

*Full Length Research Paper*

# **Morphological variability and molecular characterisation of thirty soybean genotypes using random amplified polymorphic DNA (RAPD) markers**

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The genetic and morphological diversity of soybean germplasms was studied using random amplified polymorphic DNA (RAPD) markers and morphological traits such as plant height, height of cotyledon leaves, basal leaf length, basal leaf width, basal leaf area, terminal leaflets number, terminal leaflet length, terminal leaflet width, terminal leaflet area and basal leaf petiole length. A total of 30 germplasms were investigated. The number of amplification products obtained ranged between 6 and 9. An average of five bands was obtained per primer set. The RAPD analysis of the 30 soybean germplasms revealed a total of 23 bands, amplified by 14 different oligonucleotide primers. Sixteen of these bands were highly polymorphic with percentage polymorphism at 70%, indicating the germplasms investigated had a high level of genetic diversity. The consensus tree generated from the genetic diversity analysis placed the soybean germplasms into five groups at an agglomerate coefficient of 0.6 (similarity level). Similarly, cluster analysis from the morphological characterisation revealed 5 clades with an average distance between clusters of 0.75. There was a significant lack of correlation between the RAPD markers and the morphological traits evaluated. Although, both analyses grouped the soybean genotypes into 5 clades, the composition of the individual clusters were very variable and divergent with the correlation between the largest clusters in the molecular and morphological characterisation at only 35-42%.

**Key words:** Soybean, germplasm, random amplified polymorphic DNA (RAPD), genetic diversity, morphological variability, polymorphism.

## **INTRODUCTION**

Soybean (*Glycine max*) is a leguminous plant. It originated from Asia and presently, China and United States of America are the world's largest producers

(FAO, 2011). In Nigeria, soybean cultivation started since 1928 and presently, Benue, Niger and some parts of Kaduna States are the major producing areas in the

country. As a single seasonal crop, it is grown in middle of June and harvested at the beginning of dry season (October – November) to avoid field losses (International Institute for Tropical Agriculture- IITA, 2009).

The advent of molecular biology has revolutionized the field of plant systematics and has been used successfully in establishing phylogenetic relationships at all taxonomic levels (Bohs, 2005). It has also been used in DNA fingerprinting of plant genomes (Cervera et al., 1998) and in genetic diversity studies (Isshiki et al., 2008). The use of molecular techniques in genetic diversity studies is supported by the finding that evolutionary forces such as natural selection and genetic drift produce divergent phylogenetic branching which can be recognized because the molecular sequences on which they are based, share a common ancestor (Singh et al., 2010). A number of genetic maps have been developed for soybean with several types of molecular markers. Hwang et al. (2009) mapped 1810 simple sequence repeats (SSR) site markers in some recombinant inbred populations of soybean, from which they constructed high-density linkage maps.

Random amplified polymorphic DNA (RAPD), when compared with other molecular markers, is more effective in this regard as it is simple, rapid, requires only a small quantity of DNA and it is well adapted for nonradioactive DNA fingerprinting of genotypes (Cao et al., 1999). It is also able to generate numerous polymorphisms (Williams et al., 1990). More recent studies have used molecular markers to identify genetically diverse plant introductions to be used for crosses in cultivar improvement programs (Thompson and Nelson, 1998a, b; Thompson et al., 1998; Narvel et al., 2000). These studies have had more success than conventional selection programs in producing productive lines from plant introductions crossed with elite genotypes. Khare et al. (2013) used RAPD to investigate the genetic diversity and relatedness of 38 soybean germplasms; they found 259 RAPD marker loci with 253 of them polymorphic. Perić et al. (2014) compared three methods in the assessment of diversity in soybean, and RAPD was found to be more superior in assessing differences among genetically similar genotypes. Alamri (2014) also studied inter simple sequence repeat (ISSR) and RAPD markers in detecting genetic variation in soybean. The results revealed that the levels of polymorphic loci detected with the two marker systems were in general moderate and similar. Very recently, Pakyürek and Akçin (2015) also used RAPD markers to study genetic diversity in seven different soybean cultivars, the results revealing significant polymorphisms. In Nigeria, not much has been

done on the nature of genetic diversity and characterization of *Glycine max*, especially using molecular tools. There is a need for extensive evaluation of new germplasms found in Nigeria to determine their genetic diversity.

This research work aimed to assess the morphological variability and molecular diversity of soybean (*Glycine max*.) using RAPD markers.

## MATERIALS AND METHODS

Thirty soybean germplasms (representing several independent breeding sources from IITA) used in this study were obtained from Molecular Biology Laboratory, University of Agriculture, Makurdi, Benue State, Nigeria. The soybean germplasms are shown in the Table 1.

### Planting of soybean germplasms

Three seeds of each germplasm were planted in plastic pots and thinned to two stands per pot at seven days after planting.

### Collection of DNA sample on Fast Technology Application (FTA) Plant card (Whatman, 2014)

Young leaves of soybean at 14 days after planting were excised from the plants. Depending on the size of the leaves, either whole leaves or portions of the leaves were mechanically crushed and disrupted on FTA plantsaver cards using porcelain pestle. The crushed leaves on FTA plantsaver cards were air-dried at room temperature for 1 h. Afterwards, 2 mm circles of FTA plantsaver cards containing leaf tissue were collected using a Harris Micro-Punch (Life Technologies) and was transferred into a thin-wall micro centrifuge tubes.

### Preparation of plant genomic DNA

200 µl of FTA purification reagent was added to each of the micro centrifuge tubes from above. The micro centrifuge tubes were vortex and mixed for 1–2 s at low speed. The tubes were incubated at room temperature for 5 min, and the wash solution was discarded. The washes were repeated with FTA reagents 2 more times, followed by 2 more washes with 1TE buffer (10 mmol/L Tris, 0.1 mmol/L ethylene diamine tetracetic acid (EDTA), pH 8.0). The FTA paper cards were air-dried at room temperature for 1 h, and the paper punches placed directly into PCR reagents for analysis using the method of Lin et al. (2000) and Omoigui et al. (2011).

### Polymerase chain reaction (PCR) protocol

Aliquots of DNA samples from each genotype were amplified with

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**Table 1.** List of soybean germplasms.

S/N	Genotype
1	TGX 1989-11F
2	TGX 1990-110FN
3	TGX 1989-42F
4	TGX 1990-95F
5	TGX 1989-45F
6	TGX 1989-53FN
7	TGX 1993-4FN
8	TGX 1989-75FN
9	TGX 1990-78F
10	TGX 1990-114FN
11	TGX 1987-62F
12	TGX 1448-2E
13	TGX 1989-40F
14	TGX 1990-52F
15	TGX 1989-48FN
16	TGX 1990-40F
17	TGX 1989-49FN
18	TGX 1990-57F
19	TGX 1989-68FN
20	TGX 1990-46F
21	TGX 1990-55F
22	TGX 1987-10F
23	TGX 1935-10F
24	TGX 1485-1D
25	TGX 1945-1F
26	TGX 1951-3F
27	TGX 1935-3F
28	TGX 1904-6F
29	TGX 1951-4F
30	TGX 1955-4F

each of the 14 oligonucleotide primers (Inqaba Inc. South Africa) listed in Table 2 using a thermal cycler. The PCR were carried out in 25 µl volumes containing 6.5 µl of 10 × Taq buffer, 4 µl of 2 mM dNTP mix, 1 µl primer, 1 µl of Taq polymerase, 1 µl MgCl<sub>2</sub>, 10.5 µl Nuclease-free water and 1 µl template DNA. The PCR method has an initial denaturation step at 94°C for 5 min, followed by 44 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min. A final extension step was given at 72°C for 7 min (Mundewadikar and Deshmukh, 2014). The reaction was repeated two times for each selected primer.

#### Gel electrophoresis

The PCR products were electrophoresed on 2% agarose gel in a horizontal gel electrophoresis tank. 10 µl of DNA ladder and 16 µl of PCR product were loaded on the gel and resolved for about 40 min. The gel was stained with ethidium bromide and visualized on a Benchtop UV transilluminator and photographed with digital camera for scoring.

**Table 2.** List of primers used for RAPD analysis.

S/N	Primer name	Primer sequence (5' – 3')
1	OPA-01	CAGGCCCTTC
2	OPA-02	TGCCGAGCTG
3	OPA-03	AGTCAGCCAC
4	OPA-04	AATCGGGCTG
5	OPA-05	AGGGGTCTTG
6	OPA-07	GAAACGGGTG
7	OPA-08	GTGACGTAGG
8	OPA-09	GGGTAACGCC
9	OPA-10	GTGATCGCAG
10	OPC-01	TTCGAGCCAG
11	OPC-02	GTGAGGCGTC
12	OPC-05	GATGACCGCC
13	OPC-07	GTCCCGACGA
14	OPC-08	TGGACCGGTG

#### Scoring and data analysis

The data for RAPD analysis were scored from digital photographs of ethidium bromide-stained agarose gels using the Ultraviolet Bench Top illuminator. DNA bands were considered to be similar if they occurred exactly at the corresponding position on the gel. Band size was determined by comparing with a standard DNA ladder run alongside the soybean genotypes. An RAPD band profile, presence (1) or absence (0) was recorded into a binary matrix. This matrix was subjected to genetic similarity analysis using root mean square distance (RMSD) coefficient, and clustering was done using a dendrogram constructed by an Unweighted Paired group Method using Arithmetic Averages (UPGMA) (Garcia et al., 1999).

#### Morphological characterisation

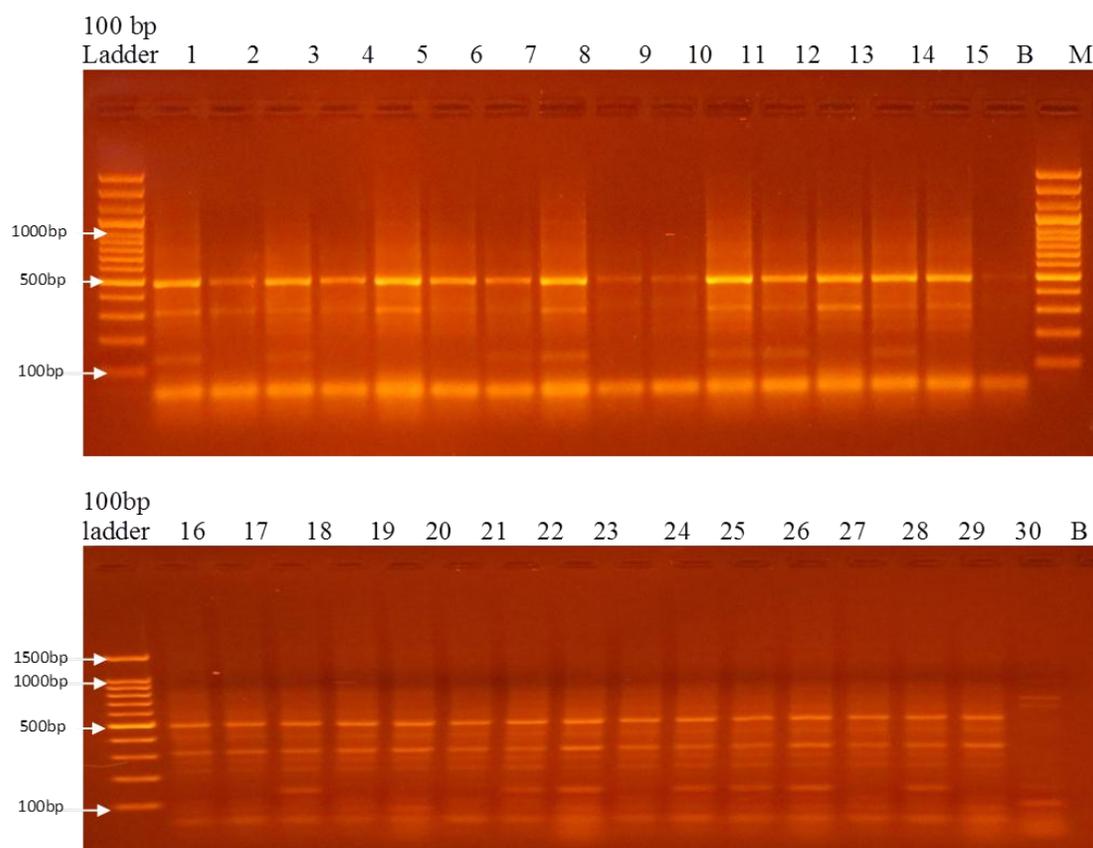
The morphological traits analyses were carried out at three weeks of planting. Parameters measured in centimetres included: Plant height, height of cotyledon leaves, basal leaf length, basal leaf width, basal leaf area, terminal leaflets number, terminal leaflet length, terminal leaflet width, terminal leaflet area and basal leaf petiole length. Plant height was measured from ground level to stem tip for three plants and mean plant height was recorded. Cotyledon height was measured from ground level to cotyledon tip, for three plants and mean cotyledon height was recorded. Basal leaf length was measured from lamina tip to the point of intersection of the lamina and stem and basal leaf width was measured from tip to tip between the widest lamina. The leaf area (basal and terminal) of each leaf was calculated using the method described by Pandy and Singh (2011). Data generated was subjected to hierarchical clustering using UPGMA, and a dendrogram was generated for the 10 traits studied using SAS 9.3.

## RESULTS

The RAPD analysis of the 30 samples revealed a total of 23 bands (Table 3). Sixteen of these bands were highly

**Table 3.** Analysis of RAPD banding pattern for soybean germplasms.

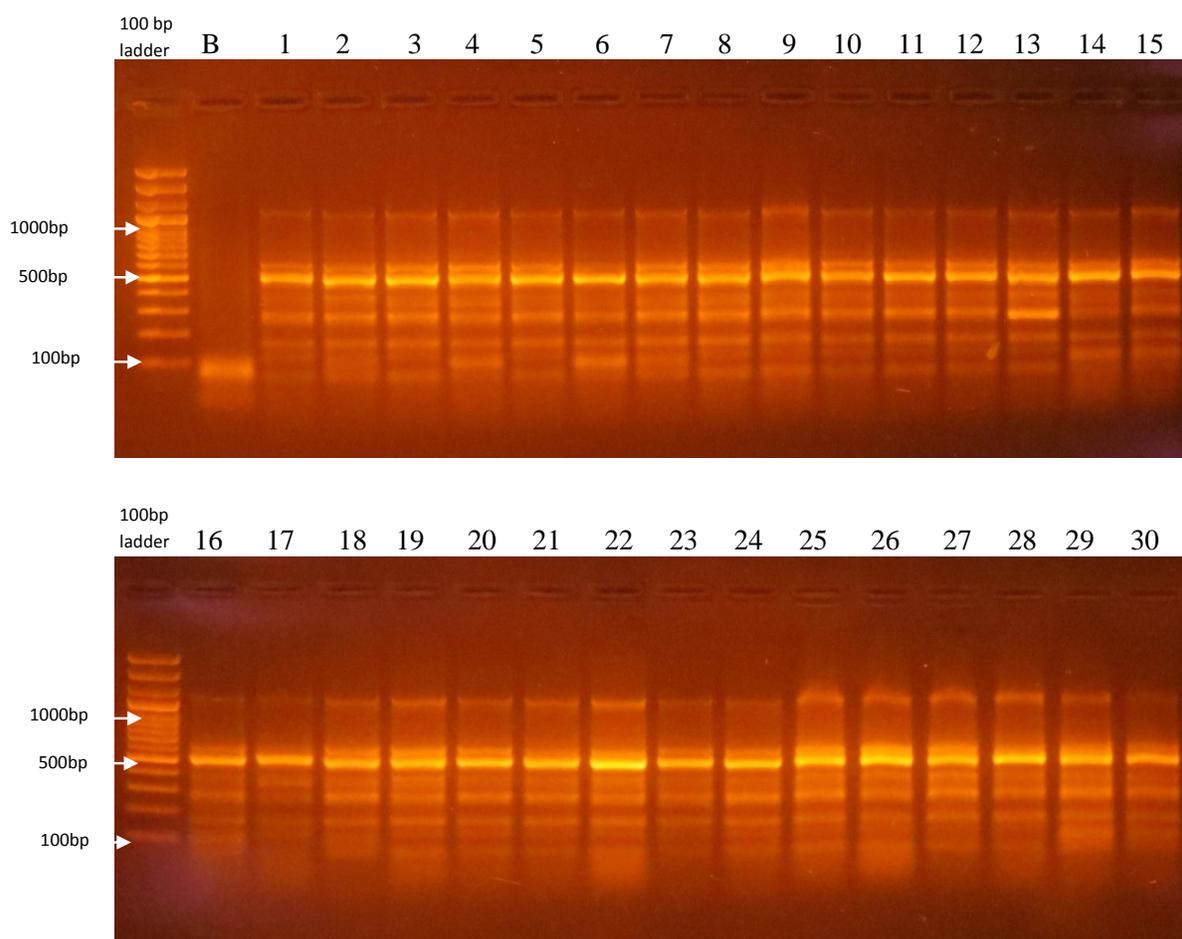
Primer set	Total number of bands	Number of polymorphic bands	Percentage polymorphic bands (%)
OPA-01, OPA-02, OPA-03 and OPA-04	6	5	83.3
OPA-05, OPA-07, OPA-08 and OPA-09 and OPA-10	9	3	33.3
OPC-01, OPC-02, OPC-05, OPC-07 and OPC-08	8	8	100
Total	23	16	70
Average	7.7	5.3	



**Figure 1.** Band profile of multiplex OPA-01, OPA-02, OPA-03 and OPA-04 Primers. 100 bp DNA ladder serves as reference point; B represents the blank; 1 to 30 corresponds to bands produced by the amplified DNA from 30 soybean germplasms studied.: 1= TGX 1935-3F; 2= TGX 1987-62F; 3= TGX 1990-78F; 4= TGX 1993-4FN; 5= TGX 1989-45F; 6= TGX 1904-6F; 7= TGX 1990-40F; 8= TGX 1989-68FN; 9= TGX 1990-52F; 10= TGX 1990-57F; 11= TGX 1989-42F; 12= TGX 1990-55F; 13= TGX 1835-10E; 14= TGX 1990-95F; 15= TGX 1990-114FN; 16= TGX 1989-53FN; 17= TGX 1448-2E; 18= TGX 1990-46F; 19= TGX 1987-10F; 20= TGX 1485-1D; 21= TGX 1945-1F; 22= TGX 1951-4F; 23= TGX 1990-110FN, 24= 1989-11F; 25= 1989-40F; 26= TGX 1955-4F; 27= TGX 1989-48FN; 28= TGX 1951-3F; 29= TGX 1989-49FN and 30= TGX 1989-75FN. The band fragments of multiplex of OPA-05, OPA-07, OPA-08, OPA-09 and OPA-10 primers ranged from 100 to 110 bp or 1.1 kbp

polymorphic with percentage polymorphism at 70%. The amplified band sizes varied between 100 and 1100 bp. The numbers of amplification products obtained were in the range 6 to 9. Multiplex of OPA-01, OPA-02, OPA-03 and OPA-04 primers produced the minimum number of polymorphic bands (6), OPA-05, OPA-07, OPA-08 and

OPA-09 primers produced the highest number of polymorphic bands (9) and multiplex of OPA-10, OPC-01, OPC-02, OPC-05 and OPC-07 primers produced 8 polymorphic bands representing 100% polymorphism. An average of 5 bands was also obtained per primer set. Figures 1 to 3 show the band profiles of RAPD markers



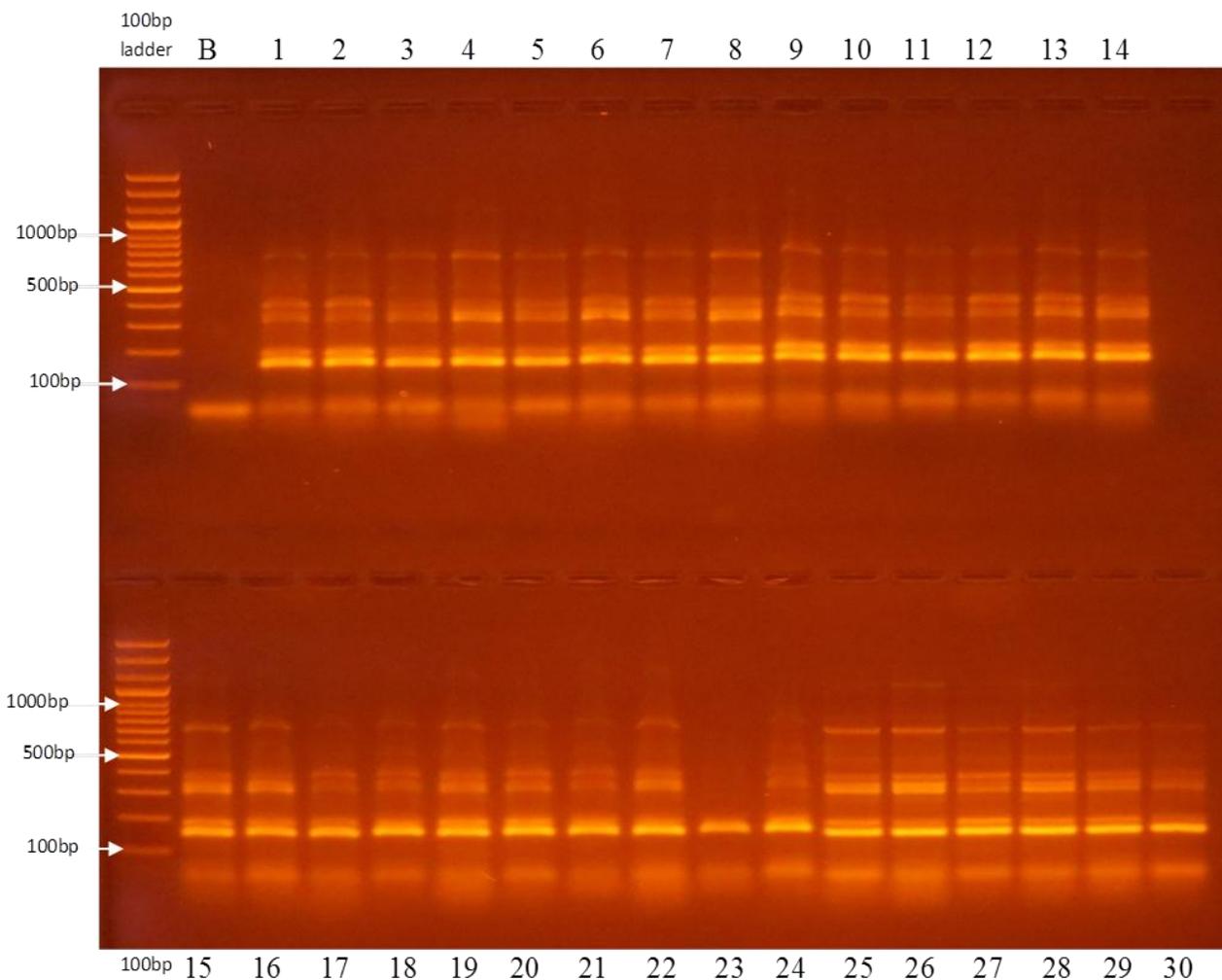
**Figure 2.** Band profile of multiplex OPA-05, OPA-07, OPA-08, OPA-09 primers and OPA-10. 1=TXG 1935-3F; 2=TXG 1987-62F; 3=TXG 1990-78F; 4=TXG 1993-4FN; 5= TXG 1989-45F; 6=TXG 1904-6F; 7= TXG 1990-40F; 8= TXG 1989-68FN; 9= TXG 1990-52F; 10=TXG 1990-57F; 11= TXG 1989-42F; 12= TXG 1990-55F; 13= TXG 1835-10E; 14= TXG 1990-95F; 15= TXG 1990-114FN; 16= TXG 1989-53FN; 17= TXG 1448-2E; 18= TXG 1990-46F; 19= TXG 1987-10F; 20= TXG 1485-1D; 21= TXG 1945-1F; 22= TXG 1951-4F; 23= TXG 1990-110FN, 24=1989-11F; 25=1989-40F; 26=TXG 1955-4F; 27= TXG 1989-48FN; 28= TXG 1951-3F; 29= TXG 1989-49FN and 30= TXG 1989-75FN. The band fragments of Multiplex OPC-01, OPC-02, OPC-05, OPC-07 and OPC-08 primers ranged from 150 bp to 700 bp.

for the thirty soybean germplasms studied. The band fragments of multiplex OPA-01, OPA-02, OPA-03 and OPA-04 primers ranged from 120 to 500 bp.

Figure 4 represents the distance matrix tree from the RAPD markers showing the degree of genetic diversity and/or relatedness of the 30 soybean germplasms. The consensus tree generated placed the soybean germplasms into 5 groups at an agglomerate coefficient of 0.6 (similarity level). Cluster 1 revealed the genetic relatedness of 5 germplasms namely, TXG 1935-3F, TXG 1989-45F, TXG 1990-95F, TXG 1990-114FN and TXG 1989-75FN. Cluster 2 comprised of six germplasms namely, TXG 1987-62F, TXG 1989-53FN, TXG 1990-46F, TXG 1989-49FN, TXG 1993-4FN and TXG 1904-6F. Cluster 3 being the cluster with the largest germplasms

comprised of 18 closely related germplasms namely, TXG 1990-78F, TXG 1989-42F, TXG 1989-68FN, TXG 1990-55F, TXG 1448-2E, TXG 1945-1F, TXG 1951-4F, TXG 1935-10E, TXG 1987-10F, TXG 1485-1D, TXG 1989-48FN, TXG 1990-52F, TXG 1990-57F and TXG 1990-40F. Cluster 4 comprised of 3 germplasms namely, TXG 1989-40F, TXG 1955-4F and TXG 1951-3F. Lastly, cluster 5 consisted of 2 germplasms (TXG 1989-11F and TXG 1990-110FN) which are outliers that are genetically different from all other germplasms.

Similarly, cluster analysis from the morphological characterisation of the soybean germplasms (Figure 5) revealed 5 clades with an average distance between clusters of 0.75. Cluster 1 comprised ten germplasms namely, TXG 1448-2E, TXG 1989-48FN, TXG 1951-3F,



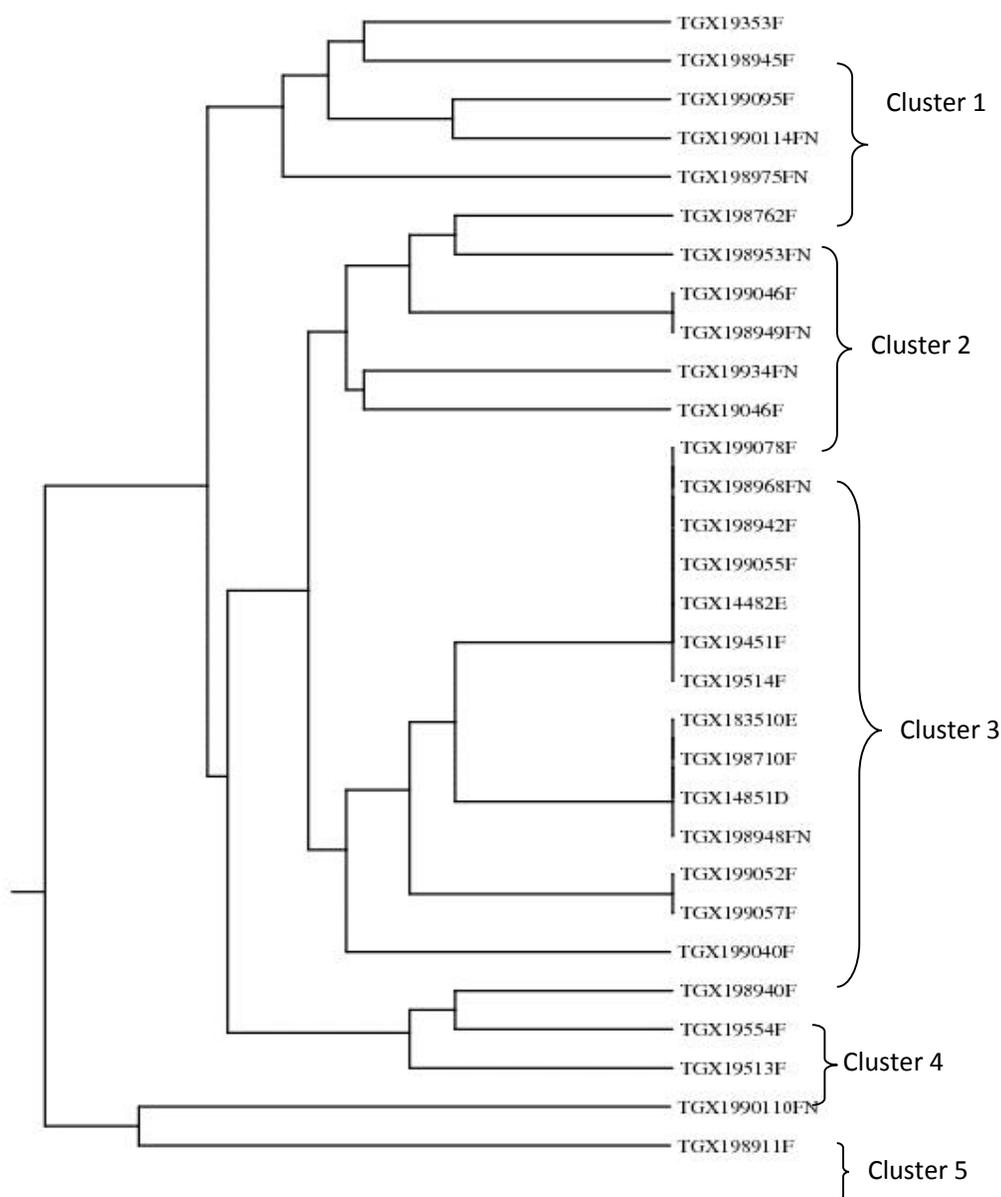
**Figure 3.** Band Profile of Multiplex OPC-01, OPC-02, OPC-05, OPC-07 and OPC-08 primers. 1= TGX 1935-3F; 2= TGX 1987-62F; 3= TGX 1990-78F; 4= TGX 1993-4FN; 5= TGX 1989-45F; 6= TGX 1904-6F; 7= TGX 1990-40F; 8= TGX 1989-68FN; 9= TGX 1990-52F; 10= TGX 1990-57F; 11= TGX 1989-42F; 12= TGX 1990-55F; 13= TGX 1835-10E; 14= TGX 1990-95F; 15= TGX 1990-114FN; 16= TGX 1989-53FN; 17= TGX 1448-2E; 18= TGX 1990-46F; 19 TGX 1987-10F; 20= TGX 1485-1D; 21= TGX 1945-1F; 22= TGX 1951-4F; 23= TGX 1990-110FN, 24=1989-11F; 25=1989-40F; 26= TGX 1955-4F; 27= TGX 1989-48FN; 28= TGX 1951-3F; 29= TGX 1989-49FN and 30= TGX 1989-75FN.

TGX1989-68FN, TGX 1990-52F, TGX 1989-45F, TGX 1935-3F, TGX 1993-4FN, TGX 1990-46F and TGX 1989-53FN. Four germplasms namely, TGX 1485-1D, TGX 1990-46F, TGX 1989-49FN and TGX 1990-55F were grouped into cluster 2. Cluster 3 consists of twelve germplasms that are morphologically related namely, TGX 1835-10E, TGX 1990-40F, TGX 1989-11F, TGX 1990-110FN, TGX 1990-57F, TGX 1945-1F, TGX 1989-40F, TGX 1990-114FN, TGX 1951-4F, TGX 1987-10F, TGX 1989-42F and TGX 1990-95F. Cluster 4 has three germplasms namely, TGX 1987-62F, TGX 1955-4F and TGX 1990-78F. Also, the dendrogram shows that cluster 5 consists of an outlier (TGX 1989-75F) that is

morphologically different from all other germplasms.

## DISCUSSION

The high degree of polymorphism revealed by the RAPD markers was further accentuated by the observation from the cluster analysis. The molecular characterization of the 30 soybean germplasms with RAPD markers revealed 70% polymorphism, indicating a high level of genetic diversity in the soybean germplasms investigated. This result also shows that the RAPD markers were highly polymorphic and informative. The result is in



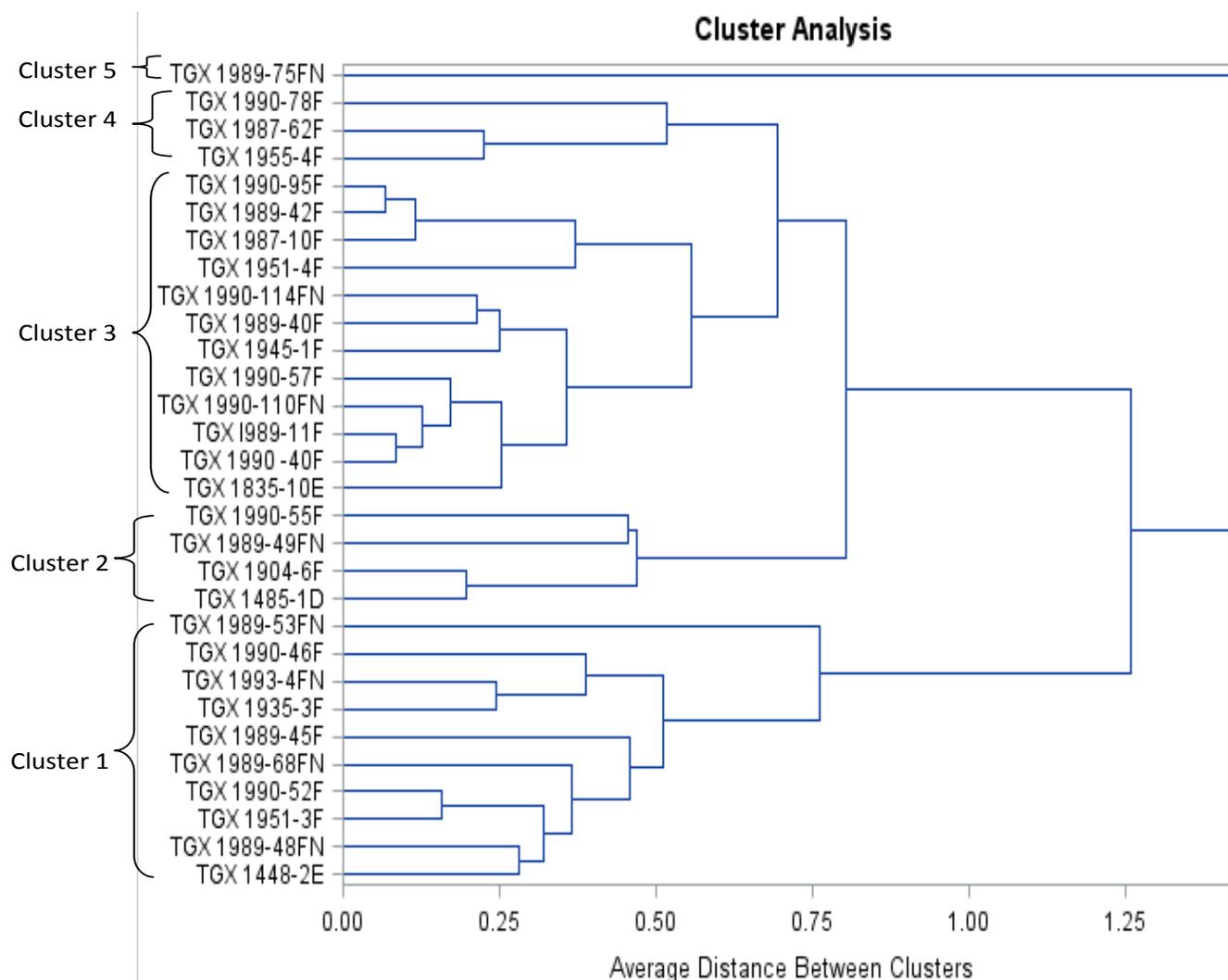
0.642

0

**Figure 4.** Dendrogram of all RAPD primers showing the genetic diversity amongst thirty soybean germplasms using UPGMA method (distance matrix using RMSD coefficient).

agreement with the report of Chowdhury et al. (2001) that RAPD markers are efficient for measuring genetic diversity and relatedness as well as identifying varieties of soybeans. This is the first report of genetic diversity analysis of advanced soybean genotypes (breeding lines) in Nigeria using RAPD markers. This result is in concordance with the previous reports of Singh et al.

(2008) who reported 89.9% polymorphism, while Li and Nelson (2005) reported 56% polymorphism in their studies using RAPD primers. However, Thompson et al. (1998) observed a low level (36%) of DNA variation among 35 soybean genotypes using RAPD primers. Conflicting reports on the extent of observed polymorphism in soybean in different studies could be



**Figure 5.** Dendrogram of morphological traits.

attributed to the unique nature of the genetic materials under investigation and sequences of the primers.

There was a significant lack of correlation between the RAPD markers and the morphological traits evaluated. Although, both analyses grouped the soybean genotypes into 5 clades, the composition of the individual clusters were very variable and divergent. For example, cluster 3 was the largest cluster in both cases with 12 and 14 members for genetic and morphological relatedness respectively but, the correlation was only 35-42% (TGX 1989-42F, TGX 1987-10F, TGX 1951-4F, TGX 1945-1F and TGX 1990-57F). Furthermore, whereas TGX 1990-110FN and TGX 1989-11F were outliers and genetically distinct and distant from the other germplasms in the RAPD dendrogram, they were shown to be morphologically related to at least 10 other germplasms in cluster 3. Also, TGX 1989-75FN was morphologically

different to all the other germplasms and hence an outlier in the dendrogram. In the genetic characterisation however, it was shown to be genetically related to 4 other germplasms in cluster 1. This lack of correlation between genetic and morphological diversity have been previously reported in other plant species (Doebley, 1989). It could well be as Zannou et al. (2008) surmised that during the process of domestication, modifications in a few genes can lead to marked phenotypic differences.

In terms of morphological characterisation, this study agrees with several earlier studies on soybean using morphological markers (Malek et al., 2014; Ojo et al., 2012; Iqbal et al., 2008; Cui et al., 2001) such as branch number, plant height, pod number and seed weight, seed shape, seed eye colour, yield, etc. which have all provided useful information for proper classification of soybean germplasms.

## Conclusion

Summarily, this study reveals the presence of significant genetic variability in the 30 soybean genotypes investigated. Genetic diversity and/or relationship assessments could provide useful information for efficient utilization of these germplasms, especially during future improvement programmes. This result also showed that the RAPD markers were highly polymorphic and informative, clearly grouping the genotypes into distinct clusters. Although, the correlation between the largest clusters in the molecular and morphological characterisation was only 35-42%, enlarging the sample size in subsequent investigations could provide a clearer picture and perhaps better information between the two. Nevertheless, advanced breeding lines of soybean which have undergone cycles of artificial selection, should not be neglected when further improvement of soybean is considered.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of the paper.

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