IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 12, Issue 7 Ser. I (July 2019), PP 34-40
www.iosrjournals.org

Maize (*zea mays* l.) Germplasmevaluation in Ibadan, Nigeria

Adeosun, F.A[1] and O. J. Olawuyi[1]
[1]*Plant genetics and Molecular Biology unit, Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria*
Corresponding Author: Adeosun, F.A

Abstract: *Maize (Zea mays L) collections were made from three researvch institutes located in Ibadan Oyo state and from ten market accessions within Ibadan metropolis for evaluation for genetic variability. Genetic resources are important for conservation and utilization of biodiversity to ensure food security. Thirty maize genotypes obtained from three maize germplasm (NACGRAB, IITA and IAR&T) in Ibadan, and ten market accessions (Bode-W, Oja Oba-W, Apata-W, Idi-Ayunre-Y, Moniya-Y, Ojoo-W, Orita Merin-Y, Bodija-Y, Bodija-W, Orita Challenge-Y) which served as control were evaluated in this study. Morphological characterisation was determined from field experiment laid out in a complete randomized design with four replicates, while extraction of DNA was carried out using molecular method. The mean square variation of maize produced significant effect ($p<0.05$) on growth, agronomic and yield characters.TZM 1351 matured earlier than other maize genotypes. OJA OBA-W produced two ears per stand, while SUWAN-I-SR-Y had the best yield traits; fresh shoot (25.50 g), dry shoot (20.75 g) ear width (11.55 cm), stover weight (3.0 g), seed weight (8.5 g) and maize husk cover (28.50 g). The highest total volume of DNA concentration (3397.8 µ/l) was recorded for MONIYA-Y, while TZM 1472 had the highest genomic DNA (2.12 ng/µl). Prin 1 accounted for the highest proportion of 37% with Eigen value of 9.32. Plant height had close relationship with most of the traits. Therefore, the variability in their performance could be useful in selection of promising maize genotypes for crop improvement and sustainable development.*

Keywords: *maize, variability, DNA, germplasm, food security*

Date of Submission: 22-06-2019 Date of acceptance: 10-07-2019

I. Introduction

Maize is an important monoecious economic crop in Nigeria accounting for over 24% of the total cereal production (Olakojo and Olaoye, 2007; FAOSTAT data, 2016, Utah, 2016). Maize adapts to different environments, and is characterized by a wide genetic base for utilization in crop improvement and plant breeding (Riera-lizarazu *et al.*, 1996; Sinletary *et al*., 2003; Rabinowicz and Bennetzen, 2006; Olawuyi *et al.*, 2015; Linda, 2016). The characterization of maize germplasm will play an essential role in meeting the demands of an increasing population. Morphological characterization is important in evaluation of growth, agronomic and yield traits of maize (Ortiz *et al*., 2010; UPOV, 2009; Olawuyi *et al*., 2015). Genetic characterization can also be estimated from various types of molecular markers of which Simple Sequence Repeats (SSRs) is included (Semagn *et al*., 2012). The SSR repeat motifs present in both coding and non-coding regions are smaller than 100bp (Toth *et al*., 2000; Ellegren, 2004; Stolle *et al*., 2013).

Despite the global production of maize, there are limited studies on characterization of favourable maize alleles based on biomarker in the germplasm. There is need therefore to identify better traits and improve conservation and management strategies of maize. This study therefore, aimed at comparing the phenotypic and molecular variability of maize using SSR primers.

II. Materials And Methods

An open field experiment was carried out at the Nursery Farm of the Department of Botany, University of Ibadan, Ibadan, Nigeria from January to April 2017.The laboratory experiment was done at the Bioscience Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. A total of forty maize genotypes collected from International Institute of Tropical Agriculture (IITA), Institute of Agricultural Research and Training (IAR&T) and National Centre for Genetic Resources and Biotechnology (NACGRAB) were evaluated in a complete randomized design with four replicates in this study. The maize seed were planted in one hundred and sixty (160)

perforated polythene bags filled with 7 kg top sandy-loam using the procedure described by Olakojo *et al*. (2001). Daily watering of the plants and other agronomic practices were also carried out.

The data of the morphological characters were determined by taking the growth characters after one week of germination till the end of the experiment which included; leaf length (cm), plant height (cm), leaf width (cm), stem length (cm), and number of leaves. At the fifth week, agronomic characters taken according to Kim (1993) rating were; plant stand, husk cover, ear aspect, tasseling length (cm), number of days from sowing to tasseling, stem lodging, root lodging, ear height (cm), silk texture, number of ears, ear at harvest and plant at harvest. On the eleventh week, yield characters taken included; fresh shoot biomass (g), dry shoot biomass (g), fresh root biomass (g), dry root biomass (g), maize Stover weight (g), weight of seed (g), maize plus husk cover weight (g), length of maize Stover (cm) and width of maize Stover (cm). The data collected were analyzed using SAS software program version 9.2 (SAS Institute 2009, Version 5) for ANOVA. The means were separated by Duncan's multiple range test (DMRT) at 5% level of probability (i.e. Range Test at $p<0.05$) and Pearson correlation co-efficient was used to establish relationship among the growth, agronomic and yield characters.

The plant materials used for molecular analysis were obtained from the maize genotypes planted on the field after two weeks of germination. Thirty-eight maize genotypes out of forty germinated and these were harvested and properly labeled in transparent cellophane nylons and were taken to the Bioscience Laboratory of International Institute of Tropical Agriculture and stored at −80 °C for DNA extraction. Plant genomic DNA was extracted by a modified CetylTrimethylAmmonium Bromide (CTAB) method (Doyle and Doyle 1990; Allen, 2006). The samples were gently mixed and centrifuged at 10000rpm for 10mins. 500ul aqueous phase of the mixture was transferred into new tubes and 500-600ul chloroform: isoamylalcohol (24:1) was added to it. This was done twice. 600ul of the upper layer was carefully transferred into fresh strips and 600ul 100% cold isopropanol was added to it to precipitate the nucleic acid after it had been centrifuged. Pellets obtained were washed using 400ul of 70% ethanol. This was done twice. Finally, pellet was allowed to air dry until ethanol evaporated completely for 50 min and 97µl of sterile distilled water was added. 3µl of RNAse was used to degrade RNA.

The agarose gel electrophoresis was carried out by dissolving1g of agarose in 100ml of 0.5×TBE buffer in a microwave oven for 5 minutes with 5µl of ethidium bromide as a staining dye. This was casted in the electrophoresis tank which contained the loaded 5µl of the dye extracted DNA and was allowed to run for 45 minutes. A negative control lacking a DNA template was used, while the gel was visualized using UV light and photographed using Gel Documentation System. The determination of Concentration and Purity of DNA Samples Using Nanodrop was measured with UV spectrophotometer. This process was initiated by using 2µl of TBE. Each DNA sample concentration was measured by applying 2µl of the samples on the lower pedester. The concentration and purity was displayed on the monitor.

Five Simple Sequence Repeat (SSR) primers comprising of forward and reverse oligonucleotide sequence were used in the amplification of 25µl containing 50ng DNA, PCR buffer (50Mm $MgCl_2$ 1.2 µl, 10× NH4 buffer 2.5ul, dNTP 2.0 µl, DMSO 1.0ul, Taq DNA polymerase 0.1µl, primer 1.5µl, H2O 14.74 µl, DNA 2.0µl). Amplification were performed in thermocycler programmed at the initial denaturing for 5 minutes, 9 cycles of denaturing was done for 15seconds at 94^{o}C , annealing for 20seconds at 65^{o}C and extension for 30 seconds, at 72^{o}C. At 35 cycles, Denaturing was done at 94^{o}C for 15seconds, Annealing was done at 55^{o}C for 20 seconds, and extension at 72^{o}C for 30 seconds the final extension was done at 72^{o}C for 7minutes and hold temperature at 10^{o}C infinity. Each 25 µl PCR reaction consisted of 10 × PCR buffer, 2.5 mM dNTPs, 25 mM $MgCl_2$, 0.2 unit of Taq polymerase, 1 µl (5 pmul/µl) of each primer and 5 µl (50 ng) of DNA. The PCR products were then loaded on 1.5% agarose gel and a 1kbplus Gene ruler from Thermo Scientific ladder. The visualization of the gel was done using Gel Doc. System.

III. Results And Discussion

The result in Table 2 and 3 shows that the genotypes and week after planting produced significant ($p<0.01$) effect on plant height, number of leaves, leaf length, leaf width and stem length, ear aspect, ear height, husk cover, plant stand, silk length, tassel length, tassel number, number of ears, stem lodging, root lodging except for plant stand at harvest with significant effect at $p<0.05$. The result of Mean Square Variation of Maize Yield Characters in Table 4 shows that genotypes produce significant ($p<0.05$) effect on fresh shoot, dry shoot, seed weight, stover length and maize husk cover. Week after planting produced highly significant effect ($p<0.01$) on all yield characters. Maize genotypes TZM 1351, TZM 408, TZM 20, TZM 37, TZM 136, TZM402, TZM 1472, TZM 1148, TZM 100 and TZM 1445 developed tassels and had early maturity traits beginning from the fifth week after planting (Plate 1). TZM 1351 matured first before others. Among the accession gotten from the market, OJA OBA-W showed a high yield trait and produced two ears per stand as shown in Plate 2. DT-SRCOF$_2$ and TZE-WPOPDTSTRC$_4$F$_2$ also

produced an ear per stand and three ears per stand respectively (Plate 2). After harvest, SUWAN-I-SR-Y and TZM 402 gave the best yield trait as shown in Plate 3. The contribution of Principal Component Axis (PCA), to the variation of the growth, agronomic and yield in the maize genotypes used in this study (Table 5) showed that plant height, stem length , ear height , tassel number , tassel length, number of leaves , fresh shoot , dry shoot , ear width , stover weight , stover length , seed weight , and maize husk cover were closely related compared to leaf width ,husk cover silk length, fresh root , dry root , seed width were closely related to one another.

In the second PCA, the number of leaves ,leaf length , leaf width , plant stand , stem lodging and root lodging were closely related compared to ear aspect ear height , husk cover, silk length and number of ear and also, fresh shoot , Fresh root, Dry shoot), Dry root , Ear width ,Stover weight , Stover width , Stover length , seed weight , maize husk cover , and tassel number , were closely related to one another. The result in principal component axis three shows that, the leaf length, fresh shoot, fresh root, dry shoot and dry root were closely related compared to Number of leaves leaf width plant stand and root lodging.

The total volume of DNA concentration and genomic DNA concentration of the thirty-eight DNA samples are shown in Table 7. MONIYA-Y yielded the highest total volume of DNA concentration (3397 µ/l), followed by genotype TZM 1278 (3299µ/l), while genotype BR 9928-DMR-SR-Y had the least at 316 µ/l. Again, genotype TZM 402 produced the highest concentration of 2.12 ng/ µl of genomic DNA, followed by TZM 1278 (1.96 ng/µl) and MONIYA-Y (1.96 ng/µl), while TZM 408 produced the least (1.36 ng/µl). The banding patterns of five SSR primers, BCM 1520, PHI 022, UCM 2281, ZCMP 7430 and ZCT197 were loaded on a Quick-load 50pb DNA ladder. Each genotype or accession of maize was combined as an individual operational unit (OUT). All the primers of SSR marker used in this study produced score-bands for each genotype. The mean polymorphic information content (PIC) was 0.53 as shown in Table 6. BMC produced the highest polymorphic information content of 71% followed by PHI 022 (57%) while, ZCMP-F 7430 had the least (41%). BMC1520 had the highest gene diversity of 0.7521 while UCM2281 had the least (0.4418). PHI 022, ZCMP-F 7430 and ZCT 197 had the least number of polymorphic bands of 3.000. The SSR primer UCM2281 gave the highest allele number and allele frequency of 6.000 and 0.7368 respectively followed by BMC 1520 (5.000) while ZCMP-F 7430 has the least allele number (3.000).

The result of this study showed variability in performances of growth, agronomic and yield traits among the maize genotypes. This agrees with the findings of Olawuyi *et al*. (2013) and Umar *et al*. (2015). The relatedness of plant height to the yield and agronomic traits i.e. the ear width, Stover weight, Stover length, seed weight and husk cover in Prin1 indicates that plant height did not only contribute to the production of green and dry matter, but also grain yield as similarly observed by Bello *et al*. (2014). The findings from this study show that the ear height of SUWAN-I-SR, TZM 402, DMR-LSR-SR-Y genotypes which matured intermediately yielded more grains than the genotypes with early maturing traits; TZM 1351, TZM 408 and TZM 20 in accordance to the findings of Wang *et al*. (2011) and Agbolade *et al*. (2016). The molecular analysis revealed that small number of microsatellite locii (5 primers) considered in this study identified 22 alleles with a mean of 4.4 per locus were polymorphic across the maize genotypes with polymorphic information content mean of 0.53 was in contrast with the findings of Weitholter *et al*. (2008) who used a larger number of primers but observed a less amount of alleles and polymorphic information content mean. The SSR primers BMC 1278, PHI 022 and ZCT 197, were polymorphic and showed a great level of gene diversity in the maize genotypes similar to the report of Terra *et al*. (2011).

IV. Conclusion And Recommendation

SUWAN-SR-I-Y performed best for both growth and yield traits and TZM 1472 for DNA yield are potential candidates for future crop breeding improvement program of maize. BMC 1520, PHI022 and ZCT 197 are also promising SSR primers that can be used in further assessment of genetic diversity in maize genotypes. Also TZM 402 could be improved for DNA hybridization purposes. Traits such as plant height, stem length, ear aspect, ear height, number of leaves, number of tassel, number of ears, fresh shoot weight, fresh root weight, dry root weight, dry shoot weight, seed weight and a high genomic DNA concentration should be considered when choosing a genotype for future breeding purposes.

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Table 1: Sources and collection of maize genotypes

Genotype From IAR&T	Genotype from NACGRAB	Genotype From IITA	Genotype From Markets
ILE-1-OB-W	DT-SRCOF2	TZM 1351	BODE-W
BR 9928-DMR-SR-Y	TZE-WPOPDTSTRC4F2	TZM 408	OJA OBA-W
DMR-LSR-SR-Y	TZE-YPOPDTSTRCO	TZM 20	APATA-W
TZE COMP5-W	NG/SA/07/154	TZM 37	IDI-AYUNRE-Y
ART 98/ SW6-OB-W	TZM 1551	TZM 136	MONIYA-Y
SUWAN-1-SR-Y	TZEEWSRBC5	TZM402	OJOO-W
DMR-ESR-SR-Y	TZL COMP/ C2	TZM 1472	ORITA MERIN-Y
ART 98/SW1-Y	TZM 1278	TZM 1148	BODIJA-Y
TZBP-SR-W	NG/SA/07/184	TZM 100	BODIJA-W
TZBP-ELD3-W	TZM 1318	TZM 1445	ORITA CHALLENGE-Y

Table 2: Mean Square Variation of Maize Growth Characters

Source of variation	Degree of freedom	Plant height	Number of leaves	Leaf length	Leaf width	Stem length
Genotypes	39	11504.08***	95.45***	3575.78***	24.23***	5778.31***
Week after planting (WAP)	5	245225.87***	861.65***	35408.92**	335.22***	172969.43***
Replicate	3	15757.71ns	18.87ns	3254.57ns	31.11ns	8323.02ns
Error	912	728.88	2.96	169.16	1.52	478.2
Corrected total	959					

Highly significant (P<0.01) =***, Significant (p< 0.05) = *, ns= not significant.

Table 3: Mean Square Variation of Maize Agronomic Characters

Source of variation	Degree of freedom	Ear aspect	Ear height	Husk cover	Plant stand	Plant stand at harvest	Silk length	Tassel length	Tassel number	Number of ear	Stem lodging	Root lodging
Genotypes	39	1.09***	53.52***	1.13***	0.98***	0.13*	39.72***	254.69***	63.65***	0.75***	2.12***	1.22***
Week after planting (WAP)	5	49.25***	3205.07***	53.73***	43.48***	33.38***	1197.54***	15868.62***	2249.35***	36.92***	101.62***	47.20***
Replicate	3	1.35ns	119.64ns	0.19ns	0.27ns	0.03ns	34.30ns	293.67ns	116.97ns	0.84ns	0.25 ns	0.97ns
Error	912	0.43	13.69	0.33	0.09	0.07	12.8	66.48	16.34	0.17	0.22	0.12
Corrected total	959											

Highly significant (P<0.01) =**, Significant (p< 0.05) = *, ns= not significant.

Table 4: Mean Square Variation of Maize Yield Characters

Source of variation	Degree of freedom	Fresh shoot	Fresh root	Dry shoot	Dry root	Ear width	Stover width	Stover weight	Stover length	Seed weight	Maize husk cover
Genotypes	39	521.28*	1031.99ns	384.63*	820.71ns	111.53ns	17.82*	8.49ns	7.17*	65.78ns	438.74*
Week after planting (WAP)	5	54136.95***	56464.80***	25639.87***	36571.60***	12.857***	1679.97***	1540.13***	2122.91***	5671.13***	52309.95***
Replicate	3	1184.32ns	385.07ns	793.56ns	290.25ns	263.72ns	21.46ns	14.25ns	8.13ns	154.51ns	1118.51ns
Error	912	318.55	772.19	239.02	625.48	88.57	11.84	8.08	4.46	49.05	293.34
Corrected total	959										

Highly significant (P<0.01) =**, Significant (p< 0.05) = *, ns= not significant

Table 5: Contribution of Principal Component Axis (PCA) to the Variation of the Growth, Agronomic and Yield in Maize Genotypes

	PRIN 1	PRIN 2	PRIN 3
PLANT HEIGHT	0.23	0.26	0
NUMBER OF LEAVES	0.07	0.36	0.18
LEAF LENGTH	0.07	0.35	0.23
LEAF WIDTH	0.12	0.34	0.19
STEM LENGTH	0.25	0.2	-0.05
EAR ASPECT	0.2	0.06	-0.3
EAR HEIGHT	0.24	0.04	-0.27
HUSK COVER	0.16	0.07	-0.37
PLANT STAND	-0.01	0.34	0.16
PLANT STAND AT HARVEST	0	0	0
SILK LENGTH	0.18	0.09	-0.3
TASSEL LENGTH	0.2	0.12	-0.17
TASSEL NUMBER	0.22	0.1	-0.12
NUMBER OF EARS	0.2	0.04	-0.32
STEM LODGING	0	0.33	0.06
ROOT LODGING	-0.02	0.32	0.13
FRESH SHOOT	0.25	-0.15	0.21
FRESH ROOT	0.19	-0.14	0.26
DRY SHOOT	0.24	-0.14	0.23
DRY ROOT	0.19	-0.13	0.27
EAR WIDTH	0.28	-0.11	0.09
STOVER WIDTH	0.14	-0.1	-0.01
STOVER WEIGHT	0.28	-0.11	0.09
STOVER LENGTH	0.27	-0.13	0.07
SEED WEIGHT	0.26	-0.1	0.08
MAIZE HUSK COVER	0.27	-0.13	0.15

Table 6: Polymorphic Information Content, Allele number and gene diversity of SSR Primers.

SSR Primers	Major Allele Frequency	Sample Size	Allele Number	Number of Polymorphic Bands	Gene Diversity	Polymorphic Information Content (PIC)	Polymorphic Information Content (PIC) %
BMC1520	0.3158	38.0000	5.0000	5.0000	0.7521	0.7099	71
PHI022	0.5263	38.0000	4.0000	3.0000	0.6260	0.5684	57
UCM2281	0.7368	38.0000	6.0000	4.0000	0.4418	0.4251	43
ZCMP7430	0.5263	38.0000	3.0000	3.0000	0.5222	0.4106	41
ZCT197	0.4737	38.0000	4.0000	3.0000	0.6219	0.5480	55
Mean	**0.5158**	**38.0000**	**4.4000**	**3.6000**	**0.5928**	**0.5324**	**53**

Table 7: Nanospectrophotometric Concentration of Extracted DNA from Maize

Samples ID	Genotypes/Accession	Total volume of DNA concentration(µ/l)	Genomic DNA concentration(ng/µl)
G1	ILE-I-OB-W	3151.0	1.92
G2	BR 9928-DMR-SR-Y	316.9	1.51
G3	DMR-LSR-SR-Y	1070.0	1.79
G4	TZE COMP5-W	1805.1	1.78
G5	ART 98/SW6-OB-W	1978.8	1.89
G6	SUWAN-1-SR-Y	1491.1	1.81
G7	DMR-ESR-SR-Y	1870.7	1.75
G8	ART 98/SW1-Y	2305.4	1.83
G9	TZBP-ELD3-W	2105.8	1.92
G10	DT-SR COF2	1655.1	1.84
G11	TZE-W	841.5	1.78
G12	TZE -WPOPDTSTRC4F2	1461.8	1.86
G13	TZE-YPOPDTSTRCO	1688.3	1.90
G14	TZM 1551	1407.5	1.86
G15	TZE EWSRBC5	1050.4	1.78
G16	TZL COMP/C2	1314.8	1.83
G17	TZM 1278	3299.1	1.96
G18	TZM 1318	1537.5	1.89
G19	TZM 1351	1543.2	1.79
G20	TZM 408	1401.3	1.36
G21	TZM 20	1527.6	1.91

G22	TZM 37	2064.7	1.82
G23	TZM 136	2469.9	1.91
G24	TZM 402	2195.1	1.84
G25	TZM 1472	1694.0	2.12
G26	TZM 1148	448.3	1.78
G27	TZM 100	958.9	1.79
G28	TZM 1445	1957.4	1.92
G29	BODE-W	1387.4	1.75
G30	OOJA OBA-W	1728.2	1.93
G31	APATA-W	1802.9	1.68
G32	IDI-AYUNRE-Y	1690.5	1.79
G33	MONIYA-Y	3397.8	1.96
G34	OOJO-W	1322.2	1.77
G35	ORITA MERIN-Y	1704.2	1.91
G36	BODIJA-Y	1087.8	1.69
G37	BODIJA-W	1358.3	1.70
G38	ORITA CHALLENGE-Y	1772.6	1.69

Plate 1: A section of the field, showing the tassels of maize genotypes with early maturing traits gotten from the International Institute of Tropical Agriculture (IITA).

(A)OJA OBA-W (B) DT-SRCOF$_2$ and TZE-WPOPDTSTRC$_4$F$_2$

Plate 2: High yield trait observed in OJAOBA-W (2 ear/stand), DT-SRCOF2 (1 ear/stand) with best leaf width and TZE-WPOPDTSTRC$_4$F$_2$ (3 ear/stand)

Adeosun, F.A. " Maize (zea mays l.) Germplasmevaluation in Ibadan, Nigeria. "IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 12.7 (2019): PP- 34-40