ISSN: 1511-3701
Pertanika J. Trop. Agric. Sci. **35 (2)**: 237 - 248 (2012)

Genetic Relationships among Soybean Accessions Based on Morphological and RAPDs Techniques

Ojo, D. K., Ajayi, A. O. and Oduwaye, O. A.*
Department of Plant Breeding and Seed Technology,
University of Agriculture,
Abeokuta, P. M. B. 2240, Nigeria
**E-mail: oduwayeoa@unaab.edu.ng*

ABSTRACT

Morphological and molecular characterization of forty soybean (*Glycine max* (L.) Merr.) accessions were assessed using 7 morphological characters and 10 Randomly Amplified DNA Polymorphism (RAPD) primers, respectively. The field experiment was carried out at two locations using randomized complete block design with three replications. The aims of the research were to analyze the morphological and molecular organization of the existing diversity and to compare the genetic relatedness among forty soybean genotypes. The data were subjected to analysis of variance and correlation analysis to determine the extent of genetic variability and correlation coefficients among the characters, respectively. Principal Component Analysis (PCA) and Single Linkage Cluster Analysis (SLCA) were employed to group the accessions. The genetic relatedness among the accessions based on RAPD molecular markers was also presented in the form of a dendrogram generated by cluster analysis using the Unweighted Pair Group method with Arithmetic Mean (UPGMA). The relative effectiveness of the RAPD markers compared to botanical descriptors in assessing the diversity among the genotypes was investigated. The single linkage cluster technique classified the 40 accessions into seven clusters while the FASTCLUS technique revealed that the number of pods per plant, pod yield per plant, 100-seed weight and seed yield per plot contributed the largest proportion of morphological variation. Out of the 100 bands generated by the 10 primers, 31 were monomorphic and 69 polymorphic. The size of the fragment varied from 250 bp to 3000 bp. RAPDs markers were highly polymorphic and more discriminatory and informative as they were able to differentiate more pairs of genotypes than the botanical descriptors. The highest yield was recorded for TGx 1834-1E (477.60g/plot) and TGx 1910-2F (459.55).

Keyword: Genotypes, morphology, polymorphism, RAPD, soybean, UPGMA

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is a principal grain legume in developing countries where it meets the expanding needs for protein, edible oil and calories. It is a good source of cheap dietary protein in Africa (IITA, 1989). It is a miraculous crop due to its extraordinary qualities; it contains about 37-42% good quality protein, 6% ash, 29% carbohydrate and 17-24% oil, comprising 85% poly-unsaturated fatty acid with two essential fatty acids (lenoleic and linolenic acid), which are not synthesized by the human body (Antalina, 2000; Balasubramaniyan & Palaniappan, 2003). Soybean is grown in the tropical, subtropical and temperate climates.

A number of tropical soybean varieties, with improved yield and agronomic characteristics, have been developed and recommended to farmers (FAO, 1999). However, the selection and subsequent recommendation for release

Received: 17 December 2009
Accepted: 8 September 2010
*Corresponding Author

of most of these varieties have been based on a subjective traditional analysis of the yield data from a number of locations, with little or no emphasis on morphological and molecular characterizations.

The assessment of the genetic relationships among the cultivated plants at morphological and molecular levels is a fundamental component of crop improvement programmes. This will provide information about genetic diversity, identification of diverse parental combinations to develop segregating progenies with maximum genetic variability for further selection, duplication in germplasm, and introgression of desirable genes or chromosome segments from diverse sources into elite germplasm (Thompson *et al*., 1998; Das *et al*., 2001; Iqbal *et al*., 2008).

Diversity, based on the morphological characters, is essential in plant breeding as it reveals important traits to plant breeders (Singh, 1989); however, the phenotypes are highly influenced by many environmental factors. In plants with a narrow genetic base in their gene pool, such as soybean, molecular descriptors can provide additional information about the characterization, degree of diversity and genetic constitution of the existing germplasm.

Currently, the rapid development of biotechnology allows easy analysis of large numbers of loci distributed throughout the genome of plants (Chakravanthi & Naravaneni, 2006). The use of RAPD technique lies in its simplicity, rapidity, requirement for only a small quantity of DNA and the ability to generate numerous polymorphisms (Chowdhury *et al*., 2001; Zenglu & Nelson, 2002; Yu *et al*., 2005). Therefore, it has been a powerful and useful technique for genetic analysis. The objectives of this study were to investigate the morphological and molecular organizations of the existing diversity in forty soybean accessions and to ascertain the genetic relatedness among the accessions.

MATERIALS AND METHODS

The forty accessions used in the study were collected from the Genetic Resources Unit of the International Institute of Tropical agriculture (IITA), Nigeria. The experimental study was conducted during the wet season of the year 2006 at two locations, namely, Abeokuta (longitude: 07^0 30'N and latitude: 03^0 54'E) and Ile-Ife (longitude: 07^o28'N and latitude: 03^o34'N), Nigeria. The randomized complete block design, with three replications, was used for the experiment. Seeds were sown by drilling in four-row plots at 0.75m between rows. Two weeks after planting, seedlings were thinned down to a within-row spacing of 0.05 m leaving a population of 480 plants per plot size of $18m^2$. No pre-emergence herbicides and fertilizer was used and weeding was carried out as required.

Morphological and physiological data were collected on ten plants per accession from the net plot for days to 50% flowering, days to maturity, number of pods per plant, pod length per plant, pod yield per plant, seed yield per plant and 100-seed weight. The data collected were subjected to statistical analysis using SAS/PC version 9. The principal component grouping of the traits was employed to examine the percentage contribution of each trait to the total genetic variation. Meanwhile, cluster analysis based on similarity matrices was also employed on agro-botanical data using the un-weighted pair group method with arithmetic mean (UPGMA) to obtain a dendrogram. The cultivars were sorted into groups by the FASTCLUS procedure of the SAS.

RAPD Analysis

Total DNA was isolated from the fresh leaves of 14 day-old soybean seedlings, grown in the green house, according to Dellaporta *et al*. (1983). Gene-based RAPD analysis was performed on forty soybean accessions. Purified DNA was quantified by spectrophotometry and by ethidium bromide staining after electrophoresis. Ten RAPD primers (Table 6) were used to generate markers as described by Tao *et al*. (1993). Each amplification was performed in a reaction volume of 25µl containing 10mM Tris-HCl pH 9.0, 50mM $MgCl_2$, 0.2mg mL^{-1} gelatine, Triton x 100.0%, 0.1mM of each of dATP, dCTP,

dGTP and dTTP (Promega), 10ng of random primer, 50ng of genomic DNA and 2 units of Taq polymerase. Amplification was carried out in a thermocycler (Mpi model), as follows: one cycle of 3mins at 94°C, 44 cycles of 20 secs at 94°C; 40 secs at 37°C and 1 min at 72°C; one cycle of 7 mins at 72°C. The amplification products were then analyzed for polymorphism after electrophoresis in 1.4% agarose gels using 2.8g in 200mls 1 X TAE buffler Pairwise comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products used to generate similarity coefficients using the statistical software NTSYS-pc 2.0 (Rohlf, 1993). GSij = Nij / (Ni + Nj- Nij), where Ni is the number of detected bands in a variety i and not in variety j, Nj is the number of detected bands in a variety j and not in variety i, and Nij is the number of bands common to the varieties i and j (Jaccard, 1908). The similarity coefficient was used to construct a dendrogram by the UPGMA, according to Sneath and Sokal (1973), Swofford and Olsen (1990) and Rohlf (1993).

RESULTS

The combined analyses of variance for days to 50% flowering, days to maturity, number of pods per plant, pod length per plant, pod yield per plant, seed yield per plot and 100-seed weight revealed highly significant ($P < 0.01$) genotypic variation for all the traits (Table 1). The effect due to location was also highly significant for all the traits. Genotype x location effects were not significant for all the traits, except for the seed yield per plot. The seed yield per plot varied from one location to another, indicating that the selection for yield per plot has to be done at each location.

Table 2 presents the mean performance of 40 soybean accessions sown over two locations. TGM 80 and TGx 1830-20E recorded the earliest flowering day (37 days) and earliest maturity day (82 days), while TGM 119 recorded the longest flowering day (47 days) and the longest maturity day (93 days). TGx 1834-1F with the highest pod yield per plant of 6.7g had 100-seed weight of 11.1g whereas TGx 1919-8F, with pod yield per plant of 4.8g had the highest 100-seed weight of 18.0g. In addition, TGM 1906-1F had the highest number of pods (23) per plant.

Table 3 shows the correlation coefficients of the pairs of six traits that were used in characterizing the forty soybean accessions. The correlation matrix showed that seed yield per plot was positively and significantly associated with number of pods per plant, pod length, pod yield per plant and 100-seed weight. Positive and significant association was also observed between the number of pods per plant, pod length, pod yield per plant and 100-seed weight. Meanwhile, days to 50% flowering and Days to maturity were both negatively associated with 100-seed weight and seed yield per plot.

The three principal components accounted for 82.7% of the total variance, with the first principal component taking 51.24%. The relative Eigen values was high (3.66) for axis 1 and low (0.72) for axis 3. The first principal component was mostly correlated with the number of pods per plant, pod length, pod yield per plant, 100-seed weight and seed yield per plot. The characters that were mostly correlated with the second principal component were days to 50% flowering and days to maturity (Table 4).

The characteristic means of the seven similarity cluster groups in the 40 soybean accessions generated by the FASTCLUS technique are presented in Table 5. An examination of the range of means revealed that the number of pods per plant, pod yield per plant, 100-seed weight and seed yield per plot contributed the largest proportion of morphological variations that existed between the cluster groups. Nonetheless, there was no significant variation between clusters with respect to other traits. Cluster V consisted of 2 accessions, which represented 5% of the total accessions. The maximum number of pods per plant (19.96), pod yield per plant (6.57g), 100-seed weight (13.13g) and seed yield per plot (468.58g) were found in cluster V. Cluster IV accounted for 2.5% of the population. This cluster showed the least value for the number

TABLE 1
Combined mean squares of seed yield and related traits for soybean accessions sown in two environments

Source	df	Seed yield per plot (g)	Pod yield/ plant (g)	Days to 50% flowering	Days to maturity	Number of pods/plant	Pod length (cm)	100 seed weight (g)
Block	2	2.51**	6.90**	6.02	2.30	23.01**	0.59	10.79
Genotypes (G)	39	2.33**	4.97**	18.26**	5.27**	39.73**	0.91**	22.90**
Location (L)	1	126.72**	81.28**	403.00**	250.10**	2247.05**	29.19**	320.31**
G x L	39	0.86*	1.14	4.26	2.42	6.16	0.38	2.71
Error	158	0.54	1.29	3.87	1.70	4.29	0.21	7.01

*, ** Significant at 5% and 1% probability levels, respectively

TABLE 2
Mean performance of 40 soybean accessions sown in two locations in 2006

S/No	Accession	Seed yield/plot (g)	Pod yield/plant (g)	Days to 50% flowering	Days to maturity	Number of pods/plant	Pod length (cm)	100 seed weight (g)
1	TGx1909-3F	443.33	6.0	43	88.5	17	4.5	12.0
2	TGx 1904-6F	379.75	5.4	42	87.0	18	4.8	9.6
3	TGx 536-02D	302.25	4.3	44	88.5	15	4.3	8.3
4	TGx 1740-2F	359.03	5.6	43	87.0	18	4.5	10.3
5	TGx 1485-1D	354.35	5.2	42	87.0	15	4.2	10.1
6	TGx 1440-1E	336.25	4.9	42	86.0	19	4.2	8.9
7	TGx 1835-10E	348.05	4.8	42	86.5	15	4.9	9.3
8	TGx 1866-2F	433.00	6.4	43	88.5	18	4.4	12.8
9	TGM 119	252.80	3.4	47	92.5	16	3.6	8.6
10	UG-5	235.05	3.1	42	85.0	13	3.6	7.3
11	TGx 1904-4F	329.70	4.4	42	85.5	15	3.7	10.9
12	TGx 1910-14F	268.25	4.0	43	87.0	13	4.2	11.3
13	TGx 1883-33F	385.78	5.3	43	86.0	16	4.4	11.4
14	TGx 1908-8F	337.00	4.5	42	85.5	16	4.4	10.6

Table 2 (*continued*)

S/No	Accession	Seed yield/plot (g)	Pod yield/plant (g)	Days to 50% flowering	Days to maturity	Number of pods/plant	Pod length (cm)	100 seed weight (g)
15	TGx 1903-5F	322.87	4.0	43	87.0	14	4.2	11.1
16	TGx 1912-13F	301.65	4.5	44	87.5	14	4.4	12.1
17	TGM 63	252.05	3.7	43	87.0	13	4.0	9.9
18	TGM 255	211.02	3.5	42	86.5	13	3.4	9.1
19	TGx 1908-1F	395.35	5.3	40	84.0	16	4.4	11.7
20	TGx 1906-1F	290.00	4.0	42	87.5	13	3.8	11.8
21	TGM 479	441.15	5.3	43	87.0	23	4.4	13.2
22	Samsory-2	352.53	5.2	43	87.5	14	4.5	11.3
23	TGx 1910-3F	425.73	5.7	45	90.0	16	4.1	11.4
24	TGx 1878-7E	326.50	4.3	43	87.0	17	4.1	12.2
25	TGx 1884-18E	395.55	5.6	42	86.5	16	4.6	12.2
26	TGx 1910-2F	459.55	6.5	42	83.5	19	4.8	14.5
27	TGx 1834-1E	477.60	6.7	44	86.5	21	5.0	11.8
28	TGx 1919-8F	343.55	4.8	44	88.0	15	4.1	18.0
29	TGx 1908-9F	447.00	6.2	43	87.0	21	4.7	14.8
30	TGx 1448-2E	344.30	4.9	43	85.0	15	4.4	11.9
31	TGx 1830-20E	419.30	5.8	37	81.5	20	4.8	11.9
32	TGx 1838-5E	417.05	5.4	39	83.0	17	4.5	11.7
33	TGx 1888-15F	292.25	3.7	42	87.5	15	3.5	9.9
34	TGx 1844-4E	365.38	4.7	43	88.5	16	4.6	11.4
35	TGM 79	359.65	5.0	43	88.5	21	4.9	11.7
36	TGx 1903-3F	409.05	6.0	43	88.5	18	4.7	13.2
37	TGM 80	360.55	4.4	37	82.0	17	4.0	9.9
38	TGx 1019-2EB	293.73	4.0	42	86.5	15	4.2	13.9
39	TGM 1197	435.00	5.4	43	86.5	20	5.0	11.6
40	TGx 1902-1E	428.05	6.1	43	87.5	19	4.4	13.0
	LSD	82.80	1.29	1.85	1.28	2.36	0.52	2.99

TABLE 3
Correlation coefficients of six traits used in characterizing 40 soybean accessions

	Days to maturity	Number of pods per plant	Pod length (cm)	Pod yield per plant (g)	100-seed weight (g)	Seed yield per plot (g)
Days to 50% flowering	0.72**	-0.20**	-0.31**	-0.23**	-0.17**	-0.37**
Days to maturity		-0.14**	-0.23**	-0.22**	-0.18**	-0.34**
Number of pods per plant			0.54**	0.68**	0.40**	0.68**
Pod length				0.63**	0.39**	0.65**
Pod yield per plant					0.52**	0.87**
100-seed weight						0.55**

** Significant at 1% probability levels

TABLE 4
Principal components analysis showing the contribution (factor scores) of each character among the 40 soybean genotypes, Eigen values and percentage total variance accounted for by four principal components

Character	Prin 1	Prin 2	Prin 3	Prin 4
Days to 50% flowering	-0.11	0.69	-0.04	0.11
Days to maturity	-0.13	0.68	-0.15	-0.06
Number of pods per plant	0.44	0.07	-0.26	-0.67
Pod length	0.44	0.02	-0.27	0.72
Pod yield per plant	0.49	0.11	-0.06	0.03
100-seed weight	0.30	0.17	0.91	0.01
Seed yield per plot	0.50	0.05	-0.07	-0.07
Eigen value	3.66	1.85	0.72	0.38
% variance	0.52	0.26	0.10	0.05
Cumulative % variance	0.52	0.79	0.89	0.94

of pods per plant (12.42), pod yield per plant (3.52), 100-seed weight (9.09) and seed yield per plot (211.02).

The ten primers were assessed for their consistent production of strong amplification and reproducible bands across forty soybean genotypes (Table 6). A total of 100 bands were generated using ten selected primers. The number of polymorphic bands produced by each primer varied from four (OPG 11) to ten (OPG 16). Out of the 100 bands observed, 31 were monomorphic for all the varieties examined in this study. The remaining 69 variable bands were reproducible and polymorphic and thus, they were regarded as informative RAPD markers for the current genetic study as these markers were able to differentiate all the accessions. Each accession could be distinguished by at least four RAPD markers. The size of each fragment varied from 250 bp to 3000 bp, depending on the primer used for the amplification.

Fig. 1 shows the morphological dendrogram. It reveals the minimum distance between the clusters and the extent of morphological relationships between pairs of genotypes within each cluster group. The diagram proposed seven groups. TGx 1740-2F (4) in group 1; TGx 1904-6G (2), TGx 1440-1E (6), TGx 1844-4E (34) and

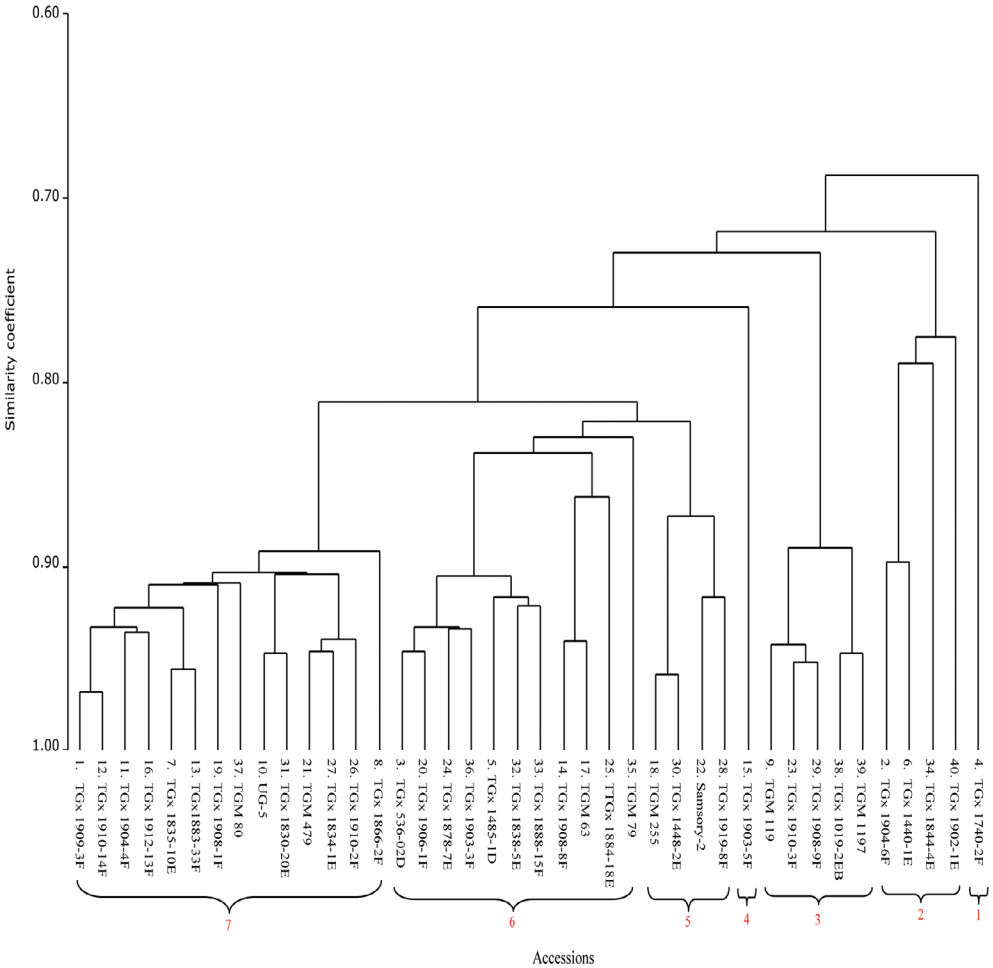

Fig. 1: Morphological dendrogram showing the similarity among 40 soybean accessions revealed by UPGMA cluster analysis

TGx 1902-1E (40) in group 2; TGM 119 (9) and TGM 197 (39) in group 3; TGx 1908-8F (14) and TGM 63 (17) in group 4; TGM 255 (18), Samsoy-2 (22), TGx 1919-8F (28) and TGx 1448-2E (30) in group 5; TGx 1903-5F (15) in group 6; TGx 1904-4F (11) and TGx 1910-14F (12) in group 7.

Fig.2 presents the molecular dendrogram, based on the similarity coefficients among the forty soybean accessions, as revealed by UPGMA cluster analysis based on the RAPD markers. The dendrogram shows a clear separation of the genotypes into five groups. For instance, TGx 1909-3F (1) and TGX 1910-8F (28) were in group 1, while TGx 1904-6F (2), UG-5 (10), TGx 1903-3F (36), TGx 1908-9F (19) were in group 2. The majority of the accessions that were evaluated were clustered in group 3, while groups 2 and 4 had five and six accessions, respectively. These groups (apart from groups 1 and 5) could be further divided into sub-groups at different similarity levels.

DISCUSSION

This study assessed the morphological and RAPD characterization of forty soybean accessions (*Glycine max* (L.) Merr. The analysis of morphological traits revealed that there is a possibility for selection among the accessions for the seven traits evaluated. In addition, there is a need to evaluate the soybean in two or more locations for seed yield to arrive at a logical conclusion with regards to the selection for this trait. According to Funnah and Mak (1980), no valid comparisons could be made regarding the relative performance of crop genotypes over all the environments in the presence of genotype by environment interaction.

The significant and positive associations observed between the seed yield per plot and the number of pods per plant, pod length and pod yield per plant indicated that these traits have influence on seed yield per plot and could be used as the selection criteria in soybean breeding programme. However, reduced days to maturity and days to 50% flowering tend to increase seed yield in soybean as it is negatively correlated to the number of pods per plant, pod length and 100-seed yield.

The result of the principal component analysis showed that different characters contributed differently to the total variation in the soybean genotypes, as indicated by the Eigen value as well as their weight and loading in different principal axes. The first principal component that accounted for the highest proportion of the total variation indicated the contribution of seed yield per plot, pod yield per plant, number of pods per plant and pod length to grain yield in soybean. If selection was to be made between the cluster groups for a future breeding exercise, these traits should then be given high priorities.

The cluster analysis had singular efficacy and ability to identify crop genotypes with the highest level of similarity through the dendrogram generated (Aliyu *et al*., 2000). The evaluation of phenetic diversity within soybean genotypes, using the cluster analysis in this study, provided seven clusters which were re-ordered into three clusters by the PCA analysis, with a lot of variations in the morphological properties. Mehetre *et al*. (1994) evaluated 51 soybean genotypes and found 10 clusters. Das *et al*. (2001) reported that the grouping pattern of the diverse genotypes suggested no parallelism between genetic divergence and geographical distribution of the genotypes. Ghatge and Kadu (1993) evaluated 58 soybean genotypes from diverse eco-geographical areas, and observed seven clusters on the basis of yield components. Meanwhile, Kumar and Nadarajan (1994) studied genetic diversity for yield components in 64 soybean genotypes, and revealed 11 clusters. Ihsan Ullah *et al*. (2007) reported 5 clusters derived from 10 genotypes on the basis of seed yield in sunflower.

Broschat (1979) considered PCA as a powerful technique for data reduction as it removes interrelationships among the components. The results reported by various researchers showed the multivariate analysis as a valid system to deal with germplasm collection. Rabbani *et al*. (1998) determined the extent of diversity and the relationship among

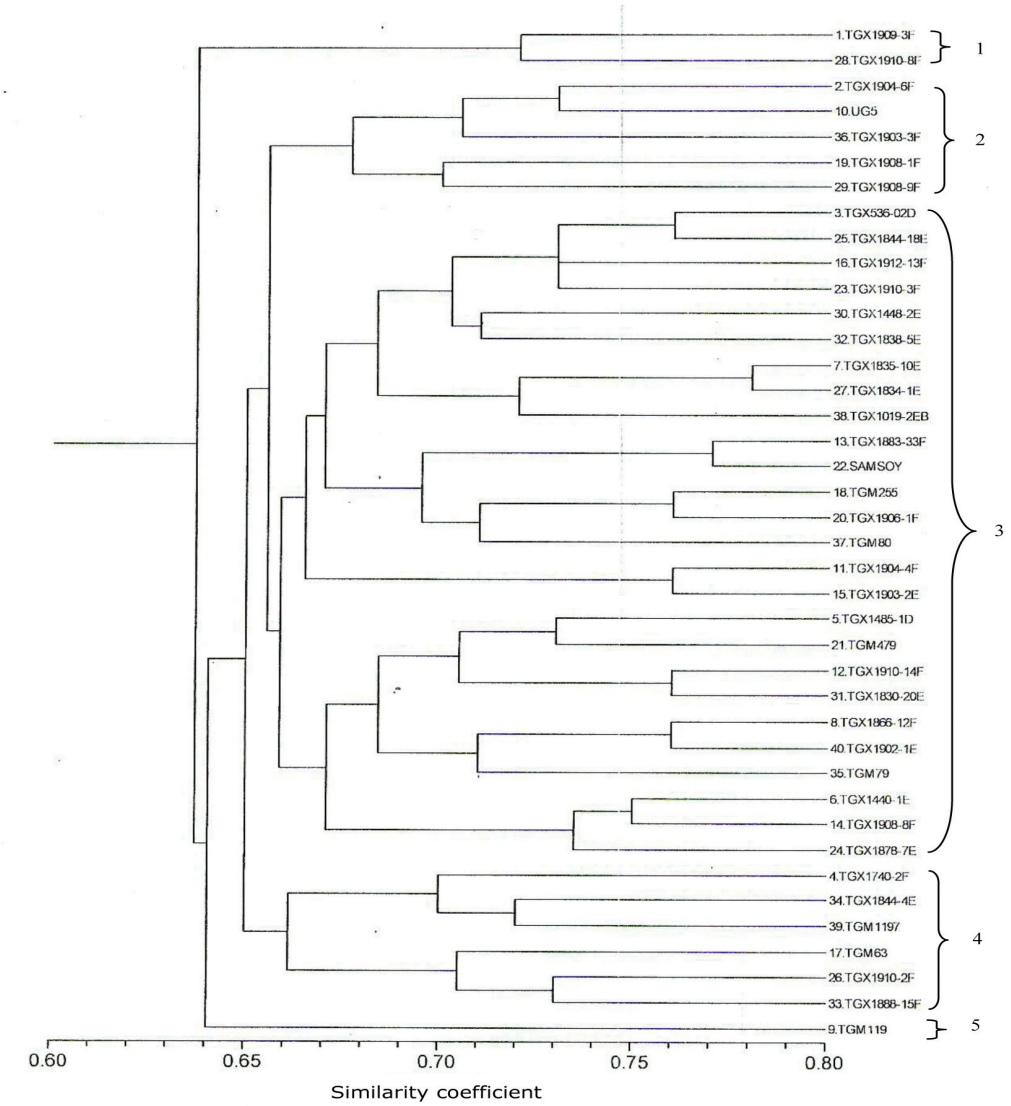

Fig. 2: Molecular dendrogram showing the genetic similarity among 40 soybean accessions revealed by UPGMA cluster analysis based on RAPD markers

Brassica juncea germplasm from Pakistan for 35 morphological characters in 52 accessions using the cluster and principal component analysis. Meanwhile, Ghafoor *et al*. (2001) studied genetic diversity in blackgram germplasm accessions. Quantitative traits were analysed for the cluster and principal component analysis. Iqbal *et al*. (2008) conducted cluster and principal component analyses on soybean germplasm. The five clusters observed on the dendrogram were reduced to only three when PC1 was plotted against PC2.

Although the morphological dendrogram generated from the similarity or distance matrices had provided an overall pattern of variation as well as the degree of relatedness among genotypes, the variations in environmental conditions such as soil types, and soil fertility

TABLE 5
Characteristics pattern of seven groups of soybean genotypes showing their mean values with standard deviations in parentheses

Character	I 14, 17, 20, 24, 25, 3, 32, 33, 36, 5	II 2, 34, 40, 6	III 13, 18, 19, 22, 28, 30, 7, 8	IV 4	V 15, 35	VI 23, 29, 38, 39, 9	VII 1, 10, 11, 12, 16, 21, 26, 27, 31, 37	Min	Max
Days to 50% flowering	42.10 (2.47)	43.84 (2.30)	41.52 (2.17)	42.00 (0.00)	42.75 (1.06)	42.75 (1.09)	42.52 (0.85)	41.52	43.84
Days to maturity	86.78 (2.63)	87.79 (3.26)	86.11 (2.22)	86.50 (0.00)	85.00 (2.12)	87.47 (0.77)	86.43 (0.98)	85.00	87.79
Number of pods per plant	18.97 (2.10)	13.63 (1.83)	17.38 (1.54)	12.42 (0.00)	19.96 (1.12)	14.33 (0.94)	15.44 (1.52)	12.42	19.96
Pod length	4.54 (0.23)	3.84 (0.28)	4.50 (0.27)	3.38 (0.00)	4.89 (0.09)	4.10 (0.28)	4.27 (0.31)	3.38	4.89
Pod yield per plant	5.88 (0.33)	3.56 (0.39)	5.15 (0.43)	3.52 (0.00)	6.57 (0.13)	4.11 (0.30)	4.69 (0.38)	3.52	6.57
100-seed weight	12.59 (1.10)	9.25 (1.69)	11.02 (0.96)	9.09 (0.00)	13.13 (1.87)	11.25 (2.17)	11.41 (2.54)	9.09	13.13
Seed yield per plot	429.87 (12.33)	252.04 (13.56)	375.13 (15.89)	211.02 (0.00)	468.58 (12.76)	295.98 (5.62)	339.51 (10.86)	211.02	468.58

TABLE 6
Nucleotide sequence of the selected primers with the number of amplified products and fragment size range (bp)

Primer	Sequence 5' to 3'	Polymorphic bands	Monomorphic bands	Fragment size range (bp)
OPG 11	TGCCCGTCGT	4	4	500 – 2000
OPI 15	TCATCCGAGG	6	5	250 – 2500
OPJ 05	CTCCATGGGG	7	3	500 – 1750
OPK 11	AATGCCCCAG	8	2	500 – 2000
OPL 13	ACCGCCTGCT	5	4	400 – 3000
OPP 12	AAGGGCGAGT	7	4	800 – 3500
OPS 18	CTGGCGAACT	8	2	300 – 2000
OPU 16	CTGCGCTGGA	10	2	500 – 2500
OPX 04	CCGCTACCGA	7	3	750 – 2500
OPY 20	AGCCGTGGAA	7	2	500 – 2000
Total		69	31	

levels (Steel, 1972), light, temperature and moisture regime (Sumerfield & Huxley, 1973; Morakinyo & Ajibade, 1998), allow for different results to be obtained using morphological grouping, particularly when the experiments were repeated in time and/or space. The observations above tend to emphasize the superiority and convincing discriminatory evidence of molecular grouping over and above the morphological grouping. The five major clusters of the RAPD dendrogram, together with their internal groups, demonstrated the polymorphic nature of the 40 soybean genotypes used in the current study.

The dendrogram obtained from the RAPD markers revealed that the markers were more discriminatory, highly polymorphic, and thus, more informative than the one obtained from the morphological characters because the markers were based on the ten OPERON primers to generate 69 RAPD bands across the 40 soybean accessions. Moreover, most of the bands were polymorphic and each band was able to differentiate between at least two of the 40 soybean accessions. Thus, differentiation among the soybean genotypes was higher using RAPD markers than the morphological characters. RAPD is a valuable tool for assessing genetic diversity levels in vegetable soybean. It detects polymorphism at the DNA level and thus is more efficient in discrimination among the varieties (Chowdhury *et al*., 2001).

In conclusion, both morphological and genetic variations were found to exist among the 40 soybean accessions that were evaluated. The number of pods per plant, pod yield per plant, 100-seed weight and pod yield per plot contributed a greater proportion of the variations that existed among the cluster groups. The dendrogram obtained from the molecular markers was more discriminatory than the one obtained from the morphological characters because the markers were based on 69 polymorphic markers produced by ten primers. The molecular dendrogram clustered the 40 soybean accessions into five groups, whereas the one based on the morphological characters had seven. The Principal Component Analysis indicated that all the 40 soybean accessions were ordered into three distinct PCA clusters.

The present study has indicated TGx 1834-1E and TGx 1910-2F as high yielding genotypes, and accessions TGM 80 and TGx 1830-20E as early maturing genotypes; however, it is unlikely that these accessions are the best to be found in the germplasm. Thus, collection, conservation and further evaluation for the selection of better germplasm are essential.

REFERENCES

Aliyu, B. N., & I. Fawole. (2000). Inheritance of Pubescence in crosses between *Vigna unguiculata* and *V. rhomboidea*. *Nig. J. Genet*., 15, 9-14.

Antalina, S. (2000). Modern processing and utilization of legumes. Recent Research and Industrial achievement for soybean food in Japan *Proceeding of RILET-JIRCAS*. Workshop on Soybean Research. September 28, Malang-Indonesia.

Balasubramaniyan, P., & S. P. Palaniappan. (2003). *Principles and practices of agronomy* (p. 45-46). India: Agrbios

Broschat, T. K. (1979). Principal component analysis in horticultural research. *Hort. Sci*., 14, 114-117.

Chakravanthi, B. K., & R. Naravaneni. (2006). SSR marker based DNA finger printing and diversity study in rice (*Oriza saiva* L.). *Afr. J. Biotechnol*., *5*(9), 684-688.

Chowdhury, A. K., P. Srinives, P. Tongpamnak, & P. Saksoon. (2001). Genetic diversity based on morphology and RAPD analysis inn vegetable soybean. *Korean J. Crop Sci*., *46*(2), 112-120.

Das, S. P., P. N. Harer, & A. B. Biradar. (2001). Genetic divergence and selection of genotypes in soybean. *Journal of Maharashtra Agricultural Universities*, *25*(3), 250-252.

Dellaporta, S., Wood, J., & Hicks, J. (1983). A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter, 1*(4), 19-21.

Food and Agriculture Organization (FAO). (1999). *Manual for training in seed production* (p. 11). Trivedi, R. K., I. A. Usman, & J. C. Umeh (Eds.). FAO: United Nations.

Ghafoor, A., A. Sharif, Z. Ahmad, M. A. Zahid, & M. A. Rabbani. (2001). Genetic diversity in blackgram (*Vigna mungo* (L.) Hepper). *Field Crops Res*., 69, 183-190.

Ghatge, R. D., & Kadu, R. N. (1993). Genetic diversity in soybean. *Ann. Agric. Res*., *14*(2), 143-148.

Ihsan Ullah, M. A., M. R. Khan, M. Ashraf, M. Y. Mirza, & M. Yousaf. (2007). Garphical analysis of multi-environment trial (MET) data in sunflower (*Helianthus annuus* L.) through clustering and GGE biplot technique. *Pak. J. Bot*., 39(5), 1639-1646.

International Institute of Tropical Agriculture (IITA). (1989). Genetic Resources for Tropical Agriculture, Pamphlet, p.32.

Iqbal, Z., M. Arshad, M. Ashraf, T. Mahmood, & A. Waheed. (2008). Evaluation of soybean (*Glycine max* (L.) Merrill) germplasm for some important morphological traits using multivariate analysis. *Pak. J. Bot*., *40*(6), 2323-2328.

Jaccard, P. (1908). Nouvelles réchérchés sur la distribution florale. *Bull. Soc. Vaudoise Sci. Nat*. *44*, 223-270.

Kumar, M., & N. Nadarajan. (1994). Genetic divergence studies in soybean (*Glycine max* (L.) Merrill). *Indian J. Genet. Plant Breed*., *54*(3): 242-246.

Mehetre, S. S., C. R. Mahajan, P. A. Patil, & D. N. Hajare. (1994). Genetic divergence in soybean (*Glycine max* (L.) Merrill.). *Indian J. Genet. Plant Breed*., *54*(1): 83-88.

Morakinyo, J. A., & S. R. Ajibade. (1998). Characterization of the segregants of an improved cowpea line IT84K-124-6. *Nig. J. Sci., 32*, 27-32

Rabbani, M. A., A. Iwabuchi, Y. Murakami, T. Suzuki, & K. Takayanagi. (1998). Phenotypic variation and the relationship among mustard (*Brassica juncea* L.) germplasm from Pakistan. *Euphytica*, *101*, 357-366.

Singh, S. P. (1989). Pattern of variation in cultivated dry bean. Annual Rep. Bean improvement. *Coop, 31*, 180-182.

Sneath, P. H. A., & R. Sokal. (1973). *Numerical Taxonomy* (P. 573). San Francisco: W. H. Freeman.

Steel, W. M. (1972). *Cowpeas in Nigeria*. (Doctoral dissertation). University of Reading, U.K.

Summerfield, R. J., & P. A. Huxley. (1973). *Day length and night temperature sensitivity screening of selected cowpea and soybean cultivars*. Reading University/IITA International Communication No.5. University of Reading, England.

Swofford, D. L., & G. J. Olsen. (1990). Phylogenetic Reconstruction. In Hillis, D. M., & C. Moritz (Eds.), *Molecular systematic* (p. 411-501). Sunderland: Sinauer Associaes.

Tao, Y., J. M. Manners, M. M. Ludlow, & R. G. Henzell. (1993). DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L) Moench) *Theor. Applied Genet*., *86*, 679-688.

Thompson, J. A., R. L. Nelson, & L. O. Vodkin. (1998). Identification of diverse soybean germplasm using RAPD markers. *Crop Sci*., *38*, 1348–1355.

Yu, C. Y., S. W. Hu, H. X. Zhao, A. G. Guo, & G. L. Sun. (2005). Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). *Theoretical and Applied Genetics*, *110*(3), 511-518

Zenglu, L., & R. L. Nelson. (2002). RAPD Marker Diversity among Cultivated and Wild Soybean Accessions from Four Chinese Provinces *Crop Science, 42*, 1737-1744.