Genetic Variation Among Three Zea Mays L. Cultivars in Iraq

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Abstract

Maize is a very fertile and widely environmental grown crop and it globally cultivated. The purpose of our research was to determine genetic variation among three Zea mays L. cultivars (Almaha, Drachma and Talar F-1). The primer ITS1 and ITS4 used as a molecular marker in a conventional Polymerase Chain Reaction (PCR) with a 290 bp amplification outcome. The nucleotide sequences of amplification products were analyzed, sequence alignment was significantly revealed which confirming Zea mays diagnosis. Furthermore, analysis of genetic relationship revealed a neighboring relationship between Talar F-1 and Almaha cultivars (93), whereas phylogeny scheme diagram clearly showed presence of Drachma cultivar in other clusters (77).

Key word: Zea mays, PCR, Sequences confirmative, Phylogenetic tree.

1. Introduction

One of the most widespread crop species is Zea mays L. possessing a wide range of morphological and physiological characteristics, it also has a wide variation in its DNA sequences. Maize growth in a variety of environmental conditions, include temperate and tropical climates all over the world because of its genetic diversity. High-yielding cultivars contribute in meet the need of food demand has ability to grow under biotic and abiotic stress and it develop and resistance difficulties in the fight against insects and infections. It belongs to the Magnoliophyta division (flowering plants), the Poaceae family (Grass family), genus Zea L., Species Zea mays (corn). However, after wheat and rice, maize is the world’s third largest crop, recognized as the “Queen of Cereals” and use in various industrial products such as glucose, starch and oil. The genus Zea mays L. has been divided into two divisions[3], the first is includes Z.diploperennis, Z.luxurians and Z.perennis, and the second which includes Z. mays ssp. Mays, Z.mays ssp. Mexicana, Z.mays ssp. Huehuetenangensis and Z.mays ssp. Parviglumis[4]. Maize genetic diversity is very important for breeding[5]. It is an essential criterion in classification of maize at molecular basis using PCR technique. Many studies were achieved on maize using several application like simple sequence repeats by [6], randomly amplified polymorphic DNA (RAPD-PCR) by [1-19]. AFLPs stand for amplified fragment length polymorphisms [7,20], also single molecular real time sequencing method (SMRT) which was used successfully in order to determine the sequences of genomic insertion and flanking of the maize line (ShSNAC1-382) of the tolerance of transgenic drought [13]. The aim of this study was estimation of the genetic diversity of three of Zea mays cultivars in Nineveh governorate depending on sequence alignment.

2. Materials and Methods

2.1. Seed collection and cultivation

Seeds were obtained from (Agricultural research center Baghdad) and cultivated during the growth season of Zea mays (February, 2020). Ten seeds of each cultivar were cultivated into each plastic pots and the cultivation period was extended spring season (March, 2020).

2.2. Molecular screening profile Genomic DNA (gDNA) extraction

gDNA was extracted from frozen fresh leaves (100 mg) of fifteen samples which divided to three groups according to originated cultivars as five samples for each cultivar, using Wizard genomic DNA purification kit (GeneAll, S. Korea), according to manufacture instructions. DNA concentration was estimated by Quanta fluorometer (Invitrogen, UK), which was...
standardized to 10 ng/µl, then it preserved at (-20) until to use [8]. DNA integrity was evaluated via Owl electrophoresis systems (Thermo,USA). Agarose powder (0.7 gm) was added to TBE (Tris-borate, EDTA-1X) buffer(Bioneer,S.Korea), Green Star DNA staining dye (Bioneer, S.Korea) was added to the mixture (50µl/ 200ml). After boiling and cooling, the mixture was placed in the electro- phoresis tank, 5µl of gDNA (10-20ng/µl) was mixed with 1µl of loading dye (methylene blue), then placed in the wells after adding TBE buffer. The power supplying was programmed at 80 volt for 1 hour of electrophoresis, DNA molecular marker 100bp.(3000-100bp.) was supplied by Bioneer (S. Korea) for utilizing in this field [8].

### 2.3. Genetic determinants amplification and sequencing alignment

Amplification mixture included, Master Mix 10µl (Wizbio,S.Korea), 1µl (10µM) of each primer set as forward and reverse (Ella-biotech,Germany) and 4µl of gDNA(10ng/µl), the final volume was set to 20µl, completed the final volume of mixture by 4µl nuclease free water. The ITS1,ITS4 primer set that used in this research was listed in table (1). Amplification steps were initial denaturation 95°C for 4 min, followed by 35 cycles of each denaturation at 95°C for 30 sec, annealing 60°C for 30 sec to hybrid the primers, 30 sec of extension at 72°C, followed by 5 min at 72°C for final extension. Gel page software (Syngene documentary system,UK) was depended for detection of the size bands. Gel purification kit was used to isolate the amplicons (Gene All,S.Korea). Sequence of products was detected using genetic analyzer (Applied Bio- systems 3500.USA). The sequence was confirmed by National Center for Biotechnology Information (NCBI), using The Basic Local Alignment Search Tool (BLAST).Whereas, the sequences of current maize cultivars were aligned via MegaX-Software application.

| Gene      | Sequence 5'-3'                  | Amplicon size (bp.) | Reference         |
|-----------|---------------------------------|---------------------|-------------------|
| ITS-1     | TCCGTAGGTGAACCTGCGG             | 290                 | White, et al (1990) |
| ITS-4     | TCTCCGCTTATTGATATGC             |                     |                   |

### 3. Results and Discussion Maize cultivars variation

The morphological characteristics showed high similarity among Zea mays cultivars, therefore it depended on molecular investigation to find real differences among maize. Molecular investigation assists to detect and classify Zea mays. Figure (1) showed the amplification products of ITS1, ITS4 gene (290bp.) for ten samples belong to maize cultivars (Almaha, Talar-f1 and Drachma). The sequence of amplification products was analyzed as a confirmative diagnosis of previous cultivars and determine the variance among them in this study.

![Figure 1](image-url)  
*Figure 1. Amplification products of ITS1, ITS4gene. Line-M,DNA marker (100bp.). Line1-10 were positive result at 290(bp.). Line 1-4 represent Almaha ,line 5-7 represent Talar-f1, and line 8-10 represent Drachma cultivar.*

The present outcomes of ITS1,ITS4 gene amplification products was agreed with many studies were achieved using conventional PCR for diagnose and classify of maize [10], who detected some of genetically modified cultivars of maize in Iraq and demonstrated 10 of all 72 cultivars. Another research was carried out by[11],who utilized DNA markers to evaluate maize lines and their hybrid based on male sterility in the cytoplasm, the type of lines and hybrid sterility were determineusing conventional PCR using particular primers for cytoplasm types C and S ,and the study of multiple PCR of genetically modified Soybean, Maize and Canola, two sites of the DNA template were extracted from genetically modified maize with the event 176 (European corn borer resistant) ,the amplification product of 152bp (expected) and 485bp (unexpected) [14].
3.1. Zea mays sequence Alignment

The sequences of ITS1, ITS4 gene amplicons was depended to confirm the diagnosis of maize. Three subspecies of *Zea mays* cultivars (Almaha, Drachma and Talar f-1) were selected for this investigation, sequence alignment revealed high identification (99%) among present cultivar sequences and subject sequences from NCBI (Figures 2, 3 and 4).

![Figure 2](image1.png)

**Figure 2.** Confirmative of ITS1-ITS4 gene sequence of *Zea mays*. Query is present sequence and subject is a comparative sequence. Identification percent 99% *Zea mays* ssp. *Mexicana*, pi 384060.

![Figure 3](image2.png)

**Figure 3.** Confirmative of ITS1-ITS4 gene sequence of *Zea mays*. Query is present sequence and subject is a comparative sequence. Identification percent 99% with *Zea mays* ssp. *Mexicana* ID: AF019817.1.

![Figure 4](image3.png)

**Figure 4.** Confirmative of ITS1-ITS4 gene sequence of *Zea mays* sequence. Identification percent 99% *Zea mays* ssp.mays USDA PI 214195.
3.2. Zea mays genetic variation

The results of Figure (5) showed the alignment of 15 sequences of ITS1-ITS4 gene of maize cultivars (Drachma, Talarf-1 and Almaha). There were a high similarity among each cultivar subspecies.

In addition, phylogenetic schematic illustration revealed two clusters, minor cluster involved four Drachma subspecies, and major cluster composed of eleven subspecies one of them belong to Drachma cultivar and five represented Almaha and five as Talarf-1 (figure 6), this means that the last two cultivars have a nearly neighboring relationship, these present results were agreed with [15], who studied the genetic diversity of 20 maize genotypes which analyzed using (SCoT) markers and the analysis of hierarchical cluster revealed that maize genotypes were divided into two main clusters (cluster1) which contains two genotypes and (cluster 2) contains 18 genotypes and were divided into two sub clusters, so that the study of genetic variation provide a good conservation of the genetic resource of maize.

The genetic variation showed a more similarity between Almaha and Talarf-1 subspecies, as they appeared in one cluster (93), whereas less similarity value was appeared clearly among the previous subspecies and Drachma cultivar, 77 (figure 7). Present outcomes were agreed with that documented by [20], who determined the distance indices among 10 cultivars of Zea mays via sequence alignment and phylogenetic tree illustration that showed the relationship among the maize cultivars.
Figure 7. Zea mays Phylogenetic tree, Talar-1, Almaha showed high similarity with one strict cluster, Drachma revealed distance in contrast with previous two cultivars.

Conclusion

Molecular screening was more accurate for classification and of Zea mays cultivars, while phenotypic diagnosis revealed weakly outcomes for classification and diagnosis of Zea mays cultivars. Phylogenetic tree showed a clear confirmative among Talar f-1, Almaha and Drachma cultivars and those known sequences (Subjects) of maize cultivars retrieved from NCBI. Similarity values was differ according cultivar origin. The present outcome assist to establish future work with more experiments focus on the yielding of subspecies of each cultivar and screening of many gene expression that have a role in growth factors activation of many process in the cell during growth season.

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