Australian Journal of Basic and Applied Sciences, 6(10): 42-47, 2012
ISSN 1991-8178

Maize (*Zea mays* L.) Genotypes Diversity Study by Utilization of Inter-Simple Sequence Repeat (ISSR) Markers

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Abstract: Analysis of the extent and distribution of genetic diversity in crop plants is essential for optimizing sampling and breeding strategies. Several methods were identified for the existence of diversity in maize for the aim of perfect selection based on great diversity among maize genotypes. In this study, Inter-simple sequence repeat (ISSR) markers were used to assess genetic diversity in a selected group of maize inbred (*Zea mays* L.) genotypes. A high level of polymorphism of 69% was detected among these genotypes. The ISSR primers showed 10 fingerprints for six genotypes out of 9 studied which are Frantic, Huediba-1, Balady, Huediba-2, Giza 2 and Mogtamaa 45-2. The maximum genetic distance of 0.48% was detected between Huediba-2 and Mogtama- 45-2. While, the minimum genetic distance of 0.16 percent was observed between Giza-2 and Var.113. The results indicated that variation can be attributed to use of ISSR.

Key words: (ISSR) markers, DNA polymorphism, Maize, Genotypes, Genetic diversity.

INTRODUCTION

Maize (*Zea mays* L.) ranks as one of the world's three most important cereal crops, it is cultivated in a wide range of environments more than wheat and rice because of its greater adaptability (Koutsika-Sotiriou, 1999). So due to its importance as food and forage crop in Sudan, it has been subjected to intensive genetic and cytogenetic studies (Asif *et al*., 2006). Several analysis of the maize genetic variability have been performed using molecular markers to assess the crop evolutionary aspects (Wu, 2000, Srdic *et al*., 2007, Abuali *et al*.,2011) or to obtain genotype characterization (Gethi *et al*., 2002) or to estimate heterosis among inbred lines (Ishag, 2004). The use of hybrid vigor or heterosis in maize culture is an outstanding successful example in applied genetics as it allowed a better understanding of the genetic phenomenon and plant breeding in general (Springer and Stupar, 2007).

Molecular markers are stable and detectable in all plant tissues regardless of the growth, differentiation, development or status of cells. Besides, they are not affected by environmental, pleiotropic and epistatic effects (Agarwal *et al*., 2008; Collard and Mackill, 2008; Moose and Mumm, 2008). For such, the use of DNA markers, compared to phenotypic aspects, becomes even more important. There are many markers available, among them are: the simple sequence repeats (SSR) markers (Tautz, 1989), randomly amplified polymorphic DNA (RAPD) (Welsh and McCleland, 1990), inter simple sequence repeats (ISSR) (Zietkiewicz et.al, 1994), amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995), single nucleotide polymorphisms (SNPs) (Chen and Sullivan, 2003) and diversity array technology (DArT) (Kilian *et al*., 2005).

These different types of molecular markers have potential to differentiate and detect differences among genotypes, in relation to cost, easiness of use, consistency and repeatability of the Results (Schlötterer, 2004; Schulman, 2007; Bernardo, 2008). One of these techniques is inter simple sequence repeats (ISSR) technique is considered simple and fast like RAPD, but has more stringency than RAPD. Also, ISSR markers are highly polymorphic, which makes them useful for studies on genetic diversity, phylogeny, genetic coding, genomic mapping and evolutionary biology (Reddy *et al*., 2002).

In the present study nine genotypes of maize from different sources were assessed for diversity by utilization of ISSR analysis.

MATERIALS AND METHODS

Seed Material:

Nine maize genotypes obtained from different sources (Table, 1). Seeds of all genotypes were sown at the experimental farm, Sudan University of Science and Technology, College of Agricultural Studies, Shambat. The

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leaf samples of maize genotypes were collected into labeled bags and stored in dry ice until DNA extraction was done.

Table 1: Genotypes name source and days to 50% flowering.

NO	Name of Genotypes	Source	Days to 50% flowering
1	Frantic	Received from ARC	62.30
2	Huediba 1	Open –pollinated variety improved by ARC	60.84
3	Balady	Local variety	50.84
4	Huediba 2	Open –pollinated variety improved by ARC	59.65
5	Giza 2	Introduced by ARC from Egypt	64.30
6	Mogtamaa 45-1	Introduced by ARC from Egypt	62.64
7	Var 113	Local material selected by ARC	58.00
8	Mogtamaa 45-2	Introduced by ARC from Egypt	59.15
9	Panama	Introduced and released by ARC	61.50

ARC : Agricultural Research Corporation, Sudan.

DNA Extraction:

DNA was extracted from fresh leaf tissue of the nine maize genotypes using modified CTAB method (Porebski *et al*., 1997). The modification was made in intention to improve the DNA quantity and the quality. In this method the fine powdered plant materials were immediately transferred into 15 ml Falcon tubes containing 5 ml of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water bath at 60ºC with gentle shaking for 60 min and left to cool at room temperature for 10 min. Chloroform: Isoamylalcohol mixture (24:1) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 4000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled Isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% Ethanol and the Ethanol was discarded. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at -20ºC for further use.

DNA Quality and Quantity:

The extracted DNA samples were observed under UV illumination after staining with Ethidium Bromide and agarose gel electrophoresis. The purity and the concentrations of the DNA were then Spectrophotometrically assessed following Sambrook *et al*., (1989) method.

PCR of the ISSR Technique:

25 ISSR primers were used in the polymerase chain reaction (PCR) in a final volume of 25 µL containing 1.0 µL DNA diluted, 0.5 µL Taq polymerase, 2.5 µL 10X buffer, 2.5 µL (2mM/µl) dNTPs, 1.5 µL (50 mM) MgCl2, 2.0 µL (10 pmol/ µl) ISSR primer and 15 µL ddH2O. The amplifications were performed in a thermal cycler following the program: 94°C for 5 min, 40 cycles (1 min at 94°C, 1 min at 43°C and 1 min at 72°C) and final elongation of 7 min at 72°C.

DNA Photograph:

5 µl of PCR product were mixed with 2 µl of loading dye and 1.4 µl of 1 Kbp DNA ladder, then were electrophoresed using 2% agarose gel at 80 Volts followed by staining with Ethidium Bromide then the separated fragments were visualized with an ultraviolet (UV) transilluminator.

ISSR Data Analysis:

The number of polymorphic and monomorphic bands were determined for each primer. Genotypes were scored (1) for present band and (0) for absent band and then entered into a data matrix. Percentage of polymorphism was calculated as the following equation: (polymorphic bands / total number of bands x 100). The tree diagram was produced by clustering the similarity data with the UPGMA method using STATISTCA-SPSS software Ver. 9 following the method as used by El-Amin and Hamza, (2012).

RESULTS AND DISCUSSION

Eight primers were used out of the 25 tested to produce 53 polymorphic bands of 69 total amplified bands, with average of 6.5 bands per primer with 69% polymorphi. The ISSR primer 810 had the highest percentage of polymorphic band with 92% (Table, 2). Júnior *et al*., (2011) results showed that ISSR primer ((GA) 8T) had same results as we obtained, with total number of bands (11), with polymorphic number of bands (10) and the percentage of polymorphic band (92%). The highest distance matrix was 0.48 between Huediba 2 and

Mogtamaa 45-2, the lowest was 0.16 between Giza 2 and Var 113.The obtained value in this study was little less than the values previously obtained by Abuali *et al*., (2011) who used RAPD markers in 27 hybrids and inbred lines of maize.

Ten DNA fingerprints were found for six genotypes out of nine studied, where three fingerprints were found in the Frantic genotype using primer 814 and primer 872. Huediba-1 had three fingerprints with primers 810, 814 and 841. Balady had only one fingerprint with primer 808. One fingerprint was obtained for Huediba-2 with primer 841, Giza 2 with primer 872 and Mogtamaa 45-2 with primer 814. According to Kachapur *et al*., (2009) appearance of fingerprints may be due to the presence of unique alleles inside those genotypes, and such alleles are important because they may be diagnostic for particular regions with a genome specific to a particular type of maize. Using the ISSR primers. Access on specific DNA fingerprints will be of high value for maize breeders working in the improvement of the crop. Also, the ISSR primers (808, 810, 814, 841, and 872) that showed specific fingerprints can be used in between to screen longer spectrum of Sudanese maize accessions.

The phylogenetic analysis of this study distinguished that Frantic, Huediba-1 and Huediba-2 genotypes are out of clusters. The first cluster contained two genotypes as sisters which are Panama and Mogtamaa 45-1. Also, Balady and Mogtamaa 45-2 genotypes as second sisters. In the other cluster there is one sister Giza 2 and Var 113 (Figure 2). Our results are in agreement with reports of Ayana *et al*., (2000) and Iqbal *et al*., (2010) who observed no genetic relationship with quantitative agro-morphological traits, which shows no correlation between molecular markers and morphological traits. Ezzat *et al*., (2010) mentioned that the differences among genotypes in agronomic traits are may due to the differences of weather patterns, soil type and the location to another. So, this may be due to the influence of different environmental conditions on the phenotypic traits, leading to apparent differences even among identical genotypes (Shehzad *et al*., 2009). So the indigenous names cannot be considered good guide to the presence of diversity (Chakauya *et al*., 2006).

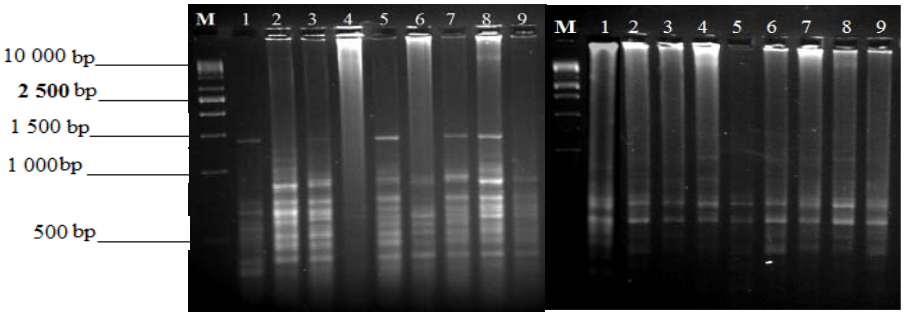

Fig 1: Amplification of the 9 maize genotypes with ISSR primers 810 and 872.

Where: M: DNA ladder (1 kbp), 1: Frantic, 2: Huediba-1, 3: Balady, 4: Huediba-2, 5: Giza-2, 6: Mogtamaa 45-1, 7: Var-113, 8: Mogtamaa 45-2 and 9: Panama.

Table 2: Polymorphism and monomorphism detected by the use of 8 polymorphic ISSR primers.

Primer name	Sequence (5'-3')	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage of polymorphic bands
807	(AG)8T	10	8	2	80%
808	(AG)8C	8	6	2	75%
810	(GA)8T	12	11	1	92%
814	(CT)8A	11	10	1	91%
841	(GA)8YC	9	8	1	89%
848	(CA)8 RG	4	1	3	25%
858	(TG)8RG	4	1	3	25%
872	(GATA)4	11	8	3	73%
Total		69	53	16	
Average		8.6	6.5	2	69%

Table 3: DNA finger prints specific for six Maize genotypes of the nine studied.

NO.	Name of Genotypes	Number of fingerprints	Primer
1	Frantic	1	814
		2	872
2	Huediba-1	1	810
		1	814
		1	841
3	Balady	1	808
4	Huediba-2	1	841
5	Giza-2	1	872
6	Mogtamaa 45-2	1	814

Table 4: Distance matrix based on ISSR data among the 9 maize genotypes. Where: 1: Frantic, 2: Huediba-1, 3: Balady, 4: Huediba-2, 5: Giza-2, 6: Mogtamaa 45-1, 7: Var-113, 8: Mogtamaa 45-2 and 9: Panama.

	1	2	3	4	5	6	7	8	9
1	0.00								
2	0.43	0.00							
3	0.35	0.23	0.00						
4	0.42	0.39	0.39	0.00					
5	0.36	0.28	0.25	0.35	0.00				
6	0.30	0.25	0.22	0.32	0.23	0.00			
7	0.38	0.32	0.29	0.30	0.16	0.19	0.00		
8	0.32	0.23	0.20	0.48	0.30	0.28	0.29	0.00	
9	0.36	0.33	0.28	0.35	0.32	0.17	0.28	0.25	0.00

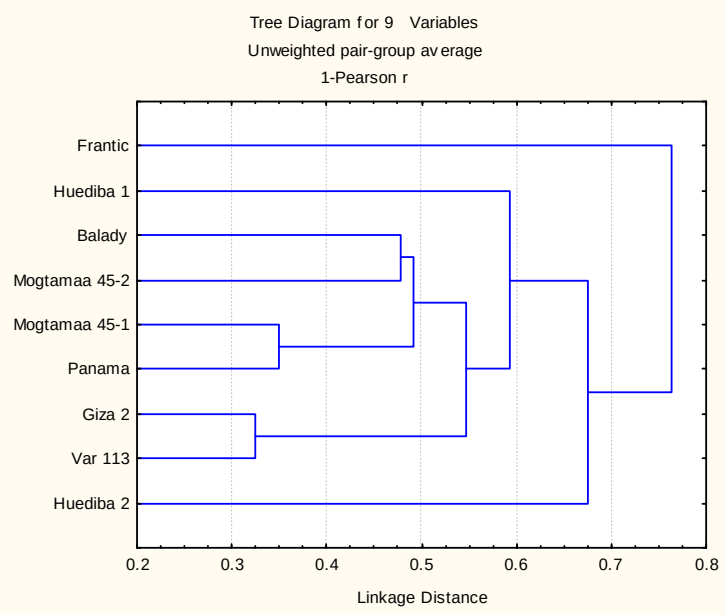

Fig. 2: UPGMA Dendrogram resulting from the analysis of 8 ISSR primers showing the relationships among the 9 maize genotypes.

Conclusions:

Genetic diversity plays a key role in crop improvement. This study was aimed at identifying genetic diversity in nine maize genotypes using 8 primers sets. The results showed that the estimation of genetic distance among maize genotypes by utilization of ISSR markers could be used as a reliable method for the characterization of maize genotypes. The DNA based markers represent a powerful tool in assessment of genetic diversity among maize genotypes. They can be used to identify diverse sources in maize germplasm collections or to select groups of genotypes with desirable characters and contrasting phenotypes, if large numbers are employed. Particularly, genetic distance estimates determined by ISSR markers may help to identify suitable maize germplasm for introgression into breeding stocks. It is recommended that these nine genotypes could be used in efficient hybridizations techniques for the object of increasing levels of genetic polymorphism in maize genotypes.

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