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PHENOTYPIC AND MOLECULAR VARIABILITY OF MAIZE (*ZEA MAYS* L.) INDUCED WITH X-RAY

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ABSTRACT

Ten genotypes of maize collected from National Center for Genetic Resources and Biotechnology (NACGRAB) were induced with X-ray for morphological and molecular assessment. The experimental design was complete randomized design with four replicates. Morphological and molecular statistical analyses of treated genotypes were conducted using SAS and Power Maker Packages, respectively while dendrogram was generated using Jaccards similarity coefficient using Unweighted Paired Group Method and Arithmetic Averages (UPGMA). The study revealed significant difference which is an indication of genetic variation of characters in treated maize. Genotype DTSR-Wco performed best in plant height (62.35 cm), leaf length (62.35 cm), number of leaves (3.15), leaf width (7.55 cm) and dry leaf biomass (0.24 g). X-ray at 90 Kv/mass, 95 Kv/mass and 100 Kv/mass decreased plant heights to 54.25cm, 53.87cm and 54.10cm respectively compared to Control. Heritability estimate was greater than 70% for all characters evaluated. Genotype TZM 1551 at 0 Kv/mass yielded the highest concentration of DNA at 2841.60 ng/ul and the highest genomic DNA concentration was obtained at 95 Kv/mass for TZM 132 with 1.91%. Primer BMC 1755 was most polymorphic with 58.77% in treated maize genotypes. The plant height was strongly correlated with leaf length (r=0.9), leaf width (r=0.76) and number of leaves (r=0.77). Principal component analysis showed close relationship between plant height (-0.03) and leaf length (0.05) compared with leaf width (-0.67) and number of leaves (0.69). Dry shoot biomass (0.05) was closely related to dry root biomass (-0.03) and dry leaf biomass (-0.04).

Key words: Mutation, Simple Sequence Repeat (SSR), Electromagnetic radiation, Electrophoresis

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INTRODUCTION

Maize is a versatile monoecious plant useful as food for humans and animals as well as raw material for many industrial uses (Olawuyi*et al*., 2010; Dwivedi *et al.*, 2016). Various techniques of inducing mutation increase variation artificially. Mutation occurs spontaneously as a result of alteration in genetic material and it changes the functions of the affected organism and its offspring (Grifith, 2005). Most mutation are harmful within the population but in some cases, mutation is beneficial in increasing agricultural values of some plants and animals.

Mutation breeding is a tool used by plant breeders for improvement of crops with narrow genetic base and plant development phenomena (Eze and Dambo, 2015). It has been widely adopted for genetic improvement of crops with desired agronomic and yield traits to enhance productivity. Electromagnetic radiation is a physical agent for inducing artificial mutation in plants which include; gamma rays, X-ray and ultra-violet ray (Eze and Dambo, 2015; Olawuyi*et al.*, 2015).

X-ray is a form of electromagnetic radiation exposed to organisms through solar system. It occupies 0.01-10 nm wave length (λ) of the electromagnetic spectrum (Sabastine, 2012). Radiation is also known to aid plant growth to a certain extent but beyond the optimum dose level, it may be lethal to plant. X-ray is also known to induce chromosomal aberration in organisms (Xing *et al.*, 2011). It has been observed that maize and other plants are negatively affected by mutagenic effects of high dose X-ray naturally sourced from the solar system. Again, the beneficial and lethal effect of X-ray have been Identified in humans medically but there is limited information on the phenotypic and genomic effects of X-ray on Maize plant. Therefore, the aim of this study was to assess the phenotypic and molecular variability of X-ray induced maize genotypes.

Simple sequence repeats (SSR) are mostly codominant and excellent markers used for carrying out studies in population genetics and mapping (Olawuyi and Onuoha, 2017). SSR makers have been used in many molecular studies due to their reproducibility, multi-allelic nature, codominant inheritance, relative abundance and good genome coverage (Anjali *et al*., 2018).

Simple sequence Repeat (SSR) works on the principle of microsatellite assay. It is based on the fact that it is highly polymorphic even between closely related lines. It requires low amount of DNA and can be automated easily for high throughput screening which can be exchanged between laboratories but are highly transferable between populations (Olawuyi*et al.*, 2019).

MATERIALS AND METHODS

Seed Collection and Experimental site

The seeds of ten maizegenotypes were collected from National Center for Genetic Resource and Biotechnology (NACGRAB), Moor plantation Ibadan, Nigeria. The genotypes; TZM 1551, TZM COMP1 C_2, TZM 132, TZE- WDTSR C_4, OBA SUPPER F_2, TZM 217, TZM 1300, DTSR- WC_O, and TZM 140 were evaluated in the screen house of the Department of Botany, University of

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Ibadan, Nigeria, while the molecular studies were carried out in the laboratory of bioscience unit of international institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Soil Sterilization, Research Design and Exposure to X-ray

The Loamy soil sample was collected, sterilized to 60^{o}C, allowed to cool and then packed into perforated polythene bags. Each bag contained 7kg of soil. The soil was watered and allowed to drain out before planting.

The experiment was laid out in a complete randomized design with four replicates. The various genotypes of maize were grouped into five and exposed to x-ray machine Silhouette VR of model E720Fx at the University College Hospital (UCH) Ibadan:

85 kv/ Mas, 90kv/mas, 95kv/mas and 100kv/mas, while unexposed (0kv/mas) was the control.

Planting

The seeds were planted in polythene bags filled with sterile soil, and spaced at a distance of 10cm between treatment and 30cm between the blocks.

All agronomic practices such as watering thinning etc. were duly carried out.

Determination of Morphological Traits

Data on growth characters (plant height, leaf length, and leaf width) were measured using meter rule while agronomic and yield related characters included; number of leaves, root lodging, number of tassels, length of tassels, dry root biomass, dry shoot biomass and dry leaf biomass.

Molecular Studies

Harvesting and Viability Test

Two weeks old fresh leaves of maize collected very early in the morning by 8:30am and were kept in cold environment at a temperature of 0°C to prevent denaturation of their DNA contents. Four leaf samples randomly collected per treatment from the four replicates were lyophilized at -80°C of liquid nitrogen. Viability test was carried out on the seeds plated in Petri dishes in order to select the viable ones.

DNA Extraction

The DNA was extracted from fresh and lyophilized young leaf sample of Maize according to Dellaporta *et al.*, (1983) protocol modified in sodium dodecyl sulphate (SDS) extraction buffer. One gram of fresh young leaves was ground using mortar and pestle followed by the addition of one litter of extraction buffer. The extraction buffer was made up of isopropanol, 10mg/mi RNASE A, β-mecaptoethanol, 1% PVP(1g of 100ml), 5M NaCl (292.2g in 1000) 1M Tris-HCl (121.4 in 1000ml, PH 7.5), 1 M EDTA (146.12g in 1000 PH 8), 20% SDS (200g SDS + 800ml of double distilled water), 70% ethanol (70ml of ethanol in 100ml of double distilled water), 25ml CIA (24ml chloroform + 1ml Isoamyl Alcohol), %M potassium Acetate (490.7 in 1000ml) stirred at 4°C, low salt TE buffer (10ml 1M Tris HCL, 2ml EDTA, 950ml of double distilled

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water and adjusted PH to 8). One litre of this buffer was prepared using 100ml Tris HCL + 50ml 0.5M EDTA + 100ml 5M NaCl +1%PVP.

DNA extraction protocol involved grinding and digesting cellular constituent in order to release the content. Detergent such as sodium dodecyl sulphate (SDS), cetyl-trimethyl ammonium bromide (CTAB) was used for removal of membrane lipids. When the DNA was released, it was protracted from endogenous nucleases by inclusion of EDTA in the extraction buffer which is necessary for chelating magnesium ion that are significant co-factor for nucleases. The Eppendorf tube were thoroughly mixed and incubated at 65°C and precipitated using chloroform and Isoamyl alcohol. Also, RNAs are normally removed using RNA degrading enzyme known as RNase A. he DNA solution was transferred to 2ml Eppendorf tube and treated with RNases (10mg/ml) for 1 hr. at 37°C and 1ml of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 10,000 rpm for 10 minutes though polysaccharide-like contaminants are more tasking to remove. Since DNA will be released along with other compound like lipids, protein, carbohydrate or phenols, it needs to be separated from another compound by centrifugation. The DNA in the aqueous phase was then transferred into new Eppendorf tubes without disturbing the interphase and ice-cold ethanol was added to precipitate the DNA in salt solution (e.g. Sodium acetate) or alcohol (100% isopropanol or ethanol), redissolved in sterile water or buffer. The pellets were washed in 70% ethanol air dried and finally dissolved 100ul of sterile double distilled water.

Agarose Gel Electrophoresis and DNA Quantification

The determination of DNA concentration extracted need to be measured using 1% Agarose gel electrophoresis and detected UV illuminator or spectrophotometer. Agarose gel checks whether the DNA is degraded or not but estimating DNA concentration by visually comparing bands intensities of extracted DNA with molecular ladder of known concentration is too subjective. Spectrophotometer measures the intensity of the absorbance of DNA solution at 260nm wavelength and also indicate the presence of protein contaminant but does not determine whether the DNA was degraded or not. This was doneto ascertain the quality and quantity of DNA extracted from the plant samples. Purity of DNA in the sample, dissolved in TE buffer was analyzed by checking the absorbance ratios at 260/280nm on Nano drop spectrophotometer followed by determination of concentration.

Primer and Primer Sequences

Polymerase chain reaction (PCR) and Amplification of DNA product

PCR reaction for SSRs was carried out in the presence of forward and reverse primers that anneal at the 5’ and 3’ ends of the DNA template. PCR fragment are usually separated on polyacrylamide gels in combination with $AgNO_3$ staining, autoradiography or fluorescent detection system. Agarose gels (usually 3%) with EtBr can also be used when differences in allele size among samples is larger than 10 bp.

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Amplification of microsatellite loci were carried out in 10ul PCR reaction containing 2.5ul of 50ng/ul total genomic DNA, 0.5 ul of 5 pMol of forward and reverse primers each, 1.0 ul DMSO, 1.0 ul of 25mM $mgCl_2$, 2.0 ul of 2.5 Mm DNTPs, 13.3ul of H_2O and 0.2 ul of Taq DNA polymerase using master Mix. Amplification was achieved using Eppendorf thermo cycler. The amplification condition was an initial step of denaturation for 5 minutes at 94°C followed by 35 cycles each consisting of denaturation step of 15 seconds at 94°C, an annealing step of 20 seconds at 55°C and an extension step of 30 seconds at 72°C. Seven minutes will be given after the last cycle of the extension step at 72°C to ensure the completion of primer extension reaction followed by cold temperature at 10°C lasting for infinity. The PCR products were separated 1.5% polyacrylamide gel at 80 volts, 300mA 60W for 1hour 30minutes. It was then visualized and photographed using silver staining under UV trans illuminator (Zang *et al.,* 2002).

Polyacrylamide gel electrophoresis (PAGE)

The reagent used was prepared from a mixture of the following; 40ml of water, 25 ml of TBE, 7.5ml of polyacrylamide, 50ul of terred and 500 ul of APS was added last. The procedure includes the glass comb rubber were washed in order to dry. The glass plate and spacer were wiped with 70% ethanol. The glass plate was lined with rubber and the spacer was placed between both spacers. The plates were carefully clipped together (the blue clip is used to clip the plate together while the white clip is used to clip the electrophoretic tank). When the glass tank was well arranged and positioned the mixed reagent was poured into it until it gets to the brim. It was then allowed for some minutes to gel after which it was then inserted into the gel tray where the DNA samples were introduced using staining dye alongside the 1kb plus ladder gene ruler from thermos-scientific for formation of bands the gels were then brought out after 1hour 30minutes and loaded into the chamber for viewing using the UV trans illuminator.

Statistical Analysis and Molecular Data Analysis

All data were subjected to Analysis of variance (ANOVA) and difference in mean was separated using DMRT at 95% probability level ($p<0.05$) while the relationship among the quantitative and qualitative traits was established using Pearson correlation co-efficient and principal component Analysis (PCA). In addition, Heritability, Phenotypic Coefficient of Variance (PCV) and Genotypic Coefficient of Variance (GCV) was also determined using SAS version 9.3 software.

Also, Molecular data generated was subjected to molecular analysis in order to generate information on total gene diversity, gene diversity per locus using Pop-Gene version 1.32 (Yeh and Boyle, 1999) and power-maker V3.25 (liu and Muse, 2005). The values obtained were used to generate dendrogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA). Cluster analysis as described by Sneat and Sokal (1973) was estimated to reveal phonetic representation of genetic relationship among the treated Maize genotypes.

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RESULTS

The analysis of variance showed that growth and yield characters were significant ($p<0.05$) across the genotype (G), treatment (T), Week after planting (WAP) and the first order interactions (G x T, G x WAP, Tx WAP), except in dry leaf biomass (DLB) where Treatment (T) and T x W were significant at $p<0.01$. The results of the second-order interaction (G x T x WAP) were significant ($p<001$) on plant height, leaf length and number of leaves while significance at $p<0.05$ were recorded in leaf width, dry root biomass (DSB) and dry leaf biomass (DLB). Disease incidence only showed significant ($p<005$) results with Treatment, WAP and G x T (Table 2).

Maize genotype DTSR-WC_O followed by TZE WDTSTR C_4 expressed the most significant ($p<0.05$) performance of plant height (62.35cm, 59.47cm), leaf length (7.55cm, 7.15cm), leaf width (62.35cm, 59.47cm), number of leaves (3.12cm, 3.02cm) respectively. The most significant number of tassels was recorded in TZM 132. Genotypes TZL COMP1 C_2 and TZM 1318 were the most disease resistant, the two with TZM 140 were least affected by root lodging while the results of disease and root lodging resistance were not significantly different among other genotypes. The genotypes, TZM 132, TZM 1318, TZE WDTSTR C_4, TZM 217, DTSR-WC_O and OBA SUPPER F_2 recorded the most significant dry shoot biomass, dry leaf biomass (including TZM 1551) and dry root biomass (except OBA SUPPER F_2). DTSR-WC_O showed highly significant tassel length (3.82cm) while the least significant was recorded in TZM 217 (0.23cm) and TZM 1300 (0.25cm) respectively (Table 3).

The performance of growth characters of maize plants subjected to x-ray treatments declined with increasing intensity of the x-ray when compared to the control, at 100Kv/MAS the least plant height (54.10cm), leaf length (54.10cm) and leaf width (2.68cm). However, the highest performances were reported at varying x-ray levels as obtained in the number of leaves (7.02cm at 85Kv/MAS), number of tassels (0.86cm at 90 Kv/MAS), most resistant disease incidence (1.25 at 90 & 95 Kv/MAS), and root lodging (0.11 at 95Kv/MAS). The results of the agronomic characters also showed the most significant performance in dry shoot biomass (0.52g at 100 Kv/MAS), dry root biomass (0.02g at 85, 90 & 95Kv/MAS), dry leaf biomass (0.25g at 100Kv/MAS) and tassel length (2.21cm at 100Kv/MAS) (Table 4).

Correlation co-efficient among characters of Treated Maize Genotypes

The result of Table 5 shows that plant height had strong positve correlation with number of leaves (r=0.77), leaf length (r=0.90) and leaf width (r=0.77). but a weak correlation with number of tassels (r=0.3413), Dry shoot biomass (r=0.16), Dry root biomass (r=0.17) and Dry leaf biomass (r=0.16). Number of leaves had a positve correlation with leaf length (r= 0.76) and leaf width (r = 0.76). Leaf leangth had a strong positve correlation with leaf width (r = 0.73). Dry shoot biomass had strong positive correlation with dry root biomass (r =0.87) and Dry leaf biomass (r=0.94) while Dry root biomass has strong positive correlation with dry leaf biomass (r=0.86).

Principal Component Analysis of Growth and Yield Characters of Treated Maize Genotypes.

The result from Table 6 delinates maize genotypes into 11 principal axis; prin 1, prin 2, prin 3, and prin 4. Prin one which constituted the highest accounted for 0.4168 of the proportion Eigenvalue of 4.5847 while Prin 11 was the least with proportion of 0.0056 and eigenvalue of 0.0056. Number of leaves from Prin 1 had the highest eigenvector of (0.6932) while dry shoot biomass was the least with (-0.0482). Also, Prin 2 produced highest eigenvector for plant height at (0.4944) while number of tassels produced the least at (0.1526). Prin 3 produced the highest eigenvector at (0.7932) while leaf length produced the least at (-0.2888). Prin 4 produced the highest eigenvector at (0.8164) for number of tassels while tassel length had the least at (-0.4772).

Heritability, Phenotypic and Genotypic Variance of Growth and Yield related characters in treated Maize genotypes

The component of variance for growth and yield analysis in maize genotypes and treated maize genotypes consisting of phenotypic variance, Genotypic variance and heritability are shown in tables 7. and 8. The phenotypic variance of both growth and yield character were higher than the genotypic variance in all characters determined. The value of the phenotypic and genotypic variance was highest at leaf lenght with values of 848.4 and 706.82 respectively but least at root lodging with the values of 0.2 and 0.4. the heritability was highest at dry root biomass with the value of 1.0 but least at Disease incident this implies that for leaf width, the proportion of phenotype resulted from the genotype. On table 4.9 the genotypic and phenotypic variances were highest in disease incident at 1112.31 and least in dry root biomass also, the heritabilitywas highest in disease incident and least in dry root biomass.

It was calculated using the following formular:

$$\text{Genotypic variance.} = \frac{\text{Genotypic MS} - \text{Error MS}}{\text{Replicate}}$$

$$\text{Phenotypic variance} = \text{Genotypic variance} + \text{Error MS}$$

$$\text{Heritability} = \frac{\text{Genotypic Variance}}{\text{Phenotypic variance}}$$

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Plate 1: TZL COMP1 C2 at 100Kv/mas of x-ray.

Plate 2: DTSR-WCo at 100kv/mas of x-ray.

Nano drop and DNA concentration of Treated Maize Genotypes.

Table 8 shows Nano-drop and DNA concentration of Treated Maize genotypes. The Nano-drop and DNA concentration for the extracted Maze genotypes were found to be at 260/280/gl. The quantity of genomic concentration was very good generally. However, the genotype TZM 132 with 100KV/MAS treatment had the highest DNA quality at 1.91g/ul from the total of 2086.60/ul of genomic DNA extracted. Though genotype TZM 1551 with 0KV/MAS produced the highest concentration at 2841.6g/ul it had a quality of 1.88g/ul. The lowest extracted total genomic DNA and concentration was recorded at 188.1g/ul and 1.43 for the genotypes TZM1551.

Frequency, Diversity and Polymorphic Information Content (PIC) of treated Maize Genotypes Using SSR Markers.

A total of eight primers of SSR marker were used to investigate the genetic diversity and molecular relationship of maize germplasm in treatment combinations. The result obtained

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shows that all primers were polymorphic across Maize genotypes with a total of three hundred and seven (307) amplified microsatellite loci, and a total of twenty-three (23) alleles were identified as shown in table 4. The percentage of allele diversity recorded was 50% while the polymorphic content information was 43%.

The number of amplified loci and number of alleles range from 23-48 and 2 to 3 with a mean of 38.37 and 2.88. Major allele frequency ranges from 0.47-0.96 with a mean of 0.80 while the allele diversity and polymorphic information content varied from 0.13 to 0.66 and 0.12 to 0.59 with mean of 0.49 and 0.43 respectively.

There were variations in major allele frequency, number of amplified microsatellites loci, number of allele and allele diversity. UMC 2281had the highest number of amplified microsatellite loci at 48 while BMC1755had the least at 23. Also, UMC 1295had the highest major allelic frequency at 0.93 while BMC1755 had the least frequency at 0.39. Polymorphic Information content was highest in BMC1755 at 0.59 while UMC 1295had the least at. Gene diversity was observed to be highest in BMC1755 with value of 0.66 and least in UMC 1295with a value of 0.12 (Table 9).

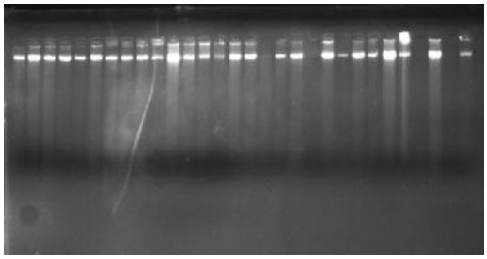

Plate 3: Genomic DNA 1-30

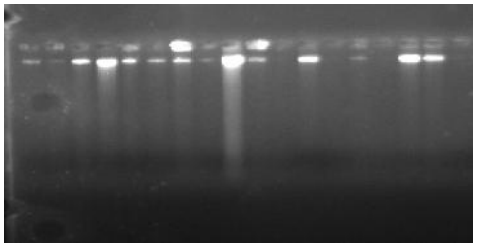

Plate 4 cont'd Genomic DNA 31-48

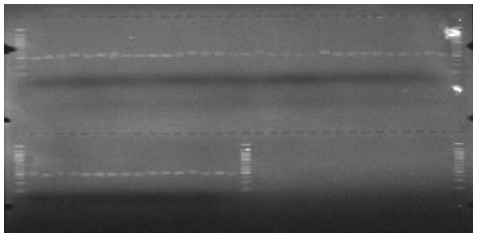

Plate 5: Polymorphic band revealing bmc1520 SSR Primer.

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Plate 6: Polymorphic band revealing bmc1755 SSR Primer

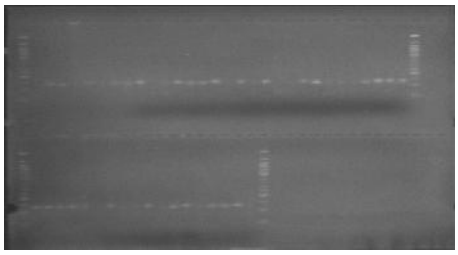

Plate 7: Polymorphic band revealing umc2151 SSR Primer

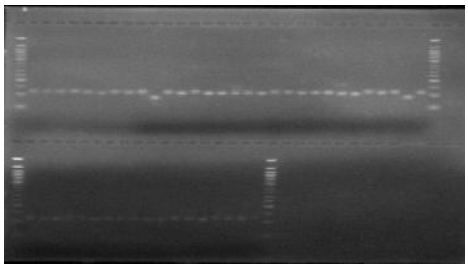

Plate 8: Polymorphic band revealing zct197 SSR Primer

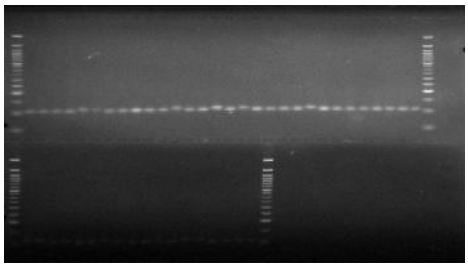

Plate 9: Polymorphic band revealing zmcp7430 SSR Primer

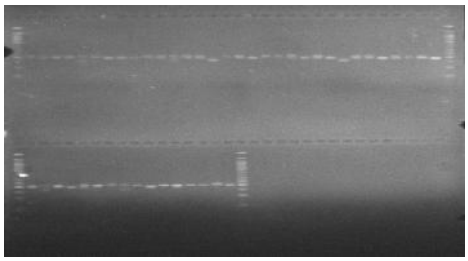

Plate 10: Polymorphic band umc2281 SSR Primer

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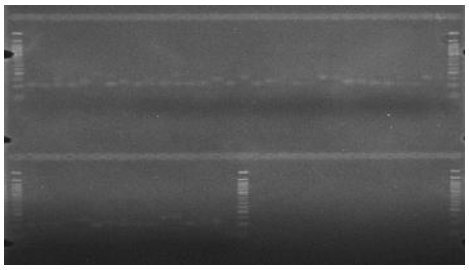

Plate 11 polymorphic band revealing unc1295 SSR primer

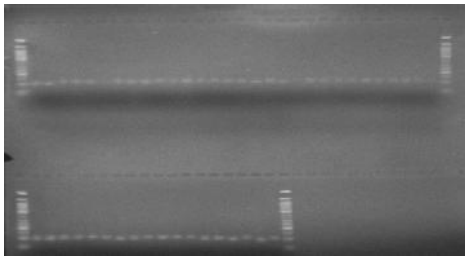

Plate 12 polymorphic band revealing umc1822 SSR primer

Dendrogram Showing the Genetic Similarity among 48 Treated Maize Genotypes

The Fifty samples comprising of ten (10) genotypes of maize were grouped into six different clusters as shown in figure 2. The genetic similarity and distances among treated maize genotypes were depicted from the dendrogram. The first cluster comprised of only G2T2 this implies that the second genotype treated with 90KV/Mas of X-ray is genetically different from all other treated maize genotypes.

The second cluster comprised of G7T1 G9T4 G10T4 G2T4 G7T4 G7T3 G1T3 G3T4 G4T4 G2T3G5T3 G1T4 G8T3 G4T3 G5T4 G3T3 G6T4 G7T4 G6T3 and G8T4. This implies that all most all the maize genotypes treated with 95Kv/Mas and 100Kv/mas are genetically similar. The third cluster comprised of G8T1 G9T2 G10T1 G7T2. The fourth cluster comprised of G5T0 G8T0 G9T0 G1T1 G4T1 G6T1 G3T2 G3T1 G2T1 G2T0 G10T0 G3T0 G7T0 G9T1 G8T2 G4T2 G4T0 G10T0 G1T2 G6T2 G5T2 G5T1. This implies that the controlled maize genotypes and those having treatment of 85 kv/mas and 90 kv/mas are genetically similar.

The fifth cluster comprised of G10T3 and the sixth cluster comprised of G6T0. This indicates that the tenth genotype treated with 95kv/mas and the sixth genotypes with no treatment are genetically different from each other and from genotypes of other clusters (Figure 1).

DISCUSSION

The options for increasing food production by at least 70% so as to cope with the rapid increase in human population are limited by the ever-changing climate condition. Induced mutations therefore unmask alleles that can be harnessed to breed superior varieties. In this study, the performance of growth and yield characters of maize which was observed to be significantly

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different is an indication of variation. This is in accordance with Bornzoue*et al.,* (2010), Delia *et al.,* (2013) and Olawuyi*et al.,* (2016) who considered genetic variation as essential for crop improvement. The genotypic and treatment reveal significant effect on the growth performance of Maize this may be attributed to genetic difference in growing types and difference on the type of adaptation.

DTSR-WC_O showed higher tolerance to the mutagenic effect of x-ray radiation with respect to plant height, leaf length, number of leaves, leaf width, length of tassels and dry leaf biomass compared to TZL COMP1 C_2 which could not tolerate the varied exposure periods. Also, with respect to dry shoot biomass and dry root biomass TZM 1318 and TZM 132 expressed tolerance to mutagenic effect of X-ray radiation.

X-ray imposed significant impact on most of the characters studied. The highest plant height was observed at 85kg. By increasing radiation dose to 90, 95, and 100 Kv/mas plant height declined by 26.5%, 27.9% and 27.0% respectively. The symptoms commonly observed in low dose irradiated are inhibition of germination, seedling growth, and other biological responses are associated with low dose radiation. Kim *et al.,* (2000) also reported that there are hypothesis that low dose irradiation could induce the growth stimulation by changing the hormonal signaling network in plant cell or by increasing the anti-oxidative capacity to easily overcome daily stress factors such as fluctuation of light intensity and temperature in the growth condition whereas high dose irradiation which resulted to growth inhibition is ascribed to cell cycle arrest at G2/M phase during somatic cell division and or various damages in the entire genome (Preusa and Britta, 2009).

It was also observed that X-radiation increased significantly the dry shoot biomass and dry root biomass. Seeds irradiated with 100kv/mas had significantly higher root weight. This agrees with the findings of Melki and Salami (2008) who observed that gamma rays (15 Gy) induced significant improvement on plant dry weight in chicken pea compared with control dose. Number of tassels and length of tassels were observed to be significantly higher at 90 and 100 kv/mas respectively.

Finding s from correlation analysis shows that the growth characters (plant height, leaf length, leaf width, and number of leaves) exhibited strong positive relationship with one another while the yield characters (dry shoot biomass and dry root biomass) exhibited strong positive relationship with each other but weak relationship with the growth-related characters. These findings are in accordance with the report of Olawuyi*et al.,* (2012). This implies that selection for any of the growth characters will probably not favor the yield characters.

The Principal Component Analysis shows that there is variation among the characters studied and the grouping of various characters into components in which prin 1 accounted for the highest eigen vector and proportion. Similar observation was also made by Olawuyi*et al.,* (2012) and Olawuyi and Onuoha, (2017). The scatter plot also shows that plant height and leaf length are

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more closely related with each other. However, leaf width and number of leaves expressed distant relationship between themselves and other characters evaluated in this study. The yield related characters were found to be closely related to each other. These differences and similarities observed among the character evaluated in this study may be due to the difference or similarities in the genes controlling the characters.

Heritability is the proportion of phenotypic variance that is due to genetic variance (Ogunniyan*et al.,* 2014). In this study heritability was greater than 70%. This implies that environmental influence was minimal on the characters studied and these characters can therefore be used for selection. This result was consistent with the findings of Galapia*et al.,* (2012); Akbar *et al.,* (2008); Rafi *et al.,* (2010).

TZM 132 at 100kv/mas of x-ray had the highest concentration of extracted DNA while TZM 1551 at 0kv/mas had the highest genomic DNA nanodrop. This implies that good quality DNA is obtained when Maize genotype was treated with 100kv/mas of x-ray.

Among noteable molecular markers used for genetic diversity studies maker assisted selctions is SSR maker (Zhao *et al*., 2011). Short repeated sequence are widely found in genome and shows great variation making them very usefull markers (Robert, 2013). All the primers used in this study were polymorphic across the the treated maize genotypes. However, the average polymorphic informaton contents was found to be below 50% this could be due to the effect of x-rays. There were also variations in major allele frequency, number of amplified microsatellite loci and number of allele and allele diversity. This information can assist breeders in selecting the appropriate breeding method to be applied to maize so as to obtain maximum yield and also maintain genetic variability.

Cluster analysis and dendrogram indicate that cluster group consist of genotypes from different treatment combination. The cluster diagram among treated Maize genotype shows that there genetic similarities and differences among treated maize genotypes. In the diagram it was observed that X-ray radiation of 85KV/ma, 90KV/ma and 0KV/ma had similar effect on maize genotypes as most of the genotypes treated with these doses were grouped into the same cluster. Also 95KV/mas and 100KV/mas had similar effect as they were grouped into the same cluster as well. This implies that low dose X-ray is sufficient to induce genetic variability in maize plant.

CONCLUSION AND RECOMMENDATION

Genotype DTSR-WC_O performed best for growth and yield and could therefore be selected for improvement of other maize genotypes.

Primer BMC1755 detected the highest polymorphic information contents compared to other primers. Hence, this primer could be subsequently used for mutagenic breeding of maize and other cereals.

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APPENDIX

The simple sequence repeat primers and their oligonucleotide sequences are shown in Table 1.

SSR PRIMER	Direction	OLIGONUCLEOTIDE SEQUENCE
umc2151	Forward	ATATGGTATTTTCTGCAGGCGT
	Reverse	AAAATCCTATACAGAAAACGGGCG
bmc1755	Forward	TGCGCACCAGCGACTGACC
	Reverse	GCGGGCGACGCTTCCAAAC
zct197	Forward	GCGAGAAGAAAGCGAGCAGA
	Reverse	CGCCAAGAAGAAACACACATCACA
zmcp7430	Forward	CGAAGCTGCTGTAAGTTTTCG
	Reverse	AAGACTTCTCGGCTCTTATCCA
bmc1520	Forward	TCCTCTTGCTGGATGTCC
	Reverse	ACAGCTGCGTAGCTTCTTCC
umc1295	Forward	GTCGATCTTCCTCCCCATCA
	Reverse	CGTTTCTATCTATGGAGTGCG
umc2281	Forward	CAATGATTGGAGCCTAACCCCT
	Reverse	ATGATGATCTGCAGAGCCTAGTCC
Umc1822	Forward	CGAAGCTGCTGTAAGTTTTCG
	Reverse	AAGACTTCTCGGCTCTTATCCA

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Table 2:Mean Square Variance of Growth and Yield related Characters at different stages in Maize Genotypes

Source of Variation	df	PH (cm)	LL (cm)	LW (cm)	NL	NT	LT (cm)	DSB (g)	DRB (g)	DLB (g)	DI	RL
Genotype (G)	9	2353.66**	2968.86**	36.29**	37.66**	40.41**	232.26**	4.88**	0.01**	0.94**	87.24ns	0.32*
Treatment (T)	4	2370.68**	4100.24**	4.78**	46.82**	9.74**	102.76**	3.71**	0.01**	0.20*	4503.40**	0.53*
WAP (W)	9	98613.34**	99979.04**	218.18**	947.97**	61.84**	630.77**	260.03**	0.80**	63.83**	38006.82**	60.59***
Replicate (R)	3	2101.62**	959.79**	23.73**	59.01**	14.79**	13.03^{ns}	1.61*	0.014**	0.69**	279.68*	0.19^{ns}
G × T	35	946.26**	1273.02**	14.73**	10.22**	9.59**	43.36**	1.63**	0.00**	0.40**	182.78**	0.29*
G × WAP	81	261.48**	283.09**	1.77**	2.28**	3.96**	37.48**	2.04**	0.01**	0.34**	88.13^{ns}	0.32***
T × WAP	36	416.43**	585.26**	2.08**	5.58**	0.91ns	16.45**	2.16**	0.01**	0.10*	4519.27^{ns}	56***
G×T× WAP	311	158.59*	172.71*	1.21**	1.28*	1.01^{ns}	6.97^{ns}	0.99**	0.00**	0.23**	182.9^{ns}	0.29***
Error	852	124.98	141.58	0.63	1.1	1.02	6.27	0.36	0	0.06	54.17^{ns}	0.16
Corrected Total	1860											

*=Significant at P<0.05, **=highly significant at P<0.01, df = degrees of freedom.

PH - Plant height, LL - Leaf length, NL- Number of leaves, NT- Number of tassels, LT- length of tassel, DSB- dry shoot biomass (g), DRB - dry root biomass (g), DLB - dry leaf biomass, DI - disease incident, RL-root length, WAP- Week After Planting

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Table 3: Genotypic effect of growth and yield characters at different stages of treated maize genotypes

Genotypes	Plant height (cm)	Leaf Length (cm)	Leaf Width (cm)	Number of Leaves	Number of Tassels	Disease Incidence	Root Lodging	Dry Shoot Biomass (g)	Dry root Biomass (g)	Dry Leaf Biomass (g)	Tassel Length (cm)
TZM 1551	50.66^{g}	6.29^{d}	50.66^{f}	2.46^{d}	0.49^{c}	4.73^{ab}	0.18^{ab}	0.32^{bcd}	0.02^{d}	0.19^{ab}	0.81^{e}
TZL COMP1 C_2	50.44^{g}	5.86^{e}	50.44^{f}	1.71^{a}	0.22^{d}	3.48^{b}	0.12^{b}	0.27^{d}	0.02^{cd}	0.17^{bc}	1.14^{bcd}
TZM 132	59.04^{bc}	6.95^{b}	60.62^{ab}	2.76^{e}	0.37^{a}	5.23^{a}	0.16^{ab}	0.48^{a}	0.02^{abcd}	0.22^{ab}	1.54^{bc}
TZM 1318	59.93^{bc}	7.03^{b}	58.93^{bc}	2.93^{b}	0.99^{b}	4.19^{ab}	0.14^{b}	0.45^{ab}	0.03^{a}	0.23^{ab}	2.02^{b}
TZE WDTSTR C_4	59.47^{b}	7.15^{b}	59.47^{b}	3.02^{ab}	0.56^{c}	4.92^{ab}	0.20^{ab}	0.45^{ab}	0.02^{abc}	0.24^{a}	1.67^{bc}
OBA SUPPER F_2	56.63^{cd}	6.71^{c}	56.63^{cd}	2.93^{b}	0.93^{b}	5.03^{ab}	0.24^{a}	0.38^{abcd}	0.02^{cd}	0.19^{ab}	1.40^{cd}
TZM 217	53.51^{ef}	6.54^{c}	53.51^{e}	2.89^{bc}	0.19^{d}	4.11^{ab}	0.16^{ab}	0.43^{abc}	0.02^{abc}	0.23^{ab}	0.23^{f}
TZM 1300	51.62^{gf}	6.57^{c}	50.53^{f}	2.40^{d}	0.03^{d}	3.44^{b}	0.18^{ab}	0.27^{d}	0.02^{cd}	0.13^{c}	0.25^{f}
DTSR-WC_O	62.35^{a}	7.55^{a}	62.35^{a}	3.15^{a}	1.01^{b}	4.84^{ab}	0.24^{a}	0.46^{ab}	0.03^{ab}	0.24^{a}	3.82^{a}
TZM 140	54.80^{de}	6.32^{d}	54.80^{de}	2.53^{d}	0.21^{d}	3.58^{ab}	0.15^{b}	0.30^{cd}	0.020^{bcd}	0.19^{ab}	0.89^{de}

Mean with same letter in the same column are not significantly different from each other at ($P<0.05$) according to Duncan Multiple range Test. (DMRT).

Table 4: Effects of X-ray on the growth and yield characters of maize.

Treatment	Plant height (cm)	Leaf Length (cm)	Leaf Width (cm)	Number of Leaves	Number of Tassels	Disease Incident	Root Lodging	Dry Shoot Biomass (g)	Dry root Biomass (g)	Dry Leaf Biomass (g)	Tassel Length (cm)
85KV/MAS	56.37^{b}	55.92^{b}	2.59^{b}	6.19^{c}	0.43^{c}	7.74^{a}	0.18^{a}	0.33^{b}	0.02^{c}	0.16^{b}	0.86^{c}
90KV/MAS	54.25^{c}	54.10^{c}	2.59^{b}	6.88^{a}	0.86^{a}	1.25^{c}	0.18^{a}	0.39^{b}	0.02^{c}	0.24^{a}	0.96^{c}
95Kv/Mas	53.87^{c}	53.87^{c}	2.89^{a}	6.90^{a}	0.58^{bc}	1.25^{c}	0.11^{b}	0.34^{b}	0.02^{c}	0.19^{b}	1.42^{b}
100Kv/MAS	54.10^{c}	54.10^{c}	2.68^{b}	7.02^{a}	0.56^{bc}	2.84^{b}	0.21^{a}	0.52^{a}	0.023^{ab}	0.25^{a}	2.21^{a}
0 KV/Mas	61.42^{a}	62.31^{a}	2.65^{b}	6.60^{b}	0.59^{b}	8.25^{a}	0.20^{ab}	0.33^{b}	0.025^{a}	0.17^{b}	1.37^{b}

Mean with the same letter in the same column are not significantly different from each other at (P<0.05) according to Duncan multiple range test.(DMRT)

Table 5: Correlation co-effeicient among characters of treated Maize Genotypes

Character	Plant Height (cm)	Number of Leaf	Leaf Length (cm)	Leaf Width (cm)	Number of Tassels	Disease Incidence	Root Lodging	Dry Shoot Biomass (g)	Dry Root Biomass (g)	Dry Leaf Biomass (g)	Length of tassels (cm)	Geno type	Treat ment	Week
PH														
NL	0.77**													
LL	0.90**	0.76**												
LW	0.76**	0.77**	0.73											
NT	0.34	0.27	0.27	0.29										
DI	0.16	0.13	0.14	0.11	-0.07									
RL	0.15	0.14	0.15	0.15	-0.08	0.06								
DSB	0.16	0.16	0.15	0.15	-0.08	0.63**	0.73**							
DRB	0.17	0.14	0.16	0.16	-0.08	0.73**	0.74**	0.87**						
DLB	0.16	0.16	0.16	0.16	-0.09	0.66**	0.76**	0.90**	0.86**					
TL	0.33	0.32	0.33	0.33	0.02	0.00	0.00	0.02	0.01	0.01				
Genotypes	0.02	0.01	0.02	0.02	0.08	-0.08	0.01	-0.01	0.01	-0.01	0.03			
Treatment	0.06	0.08	0.07	0.07	0.03	0.03	0.03	0.04	0.06	0.03	0.09	0.03		
Week	0.79**	0.80**	0.79**	0.79**	0.02	0.33	0.36	0.37	0.38	0.39	0.28	0.00	0.03	
Replicate	-0.05	-0.14	-0.049	-0.05	-0.02	0.04	-0.01	0.03	-0.02	-0.04	0.03	0.00	0.01	-0.04

PH: Plant Height; NL: Number of Leaves; LL: Leaves length; LW: Leaf Width; NT: Number of Tassels; DI: Disease Incident; RL: Root Lodging; DSB: Dry
Shoot Biomass; DRB: Dry Root Biomass; DSB: Dry Leaf Biomass; TL: Tassel Length; G: Genotype; T: Treatment; W: Weeks; R: Replicates

Table 6: Principal Components Analysis (PCA) of Growth and Yield Characters of Treated Maize Genotypes

CHARACTER	PRIN 1	PRIN 2	PRIN 3	PRIN 4
PH	-0.03	0.49	-0.21	0.16
NL	0.69	0.30	-0.06	-0.25
LL	0.05	0.46	-0.29	0.01
LW	-0.67	0.38	-0.02	-0.12
NT	0.11	0.15	0.49	0.82
DI	0.22	0.19	-0.03	0.04
RL	0.05	0.20	0.01	0.02
DSB	-0.05	0.23	0.04	0.00
DRB	-0.03	0.24	0.01	0.03
DLB	-0.04	0.23	0.03	-0.01
TL	0.00	0.22	0.79	-0.48
Eigenvalue	4.58	1.85	0.93	0.90
Proportion	0.42	0.17	0.08	0.08

PH: Plant Height; NL: Number of Leaves; LL: Leaves length; LW: Leaf Width; NT: Number of Tassels; DI: Disease Incident; RL: Root Lodging; DSB: Dry Shoot Biomass; DRB: Dry Root Biomass; DSB: Dry Leaf Biomass; TL: Tassel Length;

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Table 7:Heritability, Phenotypic and Genotypic Variance on Growth and Yield Characters in Maize Genotypes

SOURCE OF VARIATION	Genotypic Variance (σ2g)	Phenotypic Variance (σ2p)	Heritability (h2)
Plant Height	557.17	682.15	0.82
Leaf Length	706.82	848.4	0.83
Leaf Width	8.915	9.55	0.93
Number of Leaves	9.14	10.24	0.89
Number Tassels	9.85	10.87	0.91
Length of Tassels	56.5	62.77	0.90
Dry Shoot Biomass	1.13	1.49	0.76
Dry Root Biomass	0.0025	0.0025	1.00
Dry Leaf Biomass	0.22	0.28	0.79
Disease Incident	8.27	62.44	0.13
Root Lodging	0.04	0.20	0.20

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Table 8: Nano Drop and DNA concentration of Treated Maize Genotypes

Genotypes	Treatment 1		Treatment 2		Treatment 3		Treatment 4		Treatment 5	
	DNA (ul)	Conc (0KV/Mas)	DNA (ul)	Conc (85KV/Mas)	DNA (ul)	Conc (90KV/Mas)	DNA (ul)	Conc (95KV/Mas)	DNA (ul)	Conc (100KV/Mas)
TZM 1551	2841.6	1.88	985	1.81	188.1	1.43	311.6	1.47	612.7	1.83
TZL COMP1 C_2	1477.8	1.89	1040.4	1.8	957.8	1.59	1059.8	1.79	564.9	1.73
TZM 1318	1193.1	1.82	813.7	1.8	719.6	1.65	782.6	1.67	1608.6	1.89
TZM 132	2062.3	1.87	1255.4	1.85	988.5	1.81	788.3	1.8	2086.3	1.91
TZE-WDTSR	473.9	1.7	938.5	1.88	977.6	1.85	805.1	1.61	1144.7	1.67
OBA SUPPER F_2	893.3	1.8	400.8	1.69	1476.3	1.86			756.6	1.75
TZM 217	1076.2	1.82	1068.1	1.83	1122.6	1.59	1573	1.61	995.5	1.82
TZM 1300	1038.5	1.8	1161.4	1.76	936.5	1.82	630	1.85	392.8	1.58
DTSR- WC_O	848.8	1.79			226.4	1.73	1151.8	1.76	969.8	1.76
TZM 140	698.4	1.7	1423.1	1.89	820.1	1.69	1029.2	1.83	418.3	1.57

DNA (ul) = Extracted genomic DNA (ul), Conc = Concentration of Genomic DNA,

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Table 9: Frequency, Diversity of Allele and Polymorphic Information Contents (PIC) of Treated Maize Genotypes

Primers	Major Allele Frequency	Genotype No	Sample Size	Number of observations	Allele No	Availability	Gene Diversity	Heterozygosity	PIC	PIC (%)
UMC2151	0.47	3	48	32	3	0.67	0.64	0.00	0.56	56.15
BMC1755	0.39	3	48	23	3	0.48	0.66	0.00	0.59	58.77
ZCT197	0.53	3	48	30	3	0.63	0.55	0.00	0.46	45.61
ZMCP 7430	0.60	3	48	45	3	0.94	0.52	0.00	0.44	44.03
BMC 1520	0.80	3	48	46	3	0.96	0.33	0.00	0.30	29.55
UMC 1295	0.93	2	48	44	2	0.92	0.13	0.00	0.12	11.9
UMC 2281	0.50	4	48	48	3	1.00	0.54	0.08	0.43	43.16
UMC 1822	0.49	6	48	39	3	0.81	0.63	0.08	0.56	55.83
Mean	0.59	3.375	48	38.375	2.875	0.78	0.50	0.02	0.43	43.12

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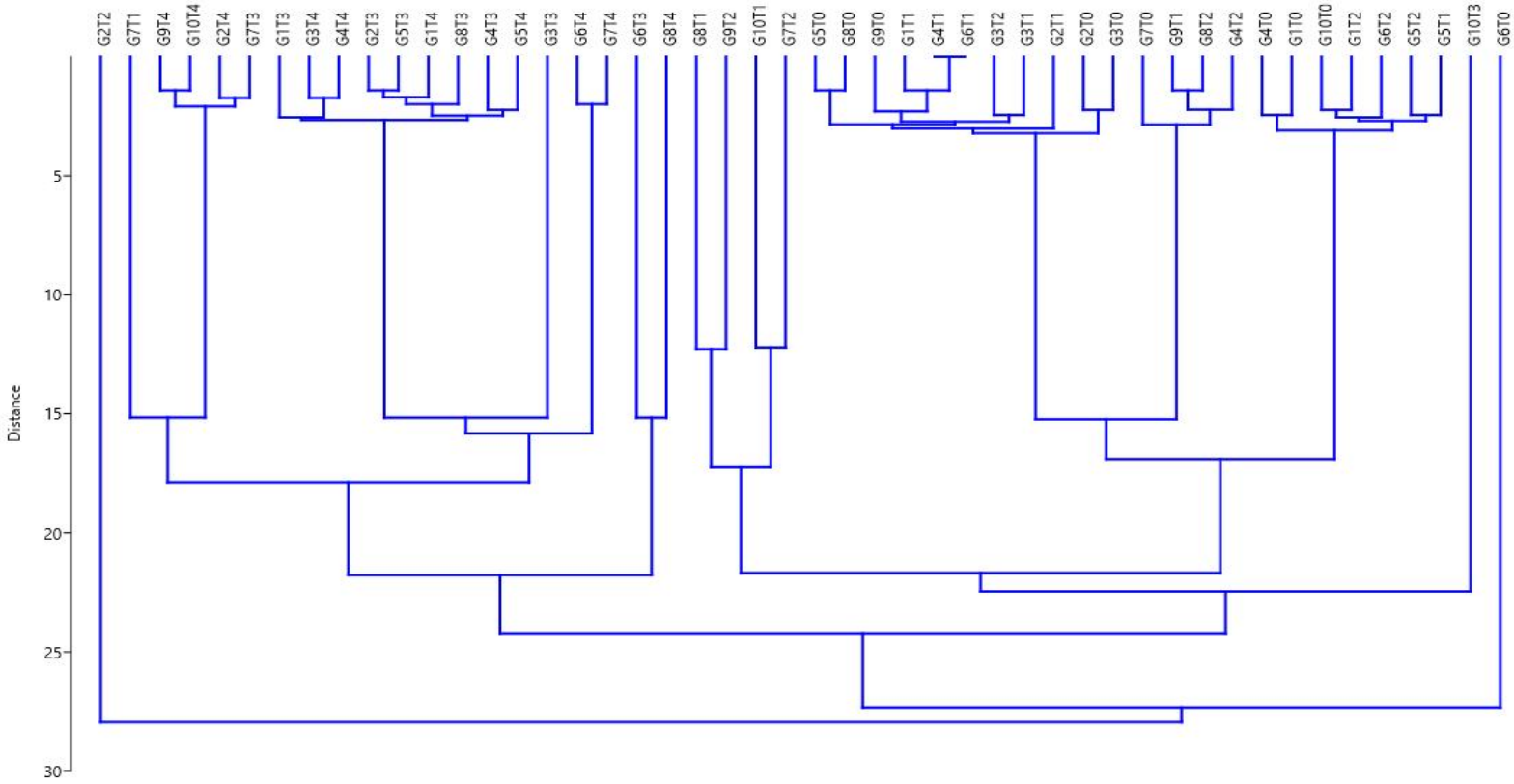

G_1=TZM 1551; G_2= TZM COMP1 C_2; G_3= TZM 1318; G_4= TZM 132; G_5= TZE-WDTSR C_4; G_6= OBA SUPPER FII G_7=TZM 217; G_8= TZM 1300; G_9= DTSR-WC$_O$; G10=TZM 140; T_1= 85KV/MAS; T_2=90KV/MAS; T_3=95KV/MAS; T_4=100KV/MAS; T_0=0KV/MAS.

Figure 1: Dendrogram showing genetic relationship among treated maize genotypes.