Pak. J. Bot., 47(1): 203-209, 2015.

GENETIC DIVERSITY OF SOYBEAN ACCESSIONS USING SEED STORAGE PROTEINS

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Abstract

Soybean, *Glycine max* (L) Merrill, is the most important grain legume in the world that has a fairly wide range of adaptations to different climatic conditions. The present study was conducted to assess genetic variations on 139 Soybean genotypes collected from different countries including Australia, Brazil, India, Japan, Pakistan, Tiwan, USA, Yugoslavia and China. A total of 17 bands have been identified for 139 Soybean genotypes which include 9 monomorphic bands and 8 polymorphic bands. Total number of bands was found highest for India (215) while these were lowest for Yugoslavia (33). Cluster analysis, clustered these accessions into 10 clusters without having any indication of grouping on the basis of their relationships to their regions. Pairwise comparisons based on Nei and Li similarities for inter-population genetic distances of soybean accessions ranged from 0.14 to 1.12. Genetic distances for soybean germplasm from different countries were found highest for Brazil (0.97±0.03) while it was lowest for Taiwan (0.91±0.02). Clustering for Soybean groups was clustered into three clusters including Korea, Taiwan in the first group while Yugoslavia and Japan were clustered in the second group. The third cluster was comprised of Soybean genotypes from China, Pakistan, USA, India Brazil and Australia. Total seed storage protein variation was partitioned by AMOVA on the basis of their origins into within-population and among-population components which revealed 10.00% of the total variation resided among countries and 90.0% within countries. Genetic patterns obtained from this study can help soybean breeders to make better plan for selecting germplasm from wide sources for a specific purposes.

Key words: Soybean genotypes, Seed storage proteins, Cluster Analysis, Principal Coordinate Analysis, AMOVA.

Introduction

Soybean, *Glycine max* (L) Merrill, is the most important grain legume in the world that has a fairly wide range of adaptations to different environment involving climatic, soil and growth conditions. Knowing the degree of genetic variability among different genotypes is of fundamental importance for efficient plant breeding programs (Rabbani *et al.,* 2010). Knowledge of genetic diversity will also allow to better understand the evolutionary relationships among accessions and to develop strategies to incorporate useful diversity in breeding programs (Pervaiz *et al*., 2010).

Traditionally, genetic diversity in soybean has been based on the differences in morphological and agronomic traits or pedigree information (Bernard *et al*., 1998) which determine the extent of phenotypic and genotypic variations in germplasm. Evaluation based on agronomic data is highly influenced by many environmental factors so biochemical markers which include seed storage protein and isozyme have been used for estimating genetic variabilities. Seed storage protein markers have been used regularly for the characterization of soybean cultivars (Natarajan *et al*., 2006). Seed storage protein markers are highly polymorphic and lack any environmental influence on their electrophoretic patterns (Gepts *et al*., 1986; Shah *et al.,* 2011). Isozymes are also used as biochemical markers which exhibit codominance at a locus making it possible to differentiate between heterozygotes (Akbar *et al*., 2012).

Seed storage protein polymorphism has been used successfully for detecting variability among soybean genotypes which show great geographical diversity (Malik *et al*., 2009). The use of polymerase chain reaction (PCR)-based markers includes random amplified polymorphic DNA (RAPD) and microsatellites are used routinely for detecting polymorphisms in soybean. RAPD markers are widely used in plants because of the simplicity of the technique (Akbar *et al.,* 2011) but they are less efficient to detect polymorphism as compared with microsatellites (Simple Sequence Repeat (SSRs)). Microsatellites have been regularly and widely used in mammalian (Weissenbach *et al*., 1992) and plant genomes (Wang *et al*., 1994, Roder *et al*., 1995; Turi *et al*., 2012). They have been reported to show a high level of polymorphism and used successfully for soybean polymorphism (Morgante *et al*., 1994, Akkaya *et al*., 1995). Their major problem with these markers lies in the high cost of development and analysis. The aim of our study is to assess the pattern of genetic diversity among 139 soybean accessions from Australia, Brazil, China, India, Japan, Korea, Pakistan, Tiawan, USA and Yugoslavia to evaluate the level of genetic variation within and among soybean genotypes using seed storage proteins.

Materials and Methods

A total of 139 soybean germplasm used for this study was collected from ten countries including Australia, Brazil, China, India, Japan, Korea, Pakistan, Tiawan, USA and Yugoslavia, representing different agro-ecological conditions (Table 1 & Fig. 1).

Soybean seed extractions were employed for this study. Seeds were crushed and ground into fine powder with a pestle and mortor. The crushed endosperm of around 0.01gm was reduced and alkylated for protein extraction (Doll & Andersen, 1980). It was solubilized and extracted with a 500µl buffer containing 5M urea, 5% β Merceptoethanol in 0.5 M Tris HCl pH (8.0), 2% SDS and 10% glycerol in a 1.5 ml polypropylene microfuge tube. The Eppendorf tubes were vortexed thoroughly using an Automatic Lab Mixer DH-10 to homogenize, and the homogenate samples were purified by centrifuging at 13000 rpm for 10 min at room temperature. The extracted crude proteins were recovered as clear supernatant, transferred into new 1.5 mL Eppendorf tubes and stored at 2°C for electrophoresis. Solubilized proteins were fractionated by electrophoresis in vertical SDS Polyacrylamide gel electrophoresis system slab gels in a discontinuous Tris HCl -SDS buffer system (pH 6.8/8.8) at 11.5% polyacrylamide concentration in Tris HCl/glycine buffer system of Laemmali (Laemmli, 1970). A 15 µL of supernatant was loaded with the micropipette into the gel wells and run at a constant current of 90 Volts at room temperature (25°C) till the tracking dye migrated to the gel bottom. Then gel was fixed in methanol and acetic acid, washed and then stained over night with glacial acetic acid, methanol and Coomassie Blue R-250. De-staining was carried out in distaining solution containing 50:10:40 ethanol, glacial acetic acid and water until bands became clear and visible. After destaining gel was stored in 10% glycerol solution and photographed or air dried at room temperature between two sheets of wetted untreated cellophane clamped in acrylic plastic frame.

The number of monomorphic and polymorphic protein bands were counted for each sample and manually scored as 1 (present) or 0 (absent). The polymorphic bands were analyzed for the level of polymorphism by counting the number of polymorphic bands and generating summary statistics on the band frequencies. To assess the variation among samples of various countries, an analysis of molecular variance (AMOVA; Excoffier *et al*., 1992) was performed using Arlequin version 3.1 (Excoffier *et al*., 2005) on all 139 samples and for countries. This analysis not only allows the partition of the total protein variation into within- and among-group variation components but also provides a measure of inter-group genetic distances as the proportion of the total protein variation residing between any two groups (called Phi statistic; Excoffier *et al*., 1992). Significances of resulting variance components and inter-group genetic distances were tested with 10,100 random permutations.

To assess the genetic associations of the soybean genotypes representing 10 countries, the binary data was processed using, ARLEQUIN (Population Genetics Analysis Program 3.0 PC, (Excoffier *et al*., 2005) and PHYLIP (Phylogenetic Inference Package, 3.68; Felsenstein, 1989). To determine genetic differentiation, all data sets were analyzed as samples within groups and between groups for genetic differentiation. The data was analyzed using ARLEQUIN to obtain standard indices for Analysis of Molecular Variance (AMOVA). A distance matrix, produced from the binary data input into the RESTDIST program of PHYLIP, was processed through the program NEIGHBOR for output as a phenogram using DRAWGRAM (PHYLIP, 3.68).

Table 1. Sampling location and variation patterns of seed storage protein in soybean genotypes collected from Australia, Brazil, India, Japan, Pakistan, Taiwan USA and Yugoslavia.

Regions	GIS information		Sample size	Presence of bands		Frequency of bands	
	Latitude	Longitude		T	P	Mean	Range
Australia	12°-37° S	115°-153°E	3	46.00	23.53	0.06	(0.022-0.065)
Brazil	01°-30° S	34°-51° W	7	109.00	17.65	0.06	(0.009-0.064)
China	31-39° N	116°-121°E	4	61.00	17.65	0.06	(0.016-0.066)
India	12°-28° N	72°-88° E	13	204.00	23.53	0.06	(0.029-0.064)
Japan	33°-43° N	130°-141° E	8	127.00	35.29	0.06	(0.047-0.063)
Korea	37°-40° N	127°-127.5° E	2	31.00	17.65	0.06	(0.032-0.065)
Pakistan	23°-37° N	61°-76° E	27	416.00	35.29	0.06	(0.031-0.065)
Taiwan	22°-25° N	120°-121° E	12	177.00	41.18	0.06	(0.034-0.068)
USA	18°-64° N	66°-165° W	61	948.00	41.18	0.32	(0.136-0.345)
Yugoslavia	44°-48’ N	20°-28’ E	2	33.00	5.88	0.06	(0.030-0.061)
Total			139	2152	47.06	0.91	(0.46-1.00)

T-total number of bands observed and P-the percentage of the polymorphic bands

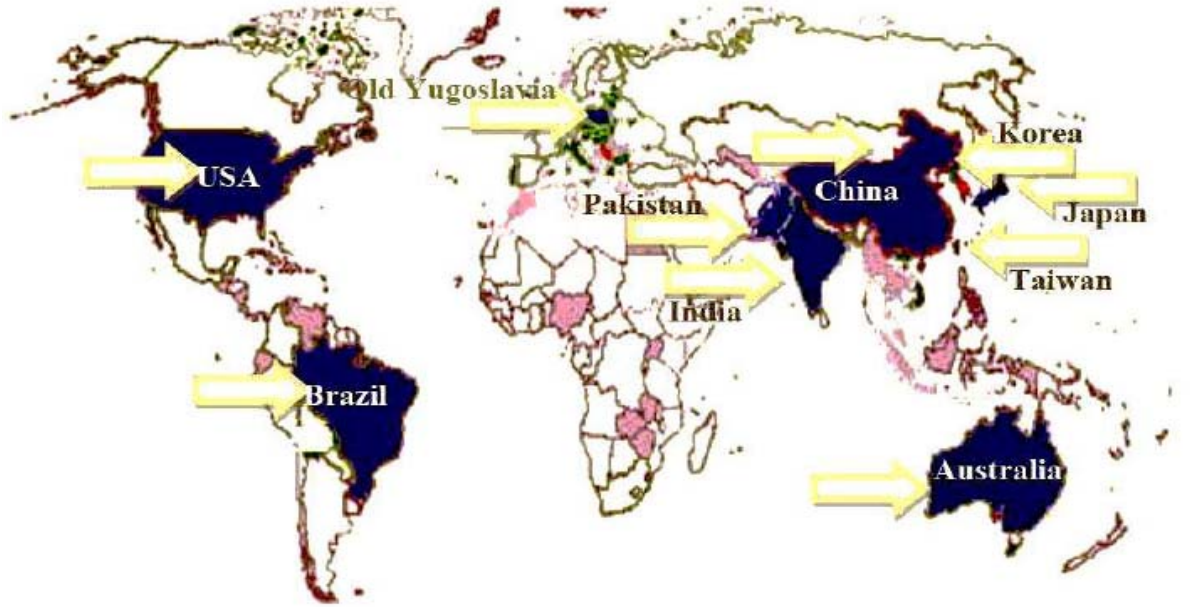

Fig. 1. Geographical regions of Soybean accessions introduced from Australia, Brazil, India, Japan, Pakistan, Taiwan USA and Old Yugoslavia.

Results and Discussion

A total of 17 bands have been identified for 139 Soybean genotypes including 9 monomorphic bands and 8 polymorphic bands ranging from 90 KDa to 14 KDa which are shown in Fig. 2. The band frequencies in the whole germplasm were observed as 0.91 ranging from 0.46 to 1.00 (Table 1). The total number of seed storage protein bands observed for different soybean groups were ranged from 31 to 948 and averaged 21.52. The percentages of polymorphic bands over the total bands detected for Soybean genotypes were ranged from 5.88 (Yugoslavia) to 41.18% (Taiwan & USA) and averaged 25.88%. The mean band frequency for each population were ranged from 0.06 (Australia, Brazil, China, India, Japan, Korea, Pakistan, Taiwan & Yugoslavia) to 0.32 (USA) and averaged 0.086 (Table 1). Thus, it was found that the correlation analysis among polymorphic bands (%) and mean band frequency for different soybean genotypes were not significantly associated with latitude and longitude. Similarly, in another study Mirza *et al*. (2007) observed a total number of 34 bands using seed storage protein in *Avena fatua*. The number of bands ranged from 24 to 34 while the percentage of the polymorphic bands ranged from 37.9 to 97%. They further observed that these variations were not associated with latitude, longitude, elevation, and sample size. In the present study, low level of polymorphism was observed using seed storage proteins which was also suggested by Hameed *et al*. (2009) who reported that low variability was associated with seed storage protein banding patterns. Similarly, Malik *et al*. (2009) proposed that cluster analysis can completely separate most of the Pakistani Soybean accessions from USA and AVRDC, but could not distinguish between the accessions from Japan and North Korea. They suggested that accessions from various sources differed considerably and it was difficult to establish any relationship between origin and clustering pattern. Other studies also revealed similar results using molecular markers which proposed that a low level of polymorphism was attributed to the self-pollinating character of Soybean and a narrow genetic base of the gene pool (Morgante *et al*., 1994).

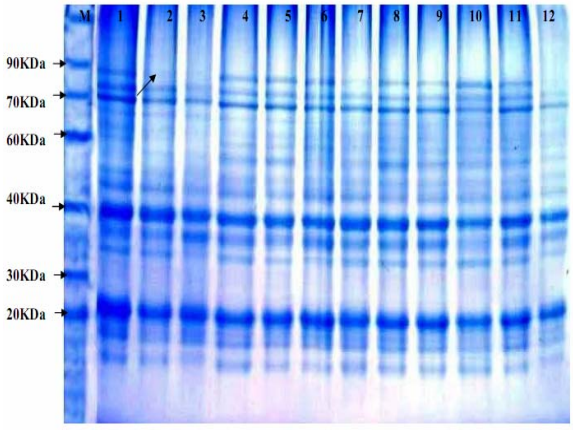

Fig. 2. Electrophoregram of seed storage proteins extracted from Soybean genotypes.

Genetic distances for soybean germplasm for different countries are shown in Table 2. Mean Genetic distances for soybean germplasm from different countries were found highest for Brazil (0.97±0.03) while followed by Australia (0.96±0.03), China (0.96±0.02) and India (0.96±0.02) while it was found lowest for Taiwan (0.91±0.02) followed by Korea (0.93±0.02). Genetic distances for whole soybean genotypes ranged from 0.83 to 1.00 and averaged 0.94 ± 0.03. The minimum genetic

distance for whole Soybean genotypes were found highest for Australian samples (0.95) while it was found lowest for USA samples (0.82). It was analyzed that maximum genetic distance was observed as 1.00 for all Soybean groups. It was noticed that sample size did not show significant effects on the values of genetic distances for the genotypes (Mirza *et al*., 2007; Shinwari *et al*., 2013). Inter-country genetic distances was found significant for soybean genotypes collected from different countries including Australia, Brazil, India, Japan, Pakistan, Taiwan, USA, Yugoslavia and China. All of the within and between-country differences were compared with spearman's rank correlation which showed positive significance for all soybean groups (Table 2). In the present study, these results were in agreement with the previous findings which proposed that the genetic distance between wild soybeans from different regions seems to reflect geographic proximity. Higher levels of allelic diversity were found in wild soybeans (28 alleles per locus) than Japanese cultivated soybean (five alleles per locus). Greatest genetic distance was between Russian accessions and those from north central China while among wild soy-beans smallest genetic distance was between Russian and north China accessions. Cultivated soybeans from Japan were most similar to wild soybeans from Japan and least similar to wild soybean from Russia and China (Kuroda *et al*., 2009).

Cluster analysis, clustered these accessions into 10 clusters without having any indication of grouping on the basis of their relationships to their origins. Cluster I comprised of 5 accessions belonging to 4 accessions from Pakistan and one from Japan. Cluster II comprised of three accessions belonging to 1 accession each from North Korea, Pakistan and USA. Cluster III comprised of three accessions belonging to two accessions from USA and one from Pakistan. Cluster IV comprised of 6 accessions belonging to 2 each from Taiwan and Pakistan while one each from USA and India. Cluster V comprised of 24 accessions belonging to 13 accessions from USA, three accessions from Brazil, two accessions each from Taiwan, Pakistan and India while only one accession each was observed for Japan and Australia. Cluster VI comprised of 30 accessions belonging to 14 accessions from USA, 4 from India, 4 from Pakistan, 3 from Brazil, 2 from China and one each from Taiwan and Thailand. Cluster VII comprised of twelve accessions belonging to five from USA, four from India and one each from Japan, Brazil and Pakistan. Cluster VIII comprised of 20 accessions belonging to 10 accessions from USA, 4 from Pakistan, two each from Taiwan and India while one each from Korea and Yugoslavia. Cluster IX comprised of 9 accessions belonging to three each from USA and Taiwan while one accession each from Australia, Japan and Pakistan. In the end the last cluster X comprised of 27 accessions belonging to 11 accessions from USA, 5 from Pakistan, 3 each from Japan and India, two from Taiwan and 1 each from Yugoslavia, Brazil and Australia (Fig. 3). Our results showed that USA, Taiwan, Pakistan, Japan, India, Brazil, China and Australia accessions were related to each other and concentrated to all the clusters. No significant relationship of the accessions was observed with any of these clusters.

Table 2. Genetic distances for soybean germplasm from different countries and significance test of inter-country genetic distances.

Locations	Sample size	Mean	Std. Dev.	Minimum	Maximum	Brazil	China	India	Japan	Korea	Pakistan	Taiwan	USA
Australia	3	0.96	0.03	0.95	1.00	**	**	**	**	**	**	**	**
Brazil	7	0.97	0.03	0.90	1.00	**	**	**	**	**	**	**	**
China	4	0.96	0.02	0.91	1.00	**	**	**	**	**	**	**	**
India	13	0.96	0.02	0.89	1.00	**	**	**	**	**	**	**	**
Japan	8	0.94	0.02	0.88	1.00	**	**	**	**	**	**	**	**
Korea	2	0.93	0.02	0.88	1.00	**	**	**	**	**	**	**	**
Pakistan	27	0.94	0.02	0.90	1.00	**	**	**	**	**	**	**	**
Taiwan	12	0.91	0.02	0.87	1.00	**	**	**	**	**	**	**	**
USA	61	0.94	0.03	0.82	1.00	**	**	**	**	**	**	**	**
Yugoslavia	2	0.95	0.03	0.85	1.00	**	**	**	**	**	**	**	**
Total	139	0.94	0.03	0.83	1.00	-	-	-	-	-	-	-	-

Table 3. Pair wise comparisons based on Nei and Li similarities for inter-population genetic distances of soybean accessions.

	Australia	Brazil	India	Japan	Pakistan	Taiwan	USA	Yugoslavia	China
Brazil	0.536								
India	0.513	0.341							
Japan	0.512	0.703	0.448						
Pakistan	0.466	0.522	0.339	0.382					
Taiwan	0.584	0.782	0.634	0.512	0.395				
USA	0.441	0.281	0.14	0.466	0.283	0.575			
Yugoslavia	0.833	0.872	0.592	0.484	0.596	0.768	0.637		
China	0.546	0.444	0.46	0.74	0.427	0.697	0.392	0.829	
Korea	0.928	1.122	0.879	0.599	0.611	0.584	0.87	0.707	0.968

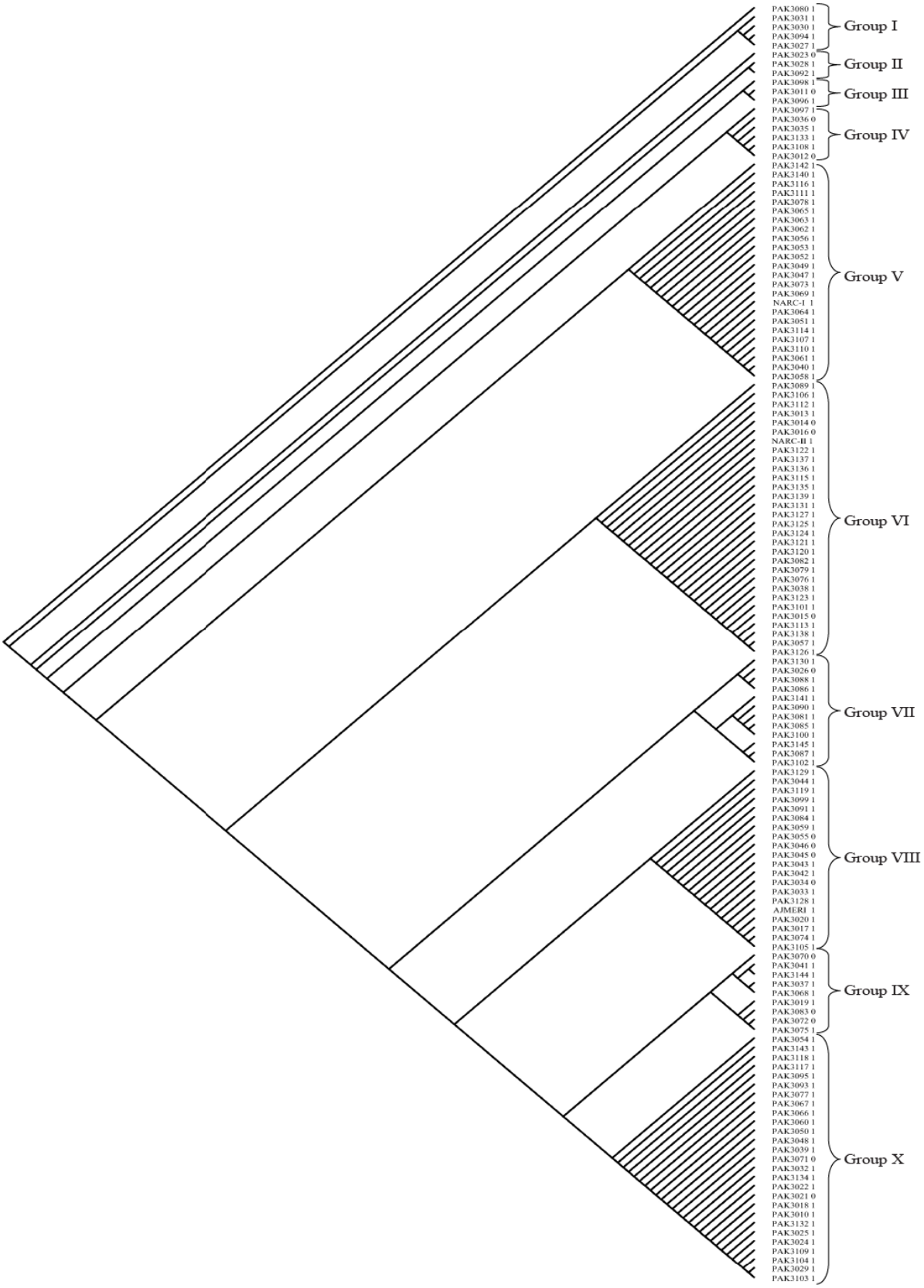

Fig. 3. Cladogram derived among soybean populations originated from Australia, Brazil, India, Japan, Pakistan, Taiwan USA and Yugoslavia using Neighbor Joining method.

Table 4. Analysis of molecular variance for detecting variations among and within different soybean genotypes collected from Australia, Brazil, India, Japan, Pakistan, Taiwan USA and Yugoslavia.

Source of variation	d.f.	Sum of squares	Variance	Percentage of variation
Among countries	9	2.262	0.012	10%
Among populations within countries	129	14.630	0.113	90%
Total	273	60.017	0.445	100

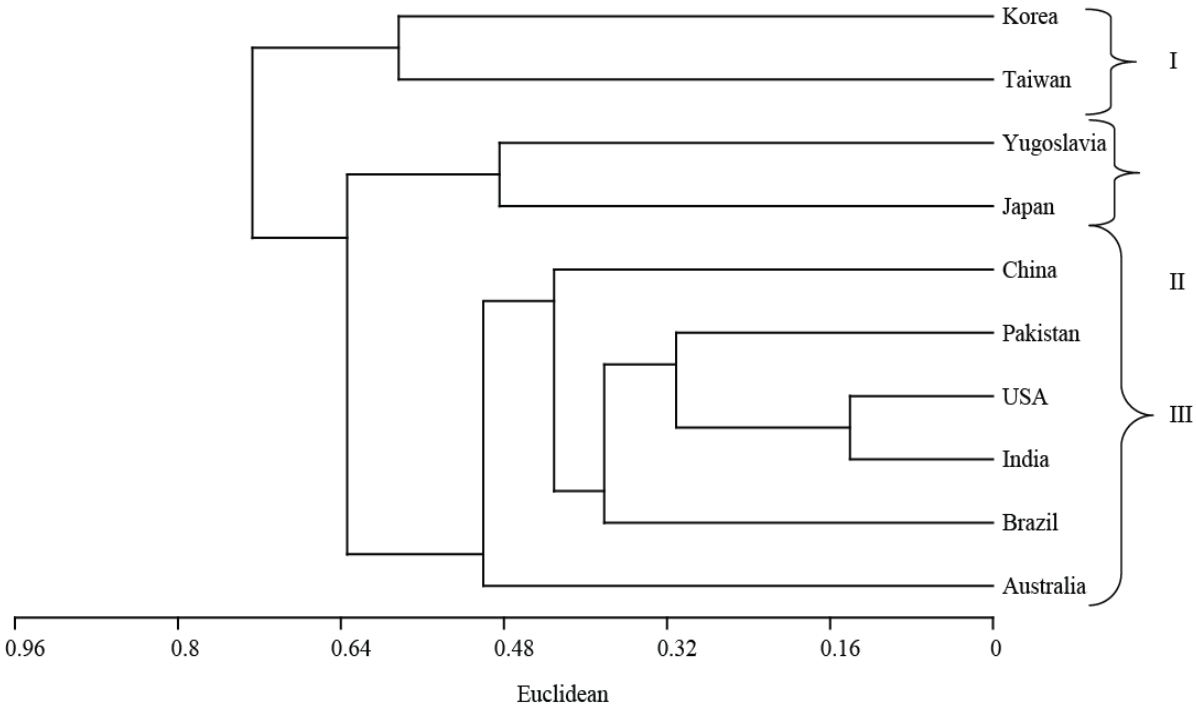

Fig. 4. Clustering of Soybean groups using Un-weighted Pair Group Method with Arithmetic mean (UPGMA).

Pair wise comparisons based on Nei & Li similarities (Nie & Li, 1979) for inter-population genetic distances of soybean accessions are shown in Table 3, which ranged from 0.14 to 1.12. Brazil & Korean genotypes were found to have highest similarity (1.12) followed by China and Korea (0.968) while lowest similarity was observed between India and USA genotypes (0.14) followed by Brazil & USA (0.281). These differences can also be visualized in the inferred genetic relationships of the Soybean groups as shown in Fig. 4. The most distant Soybean groups were observed in cluster I as Korea & Taiwan while in cluster II the other distant groups were Yugoslavia and Japan. The third cluster was the largest cluster comprising of majority of Soybean groups including Brazil, India, Japan, Pakistan, Taiwan, USA, Yugoslavia, China and Korea. Our results are inconsistent with previous findings as Griffin and Palmer (1995) clustered 1,005 domesticated soybean accessions from China, Siberia, India-South Central Asia, Japan, S. Korea, and Southeast Asia using isoenzyme markers. They indicated that groups of *G. max* from Siberia and China were closely related and accessions from Japan and S. Korea were closely related. Similarly, Perry & McIntosh (1991) evaluated 2250 soybean accessions from 78 countries based on the 17 morphological traits to determine variations among and within geographical regions. Canonical discriminant analysis clustered S. Korea and Japan accessions into one group and Chinese lines into a separate group. The consistency of results based on three different classification tools provide strong evidence that accessions from Japan and S. Korea are genetically similar and are distinct from Chinese accessions. In another study, Sihag *et al*. (2004) found that clustering pattern predicted no relationship between genetic diversity and geographic diversity. The genotypes from the same eco-geographic region were clustered in different clusters, and genotypes from different eco-geographic regions were classified into similar cluster. Total seed storage protein variation was partitioned by AMOVA on the basis of their origins into within-population and among-population components which revealed 10.00% of the total variation resided among countries and 90.0% within countries (Table 4). This difference was statistically significant (p<0.0001) based on the permutation test. Cho *et al*. (2008) analyzed polymorphism at 92 simple sequence repeat (SSR) loci. The analyzed FST values using molecular variance (AMOVA) on SSR data and indicated little genetic differentiation.

Li & Nelson (2001) utilized AMOVA program for the estimation of partition and total variance among countries, among populations (regional groups) and within populations. The differences among countries and among populations within countries were both significant but the greatest variation was found among individuals within populations which are in agreement to our results. It was proposed that identification of genetic structure is useful strategy for the establishment and management of gene banks. On the basis of these results, it was concluded that Australia, Brazil, China, India, Japan, Pakistan, Taiwan and Yugoslavia are the secondary sources of soybean germplasm and these were derived from USA germplasm banks. Genetic patterns obtained from this study can help soybean breeders to make better plan for selecting germplasm from wide sources for a specific purposes.

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(Received for publication 27 August 2013)