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MICROSATELLITE DNA MARKER FOR MOLECULAR CHARACTERIZATION OF AFRICAN MAIZE (*Zea mays* L.) LANDRACES

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ABSTRACT

Maize (*Zea mays* L) originated from Mexico but has been introduced and domesticated in various parts of the world. Maize is an important cereal crop having subsistent, commercial and industrial uses. The aim of this study was to characterize genetic diversity in some African maize landraces using microsatellite DNA markers. Maize landraces were collected from some parts of Edo State, Nigeria, while others were sourced from IITA (International Institute of Tropical Agriculture), Ibadan, Nigeria and Premier Seed Company, Nigeria. Fourteen populations of 15 plants each were characterized, the application of 7 microsatellite markers sufficiently provided information on genetic diversity of all 14 populations investigated. The study revealed a total number of 21 alleles across all loci, with a mean number of 3 alleles per locus. Polymorphic information content (PIC) ranged from 0.26 (umc1161) to 0.71 (umc1196), with a mean of 0.52, indicating that markers used were polymorphic. The dendrogram displayed two main clusters; cluster 1 had most populations from Nigeria grouped together with populations from Malawi and Togo, while populations from Guinea, Chad, Tzm-ese, Tzm-enee and Tzm-1340 from Kano, Nigeria grouped together in cluster 2. Population Tzm-1413 from Somalia in East Africa was a complete outlier, as revealed by the dendrogram. Results suggest that the populations studied were not greatly diverse but can be used to establish a field trial, where germplasm will be scored based on adaptation, tolerance and resistance to biotic and abiotic factors, which will furthermore validate the genetic variability revealed by the microsatellite markers.

Keywords: Maize, landraces, SSR, microsatellite, characterization

INTRODUCTION

Maize (*Zea mays* L.) is a cereal crop of the Poaceae family widely cultivated in most parts of the world due to its adaptability and productivity **(Gerpacio & Pingali, 2007)**. Maize introduction from the centre of origin in Mexico into different growing conditions in the tropical, sub-tropical, and temperate regions has led to the advent of hundreds of diverse landraces **(Dubreuil *et al*., 2006)**.
Maize is the most important cereal crop in Sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in SSA and Latin America **(IITA, 2015)**. Landraces are heterogeneous populations which are genetically diverse and are typically selected by farmers for their adaptability potentials **(Prasanna & Sharma, 2005)**. They are important genotypes for crop breeding owing to their ability to adapt to specific environmental conditions and the large source of genetic variability that they provide **(Paterniani *et al*., 2000)**. Maize landraces are valuable sources of genetic variability and have been intensively used in breeding programs **(Udry & Duarte, 2000)**.
Research developing new biotechnological techniques has provided increased support to assess genetic diversity at both phenotypic and the genotypic levels **(Sajib *et al*., 2012)**. The advancement in the use of molecular markers has proved to be valuable for genetic diversity analysis at DNA level in plant species **(Melchinger & Gumber, 1998)**. Unlike morphological markers, molecular markers allow great number of lines to be characterized; hence, increasing the efficiency of maize breeding programs **(Choukan *et al*., 2006)**.
Microsatellites are di-, tri- or tetra nucleotide tandem repeats in DNA sequences. These are also known as SSRs (simple sequence repeats), STRs (short tandem repeats), STMs (sequence tagged microsatellites) and VNTRs (variable number of tandem repeats). SSRs are co-dominant; highly polymorphic and specific **(Jones *et al*., 1997)**; little DNA is required; very repeatable; so cheap and easy to run; need a small amount of medium quality DNA; the analysis can be semi-automated and performed without the need of radioactivity **(Guilford *et al*., 1997)**, and are highly transferable between populations **(Gupta *et al*., 1999)**. They have been used in mapping the genome of corn **(Taramino & Tingey, 1996)**, genetic fingerprinting **(Senior *et al*., 1998)**, and to characterize landraces **(Aci *et al*., 2013)**.
Characterization of genetically heterogeneous populations using molecular markers has before now been very expensive and time consuming **(Prasanna, 2012)**. A method for microsatellite analysis of pools of individuals from a population has proven to be cost effective than genotyping multiple individuals per population, and much more accurate than genotyping only one individual per population **(Dubreuil *et al*., 2006)**. **Liu *et al*. (2005)** studied sampling method with SSR markers and showed that bulk DNA from 15 individuals could help to assess genetic diversity of maize accessions.
Studies using bulking method and SSR markers are more efficient ways to study open pollinated varieties and populations of maize, and they have allowed the elucidation of the origin of European landraces **(Rebourg *et al*., 2001, 2003)**, genetic diversity of Southwest China landraces **(Yao *et al*., 2007)**, relationship between Latin American landraces **(Warburton *et al*., 2008)**, population genetic and diversity analysis of Indian landraces **(Wasala & Prasanna, 2012)**, genetic characterization of Ghanaian landraces **(Oppong *et al*., 2014)**. Moreover, research work on measuring genetic diversity and characterizations of maize landraces of African countries are few, hence this study.
The objective of this study was to characterize genetic diversity in some African maize landraces using microsatellite DNA markers.

MATERIALS AND METHODS

Plant materials

Landraces were collected from parts of Edo State (Egor, Ehor, Ekpoma, Uromi, Sobe and Okpella), and others were sourced from IITA (International Institute of Tropical Agriculture), Ibadan, Nigeria. An OP (open-pollinated) variety (Suwan-1SR) and a hybrid variety (Oba 98) from Premier Seed Company, Zaria, Nigeria were included as controls (Table 1).

Table 1 Accessions of maize landraces used in this present study

Population	Collection Country	State/Province	Remarks
Tzm-Esu	Nigeria	Edo	White grain
Tzm-Ese	Nigeria	Edo	White grain
Tzm-Ecew	Nigeria	Edo	white/ yellow grain
Tzm-Ecene	Nigeria	Edo	White grain
Tzm-Enow	Nigeria	Edo	White small grain
Tzm-Enee	Nigeria	Edo	Yellow grain
Tzm-307	Chad	Mayo, Kebbi	White small grain
Tzm-1340	Nigeria	Kano	Reddish-black grain
Tzm-1097	Malawi		White grain
Tzm-1413	Somalia	Lower Shebelle	White/yellow/purple grain
Tzm-1276	Togo		White grain
Tzm-1545	Guinea	Baguiherd	Yellow grain

Two hundred and ten individuals were used for this study; these samples were bulked subset from 14 main populations (Table 1). Fifteen individuals were randomly selected per population for bulking. These samples were planted in a 1kg plastic bag filled with sieved topsoil in the screen house of the Department of Soil Science, Faculty of Agriculture, University of Benin, Edo State, Nigeria for young leaves to emerge for DNA extraction.

DNA extraction

Fresh young leaves 200mg (0.2g) were harvested from 15 individuals per population. Bulk samples were prepared by adding the same amount of leaf materials excised from fifteen samples in a population to form a composite sample **(Wasala & Prasanna, 2012)**. DNA was extracted from the bulked fresh leaf samples using Dellaporta method with little modification **(Dellaporta *et al*., 1983)**. DNA quality was checked by DNA quantification using a Nanodrop Spectrophotometer (Thermo scientific, ND 1000 Spectrophotometer).

Microsatellite analysis

SSR primer pairs provided by the International Institute of Tropical Agriculture were screened with the maize DNA sample using TD (touch down) SSR-1 protocol in a Thermocycler. PCR (Polymerase chain reaction) cocktail mix was prepared in 10 µl tube containing reaction mixtures, 1 µl of 10X buffer, 0.4µl of 50Mm $MgCl_2$, 0.8 µl of 2.5ml dNTPs, 0.8 µl of DMSO, 0.5 µl of forward primer, 0.5µl of reverse primer, 0.1 µl of Taq, 2.9µl of ddH_2O, 3.0 µl of diluted DNA. The amplification conditions with the touchdown thermal cycling protocol were 95°C for 1 min, 30 cycles at 94°C for 1 min, 67°C annealing temperature decreasing 0.4°C per cycle for 2 min, 72°C for 2 min, and a terminal extension step at 72°C for 1 hour.
PCR products were electrophoresed, separated on 1% Agarose gel and visualized after staining with Ethidium Bromide using Uvitech gel documentation system (ENDURO^{TM} GDS, Labnet International, Inc.).

Data analysis

Each individual band was considered as a single loci/allele. Allele/loci (bands) were scored as present (1) or absent (0). Genetic analysis was conducted based on the scoring. Average number of alleles (n_a), number of effective alleles (n_e), expected heterozygosity (H_e), Shannon's index (I), the dendrogram was constructed by the Unweighted pair-group method with arithmetic averages (UPGMA) showing distance based inter relationship among samples using Nei's distance matrix (**Nei, 1972**). All parameters were computed and analyzed using Population Genetic Analysis Software (POPGENE Version 1.31) (**Yeh *et al.*, 1999**). Polymorphic information content was calculated as:

$$PIC = 1 - \sum_{i=1}^{n} Pi^2$$

Where P = allele frequency; n = number of alleles of the jth marker and i = the ith allele of the jth marker.

RESULTS AND DISCUSSION

Seven SSRs used for this study are distributed on 5 maize chromosomes (Table 2). A total of 21 alleles were observed across all SSR loci used for the study, the number of alleles per locus varied from 2 (umc1161) to 4 (umc1196), with a mean of 3 alleles per SSR locus (Table 2). This was lower than the results reported by **Aci *et al*. (2013)** who observed a total of 87 alleles and a mean of 5.8 alleles across 18 loci on 15 Algerian accessions; **Oppong *et al*. (2014)** who detected a total of 145 alleles and a mean of 7.3 SSR alleles per locus (across 20 loci) in a study of over 500 Ghanaian maize landraces (bulked DNA), The differences in the number of alleles recorded in this study compared to other works could be due to the size of the sample studied and probably, the fewer number of loci analyzed.

Table 2 Summary statistic of the markers used in the study

Marker	Bin	N_a	N_e	H_e	I	PIC
Phi96100	2.01	3	1.98	0.52	0.85	0.49
Phi093	4.08	3	3	0.71	1.10	0.67
Phi087	5.06	3	2.52	0.63	1.01	0.43
Umc1161	8.06	2	1.35	0.27	0.43	0.26
Phi059	10.02	3	2.97	0.69	1.09	0.67
Phi084	10.04	3	1.78	0.46	0.76	0.44
Umc1196	10.07	4	3.43	0.74	1.31	0.71
Mean		3	2.43	0.57	0.94	0.52

N_a = Observed number of alleles; N_e = Effective number of alleles; H_e = Expected heterozygosity
I = Shannon's Information index; PIC = Polymorphic information content

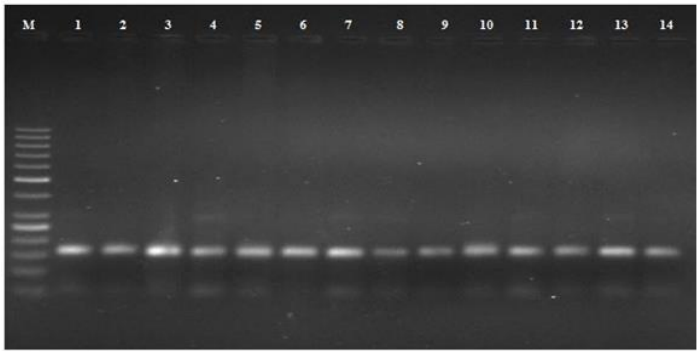

Figure 1 SSR bands for Phi084 viewed under UV light. M – 50bp DNA ladder, 1 – Tzm-esu, 2 – Tzm-ese, 3 – Tzm-ecew, 4 – Tzm-ecene, 5 – Tzm-enow, 6 – Tzm-307, 8 – Tzm-1340, 9 – Tzm-1097, 10 – Tzm-1413, 11 – Tzm-1276, 12 – Tzm-1545, 13 – Swam-1SR, 14 – Oba98

Number of effective alleles (N_e) is a function of the proportion of polymorphic loci, the number of alleles per locus, and the evenness of the allelic frequencies, thus it is a measure of the genetic information in a population or species **(Wasala & Prasanna, 2012)**. Number of effective alleles (N_e) for this study ranged from 1.35 (umc1161) to 3.43 (umc1196), with a mean of 2.43 across the loci analyzed (Table 2).The mean effective number of alleles across genotypes analyzed was lower than that recorded by **Wasala & Prasanna (2012)** and **Yao *et al*. (2007)**, of 3.85 and 3.90 respectively on 42 SSR loci each, which may be due to the number of SSR loci used as 7 SSR loci were deployed for this study. The Shannon–Weaver/wiener index is a measure of heterogeneity that include evenness and richness of species **(Hollenbeck and Ripple, 2007)**. A mean value of 0.94 for Shannon information index was detected in this study (Table 2); this was higher than the mean of 0.52 reported by **Salami *et al*. (2016)**.
H_e averaged 0.57 and ranged from 0.27 for umc1161 to 0.74 for umc1196 (Table 2). Heterozygosity mean value of 0.69 reported by **Yao *et al*. (2007)** on 42 SSR loci was higher than the mean of 0.57 recorded in this present study. **Aci *et al.* (2013)** reported a mean of 0.57; this is consistent with the heterozygosity mean obtained in this study. The genetic diversity mean of 0.46 reported by **Salami *et al*. (2016)** across 3 loci on 185 Benin landraces was lower than that observed in this study. All the SSR loci were able to detect genetic diversity which is defined as the probability that 2 randomly chosen alleles from the population are different among the maize genotypes **(Liu & Muse, 2005)**.
PIC values of each marker varied for all SSR loci, from 0.26 to 0.71, with a mean of 0.52 (Table 2). The highest mean value of 0.71 was obtained from umc1196, and the lowest value was detected in umc1161 (0.26). PIC of the SSRs was relatively high with a mean of 0.52. Similar PIC mean have been recorded by other researchers in genetic diversity and characterization studies of *Zea mays* L. **Banisetti *et al*. (2012)** reported an average PIC value of 0.49 on 22 maize genotypes, this is comparable to PIC average detected in this study. PIC average of this study indicate that markers were polymorphic and informative.
The trend across all loci studied showed marker umc1196 having the highest value across all loci for number of alleles, number of effective alleles, Shannon's information index, PIC, and heterozygosity, while umc1161 had the lowest values. **Oppong *et al.* (2014)** recorded umc1196 also having higher values than umc1161, this is an indication that although both markers are polymorphic, Umc1196 is more polymorphic compared to Umc1161.
Dendrogram displayed that the genotypes from Edo state, Nigeria fell into the same cluster (cluster 1). Genotypes from the Central part of Edo state, Nigeria (Tzm-ecew and Tzm-ecene) were more related than other genotypes in the cluster as expected. Tzm-1276 from Togo, and Suwan-1SR an open pollinated variety from Nigeria were also grouped in cluster 1, Tzm-1097 from Malawi grouped within the sub clusters of cluster 1 and displayed genetic similarity with populations in cluster 1 and 2 (Figure 2). Tzm-ese from the Southern part of Edo state and Tzm-enee from the Northern part of the state grouped together in cluster 2 displaying no genetic difference, this could be because most farmers tend to introduce their superior local genotypes to their peers during visit or farmers association meetings. Tzm-307 from Chad and Oba 98 a hybrid variety also

displayed no genetic difference as they were genetically similar based on the dendrogram. Nevertheless, it is imperative to state that 7 SSR loci might be insufficient in distinguishing the populations sufficiently.

Tzm-1413 from Somalia was a complete outlier. The outlier (Tzm-1413) as revealed by the UPGMA-based dendrogram (Figure 2) being from East Africa, a different agro-ecological zone could be the reason while they were more distantly related to all other genotypes, which were basically from West Africa.

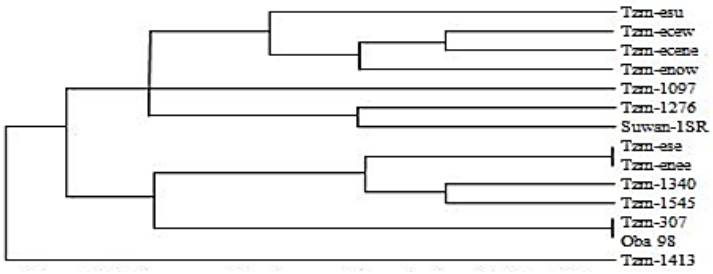

Figure 2 Dendrogram of 14 maize population based on data from SSR markers

CONCLUSION

This research has shown that the populations studied were not greatly diverse, though some of the landraces analyzed were from distinct areas where they have acclimatized; Maize remains a universal crop that possess similar traits but respond to domestication mostly based on ecology.

The result of this study will be useful to guide an oriented breeding program aimed at the improvement of populations studied. This result can thus be used to establish a field trial, where germplasm will be scored based on adaptation, tolerance and resistance to biotic and abiotic factors, which will furthermore validate the genetic divergence and similarity revealed by the microsatellite markers. Phenotypic characterization/morphological marker analysis is recommended to demonstrate the phenotypic variation (genotype + environment) of the maize germplasm studied. Genetic characterization of more numbers of maize landraces from divergent zones using high-throughput marker platforms such as SNP platforms is also recommended for a better precision.

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