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Molecular Characterization for Some Maize Genotypes Using ISSR

Abdullah Farag Mohamed Abogmiza
Botany Department, Faculty of Science,
Al-Asmarya Islamic University
Zliten City, Libya
e-mail: abdullhzliten@yahoo.com

Ahmed Fahmy Houssien AboMolecular
Molecular Biology, Genetics Department, Faculty of Agriculture, Ain Shams University
Cairo City, Egypt.

Fawzy omran mohammed Elqarnwdy; and
Hajer Mohammed Makhzoum Alsouyid
Botany Department, Faculty of Science,
Al-Asmarya Islamic University
Zliten City, Libya.

***Abstract*— Biodiversity among eight maize cultivars was assessed on the agronomical level using some yield related traits such as plant height, number of leaves/plant, number of corns/plant, grain yield/plant and on the molecular level using ISSR protocol. The agronomic traits showed a variation among the eight genotypes, as plant height ranged from (218 cm) in genotype Fardy3 to (147 cm) in Seds7 while, the number of leaves/plant ranged from 14 in Fardy3 to 7 in Seds7. In respect of number of Ears/plant, the trait ranged from 4 in genotypes Fardy3 and Fardy1 to 1 in Seds7. In grain yield/plant, the trait ranged from 419g in Fardy3 to 185g in Seds7. The results of ISSR also revealed a kind of variation in the banding patterns among the eight maize genotypes under investigation. The results of the consensus tree and similarity matrix showed that, Giza breeding program and Gimiza breeding program could be shared in common ancestors, while Fardy3 seems to be derived from different ancestors and different breeding program.**

Keywords— Maize; yield; genotype; breeding.

I. INTRODUCTION

Maize is considered a vital cereal crop all over the world as it plays a significant role in maintaining food safety and security, coping the development of human and animal consumption. Thus, improvement of grain yield is consistently one of the most important objectives for maize breeders. In maize, grain yield is a complex quantitative trait controlled by many quantitative traits loci (QTL) with a small effect [1]. A better comprehension of the genetic architecture and molecular mechanisms of yield-related traits could help to improve grain yield in maize.

Marker-assisted selection (MAS) is an efficient method of selection for yield and its related traits improvement in the process of crop breeding. The tightly linked markers that found in the genetic population and yield-related and its related traits should be identified before using in the MAS for crop breeding process [2].

QTL meta-analysis is one of the most effective methods to identify the genomic hotspot regions which control target traits more frequently and narrow down the confidence intervals of these QTLs to produce the meta QTLs (MQTLs) by integrating information from different mapping populations [3,4]. Semagn et al. [5], Wang et al. [6] and Martinez et al.[7] conducted different examples proofing that meta-analysis is the best method for characterizing candidate genes and developing molecular markers assisted selection for quantitative traits in maize. Previous studies have investigated many QTLs for yield-related traits by integrating different QTL data from maize and some of their relatives' yield-related genes using bioinformatics. Based on this strategy, two potential candidate genes for grain size and weight were identified successfully in rice [8].

As reported by Zietkiewicz et al. [9] Inter simple sequence repeat (ISSR) markers that are progressed by utilizing repeated microsatellites primers to expand inter-SSR DNA sequences. Many microsatellites anchored at 5' end or 3' end are utilized for amplifying genomic DNA that increase primer's specificity in PCR reaction as stated by Godwin et al. [10]. Unlike other PCR- based techniques, ISSR does not require any information about the targeted sequence of SSR regions [11]. Advantages of ISSR secure relatively low cost, high polymorphisms, and better reproducibility. ISSRs are highly rigid marker for achieving ISSR analysis as the random amplified markers are artificial or homoplasious bands [12, 13].

This investigation aimed to develop some molecular markers assisted selection for some yield related traits using ISSR in maize and to study the genetic relationships among eight maize cultivars.

II. MATERIALS AND METHODS

Eight maize cultivars namely, Giza1, Giza13, Giza46, Seds7, Fardy1, Fardy3, Gimiza11 and Gimiza15 were selected and obtained from Agriculture research center, Giza, Egypt. These cultivars were grown in the field experiment with three replications during the season 2017-2018. Some yield related traits such as plant height, number of leaves/plant, number of corns/plant, grain yield/plant were measured in the end of the experiment.

Genomic DNAs were essenced from young leaves of the aforementioned maize cultivars according to Janghans and Metzalat [14]. Nucleotide sequences of the 6 used primers for inter simple sequence repeat (ISSR) PCR amplification and their annealing temperatures are indicated in Table (I). PCR amplification was performed according to Zietkiewicz et al. [9] with 25 ng of template genomic DNA, 5 uM of primer, 2.5 mM of dNTP, and 1 unit of Taq polymerase, 3µl $MgCl_2$, 2.5 µl 10X buffer H_2O up to 25 µl total volume. The PCR annealing condition utilized consists 35 cycles of 94^{o}C for 30 sec, 50^{o}C for 30 sec and 72^{o}C for 1 min. Amplified products were checked on 1.2% agarose gel electrophoresis. Bands were stained using ethedium bromide in UV transilluminator. Banding patterns were scored as 1 for present band and 0 for the absent one. Similarity index and consensus tree were performed using SPSS computer program on the base on the base of banding patterns overall the used six ISSR primers.

TABLE I. SEQUENCES AND ANNEALING TEMPERATURES OF THE SIX USED ISSR PRIMERS

No	Primer sequence	Temp. (^{o}C)
1	AG(GA)8	50
2	AC (CT)8	50
3	T(CT)9	50
4	CA(GCT)6	55
5	TA(CAG)4	45
6	GG(CA)6	45

III. RESULTS AND DISCUSSIONS

A. *Yield Related Traits*

Different yield related traits were measured and used as parameters for genetic variation among the eight maize cultivars on the phenotypic level. The results are shown in (Table II) and (Figures 1-4). The results indicated that the highest in plant height was Fardy3 (218 cm) while, the lowest was Seds7 (147 cm). In number of leaves/plant, the trait ranged from 14 in Fardy3 (the highest) to 7 in Seds7 (the lowest). In respect of number of Ears/plant, the trait ranged from 4 in Fardy3 and Fardy1 (the two highest) to 1 in Seds7 (the lowest) of the trait. In grain yield/plant, the trait ranged from 419 g in Fardy3 (the highest) followed by Fardy1 (368 g) to 185 g in Seds7 (the lowest) of the trait.

TABLE II. SOME YIELD RELATED TRAITS FOR THE EIGHT MAIZE GENOTYPES UNDER INVESTIGATION

genotype	Plant height cm	N Leavs/plant	N Ears/plant	Grain yield/plant g
Giza1	174	10	2	272
Giza13	180	10	2	236
Giza46	201	11	3	315
Seds7	147	7	1	185
Fardy1	189	12	4	368
Fardy3	218	14	4	419
Gimiza11	165	9	2	218
Gimiza15	172	10	2	243

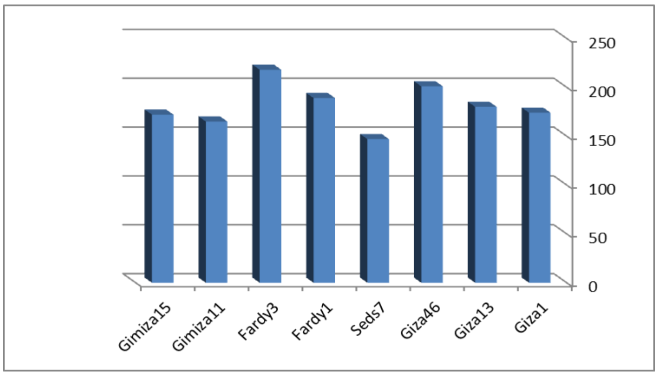

Fig.1. *Plant height/cm for the eight maize genotypes under investigation.*

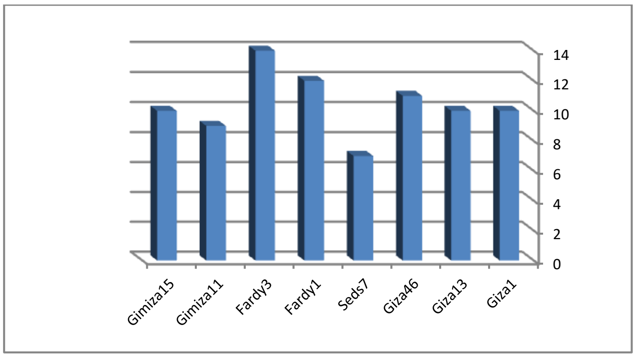

Fig.2. *N. Leaves/plant for the eight maize genotypes under investigation.*

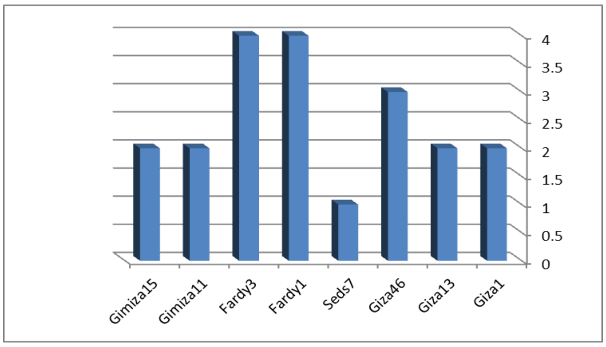

Fig.3. *N. Ears/plant for the eight maize genotypes under investigation.*

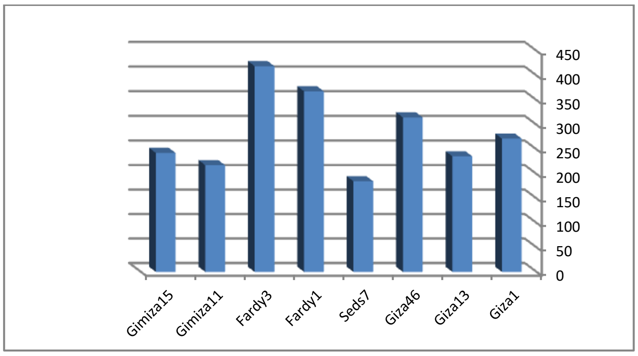

Fig.4. *Grain yield/plant for the eight maize genotypes under investigation.*

B. Molecular Study Using ISSR

The six used ISSR primers produced a total of 34 amplified fragments overall the eight maize genotypes. Out of these produced bands, 22 bands were common and 12 bands were polymorphic ones. The six used ISSR primers produced 3 positive and 3 negative markers for a particular maize genotype. The ISSR data as shown in Table (III) and Figures (5, and 6) indicated that, P1 produced 7 bands ranged from 315 to 85 bp 4 out of them were common bands (produced in all maize genotypes under investigation) and 3 were polymorphic bands. Out of the three polymorphic bands, 2 positive markers with sizes of 235 and 110 bp for genotype Fardy3 and one negative marker with size of 280 bp for genotype Seds7. P2 produced 4 bands ranged from 270 to 75 bp 3 out of them were common bands and 1 was polymorphic band. This band was negative marker with size of 215 bp for genotype Fardy3. P3 produced 5 bands ranged from 255 to 65 bp 3 out of them were common bands and 2 were polymorphic bands. Out of the two polymorphic bands, one positive marker with size of 205 bp was generated with genotype Fardy3. P4 produced 8 bands ranged from 370 to 80 bp. Five out of them were common bands and 3 were polymorphic bands. Out of the three polymorphic bands, one negative marker with size of 215 bp was showed in genotype Fardy3. P5 produced 4 bands ranged from 250 to 100 bp.

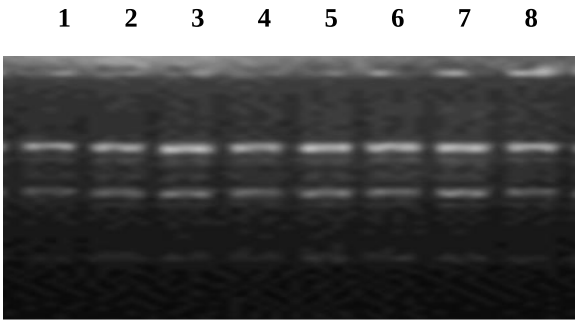

Fig.5.*Resolution of primer 3 with the eight maize genotypes under investigation*

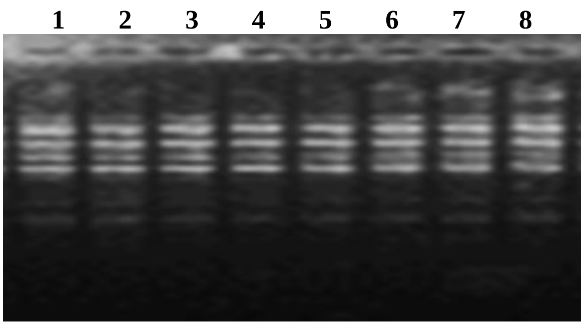

Fig.6. *Resolution of primer 4 with the eight maize genotypes under investigation.*

TABLE III: 0/1 OF THE SIX ISSR PRIMERS OVERALL THE EIGHT MAIZE GENOTYPES

P	bp	Giza 1	Giza 13	Giza 46	Seds 7	Fardy1	Fardy3	Gimiza 11	Gimiza 15
P1	315	1	1	1	1	1	1	1	1
	280	1	1	1	0	1	1	1	1
	235	0	0	0	0	0	1	0	0
	190	1	1	1	1	1	1	1	1
	155	1	1	1	1	1	1	1	1
	110	0	0	0	0	0	1	0	0
	85	1	0	1	0	1	1	0	0
P2	270	1	1	1	1	1	1	1	1
	235	1	1	1	1	1	0	1	1
	160	1	1	1	1	1	1	1	1
	75	1	1	1	1	1	1	1	1
P3	255	1	1	1	1	1	1	1	1
	205	0	0	0	0	0	1	0	0
	160	1	1	1	1	1	1	1	1
	100	1	0	1	0	0	0	1	1
	65	1	1	1	1	1	1	1	1
P4	370	1	1	1	1	1	1	1	1
	340	1	1	1	1	1	1	1	1
	295	1	1	1	1	1	1	1	1
	215	1	1	1	1	1	0	1	1
	175	1	1	0	0	1	0	1	0
	140	0	0	1	1	0	1	0	1
	90	1	1	1	1	1	1	1	1
	65	1	1	1	1	1	1	1	1
P5	250	1	1	1	1	1	1	1	1
	200	1	1	1	1	1	1	1	1
	135	1	1	1	1	1	1	1	1
	95	1	1	0	1	0	1	1	0
P6	288	1	1	1	1	1	1	1	1
	230	1	1	1	1	1	0	1	1
	175	1	1	1	1	1	1	1	1
	120	1	0	1	1	0	1	0	1
	90	1	1	1	1	1	1	1	1
	65	1	1	1	1	1	1	1	1

Three out of them were common bands and 1 was polymorphic band with size of 100 bp. Finally, P6 produced 6 bands ranged from 288 to 65 bp. Four out of them were common bands and 2 were polymorphic bands. One of them was negative marker with size of 230 bp for faedy3 genotype. As the genotype faedy3 was considered as the highest in the studied traits, all the generated ISSR markers with this genotype overall the used primers could be considered as markers for these traits.

ISSR data were used to estimate genetic similarity matrix values based on Jaccard's coefficient. The similarity values were further used to construct a phonetic dendrogram revealing the genetic relationships based on UPGMA (un-weighted pair group method using arithmetic averages). The thirty four ISSR amplified fragments out grouped the eight maize genotypes under investigations as shown in Figure (7) into two main clusters. The first main cluster included maize genotype Fardy3 and the second main cluster classified into two sub-clusters. The first sub-cluster included genotypes Giza1, Giza13, Gimiza11 and Fardy1 while the second sub-cluster included genotypes Giza46, Gimiza15 and Seds7. These results showed that Giza1 and Fardy3 were the most distantly related genotypes. On the other hand, Giza1 and Giza13 were the most closely related genotypes.

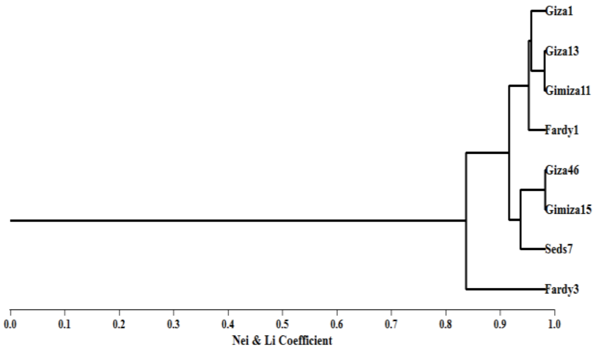

Fig.7. *Cluster analysis with UPGMA method of related eight maize genotypes using 6 primers of ISSR data based on Jaccard similarity matrix.*

TABLE IV. SIMILARITY INDEXES AMONG THE EIGHT MAIZE GENOTYPES UNDER INVESTIGATION

	Giza1	Giza 13	Giza 46	Seds7	Fardy1	Fardy3	Gimiza 11	Gimiza 15
Giza1	1.000							
Giza13	0.947	1.000						
Giza46	0.949	0.893	1.000					
Seds7	0.912	0.926	0.929	1.000				
Fardy1	0.947	0.963	0.929	0.889	1.000			
Fardy3	0.847	0.821	0.862	0.857	0.821	1.000		
Gimiza11	0.966	0.982	0.912	0.909	0.945	0.807	1.000	
Gimiza15	0.931	0.909	0.982	0.945	0.909	0.842	0.929	1.000

Genetic distances measured using Nie-72 genetic distance coefficient of the polymorphic fragments generated from ISSR marker are shown in (Table 4) indicated that (Giza13 and Gimiza11) and (Giza46 and Gimiza15) were the most closely related genotypes with distance 0.982 followed by (Giza1 and Gimiza11) with similarity value of 0.966 while Fardy3 and Gimiza11 were the most distantly related with similarity value 0.807. These results indicated that Giza breeding program and Gimiza breeding program could be shared in common ancestors, while Fardy3 seems to be derived from different ancestors and different breeding program.

These results were similar to the findings of [15] where they reported that, based on diversity, different type of markers like as morphological, phenological and agronomic characteristics are available and these have conventionally been used for estimating genetic variation. However, many traits are governed by polygenic and influence by environmental conditions and therefore, are difficult to evaluate with accuracy. Molecular markers have been proven to be powerful way in the assessment of genetic variation and in elucidation of genetic relationship within and among species. The results of Abdel-Tawab et al. [16] successfully verified the variation among nine citrus cultivars, where they used ISSR as marker to achieve their desired goals. Amaral Júnior [17] aimed to assess maize variability utilizing inter-simple sequence repeats (ISSR) markers. From 52 accessions, nine groups were established at a cutoff value of 0.36 on the abscissa in the UPGMA dendrogram. Among all the genotypes, the ancestors Tripsacum sp. and teosinte were the most divergent, that enhancees the efficiency of the ISSR technique. Teosinte was in proximity in maize more than Tripsacum sp. Although, belonging to various heterotic groups, the dent and flint types of the common maize assembled in the first group assured that the compared analysis of diversity of genetic was more noticed. In the third group, however, all the popcorn cultivars for Brazil were gathered, besides wild materials of popcorn, from different countries, thus giving a reason for breeders to be cautious and avoid the selection of materials from genetically close parents in the breeding programs.

IV. CONCLUSION

Results of ISSR have revealed a kind of variation in the banding patterns among the eight maize genotypes under investigation. The results of the consensus tree and similarity matrix showed that Giza breeding program and Gimiza breeding program could be shared in common ancestors, while Fardy3 seems to be derived from different ancestors and different breeding programs.

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