GENETIC DIVERSITY OF NIGERIAN GERMPLASM OF *GOSSYPIUM HIRSUTUM* L USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

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The genetic diversity of 18 accessions of *G. hirsutum* was analyzed using 10 RAPD markers with the aim of ascertaining the level of the species diversity at the molecular level. A total of 1,038 amplicons were generated, out of which 984 bands were polymorphic that is 94.8% and 5.2% were monomorphic. Percentage polymorphism per primer is LC 71 (50%); OPD-8 (63.6%); OPK-4 (66.7%) and others had 100% indicating high level of polymorphism. The PCA revealed that component 1 is responsible for the highest genetic variation (22.498%) and highest positive loading of 7.0593. Biplot and Cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to obtain dendrograms that separated the accessions into 4 clusters. The study concluded that genetic diversity exists in the accessions studied. The accessions are recommended for introgression breeding programmes to enhance crop performance.

Keywords: *Gossipium hirsutum*, genetic diversity, polymorphism, RAPD.

INTRODUCTION

Cotton plant belongs to the genus *Gossypium* and family Malvaceae, or mallow family. The plant is indigenous to the tropic and sub-tropic region including Africa, America and India. The plant is an essential cash crop cultivated in various countries. It provides raw material for textile industries and also for edible oil (Shashank *et al*., 2014a). The study of genetic diversity in a crop breeding programme is essential for the selection of suitable diverse parent to obtain heterotic hybrids as well as conservation and characterization of germplasm (Shashank *et al*., 2014b). Genetic diversity of cotton cultivar is measured by assessing morphological characters, biochemical and molecular markers. This provides information on choosing parents in the development of cotton cultivars and hybrids (Wu *et al*., 2006 and Ullah *et al*., 2012). Zhang *et al.* (2008) reported that DNA based marker have been subjugated broadly for molecular characterization

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and DNA finger printing of cotton. PCR and non PCR-based DNA markers such as RAPD, SSR, AFLP and RFLP have been employed in cotton genome research. Amongst these markers, RAPD provides a simple and fast approach to detect polymorphism for cultivars identification and diversity analysis (Preetha and Raveendran 2008). RAPD system of molecular markers has been utilized by many scientists. Nybom and Hall, (1991), Welsh *et al.*, (1991), Iqbal *et al.* (1997), Khan *et al.* (2000), Dighe *et al.* (2001), Rahman *et al.* (2002), Lu and Myres (2004), Mehetre *et al.* (2004a), Mehetre *et al.* (2004b), Dongre and Parkhi, (2005), Rana and Bhat, (2005) Hussain *et al.* (2007), and Sheidail *et al.* (2007) used RAPD marker technology for DNA fingerprinting in cotton and suggested that this technique is reliable for the detection of various cultivars and inter and intraspecific crosses on the base of polymorphic sequences present in their genetic makeup. The aim of this study is to measure the genetic diversity of *G. hirsitum* varieties.

MATERIALS AND METHODS

The seeds of six accessions of *G. hirsutum* used for this study were obtained from the Institute of Agricultural Research (IAR), Zaria, Kaduna State, National Center for Genetic Research and Biotechnology (NACGRAB), Ibadan, Oyo State and Dangote Ginnery, Kankara, Katsina state, Nigeria. Collections were also made of twelve accessions from other parts of Nigeria (Table 1) where *G. hirsutum* is cultivated during harvest period from the field. The accessions collected from the field were identified in the Department of Plant Biology Herbarium, University of Ilorin. The seeds were appropriately labeled to show where collections were made. The cotton seeds collected were brought for cultivation in the Botanical Garden of the University of Ilorin.

GENOMIC DNA EXTRACTION

Fresh leaves of 18 accessions of *G. hirsutum* were used in DNA extraction (Table 1). One gram of leaf tissue was frozen in liquid nitrogen and ground into fine powder. Total genomic DNA was isolated from individual accessions using CTAB method as per Doyle and Doyle (1987). The quality of DNA was assessed using 0.8% agarose gel electrophoresis. The quantity was measured using spectrophotometer at 260 nm.

PCR AMPLIFICATION

The RAPD markers, obtained from Genei Company, amplification was performed as described by Priyanka and Gohar (2012) using 10 decamer random primers (Genei, Bangalore, India). The PCR reaction was carried out in a 25 µl volume in 1 x TAE buffer containing 10mM Tris-HCl (pH 8.3), 400µM each of

dNTPs 3Mm MgCl2, 0.4 µM primers, 50ng template, 1 unit Taq DNA polymerase (Premix Taq version 2.0, Xcelris, India). The reactions were carried out on a Gradient Master Thermo cycler (Eppendorf, Hamburg, Germany) with an initial denaturation temperature of 94^{o}C for 5 min in 1 cycle, followed by 35 amplification cycles which consist of three steps: 94^{o}C for 1 minute, annealing at 40^{o}C (depending on the annealing temperature of each primer) for 50 seconds and extension at 72^{o}C for 1 min with a final extension at 72^{o}C for 10 min. the reaction was put on hold for 10 min at 4^{o}C.

RAPD GEL ELECTROPHORESIS

The amplified products along with the external size standard ladder were separated in a horizontal gel electrophoresis unit using 1.5% agarose gel in 1X TAE buffer at 110 volts for one hour (run 2/3 of gel) and stained in ethidium bromide. The banding pattern was visualized under UV light and photographed using a gel documentation system (Bio - Rad, India).

Table 1

Areas for *G. hirsutum* collection

Serial	Code for location	Collection Site	State of Collection	Accession Type
1.	KR	Kankara	Katsina	Landraces
2.	KB	Kabomo	Katsina	Landraces
3.	GS	Gusau	Zamfara	Landraces
4.	YG	Yargoje	Katsina	Landraces
5.	YB	Dangote Ginnery	Katsina	Hybrid
6.	NGB01	NACGRAB	Oyo	Hybrid
7.	NGB02	NACGRAB	Oyo	Hybrid
8.	KC	Dangote Ginnery	Katsina	Hybrid
9.	AD	Mubi	Adamawa	Landraces
10.	AD1	Mubi	Adamawa	Landraces
11.	FT	Funtua	Katsina	Landraces
12.	BK	Bakori	Katsina	Landraces
13.	ABK	Abeokuta	Ogun	Landraces
14.	GS1	Gusau	Zamfara	Landraces
15.	GS2	Gusau	Zamfara	Landraces
16.	SC8	IAR	Kaduna	Hybrid
17.	SC9	IAR	Kaduna	Hybrid
18.	KN	Gwarzo	Kano	Landraces

Table 2

List and properties of RAPD primers selected for the molecular study of the accessions of *G. hirsutum.*

Serial	Primer Name	Primer Seq 5'-3'	Annealing temp (^{0}C)
1.	OPH-20	GGGAGACATC	27
2.	LC 71	TGCCGAGCTG	27
3.	RAPD-036	GAAGAACCGC	25
4.	RAPD-31	GGGTAACGCC	25
5.	RAPD 30	GGACTGGAGT	25
6.	RADP-09	GACGGAGCAG	27
7.	OPK4	CCGCCCAAAC	27
8.	RADP-21	GACGGATCAG	25
9.	OPD-8	GTGTGCCCCA	27
10.	OPD 6	GGGAATTCGG	27

*The RAPD primers were obtained from Genei Company

DATA ANALYSIS

Bands from the molecular weight were compared. Scoring was carried out based on bands. A binary matrix reflecting the presence (1) or absence (0) of only reproducible bands was analyzed according to the method of Nybom and Bartish (2000). The percentage of polymorphic bands (%P) was calculated using Polymorphic (%) = Polymorphic bands/Total bands x 100. Principal Component Analysis and diversity indices were conducted using PAST (Paleontological Statistics), version 3.5 and Popgen 3.1 version (Rohlf, 1993). Cluster analysis was performed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA), and the relationships between genotypes were displayed as dendrogram (Sokal and Michener, 1958).

RESULTS

The RAPD diversity study of the 18 accessions of *G. hirsutum* showed high degree of polymorphism. Out of the 1038 DNA bands generated by the 10 primers, 984 bands were polymorphic (i.e. 94.8% of the bands generated were polymorphic) and 5.2% were monomorphic. Percentage polymorphism per primer ranged from 50 to 100%. Lowest polymorphism was recorded by LC 71 with 50%, OPD-8 and OPK-4 had 63.6 and 66.7% respectively. However, the remaining primers had 100% polymorphism. Polymorphic bands produced by the primers range between 1 and 17, forming a total of 48–116 polymorphic bands.

The number of DNA bands produced from the accessions by the primers ranged from 1 to 18 (Table 3). Minimum of two bands were amplified in the accessions by primer RAPD 09 while other primers amplified at least 1, 3, 4 and 7 bands respectively. Even though primer RAPD 09 recorded the least band, it produced two bands each in GS and YG. OPH 20 and RAPD 31 produced the most bands in all the accessions. Primer OPH 20 produced amplicons in all the accessions except in KR, AD and NG1. Among the DNA fragments amplified by the primer LC 71, two bands at 800 and 900 bp were produced in BK alone (Fig. 1). There was no DNA band amplified by RAPD-36 in accession KR while YG produced a single band. RAPD-21 produced only 2 bands in accession BK with KR having only a band (Fig. 2). Highest number of bands in AD1 was produced by RAPD-30 with accessions YB, NGB1 and NGB2 having only 4 bands each. Accession KB had 1 (one) band, while FT had all loci amplified by RAPD 09 however KR, AD,BK and NGB1 were not amplified by primer RAPD 09 (Fig. 3).

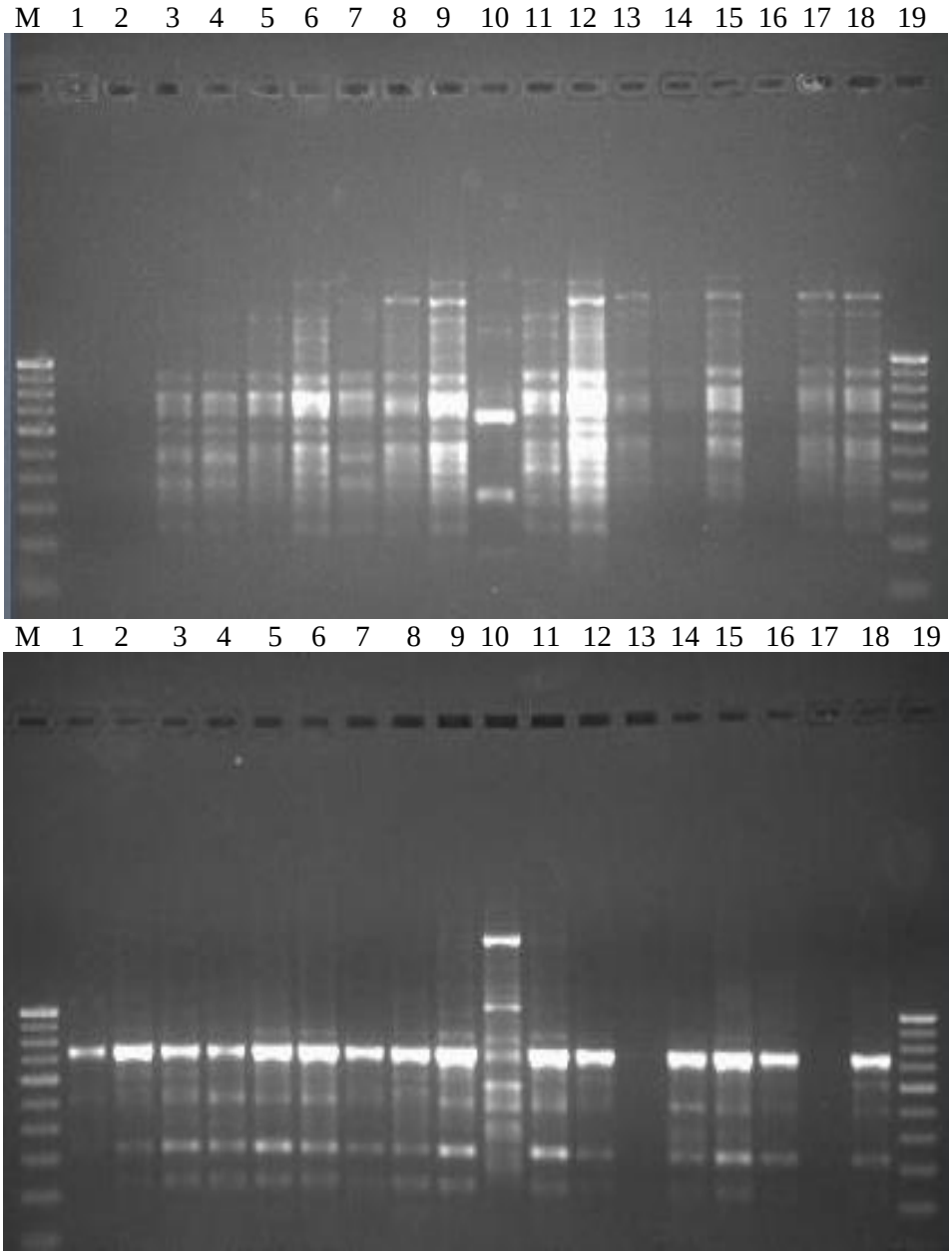
The number of bands produced by OPD 8 in KR and AD accessions are 5 and 2 respectively. However, other accessions had much more amplicons ranging from 6–9. OPK 4 amplified all the accessions in varying numbers (Fig. 4). BK was not amplified by RADP 31. OPD 6 also did not produce a single amplicon in YG (Fig. 5).

Neighbour Joining dendrogram indicated BK as the most distinct accession as it is a cluster of its own. In this dendrogram, the rest of the accessions might be split into three clusters, cluster I comprises of accessions KN, KC, GS2, YB, ABK, SC8, AD1, FT, SC9 showing their relatedness, cluster II consisted of NGB2, GS1, KB, YG, GS and cluster III included accessions NGB1, AD, KR (Fig. 6).

In the Cluster Analysis (Ward's method) dendrograms based on all 10 primers indicated BK as the most distinct accession. In this dendrogram, the rest of the accessions might be split into 3 branches: the 1st cluster comprised NGB1, AD, KR, KB, GS, YG, NGB2, GS1, the 2nd cluster consisted of AD1, SC9, YB, ABK, SC8, FT and the 3rd group included KC, GS2, KN accessions (Fig. 7).

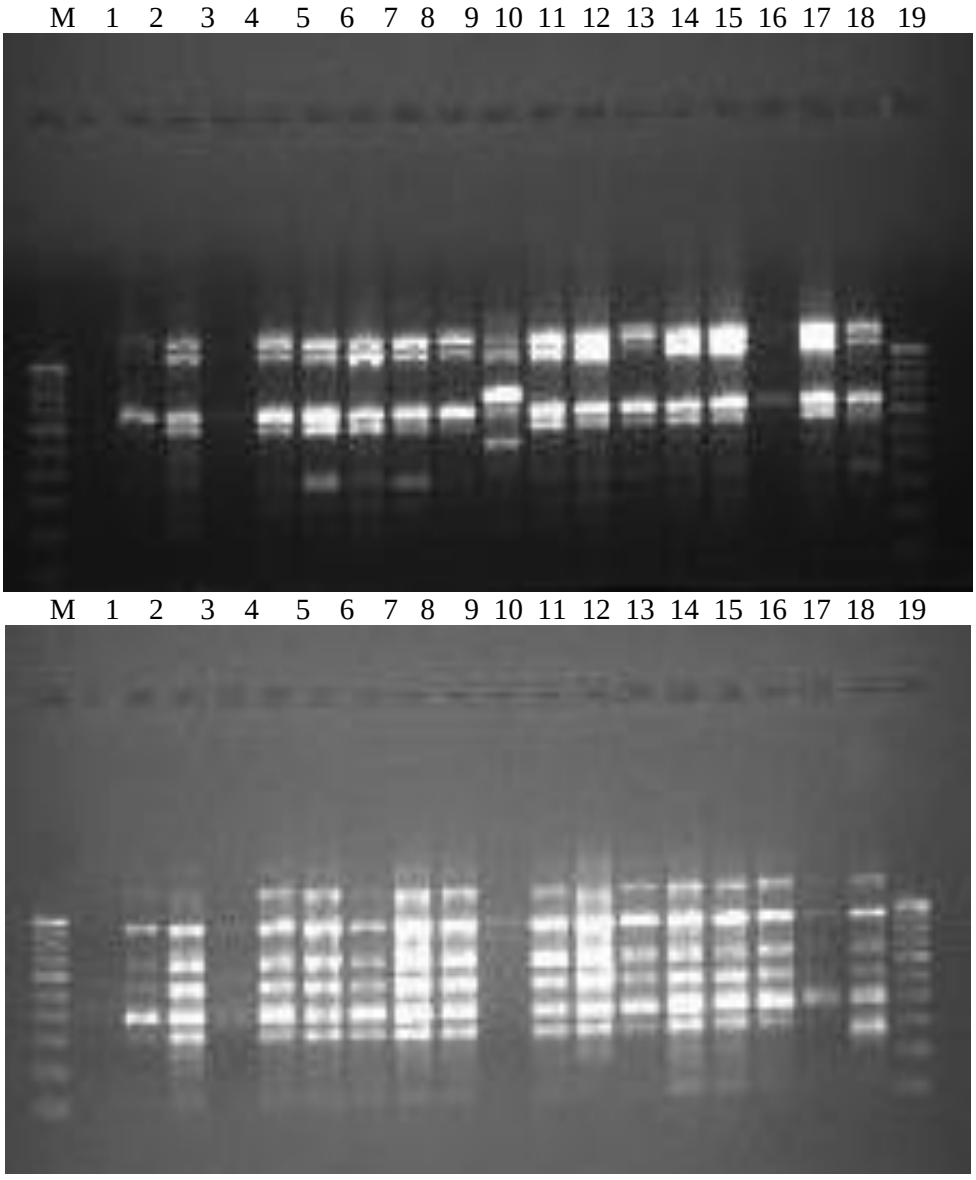
The principal component analysis revealed that four components are responsible for the observed variations in the study. The highest genetic variation was explained by component 1(22.498%) followed by component 2 (15.196%), component 3 (8.5791%) and component 4 (8.3331%) respectively (Table 4).

The Biplot analysis (Fig. 8) of the markers on the accessions placed them into four quadrants involving 10 markers. BK was only present in the first quadrant, while KR, NGB1, YG, AD, KB, GS1 were in the second quadrant indicating their genetic similarity with GS been present in both second and third quadrants sharing similarities with the accessions in the quadrants. Accessions YB, SC8, KC, AD1 and SC9 were present in the third quadrant indicating their genetic similarity with ABK in between third and fourth quadrants. NGB2, KN, GS2 and FT were present in the fourth quadrant.



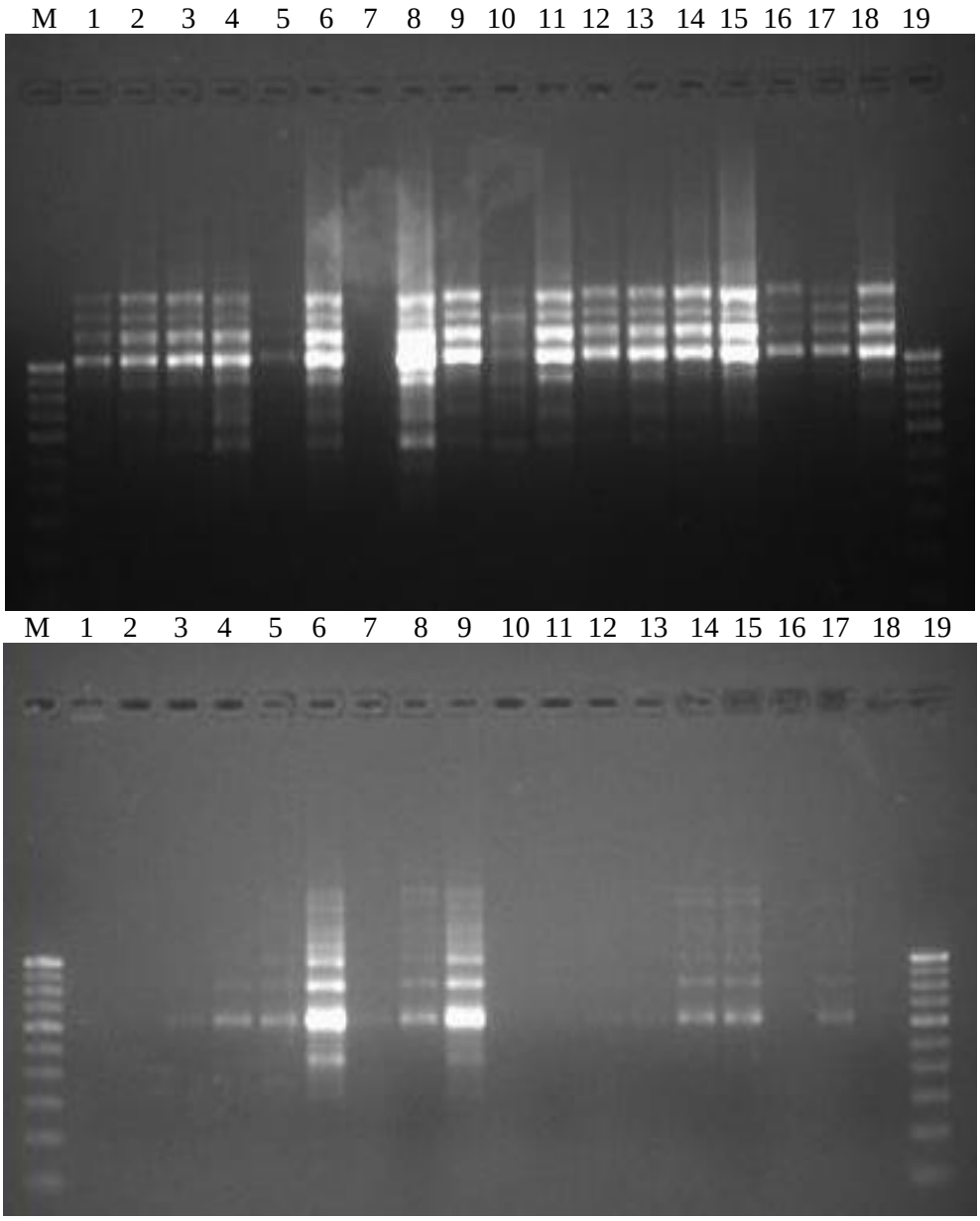
a. OPH20 b. LC 71

Fig. 1. The amplified PCR products of *G. hirsutum* by RADP primers resolved 1.5% agarose. KEY: M = 100 bp ladder, 1=KR, 2=AD, 3=GS, 4=YG, 5=YB, 6=SC9, 7=KB, 8=AD1, 9=FT, 10=BK, 11=GS2, 12=KC, 13=GS1, 14=SC8, 15=ABK, 16=NG1, 17=NG2, 18=KN.



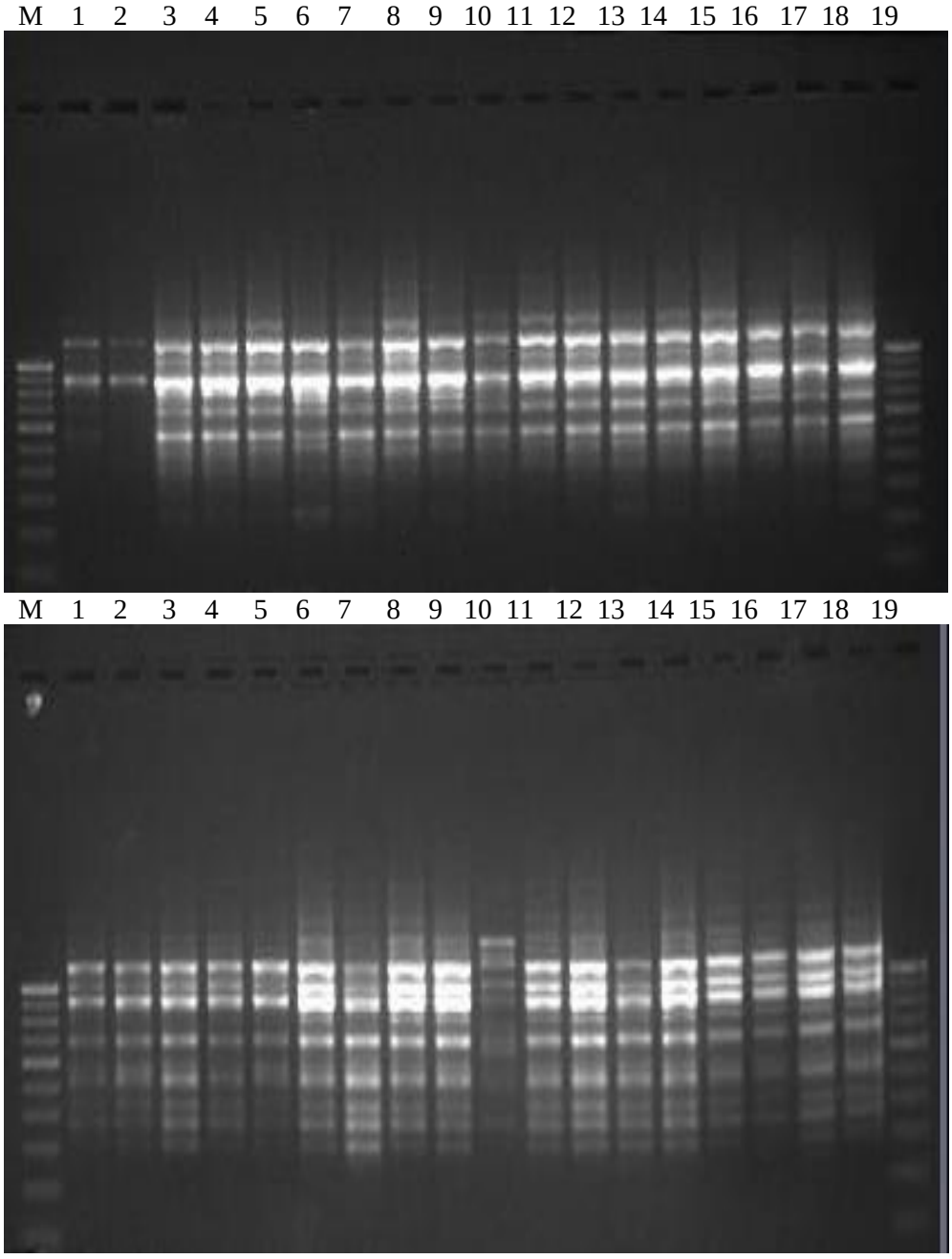
c. RAPD 36 d. RAPD 21

Fig. 2. The amplified PCR products of *G. hirsutum* by RADP primers resolved 1.5% agarose. KEY: M = 100Kb ladder, 1=KR, 2=AD, 3=GS, 4=YG, 5=YB, 6=SC9, 7=KB, 8=AD1, 9=FT, 10=BK, 11=GS2, 12=KC, 13=GS1, 14=SC8, 15=ABK, 16=NG1, 17=NG2, 18=KN.



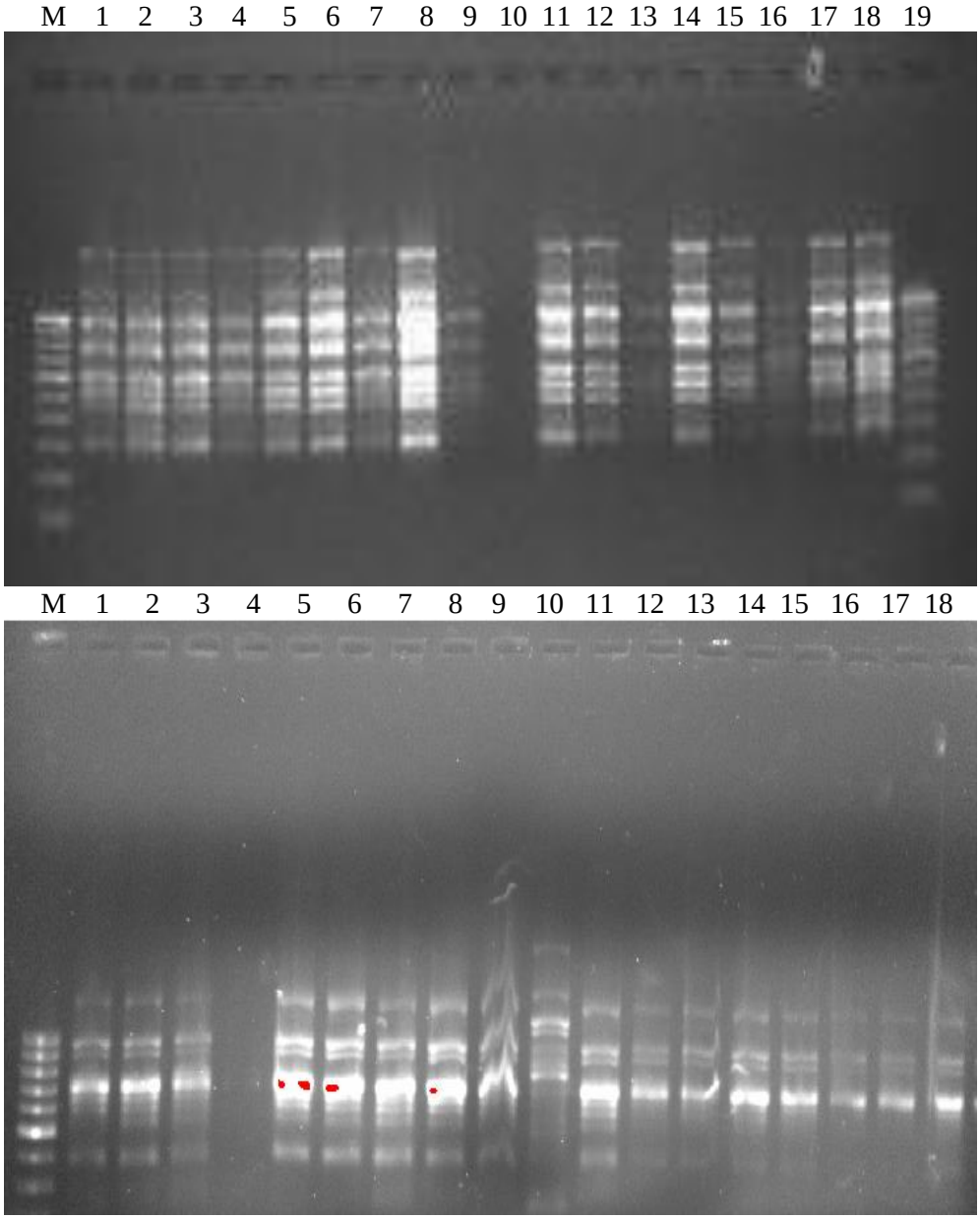
e. RAPD 30 f. RAPD 09

Fig. 3. The amplified PCR products of *G. hirsutum* by RADP primers resolved 1.5% agarose.
KEY: M = 100Kb ladder, 1=KR, 2=AD, 3=GS, 4=YG, 5=YB, 6=SC9, 7=KB, 8=AD1, 9=FT, 10=BK, 11=GS2, 12=KC, 13=GS1, 14=SC8, 15=ABK, 16=NG1, 17=NG2, 18=KN.



g. OPD 8 h. OPK 4

Fig. 4. The amplified PCR products of *G. hirsutum* by RADP primers resolved 1.5% agarose.



i. RAPD 31 j. OPD 6

Fig. 5. The amplified PCR products of *G. hirsutum* by RADP primers resolved 1.5% agarose.
KEY: M = 100Kb ladder,1=KR, 2=AD, 3=GS, 4=YG, 5=YB, 6=SC9, 7=KB, 8=AD1, 9=FT, 10=BK, 11=GS2, 12=KC, 13=GS1, 14=SC8, 15=ABK, 16=NG1, 17=NG2, 18=KN.

Table 3

Amplification parameters of RAPD markers in *G. hirsutum*

Marker ID	Amplicon Size (bp)	Band Amplication		Polymorphism		Monomorphism	
		Min	Max	Bands	%	Band	%
OPH-20	1000-100	1	14	153	100	0	0
LC 71	1200-200	3	18	66	50	18	50
RAPD-036	1000-300	4	17	117	100	0	0
RAPD-31	1200-300	1	17	145	100	0	0
RAPD 30	1200-400	1	17	109	100	0	0
RAPD-09	1000-400	2	12	48	100	0	0
OPK4	1100-300	7	18	114	66.7	18	33.3
RAPD-21	1100-100	1	17	120	100	0	0
OPD-8	1000-200	3	18	61	63.6	18	36.4
OPD 6	1100-100	1	17	105	100	0	0

Table 4

Principal component analysis using RAPD markers

PC	EIGEN VALUE	% VARIANCE
1	5.9399	22.498
2	4.01193	15.196
3	2.26504	8.5791S
4	2.20009	8.3331

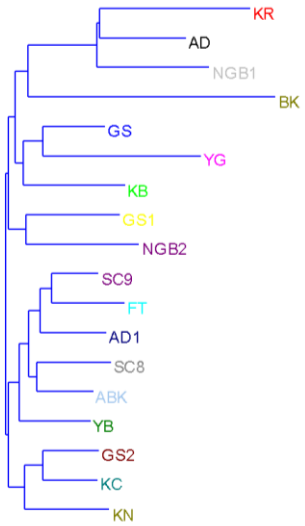


Fig. 6. Neighbor Joining Dendrogram showing the genetic similarity among 18 cotton varieties as derived from RAPD data using UPGMA.

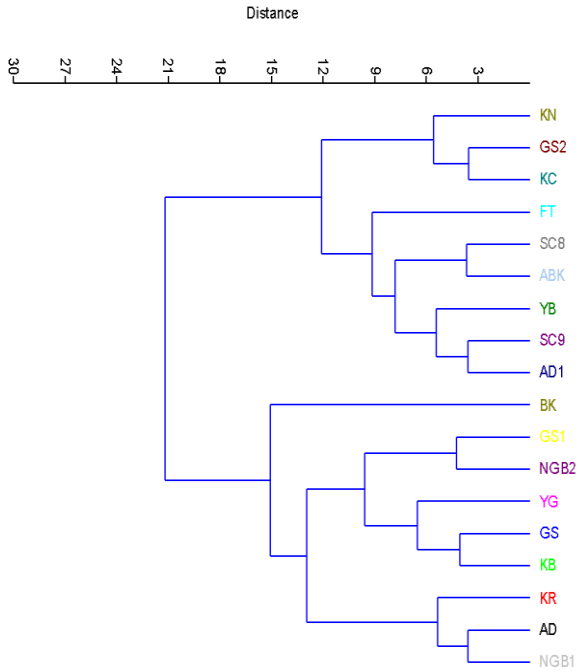


Fig. 7. Dendrogram showing of genetic relatedness of *G. hirsutum* accessions based on UPGMA.

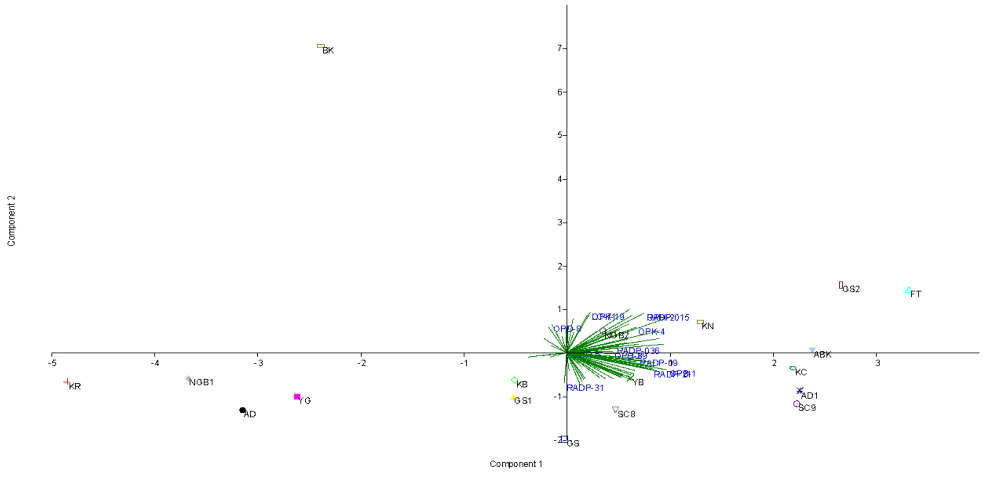


Fig. 8. Biplot analysis of the 18 G. hirsutum accessions based on ten RAPD markers amplification.

DISCUSSION

Molecular markers may or may not correlate with phenotypic expression of a genomic trait. They provide detailed information in resolving genetic diversity by identifying genetic variation in a germplasm collection. Thus, markers offer numerous advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. Additionally, they are not confounded by environmental, pleiotropic and epistatic effects (Mondini *et al*., 2009). Resolving genetic diversity through the study of morphological traits has been grossly inadequate due to environmental influence on these traits as observed by Ozrenk *et al*. (2009). The usage of RAPD markers has shown that genetic diversity exists through rapid reproduction of the random section of DNA using short oligonucleotide primers as reported by Abdulkareem *et al.* (2018).

The RAPD markers used in this study showed high polymorphism rate providing information on the species genetic diversity. In this study, 94.8% of the DNA bands generated by the 10 primers were polymorphic. These results indicated that the level of polymorphism was high among the 18 genotypes. This result is similar to the works of Punitha and Raveendran (2004) that observed a polymorphism rate of 76.3% as a result of the RAPD analysis of 11 colored and 4 white cotton genotypes using 10 primers of arbitrary sequences. Khan *et al.* (2010) found 70.2% polymorphism in 10 genotypes belonging to *G. hirsutum* using 25 decamer RAPD primers. However, in other studies conducted to determine the genetic relationships among cotton genotypes, low polymorphic level was observed in the work of Lu and Myers (2002) where 86 RAPD primers generated 13.5% polymorphism. The result of this study also differs with those of Sheidai *et al.* (2007) where 27 RAPD primers was used to determine the genetic relationship between tetraploid cotton variants cultured in Iran and a total of 19% polymorphism was obtained. The efficacy of the RAPD markers used in this work is evident in the 94.8% polymorphism obtained.

The phylogenetic results obtained from the clustering in this study indicates the genetic relatedness of the accessions could be due to similar genetic composition. The clustering was associated with points of collection for SC8, SC9, NG1 and NG2 while genetic variation was exhibited by clustering of hybrid accessions with the landraces. The clustering of various accessions and the landraces showed that they are genetically related. Sapkal *et al*. (2011) and Chaudhary *et al*. (2010) reported that presence of useful genetic diversity both in exotic and breeding line resources are evidence of genetic diversity.

Dispersal of the accessions into separate co-ordinates in the biplot analysis and different clusters indicated that they are distantly related and genetically diverse. This is as a result of the markers exploits on the genotypes to distribute the populations into different quadrants. The dispersion of the markers from the

centroid reflects their ability in delimiting the accessions. The accessions that occupied the same quadrant indicate genetic similarity while those far from the centroid are genetically distinct. Accessions that share genetic background and are closely related as delineated by the markers are either close or on the centroid. This was observed in the work of Abdulkareem *et al*. (2018).

CONCLUSIONS

The RAPD markers have proven to be useful in evaluating the genetic diversity of *Gossypium hirsutum*. This work has shown that RAPD is useful in detecting a high level of intraspecific polymorphism with 1,038 loci amplified in this work. The result of the molecular analysis revealed that RAPD markers are capable of estimating relatedness, and identifying genotypes among *Gossypium* accessions with genotype-specific RAPD marker(s). The result also revealed 94.8% polymorphism of *G. hirsitum* cultivars and also generated information about levels of genetic relatedness. The biplot and clustering analyses of the molecular data indicated the existence of intra-species diversity and also showed that the variation observed was as a result of amino acid synthesis required for survival. The genetic variability in the accessions provides justification for introgression and ideotype breeding programmes to enhance crop performance. This study indicated that the eighteen cotton accessions studied exhibited genetic diversity and relatedness.

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