satt194	0.34	1.599	0.334	0.497	0.89
6	Satt509	0.33	1.402	0.262	0.414	0.89
7	Sat_406	0.26	1.497	0.302	0.462	0.93
8	BE806308	0.33	1.540	0.311	0.464	0.89
9	Satt157	0.19	1.289	0.189	0.312	0.96
10	Satt260	0.22	1.358	0.221	0.351	0.95
11	Satt330	0.13	1.279	0.192	0.317	0.98
12	Satt331	0.29	1.506	0.290	0.433	0.92
13	Satt434	0.23	1.403	0.233	0.358	0.95
14	Satt460	0.18	1.354	0.210	0.331	0.97
15	Satt522	0.12	1.286	0.194	0.326	0.98
16	Satt545	0.20	1.353	0.222	0.356	0.96
17	Satt664	0.69	1.172	0.125	0.223	0.53
18	Satt685	0.45	1.761	0.406	0.584	0.79
19	Sat_210	Monomorphic	-	-	-	-
20	Sat_339	Monomorphic	-	-	-	-
Mean		0.29	1.402	0.245	0.383	0.897

The pair-wise comparisons of Nei's (1972) genetic distance between genotypes were varied from 0.0046 to 0.5470, which was estimated with combined data of 18 primers.

3.2 Genetic Similarity Analysis Using UPGMA

The similarity coefficients matrix was used for UPGMA cluster analysis. The pair-wise genetic similarity for 50 soybean accessions ranged from 0.304 to 0.95. The dendrogram constructed considering the genetic similarity coefficient of 0.389 among genotypes showed that the 50 genotypes produced four (IV) major clusters (Figure 2). Cluster I is the largest consisting of 40 genotypes. Cluster II having six genotypes namely PK-416, Lokon, YESOY-4, BINA-2, SY-35 and HIHS-WIHS. Cluster III having two genotypes namely BS-3 and AGS-31. Cluster IV having two genotypes namely SBG-1 and PM-78-6-3-13. Cluster I and II were divided into sub-clusters, sub-sub cluster, sub-sub-sub cluster. The nearly closest genotypes in cluster I was BINA-1 and BINA-4 with a similarity coefficient of 0.95 followed by MTD-176 and GAURAB with a similarity coefficient of 0.88. Bisen et al. (2015) analyzed 38 soybean genotypes using 16 SSR markers were found two major clusters, which were further divided into two sub-clusters. Another study by Hipparagi et al. (2017) reported three distinct clusters in 75 genotypes using 21 SSR markers and two distinct clusters reported by Hirota et al. (2012). From some other study, Tantasawat et al. (2011) found four major clusters in 25 soybean genotypes, whereas Wang et al. (2006) and Ghosh et al. (2014) found two major clusters using SSR markers. The results of the present study are consistent with the findings of Wang et al. (2006), Wen et al. (2008), Hirota et al. (2012), Ghosh et al. (2014) and Bisen et al. (2015). The genotypes used in this study belonged to different clusters because of the genetic variation among them.

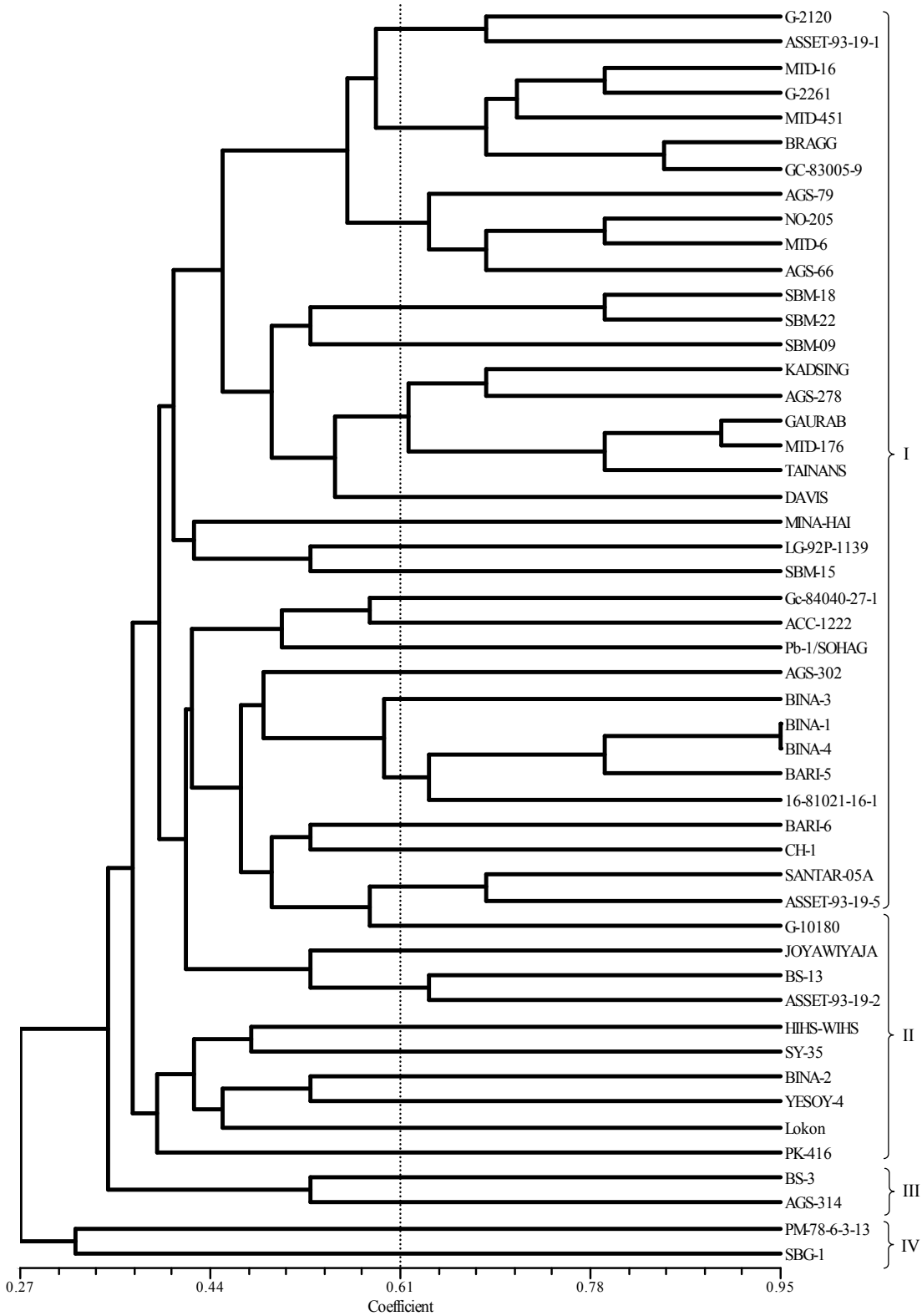


Figure 2. The dendrogram of genetic similarity among 50 soybean genotypes based on UPGMA clustering of Jaccard's similarity coefficients

Among six improve cultivated varieties, five were found in a single cluster I. The clustering of a large number of released varieties together in a single cluster indicating that those were developed from common parents. The results revealed that most released varieties are genetically dissimilar. A large number of soybean germplasm in a

single cluster I represent that the collected soybean germplasms of Bangladesh are genetically related but the genotypes of cluster II, III and IV possessing a diverse genetic backgrounds. The soybean genotypes used in the present study having the narrow genetic base. So, introduction of different exotic germplasm and utilization of wild species and wild relatives is essential for broadening the genetic diversity in Bangladesh. The genotypes having diverse genetic base may be used as a source of new alleles in soybean breeding program. The clustering analysis in the present study helps the breeder understand the genetic structure of the breeding material for effective and efficient selection. In the future, crossing must be carried out between the clusters and avoiding within the clusters as a way of widening and maintaining genetic dissimilarity among genotypes.

4. Conclusion

For the assessment of fifty (50) soybean genotypes with the use of 20 SSR markers found that there was genetic variability among these genotypes. Four major clusters were detected and the cluster I and II had been divided into sub-clusters, sub-sub cluster, sub-sub-sub cluster. The genetic diversity analysis is very important to categorize the genotypes in various clusters for the development of numerous genetic materials. Various heterotic combinations will be produced by the crossing or hybridization among different genetic materials.

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