ASSESSMENT OF GENETIC DIVERSITY AMONG SELECTED MAIZE LANDRACES AND HYBRIDS USING SSR MARKERS LINKED TO QTLS FOR DROUGHT AND NITROGEN TOLERANCE

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Abstract: In this study, seventeen quantitative trait loci (QTLs) linked simple sequence repeat (SSR) markers of maize were used to assess genetic diversity in selected 20 maize genotypes adapted to Southern Nigeria. The morpho-physiological traits considered include stay-green characteristics, days to anthesis, days to silking and anthesis-silking interval, leaf number and high nitrogen regime, ear setting and yield components, while the maize genotypes comprised of 9 landraces, 5 commercial hybrids grown by local farmers and 6 recently developed IITA hybrids. A total of 57 polymorphic alleles were amplified and the mean allele number was 3.35 and a range of 2 to 5 alleles per locus. The average polymorphic information content (PIC) value of 0.46, with a minimum and maximum of 0.16 and 0.72 were observed. The genetic similarity ranged from 0.45 to 0.91 with a mean of 0.58. The UPGMA dendrogram indicated that the 20 maize genotypes could be divided into two major groups, of which each had two subgroups. Principal component analysis also depicted diversity among the lines. Based on the grouping, lines from landraces were clustered together, while the commercial had close genetic relationship with IITA hybrids. Genetic diversity was moderately high among the landraces than in the hybrids. This study reveals the efficiency of trait-linked SSR markers to estimate the extent of the genetic variation of the maize varieties for drought/nitrogen regime adaptive traits and will be a contribution to further application of marker assisted selection in maize breeding.

Keywords: Hybrids, Genetic Diversity, Landraces, Maize, SSR linked to QTLs

INTRODUCTION

Maize is (*Zea mays* L.) is one of the most economically important tropical crops worldwide because it is a staple food for humans and animals (Reif *et al.,* 2005). Presently, maize is still largely grown by indigenous small-scale farmers in southern Nigeria using landraces that have wide adaptability to local growing environments and commercial hybrids or varieties developed from the breeding program of the International Institute of Tropical Agriculture. The high usage of commercial hybrids with enhanced yield and resistance to biotic and abiotic stresses have substantially decreased genetic diversity of landraces (Yong-Bi, 2015). The lack of protection of genetic resources of the original maize landrace varieties, is also a major contributing effect on the erosion of its genetic variability (Westengen *et al.,* 2012).

Knowledge of the genetic diversity among farmers' varieties and hybrid maize lines is useful for monitoring germplasm conservation, utilization of diverse genetic resources for cultivar improvement and protection (Smith *et al.,* 1997; Bernardo, 2002). Molecular markers provide means of measuring genetic relationships and variations among genotypes. DNA markers like microsatellites (simple sequence repeats, SSR) markers have been widely used to evaluate genetic diversity (Liu *et al.,* 2003; Menkir *et al.,* 2004; Warburton *et al.,* 2008). Furthermore, SSR markers have also been applied in the classical linkage/QTL mapping to study the genetic basis of important quantitative traits in many different population structures of various maize germplasm (Szalma *et al.,* 2007).

Several previous linkage mapping studies have identified quantitative trait loci (QTL) associated with some morphological and physiological traits in maize (Smith *et al.,* 1995; Veldboom and Lee, 1996; Li *et al.,* 2003; Zhang *et al.,* 2004; Ribaut *et al.,* 2007;

Wu *et al*., 2008). Wang *et al.* (2012) examined the QTLs for stay-green related traits such as green leaf area per plant at 30 d after flowering (GLA2), green leaf area per plant at the grain-ripening stage (GLA3), and left green leaf number per plant at the grain-ripening stage (LLN), green leaf area per plant in the whole growing period (GLA1) and total leaf number per plant in the whole growing period (TLN). Szalma *et al.* (2007) performed the QTL mapping for days to anthesis, days to silking, and anthesis-silk interval in a collection of maize inbreds and hybrids lines. A mapping study for 3 traits (plant height, ear height, and leaf number) associated with plant architecture under the high nitrogen regime and low nitrogen regime have been conducted on a group of maize genotypes (Liu *et al.,* 2003; Zheng and Liu, 2013).

To our knowledge, very little study is known on the relationship in maize landraces and hybrids using molecular characterization. The specific objectives of this study were: (a) to evaluate polymorphism of QTL linked SSR markers to assess the genetic diversity of the selected maize varieties and (b) to compare the diversity of the markers in 9 landraces with the diversity of 5 commercial hybrids and 6 hybrids from the IITA.

MATERIALS AND METHODS

Plant materials: A collection of twenty maize accessions was used for this study. A total of 5 commercial varieties/hybrids and 9 landraces adapted to the south-western part of Nigeria were collected from the local farmers from four States: Oyo, Ogun, Osun and Lagos and 6 newly developed hybrids, adapted to the south-western part of Nigeria were collected from IITA (Table1).

DNA extraction: Ten plants of each genotype were planted and at two weeks, the young leaf tissue of 8 randomly selected plants of each genotype were bulked into a single Eppendorf tube and approximately 1 g of leaf tissue from each bulk was used to isolate genomic DNA using a modified method of Dellaporta *et al.* (1993). The quality and quantity of the extracted DNA for each sample were determined using the Nano drop spectrophotometer.

SSR markers: Genetic diversity among the maize varieties was assessed using a total of 20 QTL linked SSR markers chosen from the previous linkage mapping studies of maize. These markers were selected based on tight linkage to the QTLs for the morpho-physiological traits (Table 2). The primer sequence was obtained from maize genome database (www.maizegdb.org) and synthesized (Inqaba Biotec, South Africa).

PCR Amplification of QTL linked SSRs: Each primer pair was optimized to know the accurate annealing temperature (55 to 65°C) for specific and good amplifications. PCR amplifications were performed in 25µL reaction volumes consisting of 13.85 µL sterile dd (H_20), 2.5µL 10X PCR buffer, 1.0 µL $MgCl_2$, 2.5 µL 100 ng template DNA, 0.10 1unit Taq DNA polymerase, 1.0 µL of forward primer (5pMol), 1.0 µL of reverse primer (5pMol), and 1.0 µL DMSO. Reactions were performed in an ABI 1000 thermocyler with the thermal cycling program consisted of initial denaturation at 94 °C for 2 min, 9 cycles at 94 °C for 15 s, 65 °C for 20 s, and 72 °C for 30 s, followed by 35 cycles at 94 °C for 15 s, 55°C for 20 s, 72 °C for 30 s, and a final extension at 72 °C for 7min. Samples were held at 10 °C until their removal from the thermocycler. Electrophoresis was carried out on 2% (wt/vol) SFR agarose gels. Scoring analyses were performed relative to a 50 base pairs DNA ladder from Thermo Scientific.

Data Analysis

Genetic diversity assessment

For each polymorphic SSR locus, number of allele per locus, Polymorphic Information Content (PIC), as well as total number of alleles were calculated using the formula of Smith *et al.* (1997). Variation in the number of heterozygosity within lines in the light of genetic backgrounds was examined. Genetic distances (GD) were estimated using the modified Rogers' method (Warburton *et al.,* 2008) and the genetic relationship among the genotypes was assessed using unweighted pair group method of arithmetic average (UPGMA) clustering algorithm and Principal Component Analysis (PCA) in the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) package version 2.2 (Rohlf, 1997).

RESULTS

Genotyping of maize varieties

17 out of the 20 SSR QTL linked markers produced clear and polymorphic PCR product (Figure 1) while the remaining ones were monomorphic SSRs (15 %). The 17 markers generated a total of 57 alleles among the genotypes (Table 1). Out of the 57 SSR alleles (average of 3.35 alleles per locus), 2 SSR markers produced the highest number of allele (5), while others ranged between 2-4 alleles. The polymorphic information content (PIC) of the observed alleles showed values from 0.16 to 0.72 with a mean of 0.46. Some of the SSR markers (47 %) had PIC higher than 0.5. Variation in the number of heterozygosity within lines in the light of genetic backgrounds is shown in figure 1. Observed heterozygosity within the maize landraces and recently developed IITA hybrids is higher even compared than the observed heterozygosity within the commercial maize lines.

Clustering of SSR Data

The UPGMA dendrogram (Figure 2) revealed the level of genetic relatedness among 5 commercial varieties/hybrids, 9 landraces and 6 newly developed hybrid lines. The 20 maize genotypes were clustered into 2 main groups. Group I consisted of a total of 9, with 3 and 1 landraces from Oyo and Lagos States, respectively; 2 and 1 commercial hybrids from Ogun and Lagos States, respectively and 2 IITA hybrids. Group II included 5 landraces of which 2 were from Oyo State, 2 from Ogun State, and 1 from Osun State. The remaining members of the group were 2 commercial hybrids from Ogun State and 4 IITA hybrids. The two groups were comparatively diverse since each consisted landrace, commercial hybrid and newly developed IITA hybrid.

The two IITA hybrids were identified to be similar and one landrace and one commercial hybrid collected from Lagos had very close relationship in Group I.

The generated Roger's genetic distance matrix was used to construct principal component analysis PCA) to decipher the genetic relationship among the genotypes as shown in figure 3. The PCA showed clear separation among the genotypes. The first two principal axis accounted for 12.87 % and 15.52 % variation, respectively. The PCA supports the results obtained from UPGMA cluster analysis. Genetic relationship among landraces tended to associate with commercial hybrids.

Table 1: Details of maize varieties used in this study

S/N	Maize varieties	States (town)/Institution	Kernel colour	Genetic background
1	MZY01	Oyo (Ogbomoso)	Yellow	Landrace
2	MZY02	Oyo (Ogbomoso)	White	Landrace
3	MZY03	Oyo (Lanlate)	White	Landrace
4	MZY04	Oyo (Lanlate)	White	Landrace
5	MZY05	Oyo (Iseyin)	Yellow	Landrace
6	MZS06	Osun (Ife)	Yellow	Landrace
7	MZG07	Ogun (Owode)	White	Commercial hybrid
8	MZG08	Ogun (Owode)	Yellow	Commercial hybrid
9	MZG09	Ogun (Owode)	Yellow	Landrace
10	MZG10	Ogun (Ifo)	White	Landrace
11	MZG11	Ogun (Abeokuta)	Yellow	Commercial hybrid (Oba super 6)
12	MZG12	Ogun (Abeokuta)	Yellow	Commercial hybrid (Oba super 2)
13	MZL13	Lagos (Badagry)	Yellow	Commercial hybrid (Oba super 1h)
14	MZL14	Lagos (Lagos)	White	Landrace
15	MZH15	IITA, Nigeria	White	(4)EXL05 X ADL36
16	MZH16	IITA, Nigeria	White	(1)ADL47X ADL41
17	MZH17	IITA, Nigeria	White	(3)EXL16 X EXL02
18	MZH18	IITA, Nigeria	White	(2)EXL15 X ADL35
19	MZH19	IITA, Nigeria	White	(2)EXL16 X ADL07
20	MZH20	IITA, Nigeria	White	(2)ADL32 X EXL06

Table 2: Characteristics of polymorphic SSR markers linked to QTL used in the study

SSR markers	Bin no	No of alleles	PIC	Traits reported to QTLs	Previous studies
bmc1208	5.04	3	0.54	DTA	(Szalma *et al.,* 2007)
umc1557	5.03	3	0.48	DTA and DTS	(Szalma *et al.,* 2007)
dupssr12	1.08	3	0.44	DTA and DTS	(Szalma *et al.,* 2007)
bmc1712	10.03	3	0.33	DTS, DTA and ASI	(Szalma *et al.,* 2007)
umc1221	5.04	2	0.36	DTA, DTS and ASI	(Szalma *et al.,* 2007)
bnlg197	3.06	4	0.6	DTA, DTS and ASI	(Szalma *et al.,* 2007)
umc1822	5.05	4	0.55	DTS, DTA and ASI	(Szalma *et al.,* 2007)
bnlg1520	2.09	3	0.33	DTS, DTA and ASI	(Szalma *et al.,* 2007)
umc2151	1.00	3	0.41	Stay green	(Wang *et al.*, 2012)
bmc 1429	1.00	5	0.72	Stay green	(Wang *et al.*, 2012)
bnlg 1755	4.00	3	0.53	Stay green	(Wang *et al.*, 2012)
bnlg 1337	4.00	3	0.52	Stay green	(Wang *et al.*, 2012)
umc 2281	4.00	4	0.56	Stay green	(Wang *et al.*, 2012)
bmc1375	9.07	5	0.56	Leaf number with Low nitrogen regime	(Zheng and Liu, 2013)
bmc1792	7.02	3	0.46	Leaf number and low nitrogen regime	(Zheng and Liu, 2013)
umc1295	7.04	2	0.16	Leaf number and high nitrogen regime	(Zheng and Liu, 2013)
umc 1415	8.03	4	0.37	Ear length	Veldboom and Lee, 1996
Mean		**3.35**	**0.46**		

PIC-polymorphic information content
DTA-days to anthesis
DTS-days to silking
ASI- anthesis-silking interval

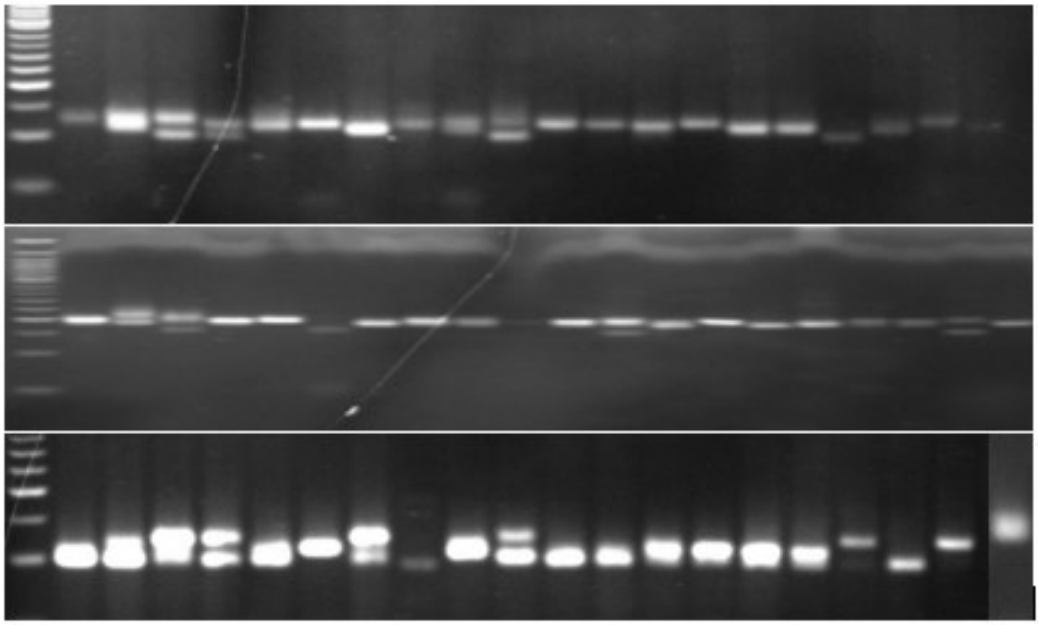

Figure 1: Gel photograph showing amplification of 3 QTL linked SSR markers (a. bmc1375 b. umc2881 and c. bmc1208) in 20 maize genotypes

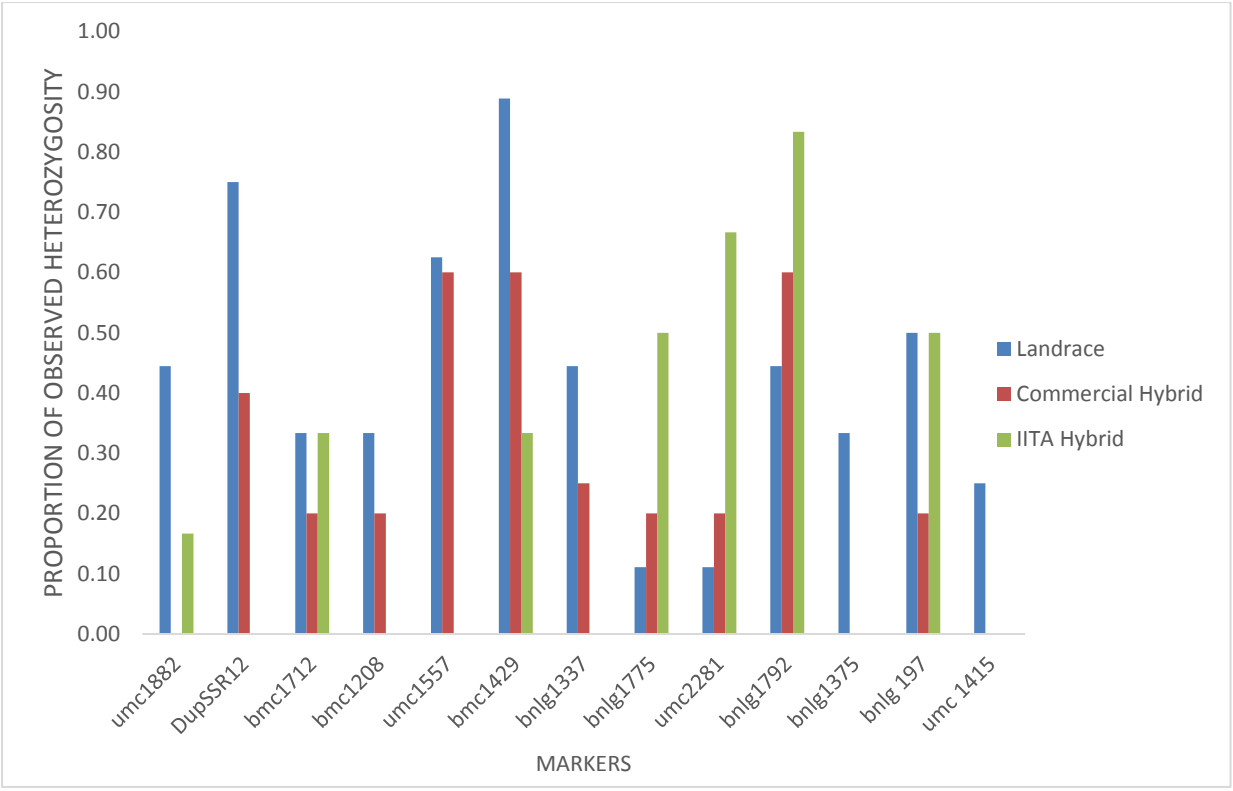

Figure 2: The proportion of observed heterozygosity of QTL linked SSR markers among landrace, commercial hybrids and IITA Hybrids

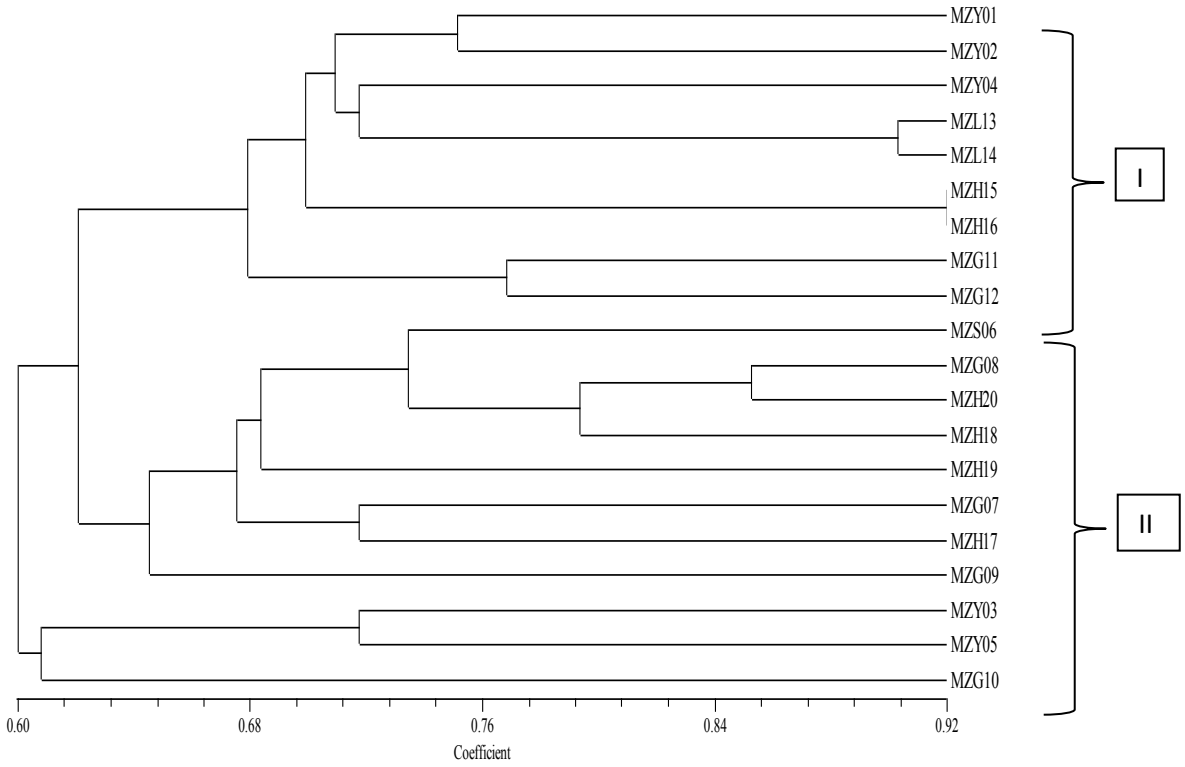

Figure 3: Dendogram of 20 maize genotypes constructed from UPGMA Cluster analysis based on QTL linked SSR markers

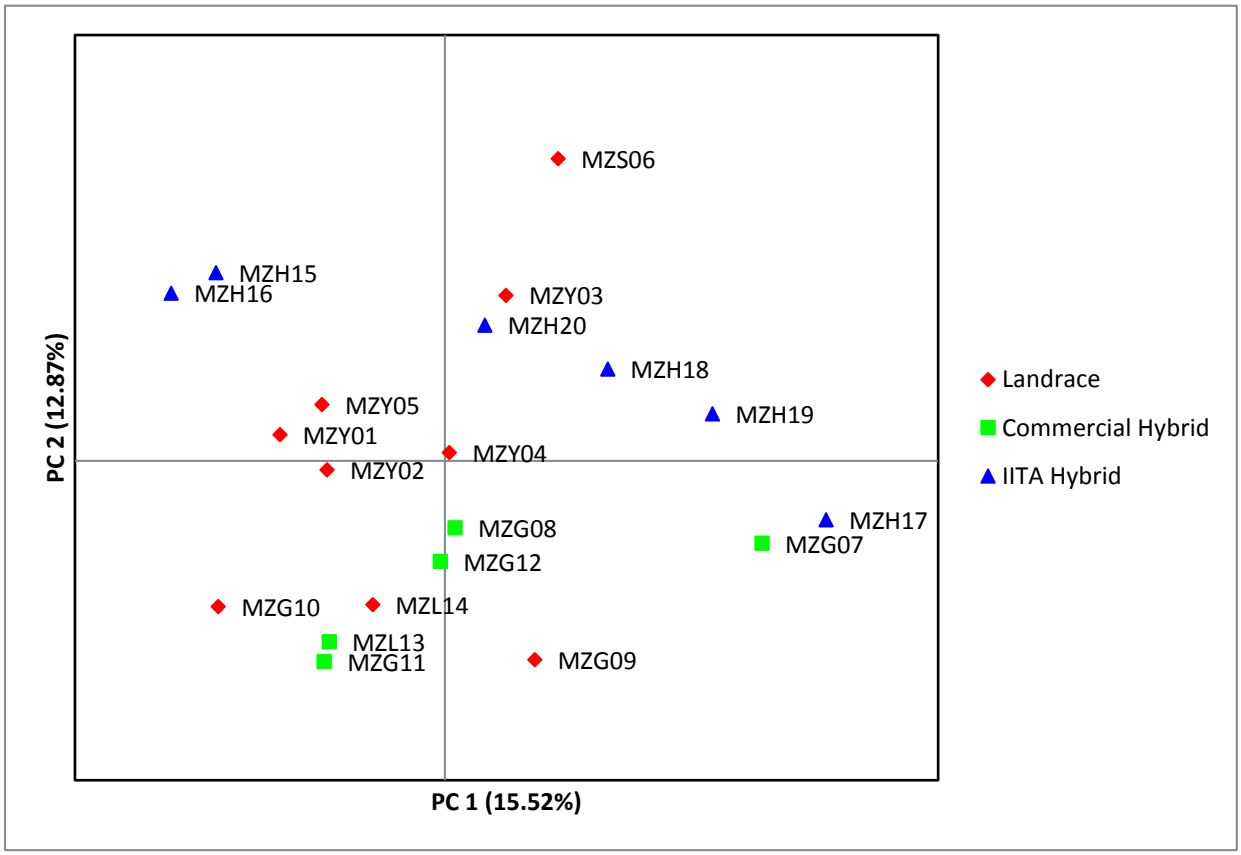

Figure 4: Principal Coordinate Analysis of 20 maize genotypes based on QTL linked SSR markers

DISCUSSION

The study of genetic diversity in specific regions of maize genome using molecular characterisation has importance for the improvement of the crop. This can facilitate the application of marker assisted selection (MAS) in maize breeding programs (Smith *et al.,* 1997; Prasanna *et al.,* 2010). QTL linked SSR markers have been used to evaluate diversity in few plant species (Ganie *et al.,* 2016). Also, quite number of genetic diversity and relationship analyses of various maize germplasm have been reported using genomic SSR analysis (Adeyemo *et al.,* 2011; Adeyemo and Omidiji, 2014). The present study represents the first report of genetic relationship in maize landraces and hybrids using QTL linked SSR loci for molecular characterization. Across the maize genotypes used in this study, we obtained a mean allele number 3.35 and a range of 2 to 5 alleles. This indicates moderate level of polymorphism of the markers among the genotypes. When compared to previous study having either genotypes, these values were close to mean number per locus generated by each marker varied from 2 to 4 with an average of 3.13 alleles per locus detected in the 13 rice varieties using the eight SSR markers tightly linked to the QTLs for aroma and cooked kernel elongation (Kioko *et al.,* 2015). However, other studies revealed lower average alleles per loci in maize inbred lines (Barbosa *et al.,* 2003; Garcia *et al.,* 2004) and in maize landraces (Molin *et al.,* 2013) using the genomic SSR markers. This implies that the genetic diversity in the set of lines studied is moderately large, and likely has been due to the landrace germplasm. SSR loci have good potential to discriminate between closely related maize genotypes and are polymorphic due to the multi-allelic variations (Veldboom and Lee, 1996; Inghelandt *et al.,* 2010; Sharma *et al.,* 2010; Yang *et al.,* 2011).

The PIC value of each locus usually demonstrates an estimate of the discriminatory power of the locus, considering the number of alleles and its relative frequencies (Smith *et al.,* 2000). In this study, the mean value of PIC of 0.46 also revealed considerable genetic variability among the 20 maize genotypes which is lower to that of 0.51 using 30 SSR markers for genotyping 79 elite maize inbred lines (Nyaligwa, 2015). The low level of PIC observed in the present study could be as a result of types of the SSR markers used and the genetic differences among the maize hybrids and landraces. In addition, some eight primer pairs (47 %) had PIC higher than 0.5, indicating their usefulness in the detection of polymorphism among the lines and also for marker-assisted selection program. Moreover, the four out of the five SSR markers linked to stay green trait QTLs (Wang *et al.,* 2012) analysed in this study showed moderate level polymorphisms among the genotypes, implying high genetic variation in the trait.

Heterozygosity provides an understanding of the information about the SSR loci and their potential to detect differences between lines based on their genetic diversity. In this study, seven out of thirteen markers that showed high allelic variants were found higher in landraces than in the hybrids. This result suggests a wide gene pool of heterozygous of the landraces which can be useful source of genes for novel alleles (McCouch *et al.,* 1997; Hossain *et al.,* 2012). The cluster analysis revealed two main groups within the maize genotypes, which was in close agreement with the results of PCA. There was no clear structure of grouping of the maize lines along genetic backgrounds (landrace, commercial and hybrids). The mixed or dispersed and close genetic relationship of the genotypes used in this study suggests that the continuous natural or human selection process of maize landraces has not brought any massive divergence from the hybrids. This may account for the large usage of hybrids by local farmers nowadays. The moderate genetic diversity further suggested that the lines used in this study may have originated from the same adapted environment.

In this study too, genotypes with lowest genetic similarity were identified MZL13 (commercial hybrid, yellow, Lagos) and MZL14 (landrace, white, Lagos). This shows similarity of commercial hybrid with landraces and the landraces may have been source population, from which inbred parents of the hybrids were extracted. The similarity between MZH15 and MZH16 at the loci assessed indicated that they had a common parent or ancestry. However, this is not unlikely due to the limited SSR markers used and narrow difference between the lines under study (Bhawna *et al.,* 2015).

CONCLUSION

The present study has demonstrated moderate amount of genetic variability among landraces, commercial hybrids and recently developed IITA hybrid lines based on a set of QTL linked SSR loci. This result indicated the intermixed grouping of the lines used. The information will be important for marker applications in maize breeding for traits such as drought and nitrogen tolerance. In addition, further analysis of molecular diversity within and among the landrace accessions adapted to the south-western Nigeria is recommended to facilitate proper classification and utilization.

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