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Genetic variations in some Egyptian *Zea mexicana* genotypes based on RAPD and AFLP markers

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ABSTRACT

Maize-Teosinte hybrids are of significant interest to maize breeders as a resource of genetic diversity during the maize domestication process as a genetic pool for maize improvement. This study examines the genetic diversity and population structure of 16 *Zea mexicana* populations which are currently active in Zea breeding programs in Egypt using Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers. RAPD analysis (14 primers) produced 141 bands; out of which, 102 (72.3%) were polymorphic. On the other hand, AFLP (5 primer combinations) yielded 276 peaks including 267 (96.7%) polymorphic ones with an average of 53.4 peaks per primer combination. A total of 11 unique RAPD markers were created by 6 primers and identified 8 genotypes. The five primer combinations generated 56 unique amplicons that successfully distinguished 12 out of the 16 genotypes tested. Furthermore, the number of observed alleles (Na), effective multiplex ratio (EMR), and polymorphic information content (PIC) indices showed higher values for AFLP (2.00, 53.4, and 0.21) than for RAPD (1.67, 7.3, and 0.15). Cluster analysis based on Nei and Li genetic distance and an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) revealed 5 main clusters representing the 16 *Zea mexicana* confirming the population structure analysis obtained. High variability of the studied teosinte genotypes using RAPD and AFLP markers will provide valuable tools for *Zea mexicana* breeding programs in Egypt.

Keywords: *Zea Mexicana*, AFLP, RAPD, molecular markers, genetic diversity.

INTRODUCTION

Both Maize (*Zea mays*) and teosinte (*Zea mexicana*) belong to the genus *Zea*. However, *Zea mays* ssp. *mays* are the only cultivated species, while the other

species are considered as wild grasses and referred to as teosintes (Sánchez *et al.*, 2011). Maize-Teosinte hybrids have been of considerable interest to both maize and teosinte breeders (Chavez *et al.*, 2012)

since they can assist in tracking the migration pathways of maize from its origin centers as well as helping in understanding the fate of genetic diversity during the maize domestication process (Varsha *et al.*, 2018). Moreover, teosinte genes have the potential to help improve productivity, disease resistance, flooding tolerance, and nutritional quality (Sánchez *et al.*, 2018; Petr *et al.*, 2018; Kyu *et al.*, 2018 and Tanvir *et al.*, 2018).

Likewise, maize-teosinte or teosinte-maize hybrids have also received attention for enhancing the fodder production potential of teosinte by taking advantage of the hybrid vigor shown by the hybrids. Therefore, Teosinte cultivation was recently utilized as a summer forage crop in Egypt (Radwan *et al.*, 2000; Fukunaga *et al.*, 2005 and Wafaa M. Sharawy *et al.*, 2011). Teosinte has the advantage of giving very high yields, due to profuse tillering capacity that is absent in fodder maize. In addition to the ability to give three cuts against one cut obtained from fodder maize (Sakr, 2017). In addition, maize-teosinte crosses like maize can be safely fed on at any stage of growth (Hassan *et al.*, 2017). Thus, the intelligent use of this valuable genetic resource depends on the understanding of the genetic diversity among and within teosinte populations (Sánchez *et al.*, 2011). Also, understanding phylogenetic relationships could be utilized for understanding maize domestication and evolution, for effective decisions on in situ conservation of teosinte species, and exploiting the potential of teosinte for further genetic enhancement of maize (Prasanna, 2012).

Molecular markers are considered the ideal choice to be used in plant genetic diversity studies because of their unambiguous genetic resource's characterization at the DNA level (Barcaccia *et al.*, 2016). Also, they are stable and detectable in all plant tissues at different developmental stages or under different environmental conditions (Violeta *et al.*, 2018). Because of the simplicity of the random amplified polymorphic DNAs (RAPDs), it has been extensively

used to differentiate and detect differences among genotypes (Suten *et al.*, 2013 and Tomkowiak, *et al.*, 2020). RAPD does not require any specific knowledge of the DNA sequence of the target organism. The identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the sequence of the primers (Franklin *et al.*, 1999). On the other hand, although AFLP is more demanding and laborious, it is a very powerful tool for assessing DNA fingerprinting because of its capacity to reveal a large number of bands in a single amplification, and the resulting higher efficiency index regardless of the plant origin or complexity (Molin *et al.*, 2013 and Mohamed *et al.*, 2016).

The present study was carried out to estimate the level of polymorphism among Sixteen *Zea mexicana* accessions using two different molecular markers: RAPD and AFLP, to identify unique DNA markers to generate a fingerprint for each genotype that acts as a unique identity for conservation, to assess the genetic relationships and population structure between these genotypes thus helping in choosing the most likely parents from a selection of candidates for future breeding programs in Egypt.

MATERIAL AND METHODS:

Study Area

The present investigation was carried out at the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.

plant material

Sixteen *Zea mexicana* accessions were provided by Forage Crops Research Department (FCRD), Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt. *Zea mexicana* genotypes and corresponding pedigree are listed in Table (1).

Table 1 The pedigree of the studied Sixteen *Zea mexicana* genotypes

Genotype	Pedigree
G1	{M × (M × K) F1} × K
G2	{M × (M × G) F1} × G
G3	{M × (M × L) F1} × L
G4	{M × (M × L) F1} × L
G5	{M × (M × K) F1} × K
G6	{M × (M × K) F1} × K
G7	{M × (M × L) F1} × L
G8	{M × (M × K) F1} × K
G9	{M × (M × G) F1} × G
G10	{M × (M × L) F1} × L
G11	{M × (M × G) F1} × G
G12	{M × (M × G) F1} × G
G13	{M × (M × K) F1} × K
G14	{M × (M × K) F1} × K
G15	{M × (M × G) F1} × G
G16	{L × (M × K) F1} × G

Where: L=local teosinte landrace, and two imported landraces from CIMMYT; K= K67-5 and G= Guatemala and M=maize (Giza 2 variety).

Genomic DNA extraction: Genomic DNA was extracted from fresh young leaves of *Zea mexicana* by DNeasy plant mini-kit according to the procedure of the manufacturer's instructions (Qiagen, Valencia, CA, USA). The extracted DNA quality and quantity were measured by a Thermo Scientific NanoDrop 2000™ spectrophotometer at 260 and 280 nm.

Random Amplified Polymorphic DNA (RAPD)

RAPD-PCR was conducted using fourteen random primers provided by Eurofins, Germany. The selected primers; names and sequences are listed in Table (2). The reaction mixture contained 2 mM of MgCl₂; 1x GoTaq® Flexi buffer, 200 μM dNTPs; 20 pM primers; 20 ng of the template DNA, 1U of GoTaq® Flexi DNA Polymerase and the reaction mixture was topped up with distilled H₂O to 25 μl; (dNTPs, MgCl₂, reaction buffer and Taq DNA Polymerase were supplied from Promega (Promega Corporation, USA). The amplification reaction was performed in a

Gene Amp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, California, USA). The reaction program was conducted as follows: denaturation cycle of 94 °C for 5 min followed by 40 cycles; 94 °C for 1 min, 36 °C for 1min, 72 °C for 2 min, followed by an extension cycle at 72 °C for 7 min. The reaction was stored at 4 °C. A volume of 15 μl of the PCR product was examined on 1.5 % agarose gel according to Sambrook *et al.* (1989) using 1Kb DNA molecular weight marker (Thermo, GeneRuler). DNA fragments were visualized and photographed using the Molecular Imager ® Gel Doc™XR.

Automated Amplified Fragment Length Polymorphism (AFLP)

Automated AFLP was carried out using the AFLP Plant Mapping Kit (PE Applied Biosystems) following the manufacturer's protocols. The *EcoRI* primers E-AACE and ACT were labeled with 5-NED

and 5-FAM respectively, whereas *MseI* primers were unlabelled (Table 3). The five primer pairs that showed the best amplification of *Zea mexicana* DNA fragments were chosen for the followed selective amplification processes. The selectively amplified products were mixed with Hi-Di formamide and GeneScan 500 ROX internal size standards (Applied Biosystems, Foster City, California, USA) before the detection with ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, California, USA) using GS STRb POP-4 polymer as a molecular sieve.

Data Analysis

Only the sharpest RAPD bands were manually scored for further analysis. AFLP's fragment sizes and genotypes were analyzed using GeneScan and GeneMapper® Analysis Software Version 4.1 (Applied Biosystems, Foster City, California, USA). Several genetic diversity parameters were measured from RAPD and AFLP polymorphism data to compare the discriminatory power of these techniques for diversity assessment of *Zea mexicana* genotypes such as the number of observed alleles (N_a), the number of effective alleles (N_e) that were calculated according to the method of Hartl and Clark (1989) and Liu and Muse (2005). Also, Shannon index (SI) was determined by the method of Shannon (1949) using POPGENE software version 1.32. Polymorphic information content (PIC) as a value of a marker for detecting polymorphism within a population was calculated using Power Marker software version 3.25 (Liu and Muse, 2005). The multiplex ratio (MR) that represents the total number of loci detected per assay and the effective multiplex ratio (EMR) were estimated according to Powell *et al.* (1996). The marker index (MI) was used to calculate the overall utility of a marker system according to the following equation: $MI = EMR \times PIC$ (Tonk *et al.*, 2011). The pairwise comparisons between the tested genotypes were used to calculate the genetic similarity using the SPSS version 16.0 program according to the method described by Nei and Li (1979) and a dendrogram was constructed using the unweighted pair group

method using the arithmetic average UPGMA. Power Marker version 3.0 software was used by Mantel test to determine the significance of the correlation between the two genetic distance matrices of both marker systems (Mantel, 1967).

The STRUCTURE program version 2.3.1 using the Bayesian clustering method was used to calculate the more likely number of clusters (K) of the individuals through maximizing Hardy-Weinberg at each K (Pritchard *et al.*, 2000).

RESULTS AND DISCUSSION

Genetic diversity

Molecular markers are widely used for evaluating plant germplasm and genetic diversity for assisting genetic polymorphism, parentage determination, germplasm characterization, gene mapping, genetic distance as well as in marker-assisted selection (Meng *et al.*, 2018). In the current study, fourteen RAPD primers and five AFLP primer combinations were used to investigate the genetic polymorphism amongst sixteen *Zea mexicana* genotypes.

RAPD Polymorphism

The selected fourteen primers amplified a total number of 141 DNA fragments with fragment sizes ranging from 250 to 3000 bp across the 16 studied genotypes showing an average of 10.1 bands per primer. The number of amplicons/primers ranged from 6 with primers OPB15 and OPO20 to 15 with primer OPA 20. On the other hand, the number of the polymorphic bands was 102 which represented a percentage of 72.3% with an average number of 7.3 per primer. The number of polymorphic amplicons varied from 3 with primers OPB15 and OPO20 to 12 with primer OPA20. Primer OPM18 was the most efficient one with a percentage of polymorphism of 100%. On the other side, primers OPB15, OPC01, and OPO20 were only able to show 50% polymorphism amplifying 6, 10, and 6 bands respectively. Representative RAPD profiles obtained by primers OPA20, OPB02, OPB04, and OPC01 are shown in Fig. (1). The high polymorphism recorded is reflected in germplasm management; to detect genetic

diversity and to reveal genetic relationships. Positive and negative unique markers (PUMs and NUMs) were used in genotype identification and in generating a unique fingerprint for each genotype. Table (2) shows a total of 11 unique RAPD markers (4 PUMs and 7 NUMs) which were created by 6 primers. PUMs were generated by 4 primers which distinguish genotypes G6, G8, G14, and G16 while, NUMs successfully identified genotypes G3, G4, G5, and G12.

The present study results had a higher number of fragments (Figure 1 and Table 2) than that recorded by Sharawy *et al.* (2011) and Vivodík *et al.* (2015) on some teosinte and maize studies and agreed with the results of Ristic *et al.* (2013) who analyzed 21 genotypes with 7 primers and obtained an average of 10.8 bands per primer. Also, the present findings are

in partial agreement with that of Bauer *et al.* (2005) who detected 78.2 % polymorphic markers amongst 11 early maturing maize hybrids using 10 RAPD primers. On the other hand, Bruel *et al.* (2006) and Sharawy *et al.* (2011) obtained a higher level of polymorphism (84.44 and 99% respectively) studying maize inbred lines using RAPD markers. The difference in the level of polymorphism obtained is mainly attributed to the degree of divergence between the different genotypes studied. However, these results confirmed that the studied *Zea mexicana* genotypes exhibit high genetic variations. Based on these results, the RAPD marker is considered efficient for determining the genetic variability between *Zea mexicana* germplasm.

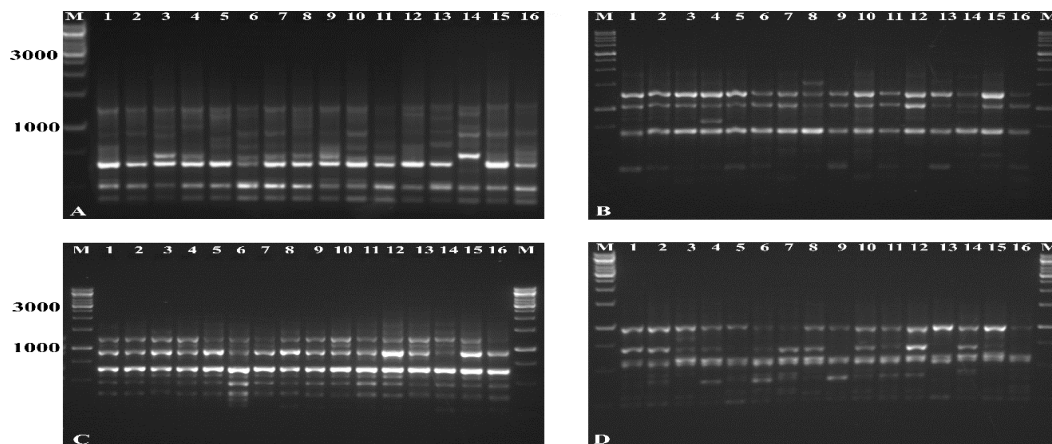


Fig. 1 Representative RAPD profiles for the sixteen *Zea mexicana* genotypes with primers OPA20 (A), OPB02 (B), OPB04 (C), and OPC01 (D) respectively, M is 1Kb DNA marker. Genotypes are numbered according to Table (1).

Table 2 List of RAPD primers with their sequences, the total number of amplified bands, number of polymorphic bands, rate of polymorphism, and the number of positive and negative primers and their corresponding genotypes.

Primer Name	Primer sequence (5'→3')	Total No. of bands	Polymorphic bands (%)	PUM		NUM	
				Number of bands/ primer	Genotypes showing positive unique markers	Number of bands/ primer	Genotypes showing negative unique markers
OPA 04	AAT CGG GCT G	8	6 (75.0%)	-	-	2	G5
OPA 10	GTG ATC GCA G	14	10 (71.4%)	1	G14	1	G12
OPA 20	GTT GCG ATC C	15	12 (80.0%)	-	-	-	-
OPB 01	GTT TCG CTC C	12	10 (83.3%)	-	-	1	G12
OPB 02	TGA TCC CTG G	10	7 (70.0%)	1	G16	-	-
OPB 03	CAT CCC CCT G	11	9 (81.8%)	1	G8	1	G4
OPB 04	GGA CTG GAG T	11	6 (54.5%)	1	G6	-	-
OPB 05	TGC GCC CTT C	7	4 (57.1%)	-	-	-	-
OPB 10	CTG CTG GGA C	9	7 (77.8%)	-	-	1	G3
OPB 15	GGA GGG TGT T	6	3 (50.0%)	-	-	-	-
OPC 01	TTC GAG CCA G	10	5 (50.0%)	-	-	-	-
OPC 09	CTC ACC GTC C	11	9 (81.8)	-	-	-	-
OPM 18	CAC CAT CCG T	11	11 (100)	-	-	-	-
OPO 20	ACA CAC GCT G	6	3 (50.0)	-	-	1	G4
Total		141	102 (72.3%)	4	4	7	4

PUM: Positive Unique Markers

NUM: Negative Unique Markers

AFLP Polymorphism

Five primer combinations were selected to measure the genetic diversity of the 16 *Zea mexicana* genotypes. A representative AFLP electropherogram generated by genotypes G8 and G10 using primer combinations E-AAC/M-CTG is represented in Fig. (2); common peaks are highlighted in black whereas grey bars represent its corresponding alleles. An example of a polymorphic peak that is found in sample G7 and not in G6 or G11 is indicated by an arrow (Fig. 2). Each of the five AFLP combination primers used in this study successfully differentiated each of the studied 16 genotypes (Table 3). They generated a total of 276 informative fragments across the 16 *Zea mexicana* genotypes, with an average of 55.2, out of them, 96.7% were polymorphic. These results are in agreement with these reported by

Legesse *et al.* (2006) who obtained a total of 334 AFLP bands, of which 275 (81.2 %) were polymorphic studying 21 CIMMYT maize inbred lines. Also, Hartings *et al.* (2008) analyzed the genetic diversity pattern in 54 Italian landraces using 10 primer combinations that produced 284 polymorphic AFLP bands with an average of 28 markers and the number of markers ranged from 12 to 46. While, Neha and Nam, (2016) reported that using eight AFLP primer combinations produced a total of 232 bands studying 78 maize lines. The polymorphism percentage is similar to that of Giordani *et al.* (2019), who obtained 97% polymorphism using 4 primer combinations studying 145 maize accessions, which was higher than that recorded by Neha and Nam (2016) who obtained a percentage of polymorphism of 67 % among maize lines.

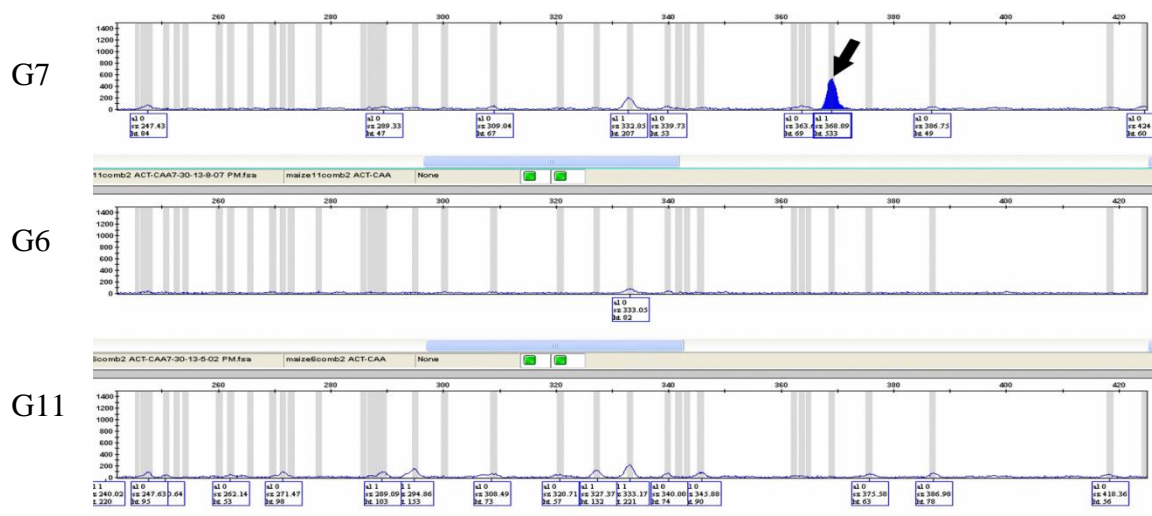


Fig. 2 Selected electropherograms of three *Zea mexicana* AFLP samples (G7, G6 and G11) using the primer combination E-ACT (FAM)/M-CAA. Arrow indicates an example of a polymorphic peak that is present in sample G7 and absent in G6 and G11.

These results confirm that AFLP analysis is clearly a powerful means of fingerprinting teosinte and effectively detect genetic variations in the 16 accessions studied. In addition, the highest number of unique markers (14) was recorded with combination E-ACT/M-CAA for genotypes G3, G4, and G8, while genotypes G1, G5, G7, G9, and G13 was characterized by the lowest number (6) using primer combinations E-AAC/M-CTA. Moreover, combination E-AAC/M-CTG produced the highest number of positive unique markers with the genotypes

G5, G7, G12, G13, and G16. Genotype G8 was successfully distinguished by primer combination E-ACT/M-CTA with a total of 10 unique markers (9 PUM and 1 NUM). While G1 was identified by 9 unique markers (6 PUM and 3 NUM). Each genotype had a unique banding profile for every AFLP primer combination. These results are in agreement with previous studies on maize using AFLP markers (Oliveira *et al.*, 2004; Legesse *et al.*; 2006 Hartings *et al.*; 2008, Neha and Nam, 2016 and Tomkowiak, *et al.*, 2020).

Table 3 AFLP analysis for the 16 *Zea mexicana* genotypes as identified by five primer combinations demonstrating the total number and the polymorphic bands, unique alleles, and the genotypes being identified by each combination.

Primer combinations name	Total No. of bands	No. of polymorphic bands		No. of positive Unique Markers	Genotypes showing positive unique alleles	No. of negative Unique Markers	Genotypes showing negative unique alleles	Total No. of Unique Markers	Total No. of genotypes identified by each primer combination
		No.	%						
E-AAC(NED)/M-CAC	43	42	(97.7)	9	G1, G4, G9, G10	3	G1	12	4
E-ACT(FAM)/M-CAA	40	39	(97.5)	10	G4, G8	4	G3,G8	14	3
E-ACT(FAM)/M-CTA	64	59	(92.2)	11	G1,G7,G10,G11, G14,G16	1	G8	12	7
E-AAC(NED)/M-CAA	59	57	(96.6)	4	G1, G7, G9, G13	2	G5, G13	6	5
E-AAC(NED)/M-CTG	70	70	(100)	12	G5,G7,G12,G13, G16	0	0	12	5
Total	276	267	(96.7)	46	21	10	6	56	12

Genetic relationships

Cluster analysis of RAPD data

The genetic similarity amongst the 16 genotypes ranged from 67.0 (between G12 and G14) to 90.0 (between G7 and G10) reflecting the high genetic variability among the genotypes under study. Dendrogram based on RAPD data matrices obtained with unweighted pair group method using arithmetic means (UPGMA) grouped the 16 *Zea mexicana* genotypes into five major groups (Fig. 4). The first one was separated from the others containing only two genotypes (G16 and G14). The second group contained three genotypes; G1, G2, and G5. Also, Genotypes G3 and G4 were grouped in a third group

according to their genetic background. Furthermore, the fourth group contained four genotypes; G9, G12, G13, and G15. The fifth group contained the remaining genotypes; G6, G7, G8, G10, and G11. These findings are in agreement with that of Bruel *et al.* (2006) and Souza, *et al.*, (2008) who constructed a dendrogram with five groups for selected maize lines based on the RAPD marker. Thus, the genetic relationships revealed by RAPD marker can be used to establish a consistent heterotic pattern between characterizing different maize lines (Carvalho *et al.*, 2004; Rinaldi *et al.*, 2007; Sharawy *et al.*, 2011; Balážová, *et al.*, 2016 and Vivodík, *et al.*, 2017)

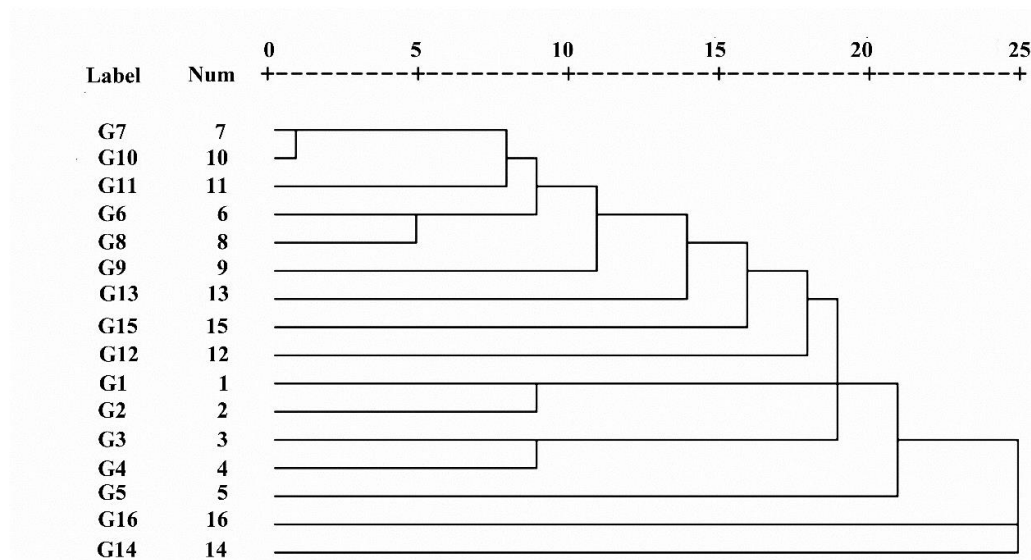


Fig. 3 Dendrogram constructed according to Nei and Li's coefficient using Unweighed Pair-group Arithmetic Average (UPGMA) and similarity matrix of the RAPD generated data for the 16 *Zea mexicana* genotypes.

.Cluster analysis of AFLP data

AFLP data created a genetic similarity that ranged from 15.0 between G8 and G11 to 64.0 between both G3 & G14 and G5 & G15 (data unpublished). AFLP dendrogram successfully separated the 16 *Zea mexicana* genotypes into five major groups (Fig. 5). The first group contained genotypes G7 and G16. The second group is comprised of three genotypes; G4,

G6, and G8. The third contained genotypes G1, G9, and G10. The fourth group is composed of genotypes G2, G11, G12, and G13. The remaining four genotypes were separated in the fifth group which had two closest pairs; (G3 and G14) and (G5 and G15). These findings are in agreement with that classifying maize lines based on AFLP markers data (Hartings *et al.*, 2008; Neha and Nam, 2016; Giordani *et al.*, 2019)

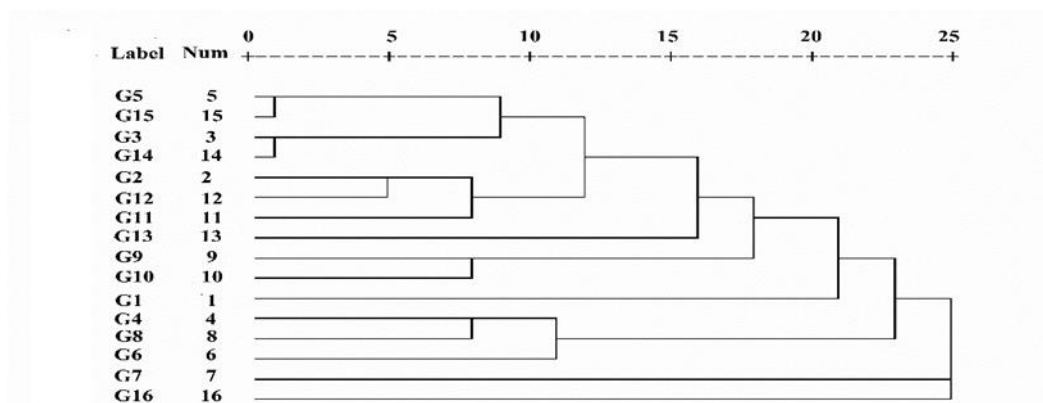


Fig. 4 Dendrogram constructed according to Nei and Li's coefficient using Unweighed Pair-group Arithmetic Average (UPGMA) and similarity matrix of the AFLP generated data for the 16 *Zea mexicana* genotypes

Cluster analysis of combining RAPD and AFLP data.

The estimated similarities generated from combining RAPD and AFLP data ranged from 52.0 for genotypes G8 and G11 to 73.0 for genotypes G9 and G10 (data unpublished). Combining RAPD and AFLP data (Figure 5) generated a more reliable relatedness representing the genetic relationships between the 16 *Zea mexicana* genotypes since combining the two molecular techniques provides extensive coverage of the *Zea* genome. Interestingly, the combined

dendrogram showed the same topography as that of AFLP, indicating that the AFLP marker would be the best-suited molecular assay to evaluate genetic relationships with high accuracy among maize inbred lines (Garcia *et al.*, 2004, Neha and Nam 2016 and Giordani *et al.*, 2019).

The incongruity between our pedigree for some genotypes and the dendrogram obtained could be due to the probability of natural selection, genetic drift, environmental effects, unintentional outcrossing, and/or mutations.

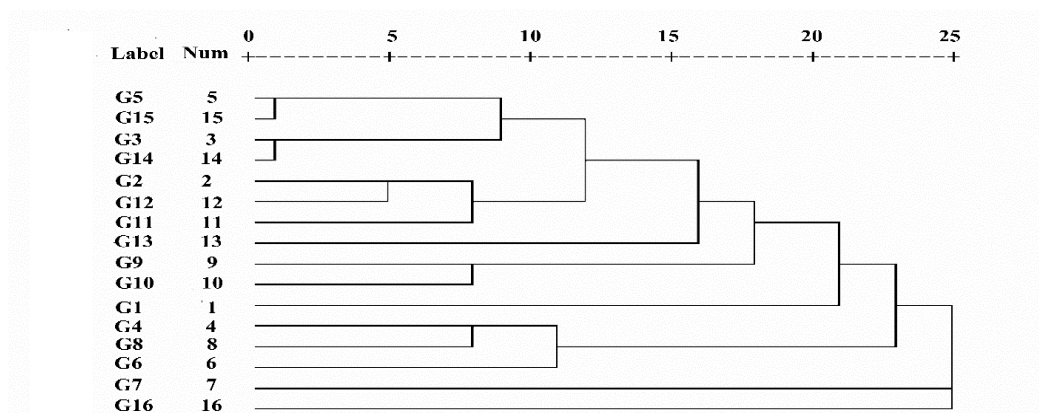


Fig. 5 Dendrogram constructed according to Nei and Li's coefficient using Unweighed Pair-group Arithmetic Average (UPGMA) and similarity matrix of the combined RAPD and AFLP generated data for the 16 *Zea mexicana* genotypes.

Population structure

Population structure has been frequently applied in many genetic studies to understand the genetic diversity among maize genotypes (Belalia *et al.*, 2019), to determine heterotic groups of maize germplasm lines (Boakyewaa Adu *et al.*, 2019), and to control false-positive associations between marker loci and phenotypic traits (Giordani *et al.*, 2019). The results of the population structure analysis (Fig. 7) confirmed that of the phylogenetic tree which showed that the most suitable ΔK (Fig. 6) was 5. Each color in the plot represents a separate population and the length of the colored segment shows the corresponding association of each sample to their respective population. The 16 lines were identified as mixed genotypes by structure analysis because these lines were derived from local teosinte landrace; Giza 2 (maize variety) and two teosinte lines from CIMMYT

(Table 1). Interestingly, genotypes G8 and G16 were separated into two groups. The third group contained G2, G11, and G15. The fourth one included G5, G3, G12, G1, G13, and G10. The remaining genotypes; G9, G7, G14, G4, and G6 constituted the fifth one. The lines were clustered based on their ancestry and selection history. However, the clustering of some lines was not based on their shared ancestry, indicating that inbred lines extracted from the same population do not necessarily have the same selection history (Boakyewaa Adu *et al.*, 2019 and Giordani *et al.*, 2019).

These results are following that of Warburton *et al.* (2011) which reported that the structural analysis of one hundred populations from six *Zea* taxa; domesticated (maize) and wild (teosinte); had high concordance to each other and were capable of establishing only five clusters.

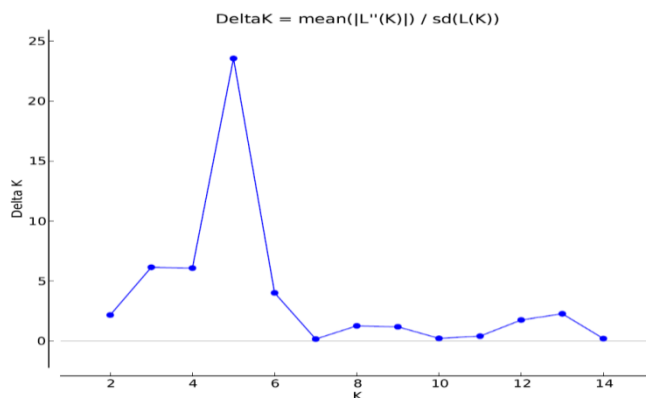


Fig.6 Bilateral chart representing the optimal number of K identified by Structure program.

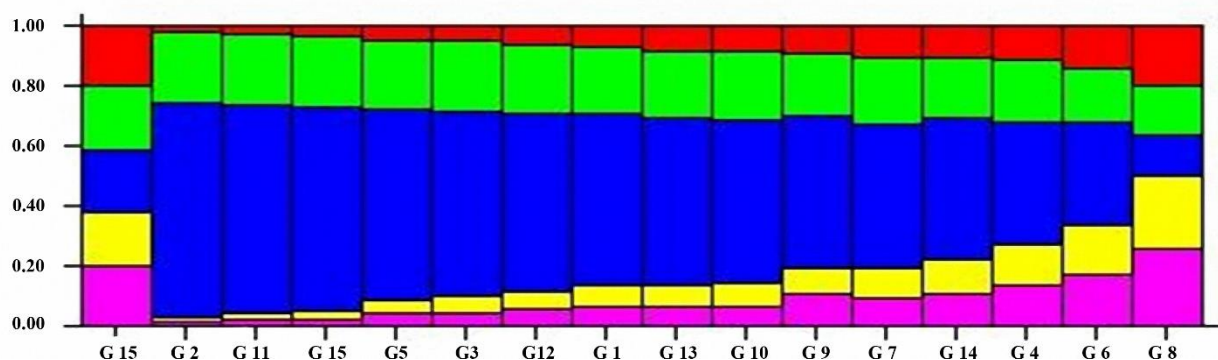


Fig.7 Population structure of the 16 *Zea mexicana* genotypes calculated using the combined data for RAPD and AFLP using K=5. Each color represents a separate subgroup and the length of the colored segment shows the estimated membership proportion of each sample to its corresponding group.

Comparing the efficiency of RAPD and AFLP in analyzing *Zea mexicana* genome:

The efficiency of both RAPD and AFLP markers to analyze the *Zea mexicana* genome was compared using fourteen RAPD primers and five AFLP primer combinations respectively (Table 4). From a total of 141 RAPD bands and 276 AFLP peaks, 102 represent 72.3% and 267 represent 96.7% fragments were found

to be polymorphic loci, respectively. Also, Tomkowiak, *et al.* (2020) compared the efficiency of AFLP, RAPD markers for determining the relationship among thirteen hybrids and nineteen inbred lines of maize. They obtained 528 AFLP markers and 234 RAPD markers using fifteen primer pairs and forty random oligonucleotide primers, respectively.

Table 4. Comparison between the efficiency of RAPD and AFLP for evaluating genetic diversity of the 16 genotypes of *Zea mexicana*.

Parameter	Marker	
	RAPD	AFLP
Number of assays screened	14 primers	5 primer combinations
Total number of bands	141	276
Multiplex ratio (MR)	10.1	55.2
Number of polymorphic bands	102	267
Polymorphism % per assay	72.3%	96.7%
Observed number of alleles (Na)	1.67 ± 0.47	2.00 ± 0.04
Effective number of alleles (Ne)	1.43 ± 0.39	1.30 ± 0.30
Effective Multiplex ratio (EMR)	7.3	53.4
Polymorphic information content (PIC)	0.15	0.21
Shannon index (I)	0.36 ± 0.29	0.33 ± 0.19
Marker index (MI)	1.10	11.21

PIC is considered as the better parameter for measuring genetic diversity than the number of alleles, taking into account the relative frequencies of each allele Aci *et al.* BMC Genetics (2018)

Moreover, Na, EMR, and PIC indices showed higher values for RAPD (1.67±0.47, 7.3, and 0.15) than for AFLP (2.00± 0.04, 53.4, and 0.21). On the other hand, the Shannon index (I) was fairly lower for RAPD (0.36± 0.29) comparing to that of AFLP (0.33±0.19). While the effective number of alleles (Ne) of RAPD was slightly higher (1.43 ±0.39) than that of AFLP (1.30 ±0.30). Moreover, the marker index (MI) of RAPD (1.1) was significantly lower compared to that of AFLP (11.21). These values agree with that obtained by Roy *et al.* (2015) and Neha and Nam (2016). Moreover, the level of polymorphism observed in this study agrees with that of Lubberstedt *et al.* (2000) and Oliveira *et al.* (2004). The

informativeness of the primer combinations can be ascertained by the PIC and MI values because the values indicate the discriminatory power of a marker system by taking into consideration the number of alleles at a locus and the relative frequencies of these alleles (Powell *et al.*, 1996). In this respect, we obtained relatively higher values of PIC (0.24) and MI (19.4) underlining the discriminatory power of the AFLP marker (Lubberstedt *et al.*, 2000 Oliveira *et al.*, 2004) and indicating the relatively high level of variability existing among the inbred lines under investigation. Moreover, Bahulikar *et al.* (2004) reported that AFLP showed a higher percentage of polymorphic loci than the ISSR marker. In contrast,

Biswas *et al.* (2011) obtained higher levels of polymorphism with ISSR than with AFLP, while Krichen *et al.* (2010) stated equal results for molecular markers. However, our results indicate that AFLP is the best-suited molecular assay for fingerprinting and assessing genetic relationships among tropical maize inbred lines with high accuracy. The correlation degree between similarity matrices obtained by both RAPD and AFLP indicated non-significant correlation values estimated through the “Manteltest” indicating that genetic diversity studies are very essential in the selection of individual genotypes among closely related groups.

CONCLUSION

Supporting the superiority of molecular marker data to define groups of populations with similar origins. The findings of the present study revealed that the two dominant markers (RAPD and AFLP) were successfully effective in measuring the genetic variation in the 16 *Zea mexicana* genotypes. Moreover, AFLPs have the advantage of having a higher marker index compared to that of RAPD, as well as its high reproducibility. The genetic diversity detected offers promising data for the development of new cultivars and the use of more than one variable marker enables a better exploring of the *Zea* genetic diversity providing a better tool for *Zea* breeding programs, registration, and varieties protection. Finally, the data obtained can be used for varietal survey and construction of germplasm collection and provides also additional information that could form the basis for the rational design of breeding programs. Supporting

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ABBREVIATIONS

(PIC) Polymorphism information content; (Na) observed alleles; (EMR) effective multiplex ratio; (RAPD) randomly amplified polymorphic DNA; (AFLP) Amplified fragment length polymorphism.

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