Nig. J. Biotech. Vol. 39(2): 97-105 (Dec 2022)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v39i2.12

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Genetic Characterization of Drought-Tolerant Maize Genotypes Based on SSR Markers

***Abegunde, O. I. [1] , Idehen, E. O. [1], [2] , Oduwaye, O. A. [2] , Fabunmi, T. O. [3] and Kehinde, O. B. [2]**

[1] Crop and Pasture Production and Sustainable Environment, World Bank Africa Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta, PMB 2240, Abeokuta, Ogun state, Nigeria.
[2] Plant Breeding and Seed Technology Department, Federal University of Agriculture, Abeokuta, PMB 2240, Abeokuta, Ogun state, Nigeria.
[3] Crop Production and Plant Physiology Department, Federal University of Agriculture, Abeokuta, PMB 2240, Abeokuta, Ogun state, Nigeria.

Abstract

Using 20 pairs of SSR primers situated on each chromosome of the maize genetic makeup, 27 drought-tolerant maize genotypes obtained from the Germplasm Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were examined for diversity. The samples' genomic DNA (gDNA) was extracted using the CTAB procedure specified by CIMMYT (2005). All 20 pairs of SSR markers used in this investigation were extremely polymorphic, resulting in 122 repeatable and polymorphic bands that were scored with an average of 12.20 alleles. Each primer yielded a main allele frequency of no more than 0.5. The average major allele frequency for the 10 primers was 0.3036. With an average of 0.8334, all markers had gene diversity values greater than 0.6. Similar to this, the Polymorphic Information Content (PIC) values had an average of 0.8164 and varied from 0.9051 (primer umc1042) to 0.6639 (Umc1669). Since primer umc1042 best identified this variability, the frequency of the main alleles generated by each primer points to the presence of significant molecular genetic variation across the samples under study. This occurrence is further supported by the gene diversity (GD) and polymorphism information content (PIC) values of the primers. Therefore, it can be inferred that the primers are all appropriate for genotyping genetic diversity. Due to their different genetic relationships, varieties IWD C2 SYN F2 and SAMMAZ 52 have the most diversified genotypes and can be utilised as parent materials in a maize breeding programme.

Keywords: Genetic diversity; drought tolerant maize; SSR markers, West and Central Africa
***Corresponding author:** ioabegunde@gmail.com

Introduction

Maize (*Zea mays* L.), a special crop with many advantages, is a necessary staple crop in the majority of homes and companies around the world (Prohens *et al.,* 2017). Due to the ongoing rise in global population, further economic and agronomic development of some of the important characteristics is imperative (Xiao *et al.,* 2017). Any known organism's genetic material degrades due to the stretching of current or old variations brought on by agricultural advancement. This may be due to the introduction of new cultivars to replace ancient landraces, increasing urbanization, the effects of climate change, and population growth (Prasanna, 2012). The genetic resources with the most favourable alleles include landraces and wild ancestors. These can be utilized to strengthen the genetic foundation of ongoing breeding initiatives.

Additionally, genetic diversity aids in defining heterotic groupings, determining the level of genetic variability, and identifying core subsets for particular features (Semagn *et al.,* 2012). Crop genetic diversity has been studied using a variety of marker approaches, including morphological, biochemical, and DNA-based markers. DNA-based markers are unaffected by agro-climatic edaphic conditions or the plant's growth stage; they are therefore preferred to morphological and biochemical markers (Govindaraj *et al.,* 2015).

Numerous maize improvement efforts have looked into the characteristics of the germplasm and identified genotypes using DNA-based markers. They facilitate selection, enable the selection of genes and genotypes associated with desirable traits, identify hybrid crossings, and do away with the subjectivism related to crossovers (Xiao *et al.,* 2017). Single nucleotide polymorphisms (SNPs), a type of DNA marker, help determine the genetic diversity of improved lines and genotypes of maize. However, because microsatellites (SSR) markers may detect several alleles per locus, they are more beneficial than biallelic SNP markers (Xu *et al.,* 2013). SSR markers have also been used successfully and efficiently to assess genetic variation in maize (Sserumaga *et al.,* 2014). Therefore, this study aimed to use simple sequence repeat (SSR) markers to determine the genetic diversity of 27 maize varieties developed for drought tolerance.

Materials and Methods

Diversity studies

Sourcing of maize genotypes

Twenty-seven open-pollinated, drought-tolerant maize genotypes sourced from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, were used for the study. Local check genotype sourced from the open market was also used as the control (Table 1).

Table 1: Description and Source of maize genotypes used in this study

Entry	Pedigree	Source	Description
1.	White DT STR Syn/TZL COMP1-W F2	07A04207	Open Pollinated
2.	TZL COMP1-W C6/DT SYN-1-W	07C05409	Open Pollinated
3.	DTSTR-W SYN2	07C05410	Open Pollinated
4.	DTSTR-Y SYN2	07C05411	Open Pollinated
5.	DT SYN2-Y	11A11988	Open Pollinated
6.	Z. DIPLO BC4 C3-W DT C1	12C24117	Open Pollinated

7.	TZL COMP4 C3 DT C2	14C31968	Open Pollinated
8.	TZL COMP3 C3 DT C2	14C31969	Open Pollinated
9.	TZL COMP3 C4	07A04207	Open Pollinated
10.	TZL COMP4 C4	07A04208	Open Pollinated
11.	ACR06 TZL COMP3 C4	11A11895	Open Pollinated
12.	ACR06 TZL COMP4 C4	11A11896	Open Pollinated
13.	AFLATOXIN SYN-W4	11A11990	Open Pollinated
14.	Synldfo/Obantanpa/TZL Comp 3 C3*2	12C24114	Open Pollinated
15.	OBATANPA/IWD-C2 SYN	12C24122	Open Pollinated
16.	AFLATOXIN SYN5	14A21603	Open Pollinated
17.	IWD C3 SYN/DT.STR SYN-W-1	14A21605	Open Pollinated
18.	OBATANPA/TZL COMP3	14A21621	Open Pollinated
19.	TZL COMP3 C5	16A21537	Open Pollinated
20.	TZL COMP4 C5	16A21538	Open Pollinated
21.	TZL COMP4 C3 DT C2/HicoryKing	17A16499	Open Pollinated
22.	DT STR-W SYN12	16A20626	Open Pollinated
23.	IWD C3 SYN/DT SYN-1-W	16A20634	Open Pollinated
24.	IWD C3 SYN F3	MK-Breeder	Open Pollinated
25.	IWD C2 SYN F2	MK-Breeder	Open Pollinated
26.	SAMMAZ 52	IB16A-20625	Open Pollinated
27.	TZB-SR		Open Pollinated
	*Local Check	Oba Super	

Molecular analysis

The molecular analysis was conducted at the African Biosciences Laboratory, and Biosciences Laboratory, both of the International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria.

Genomic DNA Extraction

Seeds of the maize genotypes (30 seeds separately) were planted in Petri dishes layered with cotton wool and moistened with Nystatin solution (to prevent the growth of fungi). The Petri dishes were placed in germination incubators (25^{o}C, 12h photoperiod, and 12h darkness). Two weeks after planting, each plant genotype's leaves were harvested and placed in appropriately labelled sample tubes fixed in ice packs. The harvested samples were immediately transferred to the laboratory. The genomic DNA (gDNA) was extracted from the samples

following the CTAB protocol described by CIMMYT (2005).

Gel Electrophoresis of Genomic DNA

The quality of the gDNA samples was checked by electrophoresis on a 1% agarose gel using the protocol described by CIMMYT (2005).

Polymerase Chain Reaction (PCR)

Simple Sequence Repeat (SSR) primers were used for this reaction. All the reaction conditions (temperatures for denaturation, annealing and extension, primer concentration, and volume of PCR master mix and distilled water) for PCR to take place were optimized using the same protocol required for PCR. PCR reactions were carried out in a final volume of 20 µl; with 4 µl gDNA, 4 µl PCR master mix, 1.2 µl primers (forward and reverse), and 10.8 µl dH_2O, annealing at optimal primer temperature as reported in Table 2. The optimized conditions were utilized for the amplification of the genomic DNA of the samples.

Table 2: The Simple Sequence Repeat (SSR) primers used for DNA amplification

ID	Name	Forward Sequence 5	Reverse Sequence	Tm
A	umc1042	AAGGCACTGCTACTCCTATGGCTA	CTGACCTTTGAATTCTGTGCTCCT	55
B	Umc2050	CTCCTGCTGTGATTCTAGGACGA	CTGGATCTCGGCATGGTCTT	56
C	Umc1669	ACGAGGGCTTCTTCTCTGAGC	GTTTCCTTCTTCATGCGACGAC	55
D	umc1805	AGTGCACCAGCTTTTAATCACCTC	TGTGACCTGTGTGGTCTGTGG	56
E	bnlg1599	TTAAATCTTCTCCGAGGCGA	GCCGATCTTGAGGAAGCC	52
F	bnlg1866	CCCAGCGCATGTCAACTCT	CCCCGGTAATTCAGTGGATA	52
G	bnlg1028	AGGAAACGAACACAGCAGCT	TGCATAGACAAAACCGACGT	53
H	umc1094	GCTACTCTCGTGGACTGGTGGT	TGAAGGCTTAGTGGTGATCCGT	57
I	Phi034	TAGCGACAGGATGGCCTCTTCT	GGGGAGCACGCCTTCGTTCT	59
J	phi1091	AAGCTCAGAAGCCGGAGC	GGTCATCAAGCTCTCTGATCG	54

Tm: Annealing Temperature (^{0}C)

Gel Electrophoresis of Amplicons

Electrophoresis of the PCR products (amplicons) was done using Polyacrylamide Gel Electrophoresis (PAGE). The picture of the gel was used for further analysis.

Data Analysis

Electropherograms of amplicons were assessed for DNA bands. Bands were scored for presence or absence using the binary codes' '1' and '0' respectively, and the sizes of the bands were estimated based on 100 bp DNA ladder. Thus, a

set of binary data was generated for each primer, and was used to create a data matrix which was analyzed using the Powermarker V2.35 software. 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 was also estimated by constructing a dendrogram through Unweighted Pair Group Method with Arithmetic Means (UPGMA) using the megaX software. Genetic distance was also computed using the mega6 software.

Results

Genetic diversity analysis of the drought-tolerant maize genotypes using SSR primers

The genetic diversity parameters of the ten Simple Sequence Repeat (SSR) primers used in this study are presented in Table 3. All 20 pairs of SSR markers were highly polymorphic, producing a total number of 122 repeatable and polymorphic bands with an average of 12.20 alleles. Primer umc1042 had 19 alleles, the highest, while primer phi1091 recorded the lowest number of alleles (six). The frequency of the major alleles produced by each primer was not more than 0.5. The frequency of major alleles produced by each primer ranged from 0.2143 in Primer Umc1042 to 0.5 in Primer Umc1669. All ten primers had an average value of 0.3036 for the major allele frequency.

All markers were considered suitable markers for genetic diversity among individuals because they all recorded gene diversity values greater than 0.6. The highest and lowest gene diversity values of 0.9107 and 0.6939 were recorded by primer Umc1042 and primer Umc1669, respectively, with an average of 0.8334. Similarly, the Polymorphic Information Content (PIC) values ranged from 0.9051 (primer Umc1042) to 0.6639 (Umc1669), with an average of 0.8164.

Table 3: Polymorphic Information Content, Gene Diversity and Major Allele Frequency of Ten SSR primers.

Primer ID	Primer Name	Sample Size	Nº of Allele	MAF	GD	PIC
A	umc1042	28.00	19.00	0.21	0.91	0.91
B	Umc2050	28.00	11.00	0.32	0.84	0.82
C	Umc1669	28.00	7.00	0.50	0.69	0.66
D	umc1805	28.00	14.00	0.29	0.86	0.85
E	bnlg1599	28.00	13.00	0.29	0.86	0.85
F	bnlg1866	28.00	10.00	0.32	0.80	0.78
G	bnlg1028	28.00	16.00	0.25	0.89	0.88
H	umc1094	28.00	10.00	0.25	0.84	0.82

I	Phi034	28.00	16.00	0.25	0.89	0.88
J	phi1091	28.00	6.00	0.36	0.77	0.73
	Total	280.00	122.00	3.04	8.33	8.16
	Mean	28.00	12.20	0.30	0.83	0.82

MAF = Major Allele Frequency; GD = Gene Diversity; PIC= Polymorphic Information Content

Evolutionary Relationships among the maize genotypes used for the study

The genetic tree generated was well-branched to form two (2) clades (Figure 1). However, these two clades did not include varieties Synldfo/Obantanpa/TZL Comp 3 C3*2 and OBATANPA/IWD-C2 SYN (which form a minor branch), IWD C2 SYN F2 and SAMMAZ 52 (which form another minor branch) and DT SYN2-Y, all of which are grouped from the two major clades. The first clade consists of 13 maize genotypes forming two main branches. The first branch had five genotypes, namely TZL COMP3 C3 DT C2, ACR06 TZL COMP3 C4, DTSTR-Y SYN2, TZL COMP3 C4 and DTSTR-W while the second branch had four genotypes: AFLATOXIN SYN5, AFLATOXIN SYN-W4, White DT STR Syn/TZL COMP1-W F2 and TZL COMP1-W C6/DT SYN-1-W, while four other genotypes (TZL COMP4 C3 DT C2, TZL COMP4 C4, IWD C3 SYN/DT.STR SYN-W-1 and Z. DIPLO BC4 C3-W DT C1) out-group from these two branches in the clade. Genotypes White DT STR Syn/TZL COMP1-W F2 and TZL COMP1-W C6/DT SYN-1-W had a genetic distance of 0.0768 between them and were the most closely related varieties.

The second clade consists of 10 maize genotypes, also forming two main branches. The first branch consisted of genotypes TZL COMP3 C5, TZL COMP4 C3 DT C2/Hicory King, TZL COMP4 C5 and IWD C3 SYN/DT SYN-1-W. Genotypes TZL COMP4 C5 and IWD C3 SYN/DT SYN-1-W formed a minor branch with a genetic distance of 0.2018 between them, while varieties TZL COMP3 C5 and TZL COMP4 C3 DT C2/Hicory King formed another minor branch with a genetic distance of 0.1134 between them. These two minor branches were closely related as they formed a cluster together to form the main branch. The second main branch consisted of ACR06 TZL COMP4 C4, DT STR-W SYN12 and OBATANPA/TZL COMP3; of these three genotypes, ACR06 TZL COMP4 C4 and DT STR-W SYN12 are the most closely related, as they cluster together to form a minor branch. Genotypes TZB-SR and Local Check also form a minor branch with a genetic distance of 0.2882 between them, and they, alongside the variety IWD C3 SYN F3 are out grouped from the two main branches.

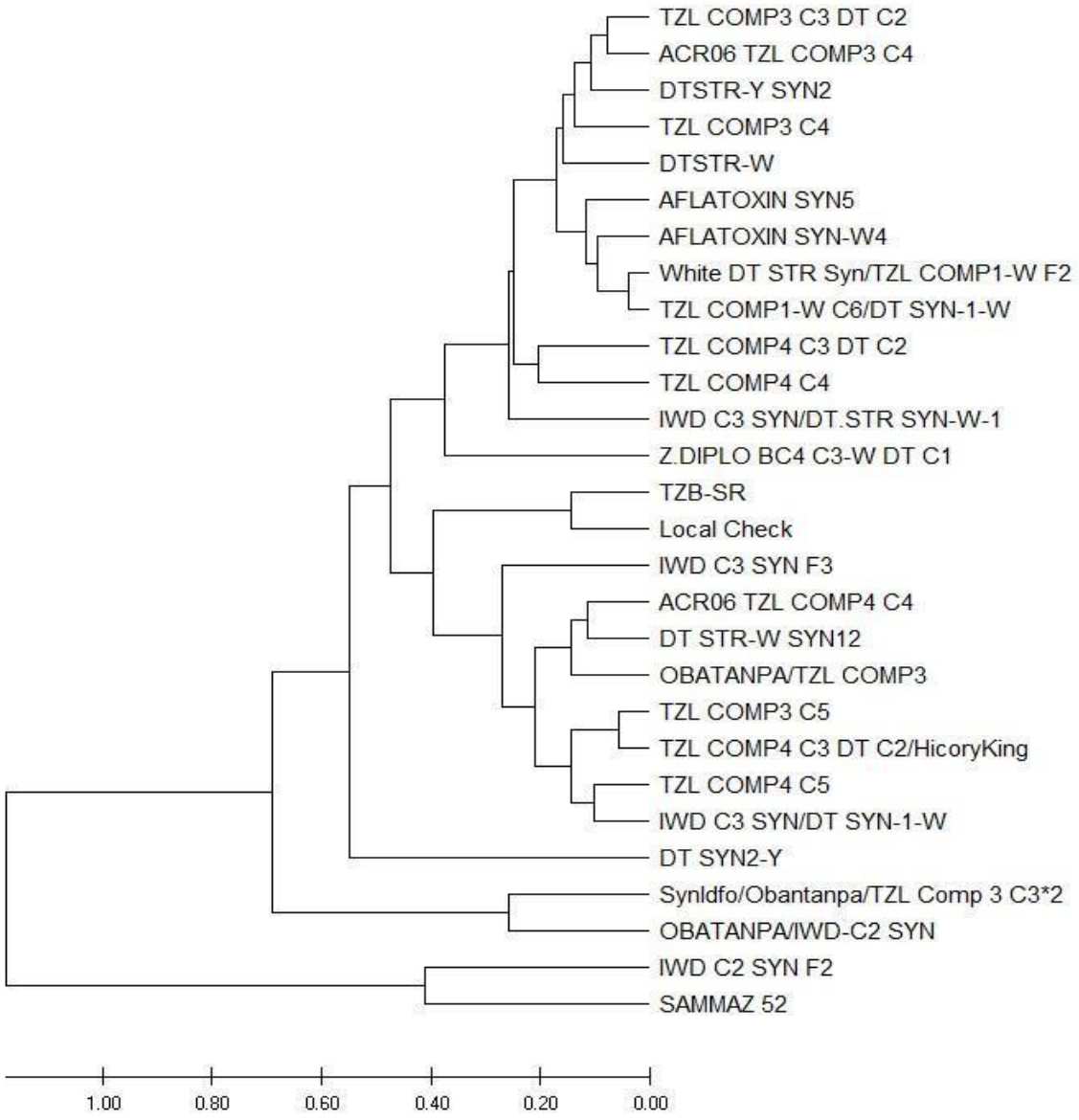

Figure 1: Relationships among the 27 maize genotypes used for the study based on the SSR markers

Discussion

Evaluation of Drought-Tolerant Maize Varieties using Molecular Technique

The advent of PCR technology has enhanced the detection of variability among crop genotypes (Shehata *et al.*, 2009). Simple Sequence Repeats (SSR) or microsatellites are PCR–based molecular markers that have features such as specificity, high polymorphism and co–dominance that make them to be more preferable to other PCR–based molecular markers such as Random

Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) (Shehata *et al.*, 2009; Zebire, 2020).

The frequency of the major alleles produced by each primer suggests the presence of high molecular genetic variability among the samples studied, and primer umc1042 best detected this variability. The primers' gene diversity (GD) and polymorphic information content (PIC) values further corroborate this fact. Thus, all the primers can be judged suitable for genetic diversity among the genotypes. In a study involving 8 maize inbred lines and 6 SSR markers, Shehata *et al.,* (2009) obtained PIC ranging between 0.42 and 0.88, averaging 0.58. This indicates that the SSR primers used were adequate in detecting genetic diversity at the molecular level in the eight maize inbred lines.

Similarly, Pandit *et al.* (2016) obtained PIC values ranging between 0.00 and 0.87 with an average of 0.65 in a study that involved 20 maize genotypes and 18 EST-SSR primer pairs, while the PIC values obtained by Vivodík *et al.,* (2021) in a study that involved 20 genotypes of old maize lines and 5 ScoT markers ranged from 0.652 to 0.816 with an average of 0.738. However, in another study by Kumari *et al.,* (2018) that involved 8 maize genotypes and 22 SSR primers, including phi 034 which was also used in the present study, low values of PIC ranged from 0.195 to 0.367 with an average of 0.297 while GD varied from 0.219 to 0.484 with an average of 0.373. Specifically, primer phi 034 had PIC and GD values of 0.250 and 0.297 compared with 0.8765 and 0.8852 obtained in the present study. According to Kumari *et al.,* (2018), this may be due to the use of maize genotype with a narrow genetic base and the use of tri/tetra/penta-nucleotide primers. But contrary to this view, this could be due to the narrow genetic base between the relatively few numbers of maize genotypes (8) used in that study compared with the wider genetic base of the 28 genotypes of maize used in the present study.

Dendrogram is a good tool for showing the relationships between the genotypes in a genetic diversity study (Pandit *et al.*, 2016). The dendrogram that was used to illustrate the genetic relationship that existed between the maize genotypes identified extensive diversity between the genotypes. Varieties IWD C2 SYN F2 and SAMMAZ 52 are the most diverse genotypes because of their distinct genetic relationship and can be used as parent materials in a maize breeding programme. On the other hand, varieties White DT STR Syn/TZL COMP1-W F2 and TZL COMP1-W C6/DT SYN-1-W in the first Claude, and varieties TZL COMP3 C5 and TZL COMP4 C3 DT C2/Hicory King in the second Claude are the most dissimilar because of the relatively short genetic distance that existed between them. They might be found to have the same genetic origin. In the study by Kumari *et al.,* (2018) which was a genetic diversity study involving 8 maize genotypes and 22 SSR primers including phi 034 which was also used in the present study, the 8 genotypes were grouped into three clusters, with 4 of the genotypes, pairing up in groups of two each, found to be genetically similar. Other studies where SSR markers have been used to identify genetic similarity and dissimilarity among maize genotypes include Souza *et al.,* (2008) and Pandit *et al.,* (2016).

Conclusion

The maize population studied exhibited some wide genetic variations, which will positively affect the decisions of breeders in future maize genetic breeding programmes, the makers used were very informative and is hereby recommended for future genetic diversity studies.

Acknowledgement

The authors are grateful to the World Bank Africa Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta,

Ogun state, for providing research support that aided the success of this research article.

Conflict of Interest

The authors declare no conflict of interest.

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