

Comparison of Population Genetic Structures between Asian and American Mungbean Accessions Using SSR Markers

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Abstract

The purpose of this study was to evaluate the genetic diversity and population structure of 65 mungbean accessions collected from East and Southeast Asia, the United States and Guatemala using 15 simple sequence repeat (SSR) markers. In total, 47 alleles were detected, the number of the alleles per locus range from two to six, with an average of 3.13. The mean major allele frequency (*MAF*), expected heterozygosity (*H_E*), and polymorphic information content (*PIC*) of the 15 SSR loci were 0.76, 0.05, and 0.28, respectively. Of the 47 alleles, 17 (36.2%) were common, with a frequency of 0.05–0.5; 16 (34.0%) were rare (frequency < 0.05) and 14 (29.8%) were abundant (frequency > 0.5). On the basis of the UPGMA dendrogram, most of the accessions were clustered into two main groups. The first group (Group I) included seven accessions and the second comprised 58 accessions, which were further divided into four subgroups. Four subpopulations were detected by model-based structure analysis. Fifty-five accessions (84.6%) showed a clear relation to each cluster based on their inferred ancestry value (>75%), while the remaining 10 accessions (15.4%) were categorized as admixtures. Mungbean accessions from US distributed to almost all clusters and 2 accessions shared genetic constituents showing it derived from mixed ancestry with Asean accessions. These results could be useful in identifying mungbean germplasms and facilitating their improvement programs.

Keywords: mungbean, genetic diversity, SSRs, population structure

1. Introduction

Mungbean (*Vigna radiata* L. Wilczek), which originated from India, belongs to the family Leguminosae, subgenus Ceratotropis. Due to its protein-rich edible seeds, its ability to fix nitrogen, drought tolerance, and early maturity, it is widely planted in various cropping systems (Tangphatsornruang et al., 2009). Mungbean, which provides two of the most important and inexpensive sources of dietary protein to the people of Asia and Africa, is a tropical legume species (Somta et al., 2008). Mungbean sprouts are a common food in some Asian countries and are an excellent source of protein, calcium, and vitamin C. It is commonly used as a vegetable accompaniment to a meal (Rehman, Ali, Saleem, & Tadesse, 2010). Mungbean is characterized by a short growth period and early maturity, which allows adaption to multiple cropping systems of the lowland tropics and subtropics (Gwag, 2008).

Evaluation of genetic diversity is very important for crop plant genetic resources. DNA-based molecular markers provide powerful tools in studying genetic diversity and population structure techniques for analyzing molecular markers such as restriction fragment length polymorphism (RFLP) (Schutte et al., 2008), random amplification of polymorphic DNA (RAPD) (Dikshit et al., 2007; Lu et al., 2009) amplified fragment length polymorphism (AFLP) (Tatikonda et al., 2009) simple sequence repeats (SSRs) (Chapuis & Estoup, 2007; Lung'aho et al., 2011), and single nucleotide polymorphisms (SNPs) (Ganal, Altmann, & Röder, 2009) are now available. SSRs or microsatellite markers, due to their codominance and high polymorphism, are particularly attractive for studying genetic structure and the relationships between species (Jegadeesan et al., 2010; Lu et al., 2009; Saxena, Saxena, Kumar, Hoisington, & Varshney, 2010; Zhang, Blair, & Wang, 2008). In the present study, genetic structure and diversity of 65 mungbean varieties collected from East and Southeast Asia, the United States and Guatemala were evaluated using 15 SSR markers.

2. Materials and Methods

2.1 Plant Material and DNA Extraction

A total of 65 accessions of mungbean collected from Korea (Nos. 1–23), the Philippines (Nos. 24–39), Vietnam (Nos. 40–43), Thailand (Nos. 44–53), Indonesia (Nos. 54–55), the United States (Nos. 56–64), and Guatemala (No. 65) were obtained from the National Agrobiodiversity Center (NAC) of Korea (Table 1). Plant young leaves were sampled and DNA was extracted using a Qiagen DNA extraction kit (Qiagen, Valencia, CA, USA) and quantified using a NanoDrop ND-1000 UV-Vis spectrophotometer (Dupont Agricultural Genomics Laboratory, Newark, DE, USA). Finally, the DNA of each sample was prepared at concentration of 20 ng/ μ L.

Table 1. The details information of 65 mungbean accessions used in this study

Serial No.	Region	Origin	IT. No. ^a	Model-subpop ^b
1	East Asia	Korea	M5	S3
2	East Asia	Korea	M6	S4
3	East Asia	Korea	M21	admixture
4	East Asia	Korea	M22	admixture
5	East Asia	Korea	M65	S3
6	East Asia	Korea	M66	admixture
7	East Asia	Korea	M106	S1
8	East Asia	Korea	M107	S2
9	East Asia	Korea	M108	S2
10	East Asia	Korea	M109	S1
11	East Asia	Korea	M205	S2
12	East Asia	Korea	M217	S1
13	East Asia	Korea	M218	S1
14	East Asia	Korea	M234	S1
15	East Asia	Korea	M325	S3
16	East Asia	Korea	M326	S1
17	East Asia	Korea	M339	S1
18	East Asia	Korea	M365	S2
19	East Asia	Korea	M371	S3
20	East Asia	Korea	M397	S1
21	East Asia	Korea	M426	S1
22	East Asia	Korea	M427	S1
23	East Asia	Korea	M429	S1
24	Southeast Asia	Philippines	M445	S3
25	Southeast Asia	Philippines	M446	S3
26	Southeast Asia	Philippines	M447	S4
27	Southeast Asia	Philippines	M448	S4
28	Southeast Asia	Philippines	M449	S4
29	Southeast Asia	Philippines	M450	admixture
30	Southeast Asia	Philippines	M451	S4
31	Southeast Asia	Philippines	M452	S4
32	Southeast Asia	Philippines	M453	S4
33	Southeast Asia	Philippines	M454	S4
34	Southeast Asia	Philippines	M455	S4
35	Southeast Asia	Philippines	M456	S1
36	Southeast Asia	Philippines	M457	S3
37	Southeast Asia	Philippines	M458	admixture
38	Southeast Asia	Philippines	M459	admixture
39	Southeast Asia	Philippines	M460	admixture
40	Southeast Asia	Vietnam	M42	S3
41	Southeast Asia	Vietnam	M43	S3
42	Southeast Asia	Vietnam	M477	S4
43	Southeast Asia	Vietnam	M478	S3

44	Southeast Asia	Thailand	M528	S4
45	Southeast Asia	Thailand	M529	S4
46	Southeast Asia	Thailand	M553	S4
47	Southeast Asia	Thailand	M562	S4
48	Southeast Asia	Thailand	M570	S4
49	Southeast Asia	Thailand	M571	S3
50	Southeast Asia	Thailand	M593	S4
51	Southeast Asia	Thailand	M611	S2
52	Southeast Asia	Thailand	M659	admixture
53	Southeast Asia	Thailand	M667	S2
54	Southeast Asia	Indonesia	M601	S4
55	Southeast Asia	Indonesia	M735	S3
56	America	USA	M637	S3
57	America	USA	M685	S1
58	America	USA	M686	S2
59	America	USA	M719	S3
60	America	USA	M721	S3
61	America	USA	M732	admixture
62	America	USA	M733	S2
63	America	USA	M736	admixture
64	America	USA	M740	S1
65	America	Guatemala	M687	S2
Total			65	

a: Introduction number of National Agrobiodiversity Center of RDA (Rural Development Administration) in Republic of Korea. b: according to inferred value defined by STRUCTURE program.

2.2 SSR Analysis

Fifteen SSR primers developed by Gwag (2008) were used for genotyping. A three-primer system (Schuelke, 2000) was used to determine the size of PCR products. In the system, unlike the normal reverse primer, the forward primer was composed of normal primer concatenate with a universal M13 oligonucleotide (TGTAACGACGCGCCAGT) labeled with one of three fluorescent dyes (6-FAM, NED, or HEX) that allowed PCR products to be triplexed during electrophoresis. PCR amplification was performed in a total volume of 20 μ L containing 50 ng of template DNA, 0.2 mM of each dNTP, 1 $\times$ PCR buffer, 1U Taq DNA polymerase, 8 pmol of each reverse and fluorescent labeled M13 (–21) primer, and 2 pmol of forward primer with M13 (–21) tail at its 5'-end. The conditions for the PCR amplification were as follows: 94°C for 3 min, 30 cycles of [94°C for 30 sec, 60°C for 45s, 72°C for 1 min], 10 cycles of [94°C for 30 sec, 53°C for 45s, 72°C for 1 min], and a final extension step of 72°C for 10 min. Microsatellite alleles were resolved using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with GENESCAN 3.7 software (Applied Biosystems) and sized precisely against 6-carboxy-X-rhodamine (ROX) molecular size standards using GENOTYPE 3.7 software (Applied Biosystems).

2.3 Data Analysis

Basic statistics for diversity measurements at each microsatellite locus, including the total number of alleles, allele frequency, major allele frequency (*MAF*), gene diversity (*GD*), and polymorphic information content (*PIC* value; Yu et al., 2003), were calculated using the genetic analysis package PowerMarker ver.3.25 (Liu & Muse 2005). The variability at each locus was measured in terms of number of alleles, expected heterozygosity (*H_E*), and genetic distance between each pair of accessions using the genetic analysis package POPGENE 1.31 (Yeh, Yang, & Boyle, 1999).

The unweighted pair group method with an arithmetic mean (UPGMA) tree from shared allele frequencies was constructed using the MEGA 4.0 program (Tamura et al., 2007), which is embedded in PowerMarker. The model-based program STRUCTURE 2.2 (Pritchard, Stephens, & Donnelly, 2000) was used to identify population structure of the accessions implementing a Bayesian clustering approach. In this model, the population genetic structure was characterized by admixture model and correlated allele frequencies. Five independent replications were performed for each run with *K* ranging from two to ten using a burn-in of 10000. The most probable value of ΔK was detected by using *K* as a criterion and the status of each location was

established based on Pritchard et al. (2000). A location would be considered to attached with one cluster by having more than 75% membership probabilities.

3. Results

3.1 SSR Polymorphisms

All 15 SSR markers showed polymorphisms and a total of 47 alleles were identified (Table 2). The size of the PCR products ranged from 110 to 321 bp (Table 2). Polymorphism of SSR markers was measured in terms of the numbers of observed alleles (NA), the number of rare alleles, major allele frequency (MAF), expected heterozygosity (H_E), and the polymorphism information content (PIC). To assess the value of PIC the following was utilized:

$$PIC = 1 - \sum p_i^2 \quad (1)$$

Here, p_i is the allele frequency of the i th alleles of the locus (Yu et al., 2003). Alleles with a frequency less than 5% were defined as rare alleles, whereas common alleles and abundant alleles were defined as those alleles with a frequency between 5% and 50% and more than 50%, respectively. The numbers of observed alleles ranged from two to six with means of 3.13 per locus while the numbers of rare alleles varied from zero to four with means of 1.07 per locus. A total of 16 rare alleles comprised 34.0% of all alleles were identified at all 15 loci. Common and abundant alleles comprised 36.2% and 29.8%, respectively, of the total.

Table 2. Overall diversity statistics at 15 SSR loci in 65 mungbean accessions

Locus	Size range(bp)	NA^a	NA^b	MAF^c	GD^d	H_E^e	PIC^f
7F	288-236	3	2	0.90	0.19	0.17	0.18
13F	171-200	2	0	0.85	0.26	0.09	0.23
14H	269-275	5	2	0.62	0.47	0.00	0.36
17H	165-181	2	0	0.93	0.13	0.11	0.13
38H	124-142	2	0	0.95	0.10	0.11	0.10
77H	219-321	3	1	0.52	0.50	0.00	0.37
87F	281-293	2	0	0.75	0.37	0.00	0.30
91F	169-183	2	0	0.76	0.40	0.02	0.38
93N	110-125	3	1	0.65	0.54	0.00	0.51
113F	165-237	2	0	0.98	0.05	0.02	0.04
142N	242-278	2	0	0.65	0.53	0.22	0.49
172H	247-250	5	3	0.80	0.32	0.00	0.27
180F	271-274	2	0	0.89	0.19	0.00	0.18
184N	289-297	6	4	0.75	0.38	0.00	0.30
198N	241-321	6	3	0.49	0.52	0.00	0.40
Total		47	16				
Mean		3.13	1.07	0.76	0.33	0.05	0.28

a: Number of alleles; b: Number of rare alleles; c: Major allele frequency; d: Gene diversity; e: Expected heterozygosity; f: Polymorphism information content.

The major allele frequency per locus and the expected heterozygosity in all the accessions varied from 0.49 to 0.98 and 0 to 0.22, with averages of 0.76 and 0.05, respectively. The genetic diversity and PIC value ranged from 0.10 to 0.54 and 0.10 to 0.51, with averages of 0.33 and 0.28, respectively. Positive relationships were found between genetic diversity and PIC values. For some loci, however, the degree of polymorphism showed no correlation with the number of alleles (Table 2).

3.2 Geographical Analysis of Diversity

As shown in Table 3, among all the accessions, the series of the average number of alleles was as follows: Korea > United States > Thailand > Philippines > Vietnam > Indonesia > Guatemala. At the countries level, the highest genetic diversity was observed in Korea ($GD = 0.37$, $PIC = 0.31$), followed by the United States ($GD = 0.36$, $PIC = 0.30$), the Philippines ($GD = 0.24$, $PIC = 0.20$), Thailand ($GD = 0.22$, $PIC = 0.19$), Vietnam ($GD = 0.16$, $PIC = 0.13$), Indonesia ($GD = 0.13$, $PIC = 0.10$), and Guatemala ($GD = 0.10$, $PIC = 0.08$).

Table 3. Number of mungbean accessions, number of alleles, major allele frequency, genetic diversity, polymorphic information content according to originated region/country.

Region	Country	NA^a	NA^b	MAF^c	GD^d	PIC^e
East Asia	Korea	23	2.47	0.73	0.37	0.31
Southeast Asia	Philippines	16	2.00	0.83	0.24	0.20
	Vietnam	4	1.40	0.89	0.16	0.13
	Thailand	10	2.07	0.87	0.22	0.19
	Indonesia	2	1.27	0.87	0.13	0.10
America	USA	9	2.27	0.73	0.36	0.30
	Guatemala	1	1.20	0.90	0.10	0.08

a: Number of mungbean accessions; b: Number of alleles; c: Major allele frequency; d: Gene diversity; e: Polymorphism information content.

3.3 Distance-based Phylogeny

The genetic relationships of populations in the present study was analysed by calculating the shared allele frequencies. A dendrogram (Figure 1) was generated using MEGA 4 (Tamura et al., 2007) embedded in the PowerMarker program (Liu & Muse, 2005). Similarity coefficients generated by PowerMarker varied from 0 to 0.8, with an average of 0.33, and were used to construct an UPGMA dendrogram using MEGA 4 software. Most of the accessions studied were clustered into two main groups by the UPGMA dendrogram (Figure 1). The first group (Group I) included seven accessions from Korea. The second group consisted of 58 accessions and was further divided into four subgroups. Subgroup GII-I included four accessions from Korea. GII-II consisted of five accessions (two from the United States and three from Southeast Asia). GII-III (26 accessions) and GII-IV (23 accessions) both consisted of accessions from East Asia, Southeast Asia, and the United States. The resulting phylogram (Figure 2) revealed a complex accession distribution pattern.

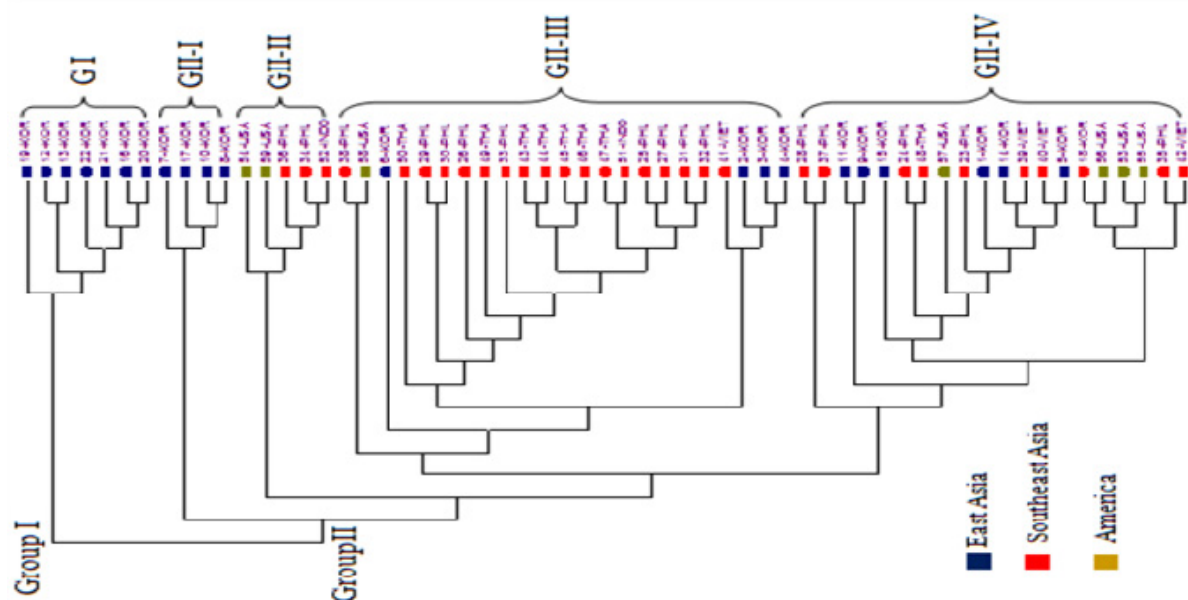


Figure 1. UPGMA dendrogram showing phylogenetic relationships among 65 mungbean accessions from East Asia, Southeast Asia and America. The colour of the blocks correspond to those of the three regions

3.4 Population Structure Analysis

A model-based approach was used to infer the population structures and detect the ancestral and hybrid forms within accessions in the present study (Pritchard et al., 2000). Results of five independent replications for each run with K ranging from two to ten were consistent, but the distribution of $LnP(D)$ revealed a continually increasing curve without a clear maximum for the true K . The ad hoc quantity (ΔK) was used to estimate the real

K value (Evanno et al., 2005). The maximum value of ΔK of the 65 mungbean accessions was identified at $K = 4$ (Figure 2). Clustering bar plots with $K = 4$ are shown in Figure 3. After analyzing the genetic background structure at different values of K , grouping patterns of accessions were observed at $K = 4$, a majority of the accessions were divided into four distinct gene pools.

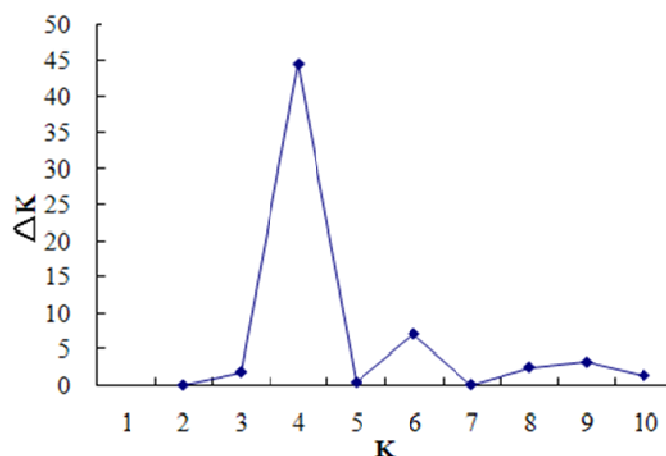


Figure 2. (Summary caption) values of ΔK , with its modal value used to detect the true K of the four groups ($K = 4$). Five independent replications were performed for each K value and averaged over the replicates using STRUCTURE 2.2 (Pritchard et al., 2000)

Of the 65 accessions, 55 (84.6%) shared > 75% ancestry with one of the four genetic populations and were assigned to that population. Ten (15.4%) were classified as admixed forms with varying levels of membership shared among the four genetic populations (Table 4; Figure 3). The first genetic population (S1) included 14 accessions (11 accessions from Korea, one from the Philippines, and two from the United States), with their memberships between 75.7% and 98.5%. The second population (S2) consisted of nine accessions (four accessions from Korea, two from Thailand, two from the United States, and only one from Guatemala). The third population (S3) was represented by 15 accessions (four from Korea, three from the Philippines, three from Vietnam, one from Indonesia, one from Thailand, and three from the United States), while the final population (S4) was represented by 17 accessions (one from Korea, eight from the Philippines, one from Vietnam, six from Indonesia, and one from Thailand) (Table 4; Figure 3). Mungbean accessions from US distributed to almost all clusters and 2 accessions shared genetic constituents showing it derived from mixed ancestry with Asean accessions.

Table 4. Distribution of accessions from different countries to each population identified by inferred value by CLUSTER program

Regions	Countries	Population 1	Population 2	Population 3	Population 4	Admixture	Total
East Asia	Korea	11	4	4	1	3	23
Southeast Asia	Philippines	1	0	3	8	4	16
	Vietnam	0	0	3	1	0	4
	Thailand	0	2	1	6	1	10
	Indonesia	0	0	1	1	0	2
America	USA	2	2	3	0	2	9
	Guatemala	0	1	0	0	0	1
Total		14 (21.5%)	9 (13.8%)	15 (23.1%)	17 (26.2%)	10 (15.4%)	65

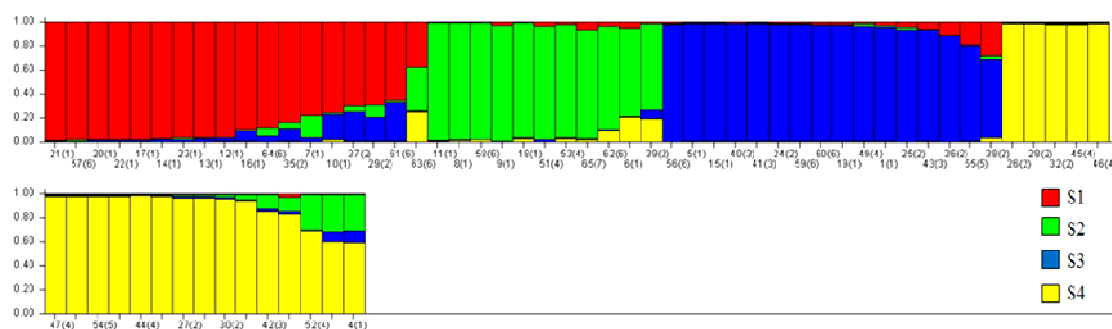


Figure 3. Model based clustering for each of the 65 mungbean accessions examined based on the 15 markers used to build the Q matrix. Each individual bar represents an accession. The different colour bars refer to four different genetic groups (S1-S4, respectively)

4. Discussion

Microsatellites have become one of the most widely used molecular markers for genetic diversity studies, linkage map construction, and marker-assisted selection (Zhao et al., 2010). In this study, a total of 47 alleles were identified among accessions studied with a mean of 3.13 per locus. Polymorphisms were identified among all the microsatellite loci. The comparatively high percentage of rare alleles (34.0%) among mungbean accessions indicates that they contributed greatly to the overall genetic diversity of the collection (Roussel, Koenig, Beckert, & Balfourier, 2004; Yifru, Hammer, Huang, & Roder, 2006).

At the countries level, the highest genetic diversity was observed in Korea, followed by the United States, the Philippines, Thailand, Vietnam, Indonesia, and Guatemala. Except the accessions from the United States, in the present study, positive correlation were identified between genetic diversity and the number of accessions which is in turn correlated with the number of alleles. Accessions from the United States possessed relatively high genetic diversity with small number of accessions indicating that mungbean germplasm from the United States are more heterogeneous than accessions from Southeast Asia.

Genetic distance-based analysis revealed that phylogenetic relationship among 65 mungbean accessions in the present study did not consistent with their geographic origin. It is reported that genetic associations in germplasm are not always associated with geographical diversity which may due to the germplasm exchange from different geographical regions (Gwag, 2008; Mohan et al., 1997). Although some of the accessions from East Asia and Southeast Asia were clustered together, a majority of the accessions were not grouped in accordance with their geographic origin. More to the point, accessions from the United States distributed in almost every subgroup of Group II which was consistent with its high heterogeneity.

A striking difference in genetic diversity was observed among the six nations. Although genetic diversity of the accessions from Korea was the greatest, the accessions from the United States, with a lower accession number (NA = 9), possessed a much higher genetic diversity than those from the Philippines (NA = 16) or Vietnam (NA = 10), indicating that mungbean germplasm in the United States are more heterogeneous than those of other nations. Germplasm collection plays an important role in diversifying crops. It is reported that wild mungbean accessions possessed a much higher genetic diversity than cultivated accessions indicating that wild accessions in germplasm collection contribute a lot to the maintainance of genetic diversity (Sangiri, C., Kaga, A., Tomooka, N., Vaughan, D., & Srinives, P., 2007). Evaluation of the genetic diversity of mungbean accessions may lay the foundation for maximizing the genetic variation in germplasm collections and facilitate their use in breeding.

The distribution of accessions from different nations in each population (S1–S4) showed that those from Southeast Asia were distributed mainly in S3 and S4, while half of the accessions from Korea were distributed in S1. Accessions from Thailand, the Philippines, and the United States were not present in S1, S2, and S4, respectively. Three accessions from Korea, four from the Philippines, one from Thailand, and two from the United States showed a complex ancestry (Table 4). The phylogram indicated that mungbean accessions came from the same geographic location did not cluster to the same group. Similarities were founded in results reported by Poehlman (1991). This may be a consequence of germplasm exchange among nations from different geographic regions (Gwag et al., 2010). Human factors are one of the reasons for the lack of relevance between genetic and geographical distance in some cases (Stankiewicz, Gadamski, & Gawronski, 2001).

In conclusion, present result pointed out that Mungbean accessions from US, with high heterogeneity, genetically related with Asean accessions.

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