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Research Article

Genetic Relationship among Maize Genotypes as Revealed by SSR Markers

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Abstract: In this investigation, nine maize genotypes were used to check the genetic relationship using 20 PCR based markers (SSR). These markers produced 55 polymorphic bands with an average of 2.75 bands per primer. Maximum genetic similarity (i.e., 0.733) was found based on Jaccard's coefficient of similarity. A dendrogram was constructed using unweight pair group mean arithmetic (UPGMA) method which clustered into two main groups based on their genetic diversity which was further confirmed by the principal component analysis. These findings clearly depicted that SSR markers could help in successful detection of genetic relationship in maize and further assist in improvement of maize productivity.

Keywords: Genetic diversity, maize, SSR markers

1. INTRODUCTION

Maize is third most important crop after rice and wheat all over the world, which is an angiosperm and monocot, C4 plant, cross pollinated and is cultivated annually. The genome of maize comprises of 10 chromosomes having approximate genetic length of 1500 cM. Due to its high nutritional value (about 50% of dietary proteins), it serves as a staple food for human consumption in many developing countries. In Africa and some of the Asian countries, 80 to 90% of the energy intake is accounted from maize consumption. Due to cross pollination it has a wide range of morphological and geographical adaptation. Based on latitude and climatic conditions, there are three categories of maize, i.e., temperate, tropical, and subtropical. Generally speaking, the classification of maize varieties are based on maturity, utilization, endosperm, kernel constitution and kernel properties (i.e., floury, waxy, dent, flint, sweet and popcorn) [1].

Genetic diversity could be assessed using morphological, agronomic, molecular and biochemical information of the organism [2-4]. Among this information, molecular level is more accurate as it provides more detailed information, very reliable, quick and no environmental impact [5-9]. Simple sequence repeats are comprised of 2-4 bases length of DNA sequence motif which are repeated many times and widely used in maize genetics studies [10]. These SSR markers are uniformly distributed, abundant, codominant, easily produced by PCR and simple interpretation [10].

Mostly DNA markers are being used for the diversity analyses which are performed by polymerase chain reactions (PCR). The main DNA markers which are driven by PCR are Randomly Amplified Polymorphic DNA (RAPD), Single Nucleotide Polymorphism (SNP), Simple Sequence Repeats (SSR), Allele-Specific Amplification (ASA), and Cleavage Amplification Polymorphic Sequences (CAPS). Among these SSR and RAPD markers are easily used by PCR reactions. Simple Sequence Repeats are the short DNA sequences of about 2-6bp in length which are distributed through out the genome of the eukaryotes. Currently these SSR markers are mostly used for genetic diversity analysis in crops like maize, rice, wheat, barley, etc. [11, 12]. The application of this technique was first reported by

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Akkaya *et al.* [13] in finger printing plant species. In this study we have reported genetic diversity analysis among nine different maize lines using twenty SSR markers distributed in whole genome of maize.

2. MATERIALS AND METHODS

2.1 Plant Material

Nine varieties of maize were collected from Biotechnology laboratory of Shenyang Academy of Agricultural Sciences, Shenyang, China and used for genetic analysis.

2.2 DNA Extraction and SSR Analysis

Total genomic DNA and amplification was done as reported earlier [14].

2.3 SSR-PCR Amplification

SSR-PCR amplifications were performed as described in Li et al [15]. The SSR-PCR products was resolved on 8% PAGE and visualized by silver staining.

2.4 Data Analysis

Data obtained after SSR-PCR amplification were scored as (1) indicating the presence or (0) absence of clear bands. The genetic similarities (GS) among maize varieties were determined as described by Nei and Li [16]. A dendogram was constructed showing the genetic relationships between genotypes using unweighted pair group method with arithmetic mean (UPGMA) [17] principal component analysis was performed using NTSYS-pc version1.80 software [18].

3. RESULTS AND DISCUSSION

Currently a wide range of genetic diversity is present in almost all species and this genetic variation might be due to the environmental conditions. This genetic diversity could be estimated by a variety of molecular techniques using standard molecular markers. Among these molecular markers, SSR molecular marker technique is most acceptable, codominant in nature, reproducible and highly polymorphic [19] which have specific primers and are species specific [20]. Some studies [21-23] suggested that these SSRs could be used for the determination of genetic diversity in the improvement of maize and could extensively be used in various applications in crop improvement [24].

Table 2 showed the amplification profile of the nine different maize lines from twenty SSR markers. The PCR products were resolved on 8% PAGE followed by sliver staining (Fig. 1). Almost all the amplified products were ranged in size of about 100-200bp. These twenty primers produced a total of 95 bands with an average of 4.75 alleles per primer. Of these 95 alleles, 40 alleles were monomorphic with an average of 2.0 allele while 55 alleles were polymorphic with an average of 2.75 alleles, respectively. Polymorphism was ranged from 25 to 83.3% with an average of 56.4%. Our findings were little lower than de Souza et al [25] who reported 89.4% polymorphism with RAPD markers while higher than Lanza et al [26] who reported 80.6% polymorphism using RAPD markers respectively. In another study, Sun et al [27] reported 100% polymorphism by microsetalites. The polymorphism level differs in corn genome with use of different molecular makers and spatial distribution [27].

Table 3 presents the similarity matrix based on Jaccord's coefficient. Maximum similarity coefficient (73.3%) was observed between variety SAAS-BL 3 and SAAS-BL 9 while the lowest similarity coefficient (26.3%) was noted between SAAS-BL 4 and SAAS-BL 7 respectively. So the similarity index based on Jaccord's coefficient was ranged from 26.3 to 73.3 % with twenty SSR markers using nine different lines of maize. This similarity was slightly lower than from earlier report of de Souza et al [25] who reported similarity range of 53 to 84% as revealed by RAPD and 11 to 82% for SSR markers based on Dice coefficient. Microsatellites had better polymorphism, variability and high diversity exploration ability [28]. Bibi et al [29] reported that genetic diversity among maize varieties could be determined using SSR markers.

Table 1. Maize SSR primers used during amplification reactions.

Sr. #	Primer	Sequence	Bin	Repeat
1	Bnlg1189	CGTTACCCATTCCTGCTACG (F) CTTGCTCGTTTCCATTCCAT (R)	4.07	AG(12)
2	Umc1775	GAGGACAACGCTGCTATTCTCG (F) GGAACTCCGTCAAAATCCCATC (R)	4.08	(CGC)5
3	Bnlg1444	GCATGGATGGAGAAAGAGGA (F) AGACGACGAAGCTTTTGCAT	4.08	AG(22)
4	Bnlg2162	GTCTGCTGCTAGTGGTGGTG (F) CACCGGCATTCGATATCTTT	4.08	AG(27)
5	Umc2041	CTACACAAGCATAGAGGCCTGGAG (F) CAGTACGAGACGATGGAGGACAT	4.08	(CAG)4
6	Umc1086	CATGAAAGTTTTCCTGTGCAGATT (F) GGGCAACTTTAGAGGTCGATTTATT (R)	4.08	(CT)12
7	Umc2285	GAAGAAGAGGGAAAGGAAGGGAG (F) AAGTAGCTGGGCTTGGAGGG (R)	4.08	(GCCGCC)4
8	Umc2286	CTAGGCGCAGAAAGAAGTGTTGTT (R) CCGAAAGAACAACAAAGGAGAGAG (F)	4.08	(ACCA)4
9	Umc2139	ATAAGGAACATCCCCACCTGTTTT (F) GGTGTGCTGGGTTCTTGTGG	4.09	(GCC)4
10	Umc1328	TACAAGGAGGAGGCCGCTGT(F) ATCCAGTCTCCGGACTTCCAAC (R)	4.09	(TGC)5
11	Umc1573	GTCCCTCCTCCTGCACACAC (F) ACGACGTCGGTACTTGCTGG (R)	4.09	(ATG)4
12	Bnlg589	GGGTCGTTTAGGGAGGCACCTTTGGT(F) GCGACAGACAGACAGACAAGCGCATTGT(R)	4.10	---
13	Umc1109	GCAACACAGGACCAAATCATCTCT (F) GTTCGGTCCGTAGAAGAACTCTCA (R)	4.10	(ACG)4
14	Umc2289	CAGCACCACCCAGTTAACCAC (F) GGCTCCGATTCACTTGATGC (R)	4.10	(CTCT)4
15	Umc1738	CCAGACATTCCCCAAACCCTA (F) CGTCGGTGTCGTACTGGTTG (R)	4.10	(CGCT)5
16	Umc2290	GAATCACAATGGATCCTACCAAGG (F) AACTGCAAGCAAAATCAACACAAG (R)	4.11	(CTT)4
17	Umc1308	GCAGATGGACACAAACAAATGAAG (F) GCTACTGATGCTGGCAATCTTACA (R)	5.0	(TG)10
18	Umc1097	CTCGTCAACGTCAACCCAAGTAAG (F) CTGTTAGATGTGCGACAACAGAGC (R)	5.0	(CA)8
19	Umc1325	ATATTGTACAGGAGCAGCTGGGAC (F) GGAGGTCATGCGTGTAAATAGGTC (R)	5.0	(CT)6
20	Umc1679	CACTGCTAAGCTGCTCCCTGTT (F) TGCTAACTAACCCTGACCCTCTCA (R)	5.01	(AAG)5

Table 2. Number of alleles detected and polymorphism produced by twenty SSR primers.

Locus	Observed No. of alleles	Monomorphic Alleles	Polymorphic Alleles	Polymorphism (%)
Bnlg1189	5	2	3	60
Umc1775	6	3	3	50
Bnlg1444	4	1	3	75
Bnlg2162	4	2	2	50
Umc2041	5	3	2	40
Umc1086	4	3	1	25
Umc2285	3	2	1	33.3
Umc2286	4	2	2	50
Umc2139	5	1	4	80
Umc1328	5	2	3	60
Umc1573	6	1	5	83.3
Bnlg589	6	2	4	66.6
Umc1109	5	4	1	20
Umc2289	4	1	3	75
Umc1738	4	2	2	50
Umc2290	6	2	4	66.6
Umc1308	6	1	5	83.3
Umc1097	4	3	1	25
Umc1325	5	2	3	60
Umc1679	4	1	3	75
Average	4.75	2.0	2.75	56.4
Total	**95**	**40**	**55**	

Table 3. Similarity matrix computed with Jaccard coefficient.

	1	2	3	4	5	6	7	8	9
1	1	0.688	0.421	0.368	0.556	0.529	0.556	0.526	0.611
2		1	0.412	0.278	0.389	0.438	0.562	0.444	0.529
3			1	0.438	0.316	0.278	0.471	0.529	0.733
4				1	0.600	0.467	0.263	0.389	0.471
5					1	0.600	0.368	0.350	0.421
6						1	0.500	0.316	0.316
7							1	0.588	0.500
8								1	0.556
9									1

Based on the amplification profile these maize genotypes were clustered through UPGMA method. The dendrogram (Fig. 2) showed two main groups. The group 1 contains three genotypes which were further divided into two subgroups while the group II contained six genotypes which were also further divided into three subgroups each having two genotypes in one subgroup. These groupings were further verified by principal component analysis (PCA) which was shown in three dimensional plots (Fig. 3). These three principal components had eigenvalues greater than unity extracted a cumulative of 97% of the variance in 9 varieties of maize. These three axes had eigen-values of 30.67, 24.46, and 14.58%, respectively. So, PCA showed the three-dimensional relationships that describe genetic variance in maize verities.

4. CONCLUSIONS

Results of this study revealed that SSR markers were very helpful in dissecting the genetic

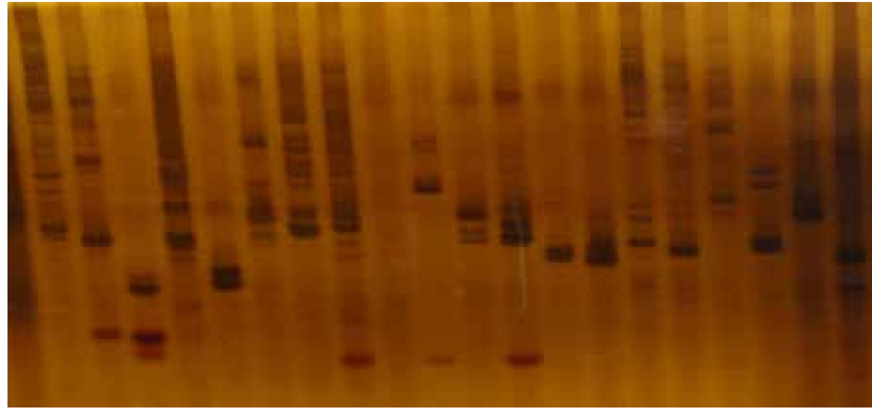

Fig. 1. Silver stained PAGE of amplification profile of SAAS-BL 1 with twenty SSR markers.

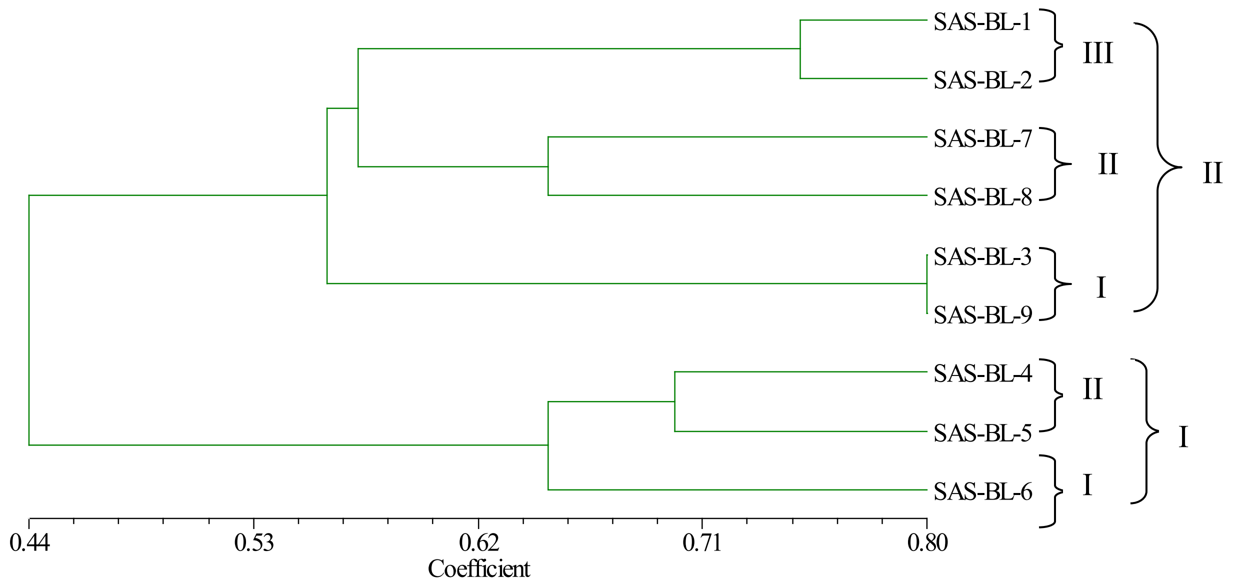

Fig. 2. Dendrogram of nine maize genotypes constructed by the UPGMA method.

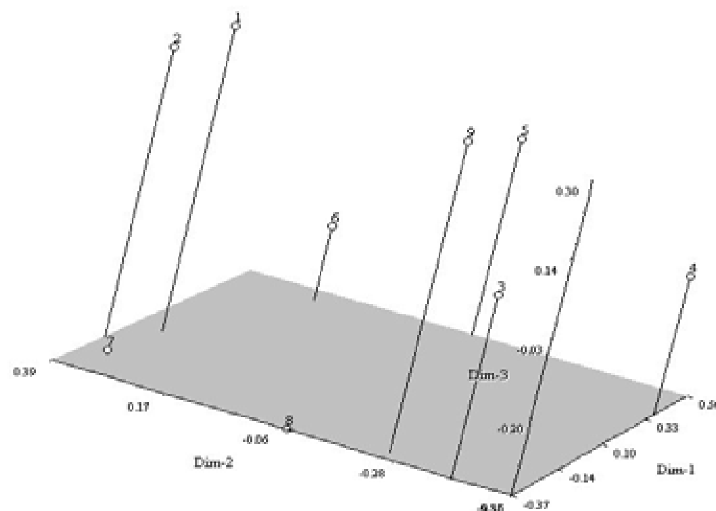

Fig. 3. Principal component analyses of nine genotypes of maize.

diversity of maize varieties. This was effective approach to study genetic variation and would be very helpful in maize genetic improvement and breeding programs.

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