Assessment of genetic diversity of maize inbred lines and hybrids in Southern Highlands of Tanzania by using Random amplified Polymorphic DNA (RAPD) markers

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ABSTRACT

Maize is Tanzania's most important food crop, providing 60% of the dietary calories and more than 50% of utilizable protein. The Southern highlands account for almost 50% of the total national maize production and contribute up to 90% of the annual purchase of maize for the National Strategic Grain Reserve. Although the study of genetic diversity of hybrid varieties in Tanzania is important for its genetic improvement, there is very limited information with this regard. Therefore, the objective of this study is to assess the genetic diversity of maize hybrids grown in Southern highlands of Tanzania by using Random Amplified Polymorphic DNA (RAPD) markers. Twelve maize samples (six inbreds and six hybrids) were collected and used in this study. DNA was extracted from young leaves and PCR was conducted using fourteen primers. A total of 123 bands were produced of which 98 (80%) were polymorphic. The range of genetic similarity was from 0.32 to 0.95 based on Jaccard's similarity coefficient. The UPGMA analysis indicated higher similarity between *the hybrids* than *the inbreds*. Cluster analysis based on Jaccard's similarity coefficient grouped the genotypes into two main clusters. One cluster contained one ibred B and the other contained the rest five inbred lines and six hybrids. Two dimensional (2D) principal coordinate analysis (PCA) grouped the varieties into two major groups. The first group contained 11 genotypes (including 5 inbred lines and 6 hybrids) while the second cluster contained only one inbred line.

Key words: Genetic Diversity, RAPD Markers, Maize, Southern highland

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INTRODUCTION

Maize (*Zea mays* L) is one of the three most important crops in the world besides rice (*Oryza sativa* L.) and wheat (*Triticum aestivum L.*). Maize was introduced in Tanzania before the First World War notably in the Arusha area but only became popular in Mbeya and Ruvuma in the 1950s and Rukwa in the 1970s (Coulson and Diyamett, 2012). It is now, by many measures, Tanzania's most important food crop, providing 60% of the dietary calories and more than 50% of utilizable protein to the Tanzanian population (Nkonya *et al*., 1997; Nkonya, 1998). In Tanzania, maize influences food security via two different channels: consumption as an important component of the nation's caloric intake, and production as an income generating activity (Nkonya, 1998). The crop is cultivated on an average of two million hectares, which is about 45% of the cultivated area in Tanzania (Lymo, 2005).

Although maize is widely cultivated crop, the Southern highlands of Tanzania (comprising Iringa, Ruvuma, Mbeya, Rukwa and Njombe) provide the most favourable climate for maize production. Currently, the Southern highlands account for almost 50% of the total national maize production and up to 90% of the annual purchase of maize for the National Strategic Grain Researve (Lymo, 2006).

The productivity trend of maize on the global, national, and regional level reveals a wide gap between potential yield and the actual yield. Global potential yield of maize is estimated at 37.5 tons per hectare though in Tanzania, yield ranges from 1.2–7.7 tons/Ha for small-scale farmers

and from 5.5–9.5 tons/Ha for large-scale farmers. Traditional seeds can produce up to 5.5 tons/Ha, while the potential of improved seeds currently available in Tanzania is 12.0 tons/Ha.

Production patterns of maize oscillate dramatically in the past 10 years. For instance, maize production has varied considerably, ranging from as high as 2,638 million tons in 2006/7, to as low as 2,107 million tons in 2009/2010. This is due to the shifting weather conditions in a given harvest year, low usage of fertilizer and pesticides, occurrence of diseases as well as non-availability of quality seeds (Tanzania Agriculture Report, 2011). Maize being staple food for most of Tanzanian, it needs greater attention to ensure its productivity remain consistently high. To ensure this, greater efforts should be done to release many varieties to increase diversity and make sure that quality seeds of those varieties reach the farmers.

Efforts have been done on breeding thus a considerable number of hybrids have been released to be used in southern highlands of Tanzania. To improve genetic diversity of local germplasm, it is important to know the extent of already existing genetic variability in the material. However, there is limited information on the genetic diversity of the maize hybrids grown in southern highlands of Tanzania.

The objective of this study is to assess the genetic diversity of maize hybrids grown in southern highlands of Tanzania using Random Amplified Polymorphic DNA (RAPD) molecular markers.

MATERIALS AND METHODS

Plant materials

Twelve seed samples from four maize varieties (UH6303, UHS5350, UH615 and H6302) were collected from Uyole Agricultural Research Institute (ARI) and Agriculture Seed Agency (ASA) in Southern highlands of Tanzania. Two categories of seeds were collected; Breeder seeds and F1 (hybrid) seeds. Out of these twelve seed samples collected, six samples were breeder seeds and six samples were hybrids.

Table 1. List of inbred lines and hybrids collected from Uyole Agricultural Research Institute and Agriculture Seed Agency

S/No.	Seed category	Variety	Sample ID*	Seed source
1.	Basic (Male)	UH6303	A	Uyole ARI
2.	Basic (Female)	UH6303	B	Uyole ARI
3.	Basic (Male)	H615	C	ASA
4.	Basic (Female)	H615	D	ASA
5.	Basic (Male)	H6302	E	ASA
6.	Basic (Female)	H6302	F	ASA
7.	F1	UH6303	G-1	Uyole ARI
8.	F1	UH6303	G-2	Uyole ARI
9.	F1	H615	H-1	ASA
10.	F1	H615	H-2	ASA
11.	F1	UHS5350	J-1	Uyole ARI
12.	F1	UHS5350	J-2	Uyole ARI

*The sample ID has been given only for the purpose of this study.

DNA Extraction and Quantification

Total genomic DNA was extracted from young leaves according to Dellaporta *et al*., (1983) protocol with minor modification as stated by Ogunkanmi *et al*., (2008). The young leaves were ground in 500µl of plant extraction buffer then transferred to new eppendorf tube and 40µl of 20% SDS was added to the tube and mixed thoroughly by vortexing. The samples were incubated in water bath at 65^{o}C for 30 minutes and 200µl of 3M sodium acetate was added to each tube and mixed thoroughly by vortexing followed by incubation in ice for 20 min. The samples were centrifuged at 14000rpm for 10min at room temperature and the supernatant was removed and transferred to new 1.5µl eppendorf tube. Equal volume of Chlorofoam:Isoamyl alcohol (24:1) was added and mixed gently by inverting the tube followed by centrifugation at 14000rpm for 10min at room temperature in a microfuge. The upper aqueous layer was transferred to a new 1.5µl eppendorf tube. An equal volume of cold isopropanol (pre-chilled in -20^{o}c freezer) was added and mixed thoroughly by gently inverting the tube then incubated at -20^{o}C for 30min. The samples were centrifuged at 14000rpm for 10min at room temperature. The DNA pellet was washed by adding 500µl of 70% ethanol and centrifuged at 14000rpm for 5min

at room temperature. This step was repeated twice. The DNA pellet was air dried for 20-60min by leaving the tubes open on its side in fume hood.

The DNA pellet was dissolved in 50µl sterile distilled water overnight. A 2.5µl of RNAse was added to the dissolved DNA and incubated at 37°C for 30min and the DNA was stored at 4°C. The concentration of DNA was measured by using Nano Drop2000 and the quality was checked on 1% w/v agarose gel by electrophoresis at 100V for 30 min. The gel was stained with ethidium bromide.

Primer Screening and Polymerase Chain Reaction

Twenty eight RAPD primers (Ascefran LLC, USA) were screened using individuals of the four varieties (UHS5350, UH6303, UH615 and H6302) in different seed categories (inbred lines and hybrids). About six inbred lines and one sample from selected hybrid were used in this process. Fourteen primers produced clear and polymorphic band patterns and they were selected for further analysis (Table 2).

Table 2. List of fourteen selected primers and their sequences

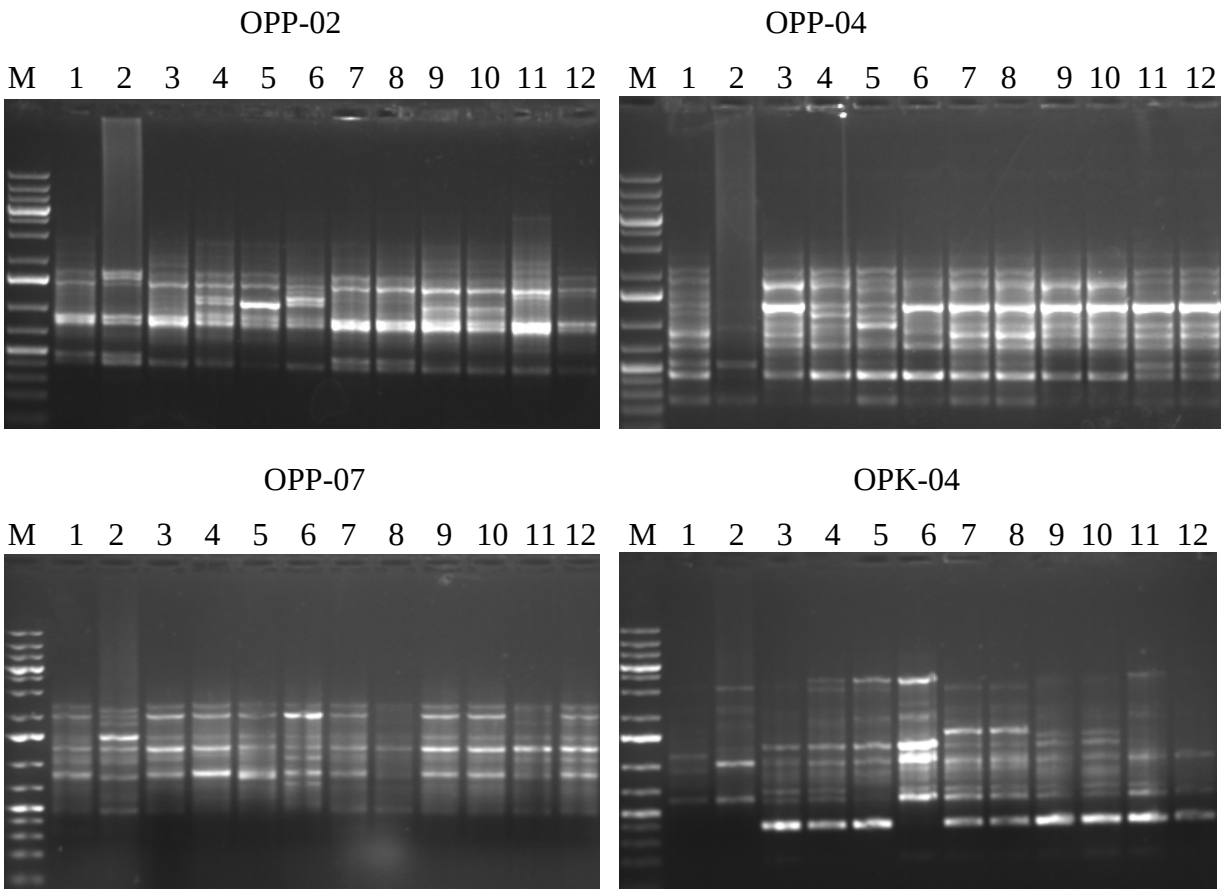
S/No.	Name	Sequence	S/#	Name	Sequence
1	OPP-02	TCGGCACGCA	8	OPK-08	AGCGAGCAAG
2	OPP-04	GTGTCTCAGG	9	OPK-10	GAACACTGGG
3	OPP-05	CCCCGGTAAC	10	OPO-04	ACGTAGCGTC
4	OPP-07	GTCCATGCCA	11	OPO-05	AAGTCCGCTC
5	OPK-03	GTCTCCGCAA	12	OPO-06	CCCAGTCACT
6	OPK-04	CCAGCTTAGG	13	OPJ-05	CCACGGGAAG
7	OPK-05	CCGCCCAAAC	14	OPJ-07	CTCCATGGGG

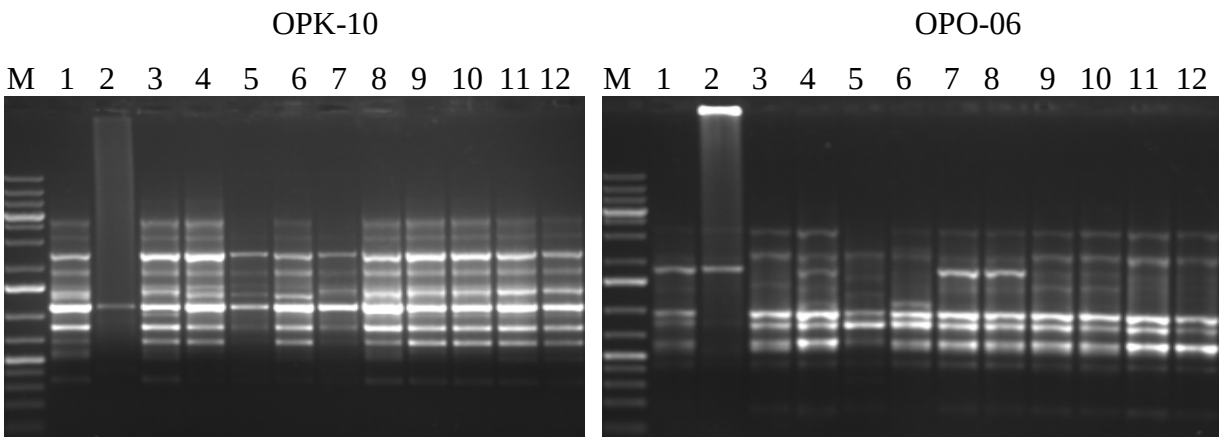
PCR was carried out in a total volume of 25 µl by mixing 10 mMTris-HCl (pH 8.6), 1x*Taq*polymerase buffer, 50 mMKCl, 2 mM $MgCl_2$, 0.27mM of each dNTP, 0.8 mM primer, 1 U *Taq*DNA polymerase (Thermo Scientific) and 50 ng template DNA.

The PCR amplification program was started by initial denaturation temperature at 94°C for 5min followed by 42 cycles denaturation temperature at 94 °C for 1 min, annealing at 38 °C for 1 min, and extension at 72 °C for 2 min, followed by final extension at 72 °C for 8 minutes. After that, the temperature was lowered to 4 °C.

Electrophoresis was done at 100 V for 75min. and the bands were visualized under UV Trans-illuminator (BioDoc-It® Imaging System) and photographed as shown in plate 1.

Plate 1: RAPD gel profile with fragments generated by primers (OPP-02, OPP-04, OPP-07, OPK-04, OPK-10 and OPO-06) in 12 maize genotypes





*The numbers listed above refer to the collection listed in Table 1 and "M" is 1kb plus DNA size marker (Thermo Scientific).

1=genotype A, 2= genotype B, 3= genotype C, 4= genotype D, 5= genotype E, 6= genotype F, 7= genotype G1, 8= genotype G2, 9= genotype H1, 10= genotype H2, 11= genotype J1, 12= genotype J2

Data scoring and analysis

The clear bands in each variety were compared and recorded as '0' (absent) or '1' (present) values. Band patterns ('0', '1' matrix) were tabulated for individual primers separately and the data were pooled to obtain a combined matrix for the varieties. The NTSYS-pc software program version 2.1 (Rohlf, 2000), was used to compute the binary data ("0", "1") and evaluate the genetic associations between genotypes. Pair-wise comparisons were made between lines based on Jaccard similarity coefficient (Jaccard, 1901) and visualized by cluster analysis, Unweighed Pair-Group Method with Arithmetic averages (UPGMA) and illustrated in a phylogram.

A principal coordinate analysis was performed based on Jaccards' coefficients for all individuals and a plot was generated using the first two principal coordinates.

The bootstrap procedure was applied to calculate variance of the genetic similarities obtained from the markers and, thus, to verify the consistency of the obtained phylogram. The variance coefficient was obtained from 1,000 bootstrap random draws using the DBOOT program (Coelho, 2001). Analysis of molecular Variance (AMOVA) was carried out by grouping the genotypes into nine populations. Population 1 contained inbred A and population 2 to 6 contained inbreds B to F respectively. Population 7 contained hybrids G1 and G2, population 8 contained hybrids H1 and H2 while population 9 contained hybrids J1 and J2.

RESULTS

In total, 14 primers amplified 123 bands in the parents and hybrids in range of 150bp (OPP-04) to 1500 bp (OPK-04). Among these; 98 fragments (80%) were polymorphic. On average, 9 bands per primer were observed with maximum of 13 bands and minimum of 6 bands. The primers OPP-04, OPK-03and OPK-05 were found to produce 100 % polymorphic fragments and the lowest polymorphism (38%) was exhibited by primer OPP-02 (Table 3).

Table 3: Band sizes, number of bands amplified per primer, number and percentage of polymorphism in maize genotypes

S/no.	Primer	Sequence	Size of Fragments in base pairs		Total no. of bands	No. of Polymorphic bands	Per cent Polymorphism
			Largest	Smallest			
2	OPP-02	TCGGCACGCA	1700	450	8	3	38
4	OPP-04	GTGTCTCAGG	2200	150	9	9	100
5	OPP-05	CCCCGGTAAC	2900	300	7	6	86
7	OPP-07	GTCCATGCCA	2400	500	10	6	60
12	OPK-03	GTCTCCGCAA	3000	400	11	11	100
13	OPK-04	CCAGCTTAGG	5000	400	13	12	92
14	OPK-05	CCGCCCAAAC	2600	400	11	11	100
17	OPK-08	AGCGAGCAAG	2000	300	6	4	67
18	OPK-10	GAACACTGGG	4000	600	11	10	91
21	OPO-04	ACGTAGCGTC	1800	200	10	5	50
22	OPO-05	AAGTCCGCTC	1750	500	6	3	50
23	OPO-06	CCCAGTCACT	3500	450	8	7	88
24	OPJ-05	CCACGGGAAG	2500	800	6	5	83
25	OPJ-07	CTCCATGGGG	2000	500	7	6	86
Total					123	98	80
Average					9	7	80

Based on Jaccard's similarity coefficients (Table 4), the similarity coefficients ranged from as low as 0.32 (between inbred B and hybrid H-2) to as high as 0.95 (between hybrids H-1 and H-2). The analysis of the molecular data revealed that there was more similarity between the F1 hybrids than the inbreds (parents). The similarity coefficients for inbreds ranged from 0.33 between B and D to 0.83 between C and D. The similarity coefficients for hybrids were high ranging from 0.72 (between G-1 and H-1) to 0.95 (between H-1 and H-1).

Table 4: Jaccard's similarity coefficient of 14 RAPD primers for twelve maize inbred lines and hybrids

	A	B	C	D	E	F	G-1	G-2	H-1	H-2	J-1	J-2
A	1.00											
B	0.43	1.00										
C	0.63	0.34	1.00									
D	0.58	0.33	0.83	1.00								
E	0.58	0.34	0.75	0.78	1.00							
F	0.55	0.35	0.67	0.73	0.64	1.00						
G-1	0.66	0.44	0.80	0.74	0.72	0.64	1.00					
G-2	0.69	0.42	0.80	0.74	0.68	0.63	0.91	1.00				
H-1	0.63	0.34	0.79	0.73	0.66	0.66	0.72	0.77	1.00			
H-2	0.61	0.32	0.84	0.77	0.70	0.69	0.74	0.78	0.95	1.00		
J-1	0.64	0.36	0.82	0.74	0.70	0.66	0.78	0.80	0.79	0.84	1.00	
J-2	0.70	0.39	0.74	0.67	0.66	0.60	0.75	0.79	0.82	0.79	0.89	1.00

The neighbor joining tree constructed for pooled data has grouped the genotypes in to two major clusters. All the genotypes were grouped in one cluster except F which was clustered separately and was loosely aggregated with other genotypes. The genotypes that were found in one cluster were further divided into two sub-clusters. The inbred C was found in one sub-cluster and the rest inbreds and hybrids in another sub-cluster. The genotypes found in another sub-cluster were further divided into two sub-clusters which were divided again in two sub-clusters. Finally, the inbred A and B as well as hybrids G-1 and G-2 were grouped in one cluster while the hybrids H-1, H-2, J-1 and J-2 were grouped in another sub-cluster as shown in figure 1.

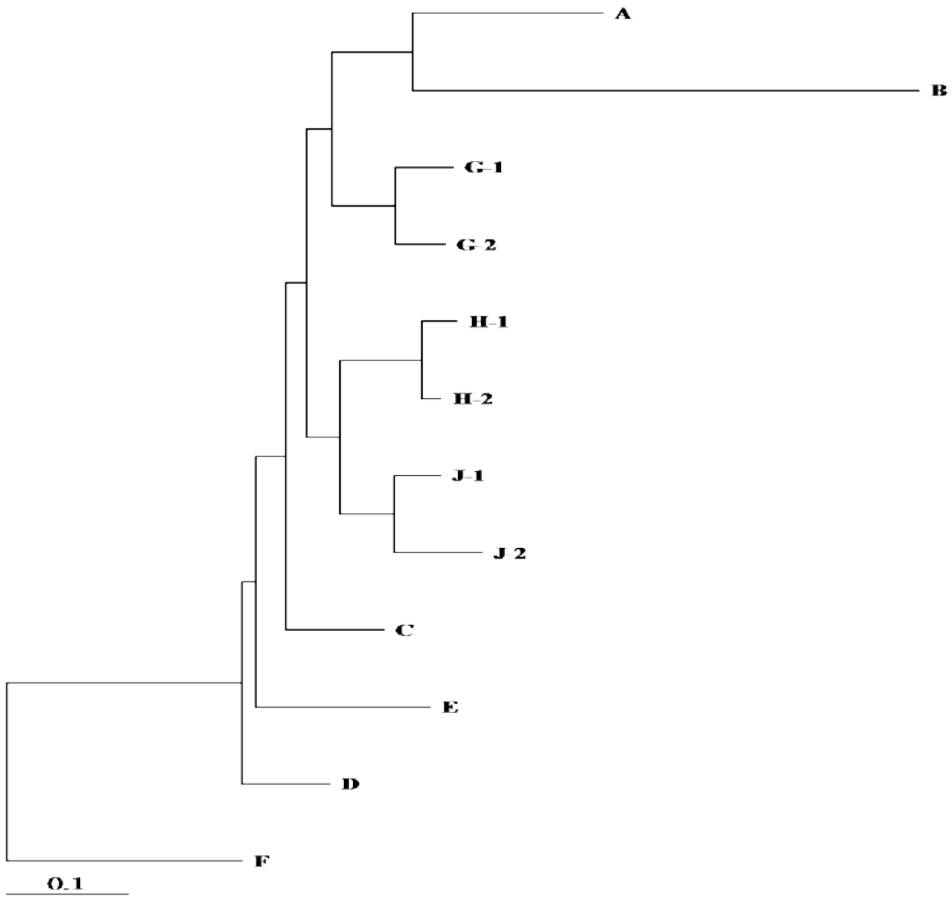


Fig. 1: Neighbor joining tree for the 12 maize genotypes using Jaccards similarity coefficient from NTSYSpc software

The phylogram constructed based on similarity coefficients using UPGMA grouped the genotypes into two major groups. The first group contained all inbred lines and hybrids except inbred B which was clustered itself in the second group. These two groups joined at 0.37 coefficient of similarity. The first group was further subdivided into two sub-clusters where the first sub-cluster contained inbred A and the rest genotypes in the other cluster. These two sub-clusters have joined at 0.61 similarity coefficient. The second sub-cluster has been sub-divided into two where the inbred F has formed one cluster while the rest genotypes fall in the other cluster. Further sub-division has lead to grouping inbred E in separate cluster and the remaining genotypes further sub-divided that the inbreds C, D, and hybrids G-1, G-2 were formed one

cluster while the hybrids H-1, H-2 and J-1, J-2 formed another clusters. The overall coefficient of similarity ranged from 0.37 to 0.95 as shown in figure 2.

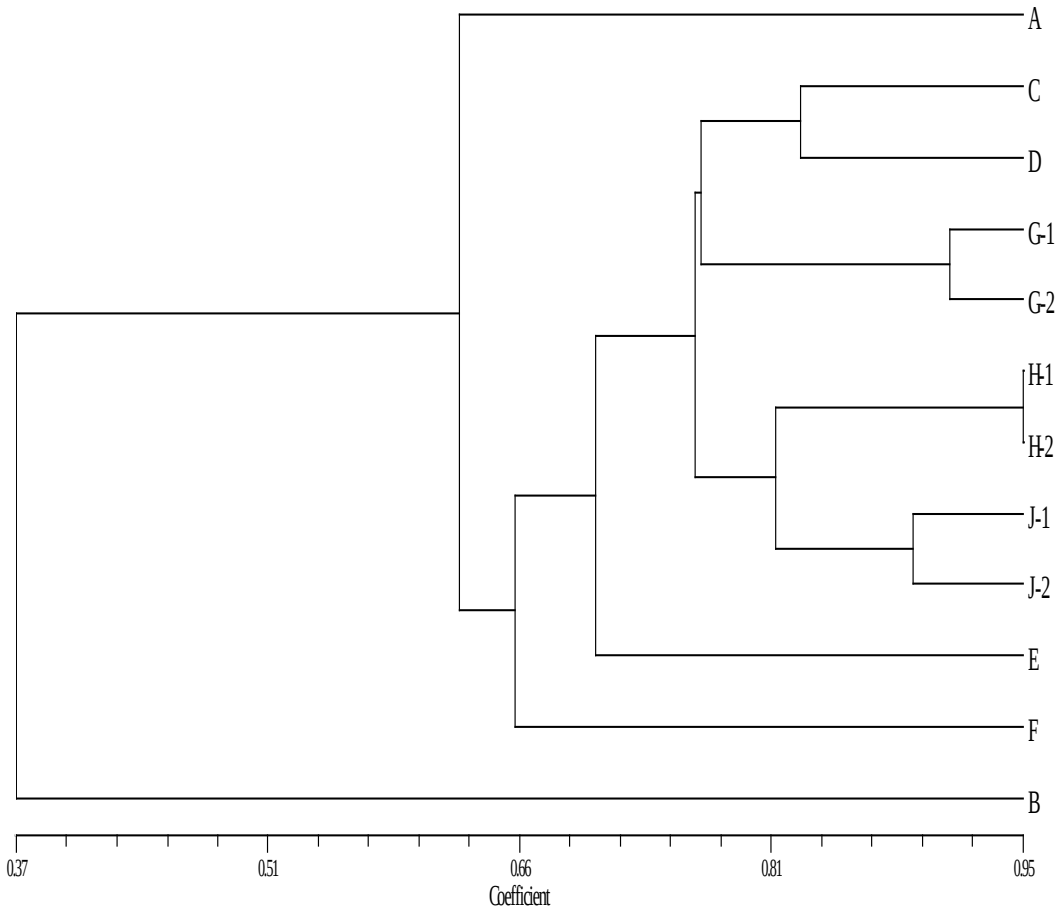


Fig. 2: UPGMA clustering for the 12 maize inbred lines and hybrids based on Jaccard similarity coefficent from RAPD data using NTSYSpc software.

Similar results have been obtained using Population Genetic (POPGENE) software to analyze the RAPD data as shown in figure 3. The results have obtained when the data was bootstrapped 1000 times. This indicates the consistence and reliability of the results obtained from this study.

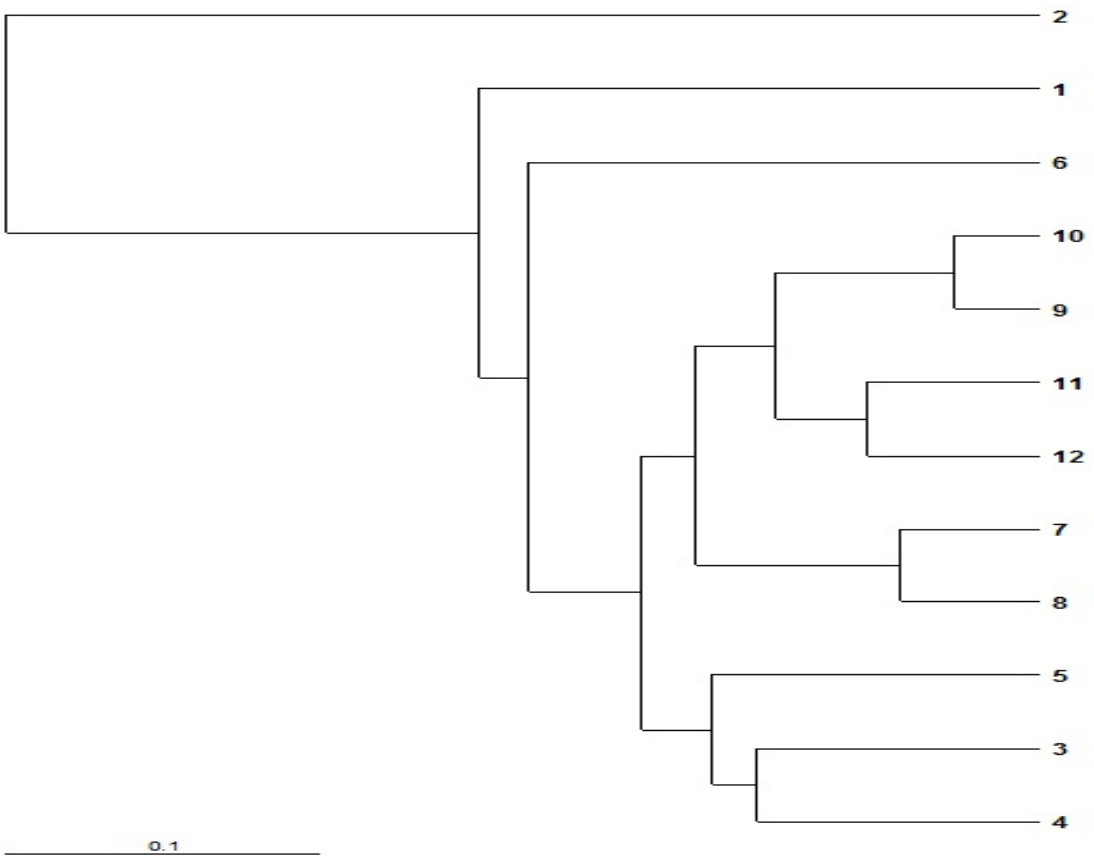


Fig 3: UPGMA phylogram clustering for the 12 maize inbred lines and hybrids (1000 bootstrap support) using POPGENE

Note: In figure four; 1=genotype A, 2=B, 3=C, 4=D, 5=E, 6=F, 7=G1, 8=G2, 9=H1, 10=H2, 11=J1, 12=J2

The Principal coordinate Analysis (PCA) indicates that the genotypes have grouped into two major groups. The genotype with identity '2' was clustered independently from the rest of the genotypes. The clustering of the genotypes are in agreement with the clustering observed in phylograms in figure 2 and figure 3.

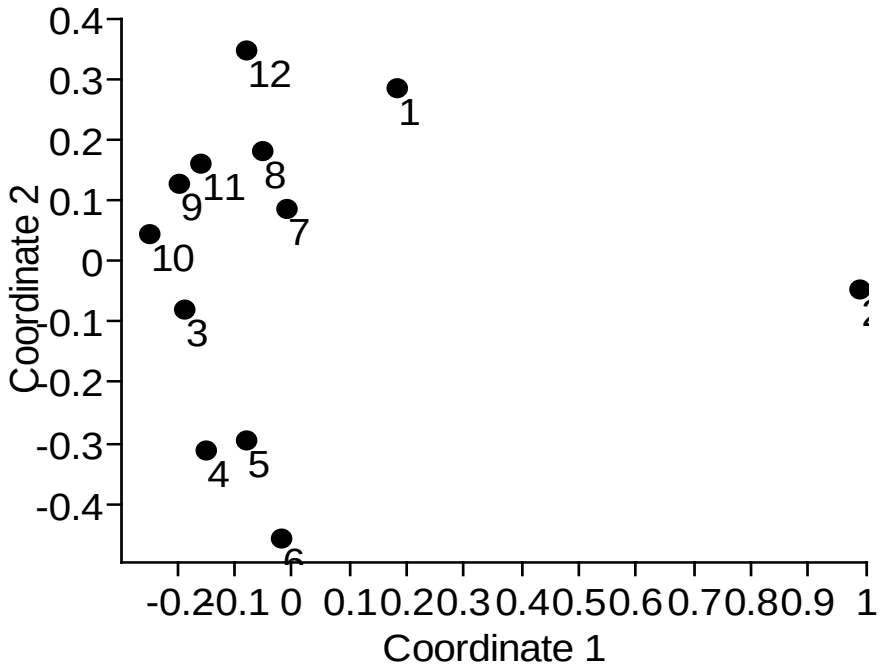


Fig. 4: Two dimensional principal coordinate analysis results for the twelve maize genotypes

DISCUSSION

Random Amplified Polymorphic DNA (RAPD) has shown to be effectively in studying genetic diversity of the studied maize genotypes. The polymorphism of 80% produced was high enough to detect the differences existing between the genotypes. This diversity was higher compared to 73.02%, but lower compared to 84.44% and 97.03% reported by Mukharib *et al*., (2013), Bruel, *et al*., (2007) and Handi, *et al*., (2013) respectively.

The similarity coefficient ranged from 0.32 (between inbred B and hybrid H-2) to 0.95 (between hybrids H-1 and H-2) indicates that there is wider diversity among the genotypes used in the study. The similarity coefficients between the hybrids (0.72 to 0.95) were high compared to the similarity between the inbred; (0.33 to 0.83).

Cluster phylogram based on similarity coefficients using UPGMA depicted that there was significant diversity among the inbred lines under the study ranging from 0.37 to 0.83 Similarity

Coefficient. Comparatively, the inbred lines A and B were grouped in one cluster but they are diverse (57.5%) as shown in similarity matrix (Table 1). The inbreds A and B were clustered with hybrids G-1 and G-2 because G-1 and G-2 are hybrids resulted from cross between inbred A and B (Table 1).

The other inbred lines; C, D, E and F are also diverse ranging from 0.83 similarity coefficient (SC) between C and D to 0.33 SC between E and F. Generally, the similarity between the hybrids ranges from 0.72 SC between H-1 and G-1 to 0.95 between H-1 and H-2. The higher similarity between hybrids H-1 and H-2 is due to the fact that these hybrids are from the same parents and the samples have taken in duplicate (Table 1).

The inbred B has clustered itself while the rest of the genotypes have clustered together forming one group. The hybrids G-1 and G-2 are the cross between A and B. The inbred B has stood alone forming its own cluster while A has clustered together with G1 and G2. This indicates that the hybrids G-1 and G-2 have drown much from A (female parent) than B (male parent).The similarity coefficients of the other inbred lines (C, D, E and F) ranged from 0.83 similarity coefficient between C and D to 0.33 between E and F. Generally, the similarity between the hybrids ranges from 0.72 between H-1 and G1 to 0.95 between H-1 and H-2. The higher similarity between hybrids H-1 and H-2 is due to the fact that these hybrids are from the same parents and the samples have been taken in duplicate.

It is important to consider that the level of polymorphism to be obtained depends on the degree of divergence between the genotypes under study (Bruel,*et al*., 2006). Another aspect to be considered is that, in this study, the primers used were rigorously pre-selected, taking into account the number and quality of the amplification products, and this may have contributed to increase polymorphism.

CONCLUSION

Based on the results from this research; the greatest similarity was observed between genotypes belonging to the same heterotic group, and the smallest similarity coefficient was observed between genotypes belonging to different heterotic groups. High rate of polymorphism (80%) has been observed in this research. The maize genotypes used were highly diverse with similarity

coefficient ranging from 0.37 to 0.95. The genetic diversity was high between the inbred lines than in hybrids.

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