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Effect of Storage Temperatures on Phenotypic and Gene Expression of Maize (*Zea mays* L.) Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. Author OJO designed the study and wrote the protocol. Authors OJO and OMC wrote the first draft of the manuscript and managed the literature searches. Author AOA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Maize is an important cereal grown globally across wide range of altitude and latitude. Temperature is one of the factors that affect the viability of maize under storage conditions.
Aim: This study, therefore, assessed the effect of different temperature storage levels on the morphological characters and molecular variability of maize genotypes.
Materials and Methods: The seeds of maize genotypes: TZLCOMP4C3, EVDT-W200STRCO, POP66SR/ACR94, POOL18SR QPM, TZM 132, TZM 1291, EVDT- Y2008 STR and TZM 1326 obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan were stored at different temperature conditions of -80°C, -20°C, 5°C, 50°C, and 25°C for 6 hours. The stored maize was sown in perforated polythene bags containing 7 kg of soil and replicated thrice in a complete randomized design. The molecular variability was also investigated on the maize genotypes stored under the varying temperatures. The effect of storage temperature was significantly higher at 50°C for all growth characters but was not significant at -80°C.
Results: The genotypic effect on the growth characters was significantly ($p< 0.05$) higher in TZM 132 with plant height (99.09 cm), leaf length (46.54 cm), leaf width (3.05 cm), number of leaves (6.25) and stem length (50.12 cm). The contribution of principal component axis (PCA) showed that

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PC 1 had the height variation with a proportion of 47.17% and eigen value of 2.83 across the growth characters. Molecular evaluation showed that EVDT-W200STRCO had the highest DNA concentration of 4885.7 ng/µl at storage temperature of 25°C, while EVDT-W200STCO at -20°C recorded the least DNA concentration of 26.60 ng/µl. The highest DNA concentration across the maize varieties were recorded at -20°C (POOL18SR QPM, TZM 132 and TZM 1326), 5°C (TZLCOMP4C3), 25°C (EVDT-W200STRCO and EVDT- Y2008 STR) and 50°C (POP66SR/ACR94 and TZM 1291). OPB 10 had the highest allelic no, gene diversity and polymorphic information content of 15, 0.97 and 97.0% respectively. The genetic distance matrix established relationship among the stored maize genotypes.

Conclusion: Maize seeds can therefore be stored at temperature range of -20°C to 50°C depending on the variety, without losing its viability and molecular constituents.

Keywords: Maize; phenotypic characters; RAPD; temperature; storage levels.

1. INTRODUCTION

Maize (*Zea mays* L.) is one of the easiest crops to grow with more widespread cultivation than any other grains [1,2,3]. Maize is a breakfast meal rich in vitamins A, C and E, carbohydrates, essential minerals, protein, dietary fiber and calories which are good source of energy [4]. In a processed form, it is a source of fuel (ethanol) and starch [5]. In developed countries, maize is consumed mainly as second-cycle, produced in form of meat, eggs and dairy products, while it is consumed directly in developing countries [6]. Maize grains are stored in gene bank by breeders to retain its viability even when kept for long time and these seeds are expected to be of highest quality [7]. Some of the factors that affect seed quality during collection, harvesting and storage include: short period after harvesting and storage in order to reduce the loss of viability and pest infestation, moisture content, temperature, relative humidity, and gaseous exchanges in the storage environment [8,9,10]. Seed longevity in storage is a genetically regulated process [11]. Maximum seed quality is defined by seed germination and vigor, reached at physiological maturity, but beyond this stage, the seed deteriorates [12]. Seed deterioration cannot be reversed, but its rate can be slowed by regulating the conditions of the storage environment [11].

Molecular characterization of maize germplasm gives an insight into genetic diversity and population structure [13]. Studies involving molecular markers provided new insights into domestication of maize [14]. DNA markers provide an effective way of identifying and characterizing germplasm accessions in order to facilitate crop improvement [15].

Some seeds are affected by environmental factors which reduce their viability, and cause variation and alteration in their genomic constituents of which temperature is included. Therefore, this study aimed at investigating the effect of storage temperature levels on the phenotypic characters, polymorphic information content and gene diversity of maize germplasm using RAPD primers.

2. MATERIALS AND METHODS

2.1 Sources of Maize Genotypes and Experimental Site

The maize genotypes: TZLCOMP4C3, EVDT-W200STRCO, POP66SR/ACR94, POOL18SR QPM, TZM 132, TZM 1291, EVDT- Y2008 STR and TZM 1326, were obtained from the National Center for Genetic Resources and Biotechnology (NACGRAB) Ibadan, Nigeria. These comprised of four landraces and four improved varieties (Table 1). The field experiment was conducted in the Department of Botany, University of Ibadan, while the molecular studies were carried out at Bioscience Unit of the International Institute of Tropical Agriculture (IITA), Ibadan.

2.2 Experimental Design and Seed Treatment

The maize genotypes were exposed to five different temperature storage levels (treatments) at -80°C, -20°C, 5°C, 50°C for 6 hours, while room temperature of 25°C served as control. This incubation was based on the standard germplasm temperature storage level for seed in some of the research institutes. The standard of International Institute of Tropical Agriculture (IITA) was used to select the varying temperature levels. Planting was done immediately with the experiment laid out in Complete Randomized Design (CRD) with three replicates.

Table 1. Sources and description of maize genotypes

Label	Genotypes	Sources	Description
A	TZLCOMP4C3	IITA	Improved variety white dent corn
B	EVDT-W2000STRCO	IITA	Improved variety white striga resistant corn
C	POP66SR/ACR94	IITA	Improved variety yellow pop corn
E	POOL18SR/QPM	GOMBE	Improved variety yellow corn
G	TZM 132	IITA	Landrace variety light yellow pop corn
H	TZM 1291	GOMBE	Landrace variety white corn
I	EVDT- Y2008STR	IITA	Landrace variety yellow corn
J	TZM 1326	IITA	Landrace variety White flint corn

2.2.1 Soil preparation, planting procedures and cultural practices

An aerated and well-drained loamy soil collected from the Department of Botany was sampled for the experiment. The soil was sieved and steam sterilized for 30 mins at temperature of 80°C. Seven kilogram of the soil was bagged into one hundred and twenty (120) black cellophane bags. Three seeds were planted at a depth of 2 cm spaced at 5 inches, but thinned to one for molecular studies after two weeks. Watering, weeding and other agronomic practices were carried out throughout the period.

2.2.2 Determination of morphological characters

Data on the growth characters were taken on weekly basis after seven days of germination, while dry biomass was determined after the eighth week of planting.

2.3 Molecular Studies

2.3.1 Harvesting

A total of 800 mg of fresh leaf samples were harvested from the nursery into properly labeled transparent cellophane nylons and carefully placed in ice cubes to avoid the denaturation of their DNA.

2.3.2 DNA extraction and Agarose gel electrophoresis

DNA was extracted according to the method developed by Dellaporta et al. [16]. The leaves of each sample were ground in pre-chilled mortal by adding little extraction buffer (sodium dodecyl sulfate-SDS). After thorough grinding, 500 µl of the extraction buffer was mixed with 5 µl of β-mercapto ethanol, and was dispensed into eppendorf tube for each of the labeled samples. The eppendorf tubes were vortexed for 10 mins before incubating in water bath at 65°C for 25 minutes. A total of 200 µl of potassium acetate (KAC) was added to each tube and well mixed before further incubation on ice for 20 minutes. The samples were centrifuged at 3500 rpm for 20 minutes. Supernatant was taken carefully to newly labeled eppendorf tubes. The supernatant was spun at 3500 rpm for 30 minutes. The supernatant was again transferred into newly labeled tubes, and then 400 µl of isopropanol was added. The samples were then incubated at -80°C, -20°C, 5°C, 50°C, and 25°C for 6 hours before spinning at 3500 rpm for 30 minutes. Supernatant was decanted, and then ethanol washed by adding 300 ul of 70% ethanol, and then spun at full speed. The ethanol was also decanted, leaving pellet in each eppendorf tube. The pellets were air dried, then dissolved by addition of Tris -Borate- EDTA buffer (Tris base, boric acid and EDTA (ethylenediaminetetraacetic acid) and kept overnight at 37°C. Agarose Gel Electrophoresis was first conducted as a quick check for the presence of DNA in the extracted plant samples.

2.3.3 Determination of Concentration and purity of DNA sample using Nanodrop

UV spectrophotometer was used for this measurement by adding 2 µl of distilled water to the DNA before RNase to dilute it. The distilled water was used as blank on the lower pedestal of the spectrophotometric machine. After blanking, each DNA sample's concentration and purity was measured one after the other by applying 2 µl of the sample on the lower pedestal.

2.3.4 DNA amplification and primer sequence

The amplifications were performed in 25 µl containing 50 Mm $MgCl_2$ 1.2 µl, 10xNH_4 buffer 2.5 µl, dNTPs 2.0 µl, DMSO 1.0 µl, Taq DNA polymerase 0.1 µl, Primer 1.5 µl, H_2O 14.8 µl, DNA 2.0 µl. Amplifications were performed in thermocycler programmed 49 cycles of denaturing for 20 sec at 94°C, annealing for 40 sec at 37°C and extension for 1 min at 72°C. PCR products were electrophoresed on 1.5% agarose gel for 2 hrs, stained with ethidium bromide and visualized under ultraviolet light. A negative control lacking template DNA was also used in each set. The following RAPD primers were used for the DNA amplification.

2.3.5 RAPD-PCR product resolution

RAPD-PCR products were resolved by electrophoresis on 1.5% agarose gels in 1 X TBE buffer. Products were visualized under UV light after staining for 30 min in a 1 µg/mL ethidium bromide, and then photographed using a UV trans illuminator [gel doc System, UVP printer (Mitsubishi)]. Products were sized against 100 bp ladder. The choice of primers OPB, OPH and OPT was based on the existing information on the crops they were evaluated for.

2.4 Data Analysis

The morphological characters were subjected to ANOVA using SAS ver. 9.1 [17] to analyze for the differences in treatment and genotypes, mean square interactions of the maize genotypes, effects of different storage temperature, genotypic performance of the morphological characters, the effect of the growth stages on the maize genotypes, was separated using DMRT at $p \leq 0.01$. Pearson correlation and contribution of principal component was also analyzed on the maize genotypes data.

The binary data generated from the molecular studies was analyzed using Numerical Taxonomic System of Statistics (NTSYS) Software (version 2.21) and Power marker software (version 3.25). Jaccard coefficient of similarity was used to estimate genetic distance and dendrogram using UPGMA between the genotypes and treatments.

3. RESULTS

The result in Table 2 shows the concentration and percentage purity (%) of forty DNA samples of maize genotypes under varied storage temperature. Optimum DNA concentration recorded in the improved maize genotypes were; TZLCOMP4C3 (4605.9 ng/µl) at 5°C, EVDT-W200STRCO (4885.7 ng/µl) at 25°C, POP66SR/ACR94 (3406.6 ng/µl) at 50°C and POOL18SR QPM (2016.9 ng/µl) at -20°C. In the landrace varieties; TZM 132 (3657.8 ng/µl) at -20°C, TZM 1291 (1861.5 ng/µl) at 50°C, EVDT-Y2008 STR (2305.7 ng/µl) at 25°C and TZM 1326 (1802.9 ng/µl) at -20°C. The highest DNA concentration across the maize varieties recorded at -20°C (POOL18SR QPM, TZM 132 and TZM 1326), 5°C (TZLCOMP4C3), 25°C (EVDT-W200STRCO and EVDT- Y2008 STR) and 50°C (POP66SR/ACR94 and TZM 1291).

The results in Table 3 show that RAPD primer OPB10 had the highest number of polymorphic bands of 15, and 100% polymorphic information content while OPH 05 and OPT 07 primers have the same number of polymorphic bands of 6.

The oligonucleotide primer OPB 10 had the highest allele number, gene diversity and polymorphic information content (PIC) of 15, 0.97 and 100 respectively (Table 4). OPB 10 had the lowest allele frequency of 0.05, while the highest allele frequency of 0.35 was recorded in OPH 05 and OPT 07 primers.

The result of genetic distance among the maize genotypes and storage temperatures is shown in Table 5. Genetic similarity was highest among treated genotypes AT2, GT3, GT4 and BT1. There is close genetic similarity between IT1, HTI and HT4. There is also similarity between CT3 and CT4; this is probably because they are the same genotype with different treatment. Samples AT1, AT2, AT3, BT1, CT1, CT2, CT3, CT4, CT5, ET1, GT1, GT2, GT3 and GT4 share the same ancestor and are closely related. Average dissimilarity between AT2 and AT3 is 0.27 with r of 0.21 and 0.32 respectively (Table 5).

The dendrogram in Fig. 1 indicate the relationship between the genotypes and treatments. There are two major clusters and 3 sub-clusters. The dendrogram show that the genotypes subjected to different temperature storage levels were seen at different clusters from each other. The treatment affected the genotypes by separating them into different cluster.

The results in Table 6 show that effect of genotype and storage temperature were highly significant ($p<0.001$) on plant height, leaf width,

number of leaves, stem length and dry biomass but significant ($p<0.05$) on leaf length for genotypes growth stages produced significant effects on all the growth characters and dry biomass. The interaction of genotypes x replicate were significantly higher for plant height, leaf width, number of leaves and stem length at ($p<0.01$). The first order interactive effects between treatments x genotypes as well as genotype x week were significantly higher for all the morphological characters and dry biomass, while Treatment x replicate produced significant effect for plant height, leaf width, number of leaves, leaf length and stem length. Again, Treatment x Week interaction was highly significant for all other growth characters and dry biomass (Table 6). The second order interaction for Treatment x genotype x week was highly significant for all the growth characters and dry biomass. The Treatment x genotype x week interactive effect was non-significant for stem length, highly significant for number of leaves and dry biomass and significant for leaf width, plant height and leaf length (Table 6).

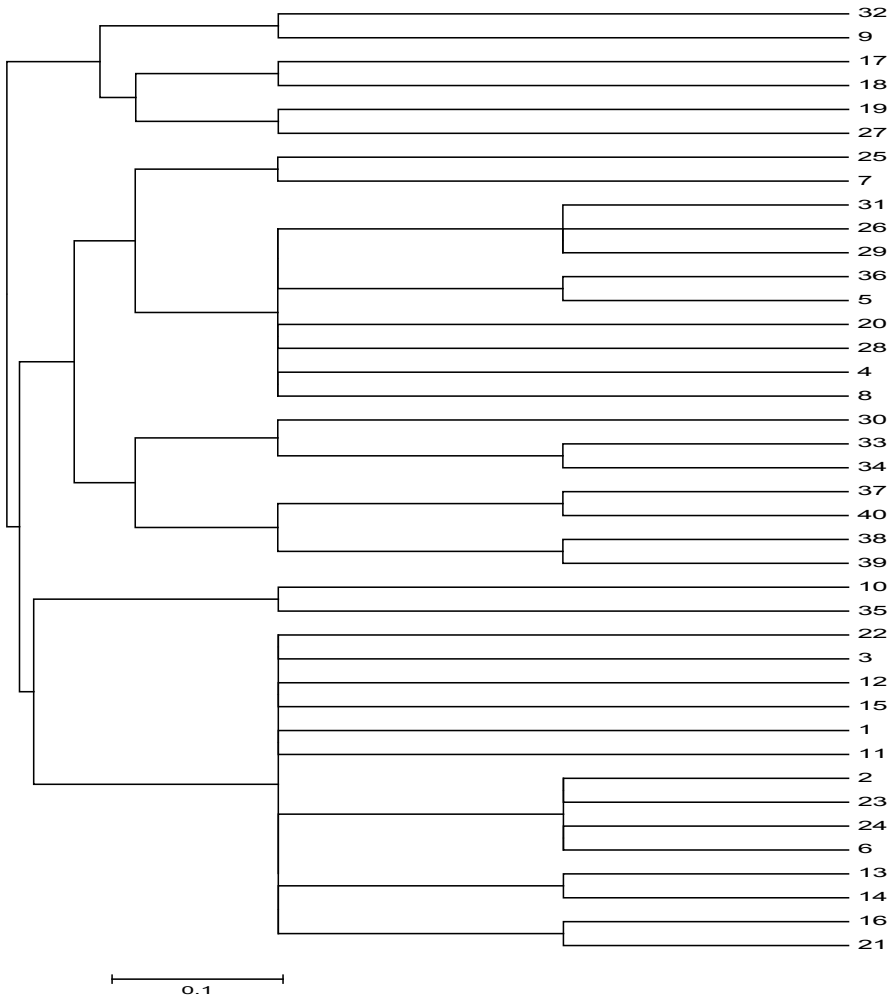

Fig. 1. The dendogram showing genetic relationship among the genotypes and storage temperature treatments

Keys;
1 = AT1, 2= AT2, 3= AT3, 4= AT4, 5= AT5, 6 = BT1, 7= BT2, 8= BT3, 9= BT4, 10= BT5, 11= CT1, 12= CT2, 13= CT3, 14= CT4, 15= CT5, 16 =ET1, 17 = ET2, 18 = ET3, 19 = ET4, 20= ET5, 21= GT1 22 – GT2 23 – GT3 24 – GT4 25 – GT5 26 – HT1 27 – HT2 28 – HT3 29 – HT4 30 – HT5 31 – IT1 32 – IT2 33 – IT3 34 – IT4 35 – IT5 36 – JT1 37 – JT2 38 – JT3 39 – JT4 40 - JT5.
A -TZLCOMP4C3, B- EVDT-W200STRCO, C- POP66SR/ACR94, E- POOL18SR QPM, G- TZM 132, H-TZM 1291, I- EVDT- Y2008 STR and J- TZM 1326.
T1- -80°C, T2- +5°C, T3- -20°C, T4- +50°C, T5- +25°C

Table 2. Concentration and purity % of forty DNA samples of maize genotypes at varied storage temperature

Description	Maize genotype	DNA Conc / purity	Temperature regime of seed treatment				
			-80°C	-20°C	+5°C	+25°C	+50°C
Improved varieties	TZLCOMP4C3	DNA Conc. (ng/µl)	2492.2	2651	4605.9	681.8	204.6
		DNA Purity %	1.94	1.93	2.07	1.85	1.26
	EVDT-W200STRCO	DNA Conc. (ng/µl)	1941.4	26.6	2271.4	4885.7	2151.1
		DNA Purity %	1.9	1.79	1.89	1.84	1.9
	POP66SR/ACR94	DNA Conc. (ng/µl)	1749.6	2048.9	2088	1110.7	3406.6
		DNA Purity %	1.96	2.07	2.04	1.77	1.94
	POOL18SR QPM	DNA Conc. (ng/µl)	1864.5	2016.9	433.9	74.6	1395.8
		DNA Purity %	2.04	1.99	1.7	1.1	2.09
Landrace varieties	TZM 132	DNA Conc. (ng/µl)	2347.9	3657.8	702.4	1114.2	2892.9
		DNA Purity %	2.11	1.97	2.11	1.87	2.1
	TZM 1291	DNA Conc. (ng/µl)	814.9	523.3	961.1	39.7	1861.5
		DNA Purity %	2.14	1.75	2.01	1.1	1.97
	EVDT- Y2008 STR	DNA Conc. (ng/µl)	237.9	352.6	1180.7	2305.7	578.6
		DNA Purity %	1.19	1.57	1.87	1.92	1.64
	TZM 1326	DNA Conc. (ng/µl)	159.3	1802.9	1580.5	988.1	902.2
		DNA Purity %	1.81	1.91	1.93		1.91

Table 3. DNA amplification and RAPD- product resolution

RAPD primers	Oligonucleotide sequence (5^1-3^1)	No of polymorphic bands	Mean total number of bands	Percentage polymorphic information content (PIC) (%)
OPB 10	AGT CGT CCC C	15	15	100
OPH 05	GGC AGG CTG T	6	6	100
OPT 07	CTG CTG GGA C	5	6	83.3

Table 4. Polymorphic information contents, Allele frequency and Gene diversity of RAPD primers

RAPD primers	Major allele freq	Sample size	Allele no	Gene diversity
OPB 10	0.05	40.00	15.00	0.97
OPH 05	0.35	40.00	6.00	0.84
OPT 07	0.35	40.00	6.00	0.82
Mean	0.25	40.00	9.00	0.88

Table 5. Genetic distance matrix showing the dissimilarity of forty maize samples under varied storage temperature

	AT1	AT2	AT3	AT4	AT5	BT1	BT2	BT3	BT4	BT5	CT1	CT2	CT3	CT4	CT5	ET1	ET2	ET3	ET4	ET5	GT1	GT2	GT3	GT4	GT5	HT1	HT2	HT3	HT4	HT5	IT1	IT2	IT3	IT4	IT5	JT1	JT2	JT3	JT4	JT5
AT2	0.21																																							
AT3	0.32	0.38																																						
AT4	0.38	0.59	0.44																																					
AT5	0.38	0.59	0.44	0.26																																				
BT1	0.32	0.08	0.26	0.77	0.77																																			
BT2	0.21	0.38	0.38	0.12	0.21	0.51																																		
BT3	0.88	1.01	0.77	0.51	0.51	0.77	0.59																																	
BT4	0.51	0.32	0.44	0.51	0.67	0.44	0.44	1.65																																
BT5	0.32	0.26	0.51	0.32	0.59	0.38	0.38	1.37	0.21																															
CT1	0.26	0.44	0.32	0.38	0.51	0.44	0.44	0.88	0.67	0.44																														
CT2	0.32	0.26	0.26	0.44	0.59	0.17	0.38	0.59	0.59	0.38	0.32																													
CT3	0.32	0.26	0.51	0.32	0.44	0.38	0.38	1.01	0.32	0.17	0.44	0.26																												
CT4	0.26	0.21	0.59	0.51	0.67	0.32	0.44	1.17	0.38	0.21	0.38	0.32	0.12																											
CT5	0.51	0.32	0.44	0.51	0.67	0.32	0.59	0.88	0.51	0.44	0.38	0.32	0.32	0.38																										
ET1	0.38	0.59	0.32	0.38	0.51	0.44	0.32	0.38	1.17	0.77	0.38	0.32	0.59	0.67	0.38																									
ET2	0.59	0.38	0.67	0.59	0.59	0.38	0.51	0.44	0.77	0.51	0.59	0.38	0.38	0.32	0.32	0.44																								
ET3	0.59	0.51	0.51	0.44	0.21	0.51	0.38	0.77	0.44	0.38	0.77	0.51	0.38	0.59	0.59	0.77	0.38																							
ET4	0.44	0.67	0.67	0.32	0.21	0.88	0.26	0.59	0.44	0.51	0.59	0.67	0.38	0.59	1.01	0.77	0.88	0.38																						
ET5	0.51	0.77	0.59	0.38	0.17	1.01	0.32	0.67	0.51	0.77	0.67	0.77	0.44	0.67	0.88	0.67	1.01	0.44	0.21																					
GT1	0.32	0.51	0.38	0.32	0.32	0.51	0.17	0.59	0.59	0.67	0.59	0.38	0.38	0.44	0.59	0.32	0.67	0.67	0.38	0.32																				
GT2	0.51	0.59	0.44	0.26	0.26	0.77	0.32	0.88	0.51	0.59	0.26	0.44	0.32	0.38	0.38	0.51	0.59	0.59	0.44	0.26	0.32																			
GT3	0.38	0.21	0.32	0.51	0.67	0.21	0.59	1.17	0.38	0.21	0.26	0.21	0.21	0.17	0.17	0.67	0.44	0.59	0.77	0.88	0.59	0.38																		
GT4	0.51	0.44	0.44	0.88	1.17	0.32	0.77	1.17	0.88	0.59	0.38	0.21	0.44	0.38	0.26	0.38	0.44	0.77	1.37	1.17	0.77	0.67	0.26																	
GT5	0.77	0.51	0.51	0.59	1.01	0.38	0.67	0.77	0.59	0.51	0.44	0.38	0.51	0.59	0.12	0.44	0.26	0.51	1.17	1.37	0.88	0.59	0.32	0.32																
HT1	0.38	0.59	0.32	0.17	0.17	0.59	0.12	0.51	0.51	0.44	0.38	0.44	0.44	0.67	0.67	0.38	0.44	0.21	0.21	0.38	0.32	0.38	0.67	0.88	0.59															
HT2	0.51	0.44	0.59	0.38	0.38	0.59	0.44	0.67	0.26	0.44	0.88	0.59	0.21	0.38	0.51	0.88	0.44	0.32	0.32	0.38	0.44	0.51	0.51	0.88	0.77	0.38														
HT3	0.44	0.67	0.51	0.21	0.21	0.88	0.26	0.59	0.59	0.67	0.44	0.67	0.38	0.44	0.59	0.59	0.67	0.51	0.26	0.21	0.26	0.21	0.59	1.01	0.88	0.32	0.32												
HT4	0.59	0.88	0.51	0.44	0.32	1.17	0.38	1.01	0.59	0.67	0.77	1.17	0.67	0.77	1.37	0.77	1.17	0.51	0.38	0.32	0.51	0.44	1.01	1.37	1.65	0.32	0.59	0.51											
HT5	0.38	0.59	0.59	0.26	0.17	0.77	0.32	0.38	0.88	0.44	0.38	0.44	0.32	0.51	0.67	0.51	0.44	0.44	0.21	0.38	0.44	0.38	0.51	0.88	0.77	0.26	0.51	0.32	0.59										
IT1	0.51	0.77	0.44	0.26	0.26	0.77	0.21	0.51	0.51	0.59	0.67	0.59	0.44	0.67	0.67	0.51	0.59	0.44	0.21	0.38	0.21	0.38	0.67	1.17	0.59	0.17	0.38	0.32	0.32	0.26									
IT2	0.44	0.38	0.51	0.32	0.44	0.51	0.38	1.01	0.21	0.26	0.77	0.67	0.26	0.32	0.59	1.01	0.51	0.26	0.38	0.59	0.51	0.59	0.44	1.01	0.67	0.32	0.12	0.38	0.38	0.59	0.32								
IT3	0.51	0.77	0.77	0.26	0.26	1.01	0.32	0.88	0.67	0.59	0.51	0.77	0.44	0.51	0.88	0.88	0.77	0.44	0.21	0.38	0.44	0.26	0.67	1.17	1.01	0.26	0.38	0.21	0.32	0.38	0.26	0.32							
IT4	0.51	0.59	0.59	0.26	0.51	0.59	0.44	0.51	0.88	0.44	0.26	0.59	0.59	0.51	0.67	0.51	0.44	0.59	0.44	0.88	0.77	0.51	0.51	0.88	0.59	0.26	0.67	0.44	0.59	0.38	0.51	0.44	0.26						
IT5	0.59	0.38	0.67	0.59	0.77	0.38	0.67	0.77	0.59	0.38	0.77	0.67	0.51	0.59	0.77	1.01	0.51	0.38	0.67	1.01	1.17	1.37	0.59	0.77	0.67	0.44	0.44	0.88	0.51	0.77	0.77	0.26	0.59	0.32					
JT1	0.51	0.59	0.44	0.38	0.17	0.59	0.32	0.38	0.88	0.77	0.51	0.59	0.59	0.88	0.67	0.38	0.44	0.32	0.32	0.26	0.44	0.51	0.88	0.88	0.77	0.17	0.51	0.32	0.44	0.26	0.38	0.59	0.51	0.38	0.44				
JT2	0.51	0.77	0.44	0.38	0.51	0.59	0.59	0.51	1.17	0.59	0.26	0.44	0.59	0.51	0.67	0.51	0.59	0.77	0.59	0.67	0.59	0.51	0.38	0.51	0.77	0.38	0.67	0.44	0.59	0.38	0.51	0.59	0.38	0.17	0.44	0.38			
JT3	0.51	0.77	0.21	0.38	0.38	0.59	0.44	0.51	0.88	0.77	0.51	0.59	0.77	0.88	0.88	0.38	0.77	0.59	0.59	0.51	0.44	0.67	0.67	0.67	1.01	0.26	0.51	0.44	0.32	0.51	0.38	0.44	0.51	0.38	0.44	0.26	0.17		
JT4	0.44	0.67	0.26	0.32	0.32	0.67	0.38	0.59	0.77	0.67	0.44	0.67	0.67	0.77	1.01	0.44	0.67	0.51	0.51	0.44	0.51	0.59	0.77	0.77	1.17	0.21	0.44	0.38	0.26	0.44	0.44	0.38	0.44	0.32	0.38	0.21	0.21	0.04	
JT5	0.44	0.51	0.38	0.44	0.44	0.51	0.51	0.59	1.01	0.67	0.44	0.67	0.67	0.77	1.01	0.44	0.67	0.67	0.51	0.59	0.67	0.77	0.77	0.77	1.17	0.32	0.59	0.51	0.38	0.44	0.59	0.51	0.44	0.21	0.26	0.21	0.21	0.12	0.08

Keys: A -TZLCOMP4C3, B- EVDT-W200STRCO, C- POP66SR/ACR94, E- POOL18SR QPM, G- TZM 132, H- TZM 1291, I- EVDT- Y2008 STR and J- TZM 1326, T1- -80°C, T2- +5°C, T3- -20°C, T4- +50°C, T5- +25°C [s]

Table 6. **Mean square interactions of maize genotypes, growth stages and different storage temperatures on growth and dry biomass characters**

Source of variation	df	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Stem length (cm)	Dry biomass (cm)
Genotypes	7	10243.79***	868.11*	3.87***	14.21***	4269.27***	31.46***
Replicate	2	736.44^{ns}	444.30^{ns}	4.30^{ns}	0.86^{ns}	386.65^{ns}	0.00^{ns}
Treatment	4	10328.74***	2296.06***	15.65***	20.30***	7470.10***	70.64***
Weeks	6	107362.97***	49253.45**	159.39***	179.84***	127906.62***	3176.57**
Genotype* replicate	14	2606.17**	601.65^{ns}	4.17***	6.78***	1153.49**	0.00^{ns}
Treatment*genotype	28	4428.76***	900.60***	3.26***	8.18***	1691.64***	21.44***
Genotype*week	42	1884.30***	626.62**	1.91***	5.27***	1408.77***	31.46***
Treatment*replicate	8	2640.58**	746.56*	3.94***	8.30***	951.18*	0.00^{ns}
Week*replicate	12	894.00^{ns}	138.68^{ns}	0.54^{ns}	1.17^{ns}	384.18^{ns}	0.00^{ns}
Treatment*week	24	1352.64^{ns}	612.56*	2.10***	2.62***	1266.29***	70.64***
Treatment*genotype*replicate	56	2441.11***	819.16***	2.05***	5.95***	1037.51***	0.00^{ns}
Genotype*week*replicate	84	726.66^{ns}	288.01^{ns}	0.59^{ns}	1.10^{ns}	404.03^{ns}	0.00^{ns}
Treatment*genotype*week	168	1081.88*	494.93*	0.97**	2.11***	645.77^{ns}	21.44***
Treatment*week*replicate	48	637.96^{ns}	444.91^{ns}	0.58^{ns}	0.95^{ns}	389.95^{ns}	0.00^{ns}
Error	335	831.327	368.84	0.61	1.11	417.05	0.00
Corrected total	838						

**p<0.05 significant, ** p<0.01 highly significant, *** p<0.001 highly significant, ns= non- significant*

Table 7. Effects of different storage temperatures on growth characters and dry biomass of maize genotypes

Treatments	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Stem length (cm)	Dry biomass (g)
-80°C	75.51^{c}	41.30^{c}	2.60^{c}	5.73^{c}	34.64^{c}	1.22^{e}
5°C	80.42^{c}	43.33^{bc}	2.71^{c}	6.11^{b}	37.04^{c}	1.31^{d}
-20°C	88.88^{b}	46.04^{b}	2.97^{b}	6.14^{b}	42.05^{b}	2.14^{c}
50°C	95.50^{a}	51.15^{a}	3.41^{a}	6.69^{a}	51.26^{a}	2.64^{a}
25°C	88.58^{b}	45.22^{bc}	2.91^{b}	6.05^{b}	36.83^{c}	2.42^{b}

Means with the same letters in the same column are not significantly different at (p>0.05) using Duncan's multiple range test (DMRT)

Table 8. Genotypic performance of growth and dry biomass characters of maize at varied storage temperature

Genotypes	Plant Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Number Of Leaves	Stem Length (cm)	Dry Biomass (G)
TZLCOMP4C3	65.31 [d]	38.90 [b]	2.63 [c]	5.28 [b]	28.34 [d]	2.39[b]
EVDT-W2000 STR CO	82.83 [c]	47.66 [a]	3.04 [a]	6.18 [a]	41.13 [bc]	2.35[c]
POP66SR/ACR 94	85.60 [bc]	45.47 [a]	3.04 [a]	6.42 [a]	38.91 [c]	2.83[a]
POOL18SR/QPM	82.69 [c]	44.51 [a]	3.17 [a]	6.34 [a]	41.07 [bc]	2.09[d]
TZM 132	99.09 [a]	46.54 [a]	3.05 [a]	6.25 [a]	50.12 [a]	1.33[h]
TZM 1291	90.31 [bc]	47.57 [a]	2.78 [bc]	6.17 [a]	39.77 [c]	1.54[f]
EVDT-Y2OO8STR	92.29 [ab]	47.32 [a]	2.95 [ab]	6.40 [a]	46.11 [ab]	1.60[e]
TZM 1326	88.10 [bc]	45.28 [a]	2.70 [c]	6.11 [a]	37.47 [c]	1.42[g]

Means with the same letters in the same column are not significantly different at (p>0.05) using Duncan's multiple range test (DMRT)

Table 9. Contribution of principal component axis (PCA) to the variation of growth and dry biomass traits of maize genotypes

Traits	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5	Prin 6
Plant height(cm)	0.47	0.09	-0.24	0.81	0.11	0.2
Leaf length(cm)	0.39	-0.35	0.08	0.13	-0.12	-0.25
Leaf width(cm)	0.5	-0.06	0.02	-0.44	-0.07	0.74
Number of leaves	0.5	-0.03	-0.26	-0.33	0.59	-0.47
Stem length(cm)	0.35	0.56	-0.11	-0.13	-0.65	-0.34
Dry biomass(g)	-0.08	0.74	0.47	0.01	0.44	0.15
Proportion (%)	47.17	23.15	9.87	7.81	6.25	5.75
Eigen value	2.83	1.39	0.59	0.47	0.38	0.35

Table 10. Pearson correlation of growth stages, morphological and dry biomass characters at different storage temperature of maize genotype

Correlation	Treatment	Genotype	Week	Replicate	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaves	Stem length(cm)	Dry biomass (g)
Treatment										
Genotypes	0.00									
Weeks	0.00	0.00								
Replicate	0.00	0.00	00.00							
Plant height	0.13	0.14	0.60**	-0.01						
Leaf length	0.08	0.05	0.55*	-0.02	0.59*					
Leaf width	0.11	0.01	0.52*	0.07	0.59*	0.56*				
Number of leaves	0.09	0.09	0.54*	-0.01	0.71**	0.60**	0.62**			
Stem length	0.07	0.07	0.71**	0.02	0.69**	0.47	0.55*	0.65**		
Dry biomass	0.09	-0.07	0.52*	0.00	0.27	0.11	0.2	0.17	0.57*	

The effect of different storage temperatures on the morphological characters and dry biomass in Table 7 shows that plant height, leaf length, leaf width, number of leaves, stem length and dry biomass at storage temperature of 50°C were significantly ($p<0.05$) higher than other temperature treatments. At storage temperatures 5°C and 25°C no significantly difference was observed in the leaf length, while -80°C, 5°C and 25°C for stem length were significantly similar to one another. On the other hand, storage temperatures of -80°C and -20°C were not significantly different for dry biomass that significantly different at 5°C, 50°C and 25°C for dry biomass.

The height of the plant, leaf width, number of leaves, stem length were significantly different for Genotype TZM 132 at $P <0.05$, Plant height for Genotype POP 66 SR/ACR 94, TZM 1291, and TZM 132 was not significantly different as well as EVDT- W2000 STRCO and POOL18SR/QPM (Table 8). The leaf length of TZLCOMP4C3 was significantly different from other genotypes, while genotypes TZM 1291 and EVDT-Y2008STR were significantly different. The number of leaves in TZLCOMP4C3 genotype was significantly different from all other maize genotypes while genotype TZL COMP4C3 and TZM 132 are significantly different. All the genotypes showed significant difference with dry biomass.

The contribution of principal component axis (PCA) to the variation of growth and dry biomass traits of treated maize genotypes is shown in Table 9. Variation was observed across the six PCA as 2.83 (47.17%), 1.39 (23.15%), 0.59 (9.87%), 0.47 (7.81%), 0.38 (6.25%), 0.35 (5.75%). The Prin 1 accounted for the highest variation with proportion of 47.17% and eigen value of 2.83. The plant height, leaf width and number of leaves in Prin 1 were closely related compared to leaf length, and stem length which are also more related than to the dry biomass. In Prin 2, plant height, leaf width, and number of leaves was more closely related when compared to stem length and dry biomass than to leaf length. While in Prin 3, plant height, number of leaves and stem length was more closely related than leaf length and leaf width than to dry biomass. The Prin 4 is more related for plant height, leaf length than to leaf width and number of leaves. Prin 5 is highly related with number of leaves and dry biomass than to plant height, while the plant height and leaf width in Prin 6 are closely related when compared to other growth characters and dry biomass.

Pearson correlation coefficient of growth characters, growth stages, genotypes, different temperature storage levels and replicate are shown in Table 10. The plant height and stem length are positive and strongly related with growth stages at $p < 0.01$, $r = 0.60$ and 0.71 respectively, while leaf length, leaf width number of leaves and dry biomass are positively associated with growth stages at $p < 0.05$; $r = 0.55$, 0.52, 0.54 and 0.52 respectively. The number of leaves and stem length are positively and strongly related with the plant height at $p < 0.01$; $r = 0.71$ and 0.69 respectively, while leaf length and leaf width are positively related to the plant height at $p < 0.05$; $r = 0.59$ respectively. Number of leaves is closely and strongly related to leaf length at $p < 0.01$ with $r = 0.60$ while leaf width is associated with leaf length at $p < 0.05$; $r = 0.56$. Number of leaves is also strongly and positively related to leaf width with $p < 0.01$; $r = 0.62$ while stem length is positively related to leaf width with $p < 0.05$; $r = 0.55$. Stem length is positively and strongly related to number of leaves at $p < 0.01$, $r = 0.65$, whereas dry biomass is positively related to stem length at $p < 0.05$, $r = 0.57$.

The RAPD-PCR banding patterns of the 40 maize genotypes are shown in Plates 1-3. The 3 primers; OPB-10, OPH-O5 and OPT-07 amplified the DNA with products ranging from 307 to 1772 bp. A total of 27 bands were generated from 3 primers. The maximum of major band was observed with primer OPB-10 having 15 bands, while minimum number of 6 bands each were observed in primers OPH -05 and OPT-07 (Plates 1-4). Monomorphic bands were seen in primer OPT-07 (Plate 2) while the distinct polymorphic band produced a primer of OPB-10 (Plate 1). Plates 4, 5 and 6 show maize genotype at 3 weeks after planting seed stored at temperatures of -80°C, 5°C and 25°C respectively.

4. DISCUSSION

Maize has diverse utility and is important to store it for a long or short period of time without losing its viability and genome. From this study, the effect of varied temperature storage levels on the morphological characters and molecular variability of maize genotypes. Bano et al. [18] reported that multidisciplinary approach which may include physiology, genetics and molecular biology will be the best way to understand responses of maize to low temperature stress at emergence stage. In another study, it was

affirmed that evaluating maize hybrids for tolerance to high temperature stress is an essential step towards stabilizing yields [19]. The findings from this study also revealed that interaction among the genotypes treatment and growth characters produced significant effect on plant height and increase in temperature affects plant growth as similarly reported [20].

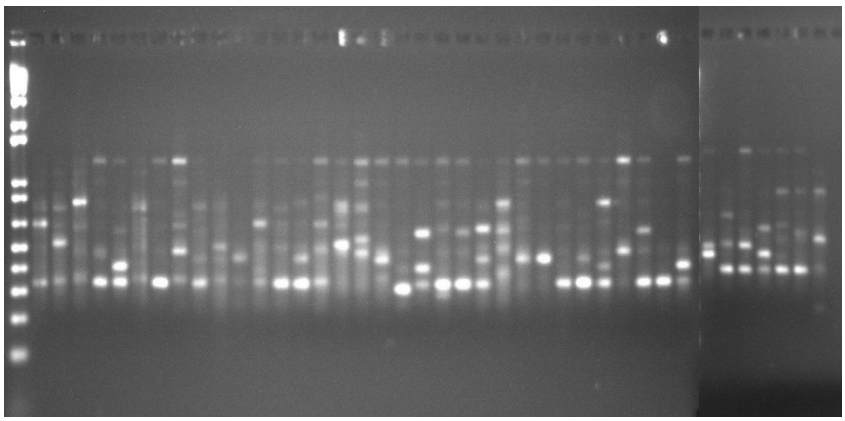

Plate 1. OPB -10 RAPD-PCR primer polymorphic bands

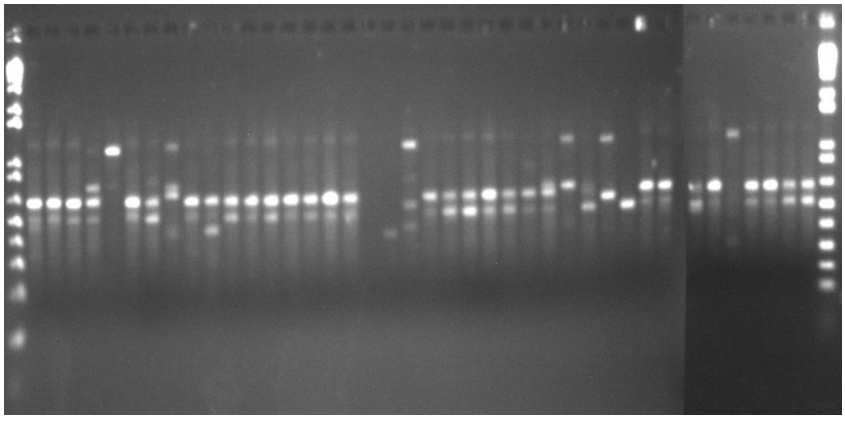

Plate 2. OPH – 05 RAPD- PCR primer polymorphic bands

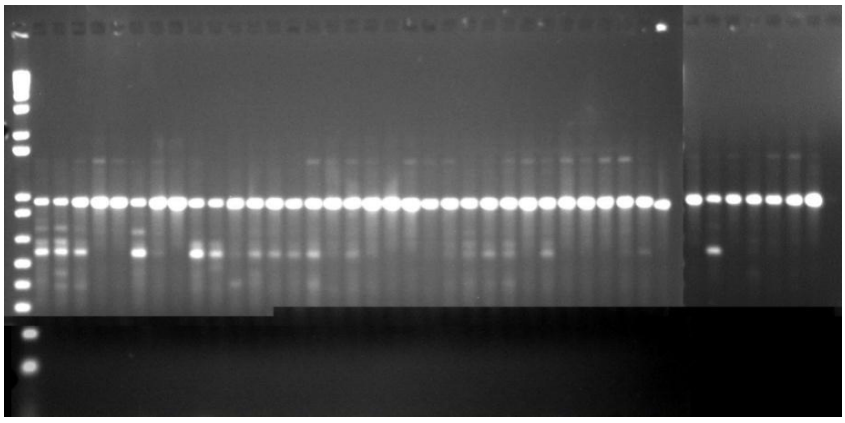

Plate 3. OPT- 07 RAPD- PCR primer polymorphic bands

Plate 4. Maize genotypes stored at -80°C (T1) 3 weeks after planting

Plate 5. maize genotypes stored at 5°C (T2) 3 weeks after planting

Plate 6. Maize genotypes stored at 25°C (T5) 3 weeks after planting

The interaction between temperature treatments and genotypes were significant for the growth characters which include the plant height, leaf length, leaf width, number of leaves, stem length and dry biomass in accordance with Ihsan et al. [21] who confirmed significant genetic differences for morphological traits between maize genotypes. Also, Abayi et al. [22] observed significant genetic variation in important agronomic traits. This could be due to genetic

variation among different maize genotypes and difference in their adaptation to high temperature which affects the growth and development of the crop as previously reported [19]. Effect of the treatments which were significant at 50°C for all the growth characters were in accordance with the result of an earlier study [23] which reported that quality of seed improved when stored at high temperature of 35°C. The low significance at low temperature of -80°C was in agreement with a research finding which reported that cold tolerance is complication phenomenon because multiple genes are involved to control chilling stress [24].

Maize genotypes TZM 132, performed best with highly significant effect for all the growth characters. There was genetic variation among the maize genotypes with varied response to different temperature storage levels which could be due to genetic variation of maize genotypes in tolerating high temperature among maize genotypes [19].

The variation of maize genotypes to growth characters with response to PCA shows that proportion and eigen vector for prin 1 which accounted for the variation of maize growth characters agreed with similar observation made by Olawuyi et al. [25]. This could help in selection of better genotypes under different temperature storage conditions.

The gene cluster representation showed diverse variation among treatment of maize genotypes. The response of individual genotypes to treatment showed how the genetic relationships were classified in agreement with the report which established cluster analysis as a method of classification based on stress tolerance and susceptibility to grain yield in both normal and stress conditions [26].

5. CONCLUSION

The highest DNA concentration of 4885.7 ng/µl was recorded for EVDT-W200STRCO genotype at room temperature (25°C). The first order interactive effects between treatments x genotypes were significantly higher for all the morphological characters and dry biomass while the second order interaction for treatment x genotype x week was highly significant for all the growth characters and dry biomass. The effect of different storage temperatures on the morphological characters and dry biomass showed that plant height, leaf length, leaf width, number of leaves, stem length and dry biomass at storage temperature of 50°C were significantly higher than other temperature treatments. The growth characters were higher in TZM 132 than other genotypes. Therefore, maize seeds can therefore be stored at temperature range of -20°C to 50°C depending on the variety, without losing its viability and molecular constituents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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