RESEARCH ARTICLE

Genetic Diversity Among Ten Maize Genotypes Using Simple Sequence Repeat DNA Markers

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ABSTRACT

Genetic diversity among ten maize genotypes (seven inbred lines and three testers) was investigated using ten simple sequence repeats (SSRs). Primers (bnlg128, bnlg1839, Umc1117, bnlg1144, and bnlg1152) generated the highest number of bands (4 bands) for inbred lines while the primer bnlg128 showing the highest number of bands (3 bands) for testers. The primer bnlg128 shows the highest effective number of alleles (n_e) for inbred lines and testers. Among the studied primer bnlg1839 in inbred lines and primer bnlg128 in testers showed the maximum polymorphism information content (PIC) and the greatest diversity. Using UPGMA cluster analysis, the seven inbred lines were grouped under three clusters, while grouped the testers under two clusters. Most of the inbred lines which were derived from the same source population were grouped in the same cluster based on the SSRs DNA markers, indicating high genetic differentiation among their source populations. Results showed that the SSRs were informative in detecting genetic differences among the maize inbred lines and testers, as exhibited by the high average of Shannon's information index (I), Nei's expected heterozygosity (Nei's), and PIC. The results suggest that the studied genotypes are diverse and may be utilized for further breeding programs.

Keywords: Genetic diversity; Maize genotype; PCR; Simple sequence repeats

INTRODUCTION

Maize (*Zea mays* L.) considered one of the most important cereal crops in the world and Iraq, after wheat, barley, and rice. It plays a major role in meeting the demand for food, feed, livestock, and industrial raw materials (Ali et al., 2014).

The increasing usage of maize as a staple food reflects the maximum yield per hectare compared with wheat and barley. In Iraq according to statistics from the food and agriculture organization (Faostat, 2018), the cultivated area in the whole year (2017) reached up to (52012) hectares, producing (185291) tons.

Hybrid maize has greater yield potentiality than those of synthetics and composites (Pandey and Gardner, 1992, Vasal et al., 1995). The hybrid varieties are currently planted in the country. The most limiting factor of maize research in Iraq especially in Kurdistan is that the development, improvement, and maintenance of parental/inbred lines. On the other hand, the difficult of imported hybrid seed is the involvement of high price and uncontrolled quality. Moreover, the farmers cannot get the seeds timely. One important approach to improve this situation is the development of inbred lines which might produce high yielding hybrid varieties. The genetic diversity between the genotypes is important because the genetically diverged

parents are able to produce high heterotic effects (Falconer, 1960, Arunachalam, 1981, Mian and Bahl, 1989). Maize breeders are consistently emphasizing the importance of diversity among parental genotypes as a major factor contributing to heterotic hybrids (Ahloowalia and Dhawan, 1963, Ihsan et al., 2005, AbdulHusseinAl-Badeiry et al., 2014).

Genetic diversity defined as the probability that two randomly chosen alleles are different in sample characters by the forces of evaluation such as mutation, gen flow, genetic drift, and selection in heterogenous environment (Hallauer et al., 2010). Various approaches have been used to assess genetic diversity in crop germplasm including morpho-agronomic, pedigree biochemical, and DNA-based on molecular technique (Govindaraj et al., 2015, Kashiani et al., 2012).

The most common molecular markers used to assess genetic diversity in maize include restriction fragment length polymorphism, random amplified polymorphic, microsatellite or simple sequence repeats (SSRs), amplified fragment length polymorphism, and single nucleotide polymorphism (Molin et al., 2013). Among available DNA-based markers sample sequence repeats SSR have been successfully used to genotype diverse maize (Semagn et al., 2012).

Polymerase chain reaction (PCR) development has contributed toward the efficiency in detecting genetic diversity using molecular marker (Kalia et al., 2011). Maize represents an important resource for studies of genetic diversity. Several studies have been used SSR markers to characterize maize such as (Sharma et al., 2010) in India; (Beyene et al., 2006) in Ethiopia; (Smith et al., 1997) in USA; (Nikhoul et al., 2013) and (Choukan et al., 2006) in Iran; and (Senior et al., 1998) (Salami et al., 2016). Aci et al. (2013) used 18 SSR markers to study genetic diversity among 15 corn inbred lines. They found a clear grouping of inbred lines into three main clusters. Nikolić et al. (2015) who investigated genetic diversity among 29 corn inbred lines using 25 SSR markers indicated that the lines could be assigned into two different heterotic groups. Diwan et al. (2015) who analyzed genetic diversity among 14 sweet corn genotypes using 10 SSR DNA markers were able to cluster the genotypes into two main clusters. In another study involving 15 sweet corn inbred lines using 100 SSR markers, Lopes et al. (2014), were able to group the inbred lines into four major groups. Maize genotypes developed in the previous studies were significantly different for all traits measured and displayed wide genetic variability based on phenotypic evaluations. The aim of the present study determines genetic variation among ten maize genotype using ten simple sequence repeats (SSRs) DNA markers for informative among those markers and genotypes (inbred lines/testers).

MATERIALS AND METHODS

A series of ten different maize genotypes from different source populations were used as plant materials in this study [Table 1] at the Faculty of Agriculture Engineering Science/Salahaddin University.

Seeds from each genotype were germinated and the seedlings were then grown to the Two-three leaf stage. Genomic deoxyribonucleic acid was extracted from young leaves using the CTAB procedure (Saghai et al., 1994) with minor modification. Ten SSRs DNA primers distributed

throughout the maize genome were chosen from the website, maize genomic database (<http://www.maizegdb.org>) based on their polymorphism information contents (PIC) and also QTL information reported by previous investigators [Table 2].

PCR amplification was carried out in 20 µL reaction mixer that include 2µL DNA template, 0.5 µM of each primer (forward and Reverse), 4 µL hot start red load ×5 Master Mix (3.5 mM MgCl₂ final concentration), and fill to 20 ul with distilled water.

The PCR conditions are as follows: Initial incubation at 95°C for 5 min followed by 30 cycles of reaction with step of denaturation at 95 °C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 1 min, and the 30 cycles were followed by a step of final extension at 72°C for 7 min. PCR product was checked for amplification in a 1.5% agarose gel stained with ethidium bromide. The optimum size of the product was ascertained comparing it with 100 bp DNA ladder.

Statistical Analysis

Gel images were scored manually and repeated twice to limit errors in scoring. The data were scored as “1” (present) or “0” (absent), while bands that were diffused or too difficult to score were considered as missing data (“9”). The resulting matrix was then analyzed with a range of statistical and multivariate methods to figure out the genetic structures among and within the genotypes studied.

Descriptive statistics for each inbred line including allele frequency, average number of alleles per locus (n_a), effective number of alleles (n_e), observed and expected homozygosity (Hom_o and Hom_e , respectively), observed and expected heterozygosity (H_o and H_e , respectively), Shannon's information index (I), and Nei's expected heterozygosity (Nei) were estimated using the Population Genetic Analysis software (POPGENE) Version 1.3.1 (Yeh et al., 1999).

Furthermore, the PIC which is allelic diversity at a locus and inbreeding coefficient (F) (Keller and Waller, 2002) was estimated for each SSR marker using the Power Marker software version 3.25 (Liu and Muse, 2005). PIC was calculated for each primer as:

$$PIC = 1 - \sum_{i=1}^n p_{ij}^2$$

Where, P is the relative frequency of the jth pattern for SSR marker i.

RESULTS AND DISCUSSION

Genetic diversity among the genotypes was investigated using 10 SSR DNA Markers. The highest average number

Table 1: List of maize genotypes taken for the study

Genotype No.	Name	Source/Pedigree
Lines		
L1	890	S5340
L2	Kr640	Idt
L3	3007	ERLEVO
L4	LH74	SSS
L5	232Dss	SSS Dekalb
L6	ZP434	Monsanta symmet
L7	Mst4279	Monsanta symmet
Testers		
T1	Talar	Variety
T2	5012	Monsanta symmet
T3	Cades	Hybrid (commercialcommercial)

Table 2: Simple sequence repeat (SSR) DNA primers used to analyze genetic diversity on 10 maize genotypes and their chromosomes, Bin numbers, repeat motifs, and sequences (<http://www.maizegdb.org>)

No.	Locus	Chr.	Bin	Repeat Motif	Direction	Sequence
1	umc1166	1	1.02	(CT)10	Forward	CGATCAGATCATACACAACCTTGC
					Reverse	GAGGATCGATTCTTGGCGAGT
2	bnlg2077	2	2.07	AG(33)	Forward	GACCAGAGGATGGGGAAATT
					Reverse	GTAGGCACATGCACATGAGG
3	bnlg1144	3	3.02	Unspecified	Forward	TACTCGTCGTGTGGCGTTAG
					Reverse	AGCCGAGGCTATCTAACGGT
4	umc1117	4	4.04	(TCGCA)4	Forward	AATTCTAGTCCTGGGTCGGAATC
					Reverse	CGTGGCCGTGGAGTCTACTACT
5	bnlg1006	5	5.00	AG(20)	Forward	GACCAGCGTGTGTGATCCC
					Reverse	GGAGACCCCGACTCTCTCTC
6	phi452693	6	6.04	AGCC	Forward	CAAGTGCTCCGAGATCTTCCA
					Reverse	CGCGAACATATTCAGAAGTTTG
7	bnlg1022	7	7.02	AG(12)	Forward	GTGTTGTCGATCCACTCCCT
					Reverse	GCAAAGATCTGTGAGGGGAC
8	bnlg1152	8	8.06	AG(18)	Forward	CGCTACCGATTGTTGAATTG
					Reverse	AAAGTCGTCCGGTCAAATTG
9	bnlg128	9	9.07	AG(15)	Forward	CACCTGGAGGGACCCATTCC
					Reverse	AGGACCACAGGATCCATCATCCT
10	bnlg1839	10	10.07	AG(24)	Forward	AGCAGACGGAGGAAACAAGA
					Reverse	TCTCCCTCTCCCTCTTGACA

Chr.: Chromosome, Bin: A sub-region on corn chromosome

of alleles (n_a) among the seven maize inbred lines were obtained from bnlgl28, bnlgl1839, Umc1117, bnlgl1144, and bnlgl1152 (4band), while the primer bnlgl28 showing the highest average number of alleles (n_a) for testers. Whereas the least number for inbred lines was obtained from bnlgl1006 and bnlgl1022 (2 band), while the primers Phi45269, bnlgl1022, and Umc1117 show the least number for testers (1 band) [Table 3]. The primer bnlgl28 shows the highest effective number of alleles (n_e) for inbred lines and testers with (3.77) and (3.00), respectively, while the lowest effective number of alleles for inbred lines was (1.32) recorded by primer bnlgl1006, and for testers all primers give (1.80) value with exception of bnlgl28. Mora et al. (2013) found effective number of alleles of 4.8 when assessing 28 Brazilian popcorn populations with 11 SSR markers. In this study, the mean value of observed homozygosity (H_o) was found to be high (1.00) among the genotypes (inbred lines, testers). This indicates that the inbred lines studied are homozygous at almost all loci amplified and can be maintained by self-pollination. In addition, the mean value of observed heterozygosity (H_e) for inbred lines and testers was found to be low, with a value of 0.00, which was lower than the mean values found by (Kashiani, 2012), (Sserumaga et al., 2014), and (Lopes et al., 2015).

Expected heterozygosity (H_e) which is an indication of gene diversity at the loci amplified for the SSR marker bnlgl28 was found to have the highest value for inbred lines and testers with 0.79 and 0.80, respectively, were similar to Enoki et al. (2002) in their studies of genetic diversity among maize

inbred lines. In the present study, the average number of alleles (n_a) and the number of effective alleles (n_e) estimated per locus per inbred line were both >1, which lead to very high differences between the respective observed and expected heterozygosity. In an investigation on genetic diversity among 79 corn inbred lines, Nyaligwa et al. (2015) reported that the mean numbers of observed and effective alleles were 4.7 and 2.4 per marker, respectively.

Genetic diversity which was measured by Shannon's information index (I) and overall Nei's expected heterozygosity (Nei), gave an average values of 1.03 and 0.57 for inbred lines, and for testers gave a value of 0.49 and 0.33, respectively, indicating that the maize genotypes studied became differentiated at the DNA level. The SSR DNA Markers bnlgl28 in the inbred line and testers was found to have the highest Shannon's information index (1.35 and 1.10, respectively) and Nei's expected heterozygosity (0.73 and 0.67, respectively), these finding indicate the presence of different heterotic group among these SSRs DNA markers, while SSRs DNA markers bnlgl1006 in the inbred lines were found to have the lowest Shannon's information index and Nei's expected heterozygosity (0.41 and 0.24, respectively), while for the testers the SSR DNA Markers Phi45269, bnlgl1022, and Umc1117 showed the lowest Shannon's information index and Nei's expected heterozygosity (0.00). This result shows that the SSR DNA Markers were able to detect the presence of diversity among the genotypes evaluated. Consequently, the SSR DNA markers could be useful for selecting plant materials in breeding programs by defining the genetic similarities and differences among them.

Table 3: Genetic information revealed by 10 simple sequence repeat primers amplified on ten maize genotypes

Traits/Locus	n_a	n_e	Hom_o	Hom_e	H_o	H_e	I	Nei's	PIC
Inbred lines									
bnlg128	4	3.77	1.00	0.21	0.00	0.79	1.35	0.72	0.69
bnlg1006	2	1.32	1.00	0.74	0.00	0.26	0.41	0.78	0.33
bnlg1839	4	3.27	1.00	0.25	0.00	0.75	1.28	0.78	0.74
bnlg2077	4	2.58	1.00	0.34	0.00	0.66	1.15	0.72	0.58
Phi45269	3	2.33	1.00	0.38	0.00	0.62	0.96	0.74	0.65
bnlg1022	2	1.69	1.00	0.56	0.00	0.44	0.60	0.70	0.27
Umc1117	4	3.27	1.00	0.25	0.00	0.75	1.28	0.58	0.65
bnlg1144	4	3.27	1.00	0.25	0.00	0.75	1.28	0.46	0.61
bnlg1152	4	2.58	1.00	0.34	0.00	0.66	1.15	0.46	0.70
Umc1166	3	1.81	1.00	0.52	0.00	0.48	0.80	0.66	0.47
Mean	3.40	2.59	1.00	0.38	0.00	0.62	1.03	0.57	0.57
St. Dev	0.83	0.81	0.00	0.17	0.00	0.17	0.32	0.16	0.54
Testers									
bnlg128	3	3.00	1.00	0.20	0.00	0.80	1.10	0.67	0.68
bnlg1006	2	1.80	1.00	0.47	0.00	0.53	0.64	0.44	0.21
bnlg1839	2	1.80	1.00	0.47	0.00	0.53	0.64	0.44	0.64
bnlg2077	2	1.80	1.00	0.47	0.00	0.53	0.64	0.44	0.57
Phi45269	1	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.50
bnlg1022	1	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.32
Umc1117	1	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.64
bnlg1144	2	1.80	1.00	0.47	0.00	0.53	0.64	0.44	0.64
bnlg1152	2	1.80	1.00	0.47	0.00	0.53	0.64	0.44	0.57
Umc1166	2	1.80	1.00	0.47	0.00	0.53	0.64	0.44	0.41
Mean	1.80	1.68	1.00	0.60	0.00	0.40	0.49	0.33	0.52
St. Dev	0.63	0.60	0.00	0.29	0.00	0.29	0.37	0.24	0.93

n_a : Average number of alleles, n_e : Number of effective alleles, Hom_o : Observed homozygosity, H_o : Observed heterozygosity, Hom_e : Expected homozygosity, H_e : Expected heterozygosity, I: Shannon's information index, Nei: Nei's expected heterozygosity, PIC: Polymorphism information content, S.D.: Standard deviation

PIC demonstrates the informativeness of a marker system and its potential to reveal differences among genotypes based on their genetic relationships. The PIC values based on data obtained from ten markers for provinces assessment showed that the amount of PIC for inbred lines and testers varied from 0.27 to 0.74 and 0.21 to 0.68, respectively, with average value of PIC for the SSR loci was 0.57 and 0.52, respectively, which indicates that informativeness of the markers used was high, based on the scale reported by Botstein et al. (1980) that a PIC value of ≥ 0.5 accounts for a highly informative marker, $0.50 > PIC > 0.25$ for informative marker, and $PIC \leq 0.25$ for a slightly informative marker. Similar finding were reported by Adeyemo et al. (2012) with average value of 0.56. The highest amount of PIC for inbred lines and testers was 0.74, (0.68) and it was related to the bnlg1839 and bnlg128, respectively, PIC for inbred lines which was of almost the same magnitude ($PIC = 0.73$) as reported by Ozturk et al. (2019) that used SSR DNA markers amplified in detecting genetic variation among the ten maize genotypes. The lowest amount of PIC for inbred lines and testers was equals to 0.27 and 0.21 which related to the bnlg1022 and bnlg1006, respectively [Table 3]. In general, for inbred lines and testers 7 markers were highly informative ($PIC \geq 0.50$), 3 markers were moderately informative ($0.50 > PIC > 0.25$).

The high level of polymorphism indicates that variations among the maize genotypes were considerably high. Exploitation of heterosis is more effective when genetic diversity among the genotypes is high.

Genetic similarity coefficients among the three maize testers were calculated according to Shannon's [Table 4]. The highest similarity value was 0.80 between the testers 5012 and Talar, which shows strong similarity between them. In contrast, the lowest genetic similarity (0.30) was found between the Testers Cades and Talar showing that they are dissimilar from each other may be because they exhibit different morphological features and traits or different geographical origins.

The highest genetic similarity (0.60) was found between the lines 232Dss and LH74, indicating high similarity between them. While the lowest genetic similarity (0.00) was found between ZP434 and 890, this indicates high genetic diversity among them as they possessed dissimilar bands at the loci amplified Table 5.

The dendrogram of seven maize genotypes was constructed based on molecular genetic similarity coefficients values using UPGMA cluster analysis [Figure 1]. Genotypes that

were clustered together were said to be genetically identical and were genetically different from other genotypes that formed other clusters. The tree constructed showed that the genotype could be separated in to three clusters. Maximum genotypes (L4, L5, L6, and L7) were distributed under Cluster III, this indicates that these genotypes were similar, followed by Cluster I and Cluster II containing two (L1 and L2) and one (L3) genotypes, respectively. The most diverse genotype was L3. This indicates that is possessed the most

Table 4: Genetic similarity coefficients among 3 maize testers using shannon's similarity coefficient

Testers	Talar	5012	Cades
Talar	1.00		
5012	0.80	1.00	
Cades	0.30	0.40	1.00

Table 5: Genetic similarity coefficients among seven maize inbred lines using Shannon's similarity coefficient

Inbred lines	890	Kr640	3007	LH74	232Dss	ZP34	Msl4279
890	1.00						
Kr640	0.40	1.00					
3007	0.30	0.40	1.00				
LH74	0.30	0.50	0.40	1.00			
232Dss	0.30	0.30	0.30	0.60	1.00		
ZP434	0.00	0.10	0.20	0.40	0.30	1.00	
Msl4279	0.30	0.30	0.30	0.50	0.40	0.40	1.00

Figure 1: Dendrogram showing distribution of seven maize inbred line {L1 (890), L2 (Kr640), L3 (3007), L4 (LH74), L5 (232Dss), L6 (ZP434), L7 (ZP434)} among three clusters

Figure 2: Dendrogram showing the distribution of three maize tester {T1 (Talar), T2 (5012), T3 (Cades)} among two clusters

dissimilar genetic characteristics compared to the other genotypes evaluated. Therefore, highest diverse genotypes can be used as parents in breeding program. Kumari et al. (2018) constructed dendrogram using similarity coefficient values divided eight genotypes in three divergent clusters. (Kumar et al., 2016) reported 13 genotypes grouped into five clusters. While the phylogenetic tree for testers grouped the genotypes in two clusters on the basis of similarity coefficient values at 0.57 similarity coefficient. In the cluster II had maximum two genotypes (T1 and T2) followed by cluster I containing one genotype (T3) [Figure 2].

CONCLUSION

Genetic diversity was studied to find out the more diverse inbred lines in maize which might be used in hybridization program. Seven maize inbred lines were grouped into three different clusters while grouped the testers in to two clusters. The crosses involving parents/inbred lines from most divergent clusters are expected to manifest maximum heterosis and generate wide variability in genetic architecture.

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