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Original article

Simple Sequence Repeat (SSR) marker-based Genetic diversity assessment of some Maize (*Zea mays* L) landraces in Nigeria

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ABSTRACT

Genetic diversity can be enlarged by combining desired traits from different local and wild populations of different geographical origins into the breeding lines. This study is aimed at assessing the diversity among 23 maize landraces using 10 simple sequence repeats (SSR) markers. The study identified a total of 19 alleles among the 10 primers used. The mean allelic frequency was 0.8304 and the highest allele per locus was recorded in the primer pairs bnlg 105, bnlg 1754, Phi 083 andP-umc1682F - 2324R, as they also showed the lowest allele no (1.000) respectively. The primer Umc 1568 showed the highest percentage polymorphism (65%) having a total number of 15 bands, with a mean value of 0.73 and PIC value of 0.68 more than other primers. A dendrogram constructed from pooled data revealed 13 clusters showing some level of polymorphism. Some landraces (JG-02, NG-02, KW-03, KD-02,NG-03 and JS-0) were relatively distinct from others at 4999.50 exhibiting genetic divergence while others from different origins were clustered together revealing genetic similarity. The results revealed that SSR markers can efficiently evaluate genetic variation in maize germplasm which could be efficiently used in current and future breeding programmes

Keywords: Maize germplasm, Molecular markers, Primers, Dendrogram, Genetic resources
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INTRODUCTION

Maize (*Zeamays* L.) is ranked third after wheat and rice in the world production of cereal crops and is widely cultivated throughout the globe in a wide range of agro-ecological environments (22). The demand for maize global production as a source of food, forage, oil, and biofuel is increasing for the ever-increasing world human population. However, the number of maize landraces decreases in farmers' fields over time, threatening the availability of genetic resources for the future.

Genetic diversity is the foundation for crop improvement, and it plays an important role in breeding programs (3). Information on genetic diversity and relatedness among maize inbred lines have been useful in selecting parental combinations for developing superior hybrids and for assigning inbred lines into heterotic groups (10). Morphological markers have been widely used to assess genetic diversity because they are cheap, rapid, and easy to measure (20). However, they are highly influenced by the environment and several other factors limit their ability to estimate genetic diversity (17). Molecular markers are a useful tool for assessing genetic diversity because they are stable, polymorphic, readily available in the genome, and are not sensitive to environmental factors (14). Several types of molecular markers have been used to assess genetic diversity and group maize inbred lines into heterotic groups (13)

The development of molecular markers to measure the relationships between plants and genetic diversity depends on polymorphisms found in DNA (19). Polymerase chain reaction (PCR)-based approaches are commonly used to assess the genetic diversity of maize genetic resources. Simple sequence repeats markers (SSR), also known as micro-satellites, were used to characterize and differentiate the Bulgarian maize germplasm collection (16), the isolation-by-distance and altitude of maize landraces in the Western Highlands of Guatemala (22), and also for the assessment of genetic diversity among maize inbred genotypes developed in Italy (Losa *et al.,* 2011). The objectives of this study were, therefore, to assess the genetic diversity among some maize landraces grown in northern part of Nigeria using Simple Sequence Repeats molecular marker (SSR).

MATERIALS AND METHODS

Germplasm Collection of Maize (*Zea mays*) Seeds

A germplasm collection exploration was undertaken to collect the maize seeds from local farmers and markets in collaboration with Agricultural Development projects (ADP) extension officers to all areas of production (i.e. some major maize producing areas of the country) to collect the known genotypes of the crop in Nigeria. Germplasm collection mission was undertaken to north central, northwest, part of Nigeria, representing the major maize producing areas of the country which cut across seven states. The states visited includes, Niger, Nassarawa, Kwara, Plateau, Kaduna, Jigawa and Bauchi respectively.

Raising Seedlings for Sample collection

The experiment was carried out at Tsaragi Research Farms, Ilorin Nigeria. The accessions were grown in a complete randomized design with three replicates Each accession was grown in single row plots, with inter and intra-row spacing of three (3) feets each (16).

Molecular Analysis

The molecular work was carried out at Bioscience Center, International Institute for tropical Agriculture (IITA) Ibadan, Nigeria.

Sample Collection

The leaves of each of the 23 maize accessions were collected from young (2 weeks). The leave samples were dried

using silica gel and taken to IITA, Ibadan for analysis.

DNA extraction

Total nucleic was extracted using a modified CTAB extraction protocol. Briefly, approximately 100mg the young leaves of the maize samples were grinded in 1ml CTAB extraction buffer (2% CTAB (Cetyltrimethyl ammonium bromide) powder (w/v), 100mM Tris-HCl, 20mM EDTA, 1.4M NaCl and 0.2% β-Mercaptoethanol (v/v) (Add just before use)) in a sterile mortal and pistil. sap was poured into new sterile tube and vortex briefly followed by an incubation at 60^0 C for 10mins. Immediately, tubes were placed on ice and add equal volume of Phenol, Chloroform and Iso-amyl alcohol at 25:24:1 was added followed by vortex and centrifuge at 12000 rpm for 10mins. 450µl of the supernatant was collected into new sterile tube to which 300µl of cold Isopropanol was then added and Mixed gently and incubate for 1hr at -20^o C. Samples were then Centrifuge at 12000g for 10mins. to sediment the nucleic acid followed by Decanting the supernatant gently and ensured the pellets were not disturbed. pellets were then washed by adding 500µl of 70% ethanol and centrifuge at 12000 rpm for 5mins. This was repeated twice. Ethanol was then decanted and the RNA air dried at room temp. Pellets were then suspended in 50µl TE buffer or sterile water treated with EDPC till further use/storage.

Polymerase Chain Reaction (PCR)

Using 10 selected maize SSR specific primers, PCR was set up to detect the extent of diversity in the 23 maize landraces. Reaction cocktail for each SSR primer pair consisted of 2.5 µl of 5x PCR SYBR green buffer (2.5) , 0.75 µl of 25mM $MgCl_2$, 0.25 µl of 10 mM of dNTPs mix, 0.25 µl of 10 pmol each forward and reverse primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 2µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a touchdownof 50°C -60°C (with a temperature increase by 1°C after every 5 cycles) and a final termination at 72°C for 10 mins. And chill at 4^oC.

Band separation

The separation of bands as produced by each primer was done in a 1.5% Agarose gel. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Seven µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was subjected to electrophoresis at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

Data analysis

Binary data was then generated for each primer set using 1 (presence of positive amplification at a particular band size) and 0 (absence of positive amplification at a particular band size) the generated binary data was the used to create a data matrix which was analyzed using the Powermarker V2.35 software (Kejun and Spencer. 2005). Genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content were then generated using the power marker software. The genetic relationship among treated samples were also estimated by constructing a dendrogram through unweighed pair group method with arithmethmetic Averages (UPGMA)

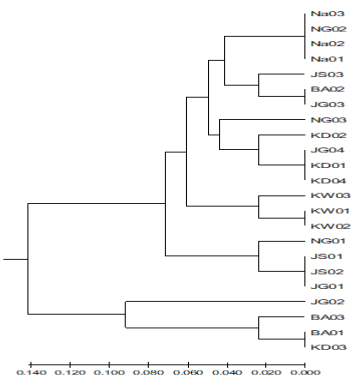


Figure 1: Dendrogram of twenty-three maize accessions based on SSR markers according to the unweighted pair group mean algorithm (UPGMA) method based on a similarity matrix by MEGA 6 software

RESULTS

The genetic distances among the twenty-three maize landraces were obtained using the unweighted pair group method of arithmetic averages (UPGMA) as indicated in Figure 1 below. The obtained dendrogram grouped the landraces into five main Clusters at the molecular distance between 0.00-0.14, Based on the clustering, Cluster I had the highest number of clusters with a total number of twelve genotypes, which was further subdivided into two sub clusters; sub cluster IA containing 7 genotypes which are Na03, NG02, Na02, Na01, JS03, BA02 and JG03, and 5 genotypes in the sub cluster IB which includes NG03, KD02, JG04, KD01, KD04 (Figure 1).

Cluster II consists of three genotypes which are KW03, KW01 and KW02, Group III had four genotypes which includes NG01, JS01, JS02 and JG01 respectively. Group IV includes the genotype JG02 which is genetically distinct from all other genotypes and group V genotypes includes BA03, BA01 and KD03, (Figure 1).

Genotypes revealed varying degrees of genetic diversity ranging from 0.00 to 0.402 in Jaccard coefficient (Figure 2). The least similarity coefficient of zero (0) was observed among the landraces

Na02, Na01 and Na03 all in sub cluster 1A, in addition, the accession NG02 is highly related to the accessions Na01, Na02 and Na03 with high similarity coefficient of zero (0), indicating high genetic relatedness and low genetic diversity between the landraces. The highest diversity (least similarity coefficient, A nd BA01, JG04 and BA03, Kd03 and JS01, KD03 and JS02, KD03 and KW02, KD03 and kw02, KD04 and BA03, which confirms that these genotypes were genetically diversified, (Figure 2). The 23 maize land races yielded as many as 19 alleles, with an average allele frequency of four (4). The SSR primers sets bnlg 105, bnlg 1754, Phi 083 and Pumc1682F-2324 R demonstrated fewer allele numbers, (Table 1).

The PIC values ranged from 0.00 to 0.6848, (Table 2). PIC values revealed that bnlg182 and umc1568 were the most efficient in detecting the polymorphic information content among the 23 studied Maize landraces

(0.402) was found between accessions BA02 and JS01, BA02 and JS02, BA03 and Na01, BA03 and Na02, BA03 and Na03, KW01 and BA03, KW02 and BA03, KW03 Na03 with high similarity coefficient of zero (0), indicating high genetic relatedness and low genetic diversity between the landraces. The highest diversity (least similarity coefficient, (0.402) was found between accessions BA02 and JS01, BA02 and JS02, BA03 and Na01, BA03 and Na02, BA03 and Na03, KW01 and BA03, KW02 and BA03, KW03

Figure 2: Jacards similarity coefficient of the date palm accessions Based on SSR Marker

	Na01	Na02	Na03	NG01	NG02	NG03	JS01	JS02	JS03	BA01	BA02	BA03	KW01	KW02	KW03	JG01	JG02	JG03	JG04	KD01	KD02	KD03
Na01																						
Na02	0																					
Na03	0	0																				
NG01	0.101	0.101	0.101																			
NG02	0	0	0	0.101																		
NG03	0.101	0.101	0.101	0.101	0.101																	
JS01	0.16	0.16	0.16	0.048	0.16	0.16																
JS02	0.16	0.16	0.16	0.048	0.16	0.16	0															
JS03	0.048	0.048	0.048	0.16	0.048	0.16	0.228	0.228														
BA01	0.308	0.308	0.308	0.308	0.308	0.16	0.402	0.402	0.228													
BA02	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.048	0.16												
BA03	0.402	0.402	0.402	0.228	0.402	0.228	0.308	0.308	0.308	0.048	0.228											
KW01	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.16	0.308	0.101	0.402										
KW02	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.16	0.308	0.101	0.402	0									
KW03	0.16	0.16	0.16	0.048	0.16	0.16	0.101	0.101	0.228	0.402	0.16	0.308	0.048	0.048								
JG01	0.16	0.16	0.16	0.048	0.16	0.16	0	0	0.228	0.402	0.16	0.308	0.16	0.16	0.101							
JG02	0.228	0.228	0.228	0.228	0.228	0.228	0.308	0.308	0.16	0.16	0.101	0.228	0.228	0.228	0.308	0.308						
JG03	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.048	0.16	0	0.228	0.101	0.101	0.16	0.16	0.101					
JG04	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.16	0.308	0.101	0.402	0.101	0.101	0.16	0.16	0.101	0.101				
KD01	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.16	0.308	0.101	0.402	0.101	0.101	0.16	0.16	0.101	0.101	0			
KD02	0.048	0.048	0.048	0.048	0.048	0.048	0.101	0.101	0.101	0.228	0.048	0.308	0.048	0.048	0.101	0.101	0.16	0.048	0.048	0.05		
KD03	0.308	0.308	0.308	0.308	0.308	0.16	0.402	0.402	0.228	0	0.16	0.048	0.308	0.308	0.402	0.402	0.16	0.16	0.308	0.31	0.23	
KD04	0.101	0.101	0.101	0.101	0.101	0.101	0.16	0.16	0.16	0.308	0.101	0.402	0.101	0.101	0.16	0.16	0.101	0.101	0	0	0.05	0.31

Table 1: Allelic frequency and Allelic Number of the 10 Studied Primers

S/N	Marker	Major Allele Frequency	Allele No	Availability
1	bnlg105	1	1	1
2	bnlg 1754	1	1	1
3	Phio72	0.86	2	1
4	Phi 034	0.82	2	1
5	Umc 1568	0.34	4	1
6	Phi 083	1	1	1
7	Phi 085	0.7	2	1
8	bnlg182	0.74	3	1
9	umc1682F	1	1	1
10	bnlg176	0.83	2	1
	Mean	**0.8304**	**19**	**1**

Table 2: Polymorphism obtained from 10 SSR primers among the 23 Maize landraces Studied

Primer Name	Forward Reaction	Reverse Reaction	Polymorphic Band	Polymor Phism (%)	Gene Diversity	PIC
bnlg105	GACCGCCCGGGACTGTAAGT	AGGAAAGAAGGTGACGCGCTTTTC	0	0	0	0
bnlg1754	CCATCGCTGTACACATGAGG	TACCCGAAGGATCTGTTTGC	0	0	0	0
Phi072	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT	2	8.7	0.22	0.2
Phi034	TAGCGACAGGATGGCCTCTTCT	GGGGAGCACGCCTTCGTTCT	3	13	0.3	0.24
Umc1568	AAGTCCAGCCAAGTTCATCAAAGA	ACTGTAACTAAACTGGGTGTGCCC	15	65	0.73	0.68
Phi083	CACCCGATGCAACTTGCGTAGA	TCGTCACGTTCCACGACATCAC	5	21	0	0
Phi096	TCCACCATTTGACACTTAGGCA	GCGTAGGACGACCGTTGAA	4	17	0.4	0.33
Bnlg182	AGACCATATTCCAGGCTTTACAG	ACAACTAGCAGCAGCACAAGG	6	26	0.42	0.4
umc1692	AGAGACGAACTGAAGCCTGAAGTG	GATGTCCACGTCCTGGTAGAAGTT	0	21	0	0
bnlg1176	ACTCCTCAAAACCTAGGTGACA	CACCGATGATGGTGAGTACG	4	17	0.3	0.3

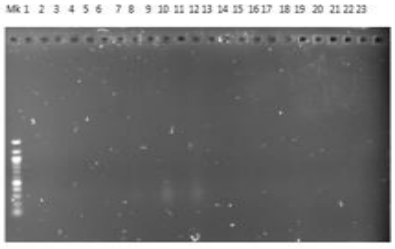


Plate I. Gel image for bnlg105 primer

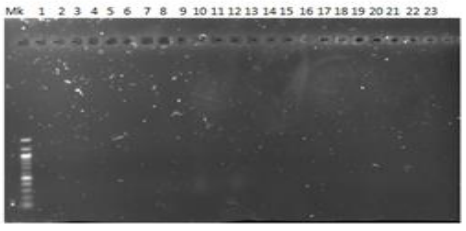


Plate II. Gel image for bnlg 1754 primer

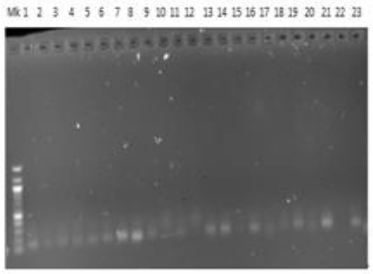


Plate III. Gel image for Phio72 primer

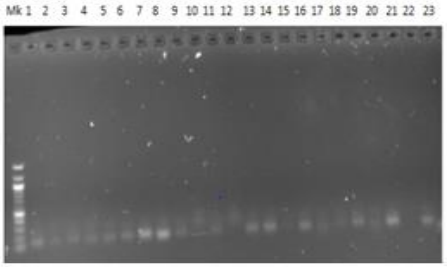


Plate IV. Gel image for Phi 034 primer

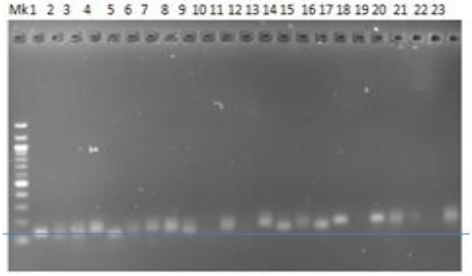
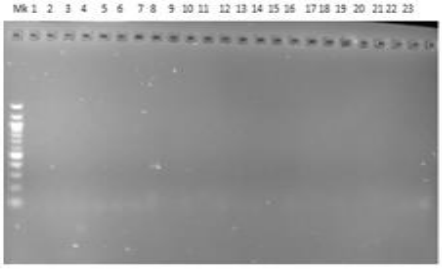


Plate V Gel image for U mc 1568 primer



PlateVI. Gel image for Phi 083 primer

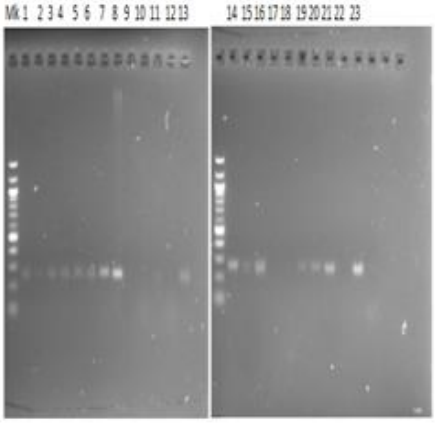


Plate VII. Gel image for Phi 085 primer

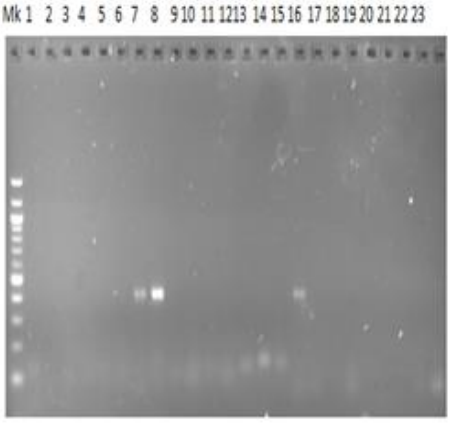


Plate VIII. Gel image for bnlg182 primer

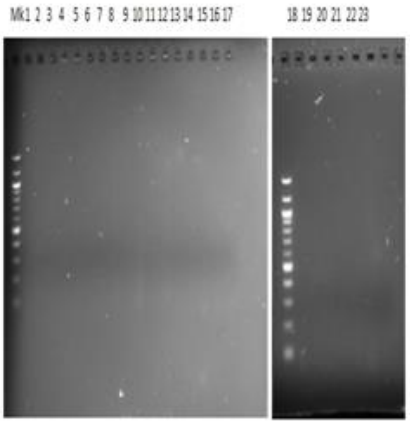


Plate IX Gel image for umc1682F primer

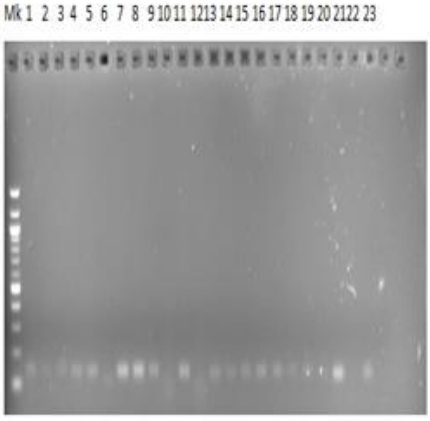


Plate X Gel image for bnlg176 primer

DISCUSSION

The advent of the molecular marker technique has made the evaluation of genetic diversity easier because environmental factors do not control them. Several types of these markers have been used to assess the level of genetic diversity in different crops ranging from dominant to co-dominant markers.

The allele number of 4.0 observed in one of the primers is analogous to the 4 to 17 alleles number per loci as observed in some studied maize populations as reported by (1) who also reported that the difference in allele numbers might be as a result of the difference in the methodology used in the detection of polymorphism and plant genetic material used.

6.3 allele number as recorded by Pejic *et al.,* 1998 in their study of Genetic diversity of some maize inbred lines in United states corn belts using 27 SSRs, this has also been supported by (25) who also reported 7.4 alleles in each locus in 155 inbred using 79 SSRs. though disagrees with the study as reported by (8) who identified 127 alleles from 30 evaluated loci in their genetic diversity and structure study with 31 popcorn accessions using 30 primers, the variations in the allele number might be as a result of the number of primer used .The lowest gene diversity of 0.22 recorded by the primer umc 1658 in this study, is an indication of low genetic diversity observed among the landraces and perhaps might be an indication that the markers detecting fewer alleles had less gene diversity in comparison to those detecting more alleles, this has also been supported by (4) in their study on genetic diversity assessment among exotic and native maize inbred lines of Bangladesh using Microsatelite marker

The results from the dendrogram indicated the clustering of some landraces collected from the same origin together e.g the accession KW-01 and KW-02, Na-01, Na-02 and Na-03, the clustering together of the landraces are indications that genetic divergence followed geographical separation. This has also been reported on other crops like Sesame (*Sesamum indicum* L) where (2) reported in their study on genetic diversity of sesame accessions in Nigeria and opined that some accessions have adapted well to their local environments through gene rearrangement due to long periods of

cultivation, making them to become ecotypes.

Molecular characterization is the most effective and reliable method to detect and quantify variation within and among germplasm collections. Genetic variation is detected using SSR markers in maize that could easily detect the phenotypic variations and desirable biological functions (5). Similarly, 65 inbreds lines were investigated using 60 SSRs by (9). (12) investigated diversity in six inbreds lines using 44 SSRs. Moreover, 95 inbred lines were investigated using 1,536 SNPs by(15).

In the present study, genetic diversity among 23 inbred lines was assessed using SSR Marker with a Polymorphic Information Content (PIC) values ranging from 0.2-0.68, demonstrating a good discriminatory power of the markers in assessing genetic diversity among the landraces, This supports the opinion of (7) who reported that the PIC values can be classified into three categories (i) if the PIC value of the marker is more than 0.5, the marker is considered as highly informative, (ii) if the PIC value ranged from 0.25 to 0.5, the marker is moderately informative, and (iii) if the PIC value is less than 0.25, then the marker is slightly informative. Several loci showed PIC values greater than 0.70, indicating that the SSR markers used in the present study were highly informative.

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