THE GENETIC DIVERSITY OF MAIZE INBRED LINES GROWN IN IRAQ USING SIMPLE SEQUENCE RECURRENT (SSR) MARKERS

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ABSTRACT

This study was conducted to determine the genetic variability among maize inbred lines grown in Iraq by using Simple Sequence Recurrent (SSR) markers. For estimating the genetic relationships, 10 SSR primers were used with 10 maize inbred lines. Two hundred and sixteen alleles (bands) with a range of 13 to 41 were identified for the SSR loci. Polymorphism information content (PIC) of the 10 SSR loci ranged from 0.60% to 100%. Primers 'umc1566', 'umc1542', and 'umc2189' showed the maximum PIC value; by contrast, 'umc2225'showed the lowest PIC value. The 10 maize inbreds were clustered based on the matrix of genetic similarity using the UPGMA algorithm. Cluster analysis placed the inbreds lines in three clusters based on SSR data. The studied inbreds lines divided to groups and subgroups with cluster analysis that revealed agreement with their geographical origin. The results indicated that SSR marker has a high degree of polymorphism that allows efficient identification of maize genotypes, and this could be used in determining their heterotic groups.

Keywords: *Zea mays* L., maize polymorphism, genetic variation, molecular markers

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التنوع الوراثي لسلالات الذرة الصفراء المزروعة في العراق باستخدام بادئات التكرار التسلسلي البسيط

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المستخلص

أجريت هذه الدراسة لتحديد التباين الوراثي بين سلالات الذرة الصفراء المزروعة في العراق باستخدام بادئات تكرار التسلسل البسيط Simple Sequence Recurrent (SSR). ولغرض تحديد العلاقات الوراثية، تم استخدام 10 بادئات من SSR مع 10 سلالات نقية من الذرة الصفراء. تم تحديد مائتين وستة عشر أليل (حزمة) بمدى يتراوح من 13 إلى 41 لمواقع بادئاتSSR. تراوح محتوى معلومات تعدد الأشكال Polymorphism information content(PIC) لمواقع بادئاتSSR العشرة من 60% إلى 100%. أظهرت البادئات "umc1566"، و"umc1542"، و"umc2189" الحد الأعلى لقيمة (PIC)، وعلى النقيض من ذلك، أظهر البادىء "umc2225" الحد الأدنى لقيمة (PIC). تم تجميع السلالات العشرة للذرة الصفراء باستخدام مصفوفة التشابه الوراثي وحسب خوارزمية UPGMA. وضعت هذه المصفوفة سلالات الذرة الصفراء في ثلاث مجموعات بناءً على بيانات بادئات SSR. كذلك قسمت السلالات إلى مجموعات رئيسية ومجموعات فرعية وهذا توافق مع أصولها الجغرافية. أظهرت النتائج إلى أن بادئاتSSR تمتاز بدرجة عالية من تعدد الأشكال مما يسمح بتشخيص التراكيب الوراثية للذرة الصفراء، ويساعد في تحديد مجموعاتها الهجينة.

الكلمات المفتاحية: الذرة الصفراء، تعدد الأشكال للذرة الصفراء، التباين الوراثي، المعلمات الجزيئية

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INTRODUCTION

Maize (*Zea mays* L.) is the third most important food crops among cereals grown worldwide and is grown by almost all farmers. It is cultivated for human, animal, and industrial purposes. As a result of this importance, the government gave attention to the production of this crop by providing some basic production requirements at subsidized and reduced prices (3, 5, 11). Maize is one of the Poaceae family; its environmental requirements gave the possibility for successful cultivation and production of this plant in Iraq (12, 25). To produce new cultivars under different environmental conditions, the importance of crop diversity cannot be ignored (4, 13). Exploration of genetic diversity to obtain useful variation has great potential for crop breeding programs. Maize is cross pollinating crop in nature and heterozygous, hence possesses broad genetic diversity, but this is reduced by selection. Limited genetic diversity would negatively impact maize breeding (22). Evaluation of genetic diversity is a pre-requisite for the production of diverse inbred lines and has a pivotal importance for the development of new maize hybrids (16). The success in identifying heterotic hybrids in maize hybrid breeding depends on the availability of genetically diverse maize inbred lines developed from different heterotic gene pool (27). Knowledge of the genetic diversity and relationships among maize inbred lines could facilitate germplasm management and plant breeding programs (18). In ancient times, landraces were more popular due to the presence of high genetic variability; however, it has been replaced by improved and uniform cultivars with a higher yield. Molecular markers have proven to be a valuable tool for genetic diversity analysis of many crop species. DNA markers play an important role in identifying diverse germplasms because of high precision and accuracy (23). Genotyping techniques by using genetic markers have allowed assessment of the genetic diversity among maize inbred lines to synthesize most heterozygotic hybrid combinations (35). Among the various types of DNA-based markers, microsatellites or simple sequence recurrent (SSR), which are short sequences containing tandemly repeated copies of one to six nucleotide fragments, are currently considered as the molecular markers of choice and used extensively to assess genetic diversity (34). SSRs rapidly adapted by plant researchers due to their simplicity, high levels of polymorphism, high reproducibility, and co-dominant inheritance patterns (21). The SSR markers have wide applicability for genetic analysis in crop plant improvement (15). Nearly 1000 SSR markers are available in maize under public domain facilitating their utilization for diverse purposes in genetics and plant breeding and used as an important tool for purity identification of maize hybrid (24). Almeida *et al*., (6) used SSR markers and reported low genetic diversity (0.22 to 0.33) in normal maize and sweet corn compared to the main populations of International Maize and Wheat Improvement Center (CIMMYT), which displayed genetic distances of 0.45 to 0.61 with SSR markers. Pandit *et al*., (29) evaluated 18 primer pairs that produced 92 different markers with polymorphism information content (PIC) value ranging from zero to 0.87; three different dendrograms based upon the dissimilarity coefficients were constructed. The information about genetic diversity of maize inbred lines revealed by genetic markers could be useful in planning strategies for future maize breeding programs. The aim of this study was to determine the genetic diversity of maize genotypes that are grown in Iraq using SSR primers.

MATERIALS AND METHODS

Plant materials: Ten maize inbred lines (SY-16, Zm-17, EXZ-34, Dr-B-5, O-22, MGw-14, SYn-7, EXw-40, S-90, and Dr-C5) were obtained from Maize Research Center in Baghdad and used for determination the genetic diversity among them.

DNA extraction

Total genomic DNA was extracted from fresh leaves at 4-5 leaf stage seedlings, using cetyltrimethylammonium bromide (CTAB) method according to (19). The concentration of genomic DNA was determined based on optical density readings. The final concentration of all the samples was adjusted to 25 ng/µl for amplification by Polymerase Chain Reaction (PCR).

Microsatellites amplification

The method described by (36) was followed with some modifications. PCR was performed using15µl reaction volume consisting of2 µl of 25 ng template DNA mixed with 13 µl of master mix(1.5 µM of $MgCl_2$, 0.2 mM of dNTP mix (Promega), 0.2µMof SSR forward and reverse primers, 0.625U of Taq polymerase (Promega) and 1×PCR reaction buffer (Promega).The PCR profile was programmed with initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min.; this was followed by one final extension cycle at 72°C for 5 min, and an indefinite hold at 4°C.A negative sample with water instead of the genomic DNA was added to check the possibility of contamination in the amplification reactions. Amplifications were performed twice to ensure accuracy. The molecular size was estimated using a 100 bp ladder. The amplification products were separated on 1.4% agarose gel according to (32).

SSR primers and data scoring

Fifteen SSR primers were screened and only 10 were selected for PCR analysis based on their high polymorphism information content from previous studies (9) to determine the genetic variability among maize inbred lines (Table 1). For each primer, the gel was analyzed by scoring the presence or absence of ISSR bands. Presence of an amplified fragment was scored as 1, while absence was scored as zero.

Data analysis

The binary matrix based on marker scores was subjected to cluster analysis. Only clear and unambiguous bands of SSR markers were scored. SSR markers were subjected to cluster analysis based on similarity coefficient values. Dendrogram was constructed based on the similarity coefficient matrix data by applying unweighted pair group method with arithmetic average (UPGMA) cluster analysis using the NTSYSpc program version 2.1 by (31).

Table 1. SSR primer pairs sequences and their annealing temperatures

No.	Primers names	Repeats	Primer Sequence 5`-3`	Annealing Tempe. (°C)
1	umc1566	(GCC)6	F- CGTCTACCTAACCCACCCTC R- AGGCTGAAGAGGAAGTCGAC	55
2	umc1542	(AG)10	F- CAAAGACGACGTTCCTGCAT R- CCCTGACCATCGATCTGCTA	55
3	umc2189	(CAG)4	F- AGTACAGTACACCAATGGGC R- CGACTACAAGCCTCTCAACT	55
4	umc2225	(AGAGAGA GAGAGAG)4	F- AAGGGAACAATCGGAAGGGT R- GCATGCGATTTTACCGGGTT	55
5	bnlg1633	(AG)16	F- GTACCTCCAGGTTTACGCCA R- TCAACTTCTCATGCACCCAT	55
6	bnlg2235	(AG)23	F- ATCCGGAGACACATTCTTGG R- CTGCAAGCAACTCTCATCGA	55
7	bnlg1526	(AG)15	F- ACGAGCGAGTGGAGAATAGG R- AGCCCAGTACGTGGGGTC	55
8	bnlg1017	(AG)18	F- ATTGGAAGGATCTGCGTGAC R- CAGCTGGTGGACTGCATCTA	55
9	bnlg1767	(AG)16	F- AATTTCACGGTAGGGACACG R- AATCCGCGTGTTTTCATAGG	55
10	Phi 031	GTAC	F- GCAACAGGTTACATGAGCTGACGA R- CCAGCGTGCTGTTCCAGTAGTT	55

RESULTS AND DISCUSSION

Amplification products of the ten primers were selected out of the fifteen used in this study; the ten primers generated polymorphic amplification fragments that were obvious and highly reproducible. The other five were ignored because either they produced monomorphic or unclear bands. The ten primers successfully amplified the DNA of all the genotypes; the fragment size ranged

between 100 to 1100 bp for all genotypes. The total number of markers ranged from 13 to 41, and the number of the scored markers with different size ranged from 2 to 15.The genetic diversity among the 10 selected maize genotypes was explored and showed polymorphism in the final analysis. A total of 216 different alleles were amplified by the 10 SSR primers yielding an average of 21.6 SSR alleles per locus. SSR primer 'bnlg1017' generated efficient marker profiles for all 10 maize genotypes. Primer 'bnlg1017' showed the highest number of polymorphic alleles (41), followed by primer 'bnlg2235', which scored 27 polymorphic alleles. The lowest number of alleles was recorded with primers'umc1566' and 'bnlg1767' that each produced only 13 polymorphic alleles (Table 2). The polymorphism information content (PIC) ranged from 60% to 100%. Primers'umc1566', 'umc1542', and 'umc2189' showed the maximum PIC value (100%), followed by 'bnlg2235' (85.72%). By contrast, primer'umc2225'showed the lowest PIC value (60%). Regarding primer 'bnlg1017', the genotypes'Zm-17' and 'O-22' produced unique banding patterns (Figure 1), while for primer 'umc2225', genotypes'MGw-14', 'SYn-7', ' EXw-40' and 'S-90' shared the same banding patterns. The amplification products that obtained from the ten primers were analyzed pair wise to compare the genetic relationship among maize genotypes. Comparison of SSR profiles revealed the similarity coefficient matrix that is presented in (Table 3). Depending on that, a dendrogram was generated from SSR markers to determine the genetic diversity of the 10 maize genotypes that used in this study (Figure 2). The dendrogram recognized three main clusters. The first one included three genotypes 'SY-16', 'EXw-40', and 'S-90', with 93.7% similarity. The second cluster included two genotypes, 'MGw-14', and 'SYn-7' with 95.6% similarity. The third cluster included three genotypes, 'Zm-17', 'EXZ-34', and 'Dr-B-5', with 92.5% similarity. The genotype 'Dr-C5' was genetically separated from the other studied genotypes. Genetic diversity is a very important phenomenon for the success of crops in the environment. If diversity is not observed in the genetic material, it is impossible to obtain the best performance of the plant with other desirable parameters because selection is based on genetic variability (26). It is necessary to evaluate germplasms using molecular tools such as SSR markers because selection based on phenotypic parameters is suboptimal due to certain limitations due to environmental variations (17).

Table 2. Number of different size markers, number of monomorphic markers, % of monomorphic markers, and % of polymorphic markers generated by each of the ten selected primers with the ten genotypes of maize.

Primers names	Total No. of bands	No. of bands/ genotype*	No. of different size markers	No. of monomorphic markers	% of monomorphic markers	% of polymorphic markers
umc1566	13	1.3	2	0	0%	100%
umc1542	15	1.5	3	0	0%	100%
umc2189	16	1.6	2	0	0%	100%
umc2225	24	2.4	5	2	40%	60%
bnlg1633	26	2.6	6	2	33.33%	66.67%
bnlg2235	27	2.7	7	1	14.28%	85.72%
bnlg1526	22	2.2	6	1	16.67%	83.33%
bnlg1017	41	4.1	15	4	26.67%	73.33%
bnlg1767	13	1.3	4	1	25%	75%
Phi 031	19	1.9	3	1	33.33%	66.67%
Total	216	-	53	12	-	-

*** Calculated by dividing the total number of bands produced by each primer on the total number (10) of genotypes**

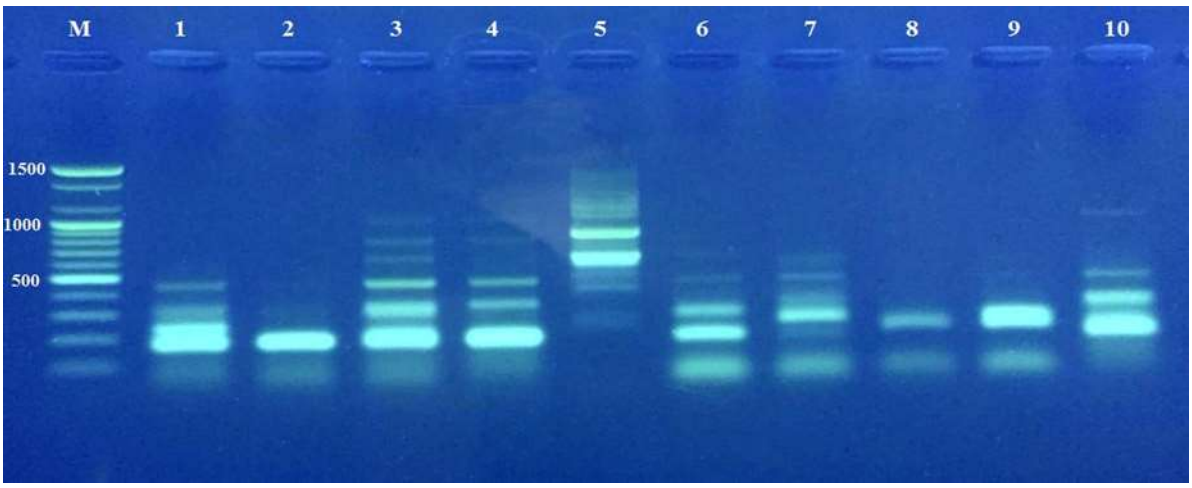

Figure 1. Example of agarose gel electrophoresis showed the SSR pattern of different corn genotypes with primer bnlg1017: lane M, 1Kb DNA marker; lane 1, SY-16; lane 2, Zm-17; lane 3, EXZ-34; lane 4, Dr-B-5; lane 5, O-22; lane 6, MGw-14; lane 7, SYn-7; lane 8, EXw-40; lane 9, S-90; lane 10, Dr-C5

Table 3. Similarity coefficient matrix of pairwise comparisons for 10 maize genotypes

Genotypes	SY-16	Zm-17	EXZ-34	Dr-B-5	O-22	MGw-14	SYn-7	EXw-40	S-90	Dr-C5
SY-16	1.000									
Zm-17	0.906	1.000								
EXZ-34	0.881	0.925	1.000							
Dr-B-5	0.893	0.925	0.925	1.000						
O-22	0.862	0.868	0.868	0.881	1.000					
MGw-14	0.900	0.931	0.906	0.893	0.862	1.000				
SYn-7	0.893	0.912	0.887	0.887	0.868	0.956	1.000			
EXw-40	0.906	0.900	0.875	0.875	0.868	0.906	0.925	1.000		
S-90	0.918	0.900	0.862	0.887	0.868	0.906	0.900	0.937	1.000	
Dr-C5	0.856	0.875	0.825	0.875	0.843	0.881	0.862	0.862	0.900	1.000

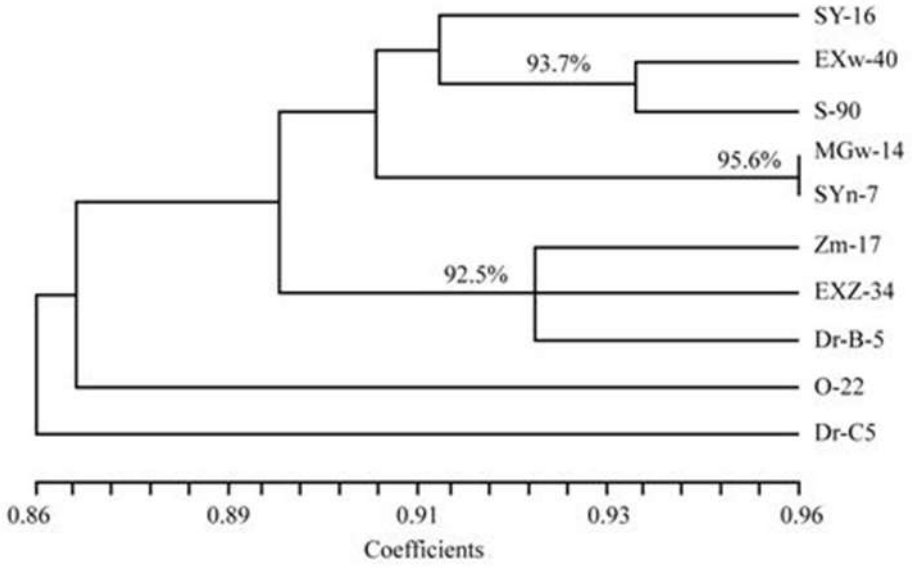

Figure 2. Dendrogram generated from SSR markers data showing the genetic relationship among 10 maize genotypes

The importance of determining the genetic relationship via molecular tools is to select the best parental inbreds necessary to create genotypes with new genes pool and predicting the best hybrid (8). The results of this study indicated that some inbred lines could be used in a breeding program to develop hybrids of high grain yield; this in agreement with (2). Adu *et al.*, (1) indicated that the SSR used were very polymorphic for diversity studies.

The high polymorphism rates found in SSR primers used in this study indicated that molecular characterization of genotypes using ISSR markers is an efficient strategy. This level of polymorphism is similar to results found in several studies (21, 25, 28, 29, 33). The moderate to high PIC values obtained here indicated the importance of DNA markers in germplasm analysis, gene mapping, and molecular breeding. The cluster analysis based on the UPGMA method was used to group genotypes based on similarity in dendrogram. The results obtained from the molecular characterization indicated the existing of genetic similarity coefficient that ranged from 0.825 to 0.956 among the studied genotypes. The genotypes that located in different clusters should be considered for genetic enhancement. For example, crossing the inbred lines Dr-C5 or O-22 with EXw-40 or S-90 as they are far related; this could produce hybrids with enhanced characters. Hybridization is the appropriate mean to increase corn yield of hybrids that produced from inbred lines (9, 10). Genotypes located in the same group are more closely related and care should be taken when using similar genotypes in crossing for hybrids production. Therefore, the revealed genetic diversity among the studied genotypes could consider as a source that could be applied for successful future development of maize hybrid breeding programs in Iraq. Junior *et al*., (20) identified different clusters of maize genotypes using ISSR markers. The division of accessions in different groups showed that they are diverse and suitable for breeding purposes. Also, (14) indicated that wide genetic diversity of maize populations revealed by SSR markers could be used as genetic resources in future breeding studies.

CONCLUSIONS

The ten primers that applied in this study were successfully amplified the DNA of maize genotypes. They revealed the usefulness of Simple Sequence Recurrent (SSR) markers as efficient tool for determining the genetic diversity among maize inbred lines that can be applied in breeding programs for the development of maize hybrids. In addition, SSR markers were highly polymorphic and could be utilized in studying the genetic relationships and diversity of other plants that grown in Iraq. Also, the obtained results could be used for establishing a genetic database for maize genotypes in the region.

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