Biochemical Study of maize (*Zea mays* L.) genotypes through total seed protein by SDS - PAGE

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Abstract

Maize (*Zea mays* L.) is one of the world's most important cereals, serving as a primary source of subsistence and industrial products. The remarkable phenotypic diversity exhibited by different maize genotypes depends on their complex biochemical compositions. In pursuit of a deeper understanding of the genetics and biochemistry of maize, this research explores the overall seed protein profiles of diverse maize genotypes using Sodium Dodecyl Sulfate-Polyacryla-mide Gel Electrophoresis (SDS-PAGE). Primary objectives include comprehensive analysis and comparison of total seed protein composition between distinct maize genotypes, identification of protein differences associated with phenotypic differences, and insight into the genetic and environmental determinants of maize seed protein composition. Through state-of-the-art analytical techniques, including SDS-PAGE, This research examined protein supplements found in corn seeds. The results promise to reveal the genetic and environmental factors that shape the biochemistry of corn. The patterns of the total protein gave a number of 12 main bands, whose weights ranged between 17 kDa and 245 kDa. The total number of bands reached 108 bands, including 8 homogeneous bands and 6 heterogeneous bands. . The percentage of variation (difference) between the compositions was 32%, which indicates that the percentage of similarity (similarity) was 68%. The level of similarity is higher than the level of dissimilarity between the studied structures and this is due to the geographical origin of the structures and their genetic material, or that the plant is the result of hybridization between species, which ultimately benefits humanity's quest to achieve sustainable agriculture and improved nutrition.

Keywords: *Zea mays*, SDS-PAGE, genotypes

Introduction

The maize plant (*Zea mays* L.) has long been a cornerstone of global agriculture, serving as a vital source of food, fodder, and industrial products [1]. Its versatility and adaptability have made maize a staple crop in many parts of the world, contributing significantly to global food security [2]. Within the realm of maize research, one area

of particular interest revolves around the biochemical characterization of maize genotypes, with a specific focus on their total seed protein composition [3]. This research endeavors to delve into the intricate world of maize biochemistry, unveiling the unique protein profiles that underpin the phenotypic diversity observed in different maize genotypes [4].To embark on this scientific journey, we must first establish the foundational importance of maize as a crop and the significance of understanding its biochemical composition. Drawing upon seminal scientific sources and the pioneering work of esteemed researchers in the field, we set the stage for a deeper exploration of maize genetics and biochemistry [5].

Maize, also known as corn, is a cereal grain of utmost agricultural importance worldwide. With its origins dating back to ancient civilizations in the Americas, maize has evolved from a humble wild grass to one of the most widely cultivated crops globally [6] . The maize plant exhibits remarkable genetic diversity, manifested in an array of phenotypes that vary in color, size, shape, and adaptability to diverse environmental conditions [7] .This technique was used by to diagnose 7 varieties of bread wheat (Triticum aestivum) [8] . While In addition, the technique was also used in the genetic characterization of 22 tomatoes (*Lycopersicon esculentum* L.) Genotypes [9] . At the heart of maize's remarkable diversity lies its intricate biochemical composition. The plant's genes orchestrate the synthesis of a multitude of proteins, each with its unique role in plant development, growth, and adaptation to environmental challenges. The total seed protein composition, a complex ensemble of proteins, encapsulates the genetic makeup and environmental interactions of maize genotypes [10] .This research is driven by a compelling rationale—to unravel the biochemical intricacies that define maize genotypes through the study of their total seed protein profiles. The primary objectives of this research are as follows:

To comprehensively analyze and compare the total seed protein composition of distinct maize genotypes.

To identify variations in protein profiles that may underlie phenotypic differences among maize genotypes [11] .To gain insights into the genetic and environmental factors influencing maize seed protein composition [12]. The significance of this research extends beyond the realm of basic science. By elucidating the biochemical intricacies of maize genotypes, we aim to Contribute to a deeper understanding of the genetic and environmental factors shaping maize biochemistry [13] . Provide valuable insights for crop improvement programs by identifying protein markers associated with desirable traits.Enhance our knowledge of the intricate interplay between genotype, environment, and protein composition in maize [14]. The biochemical study of maize genotypes through the analysis of their total seed protein profiles holds profound implications for both agricultural science and food security[15] . As the global population continues to burgeon, the need to optimize crop performance, nutritional content, and environmental resilience becomes increasingly imperative [16] . This research aligns with the broader goals of enhancing crop productivity, improving nutritional quality, and mitigating the impacts of environmental stressors on maize cultivation [17].

In this following study, we explore biochemistry for ten genotypes of maize, drawing inspiration from the rich tapestry of maize research. By leveraging cutting-edge techniques and methodologies, we aim to uncover the biochemical secrets that make each maize genotype unique. Through this journey, we aspire to contribute to the advancement of our understanding of maize genetics and biochemistry, ultimately benefiting both global agriculture and the well-being of humanity.

Materials and Methods
Plant Material

Seeds of ten maize genotypes Sumer, Fajr1, Oryx, Baghdad3, and Nahrain hybrids were obtained from the Research and Development Department of the Ministry of Agriculture while DKC 6777, ZP.glorya, PIOWEE R, KWS and Syngenta were obtained from a commercial supplier. The genetic composition of each variety was determined through Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Protein Extraction

Total seed protein was extracted using the Tris-HCl/Phenol method [9]. With some simple modifications, the seeds of each variety were ground, and the seed powder weighing 0.02 grams was taken, then it was placed in an absorbent tube, and 200 microliters of protein extraction buffer solution (25 mM Tris base, PH 8.8) was added to it. The powder was crushed using a micropestil to facilitate mixing of the powder with the solution, and mixed with a vortex for a minute to ensure complete mixing. The mixture was then stored in the refrigerator at 8°C for an entire night. To extract the protein, then centrifuge at 10,000 rpm for 15 minutes and collect the supernatant. Then the extract of the protein mixture was dissolved in an equal volume of buffer solution (0.06 Tris base, pH 6.8.2% SDS, 10% glycerol, 0.025% bromophenel_blue) and incubated at 60-70°C in a water bath for 10 minutes, then cooled immediately on ice for 5 minutes and centrifuge the mixture. The homogenates were centrifuged at 10,000 rpm for 15 min to separate the protein-containing phenol phase from debris. The upper phenol phase containing the proteins was carefully collected and transferred to new tubes. An equal amount of methanol saturated with ammonium acetate was added to precipitate the proteins. Protein pellets were collected by centrifugation, washed with ice-cold acetone, and air dried. The supernatant was Used for loading onto the gel. A current of 1.5 mA per well with a voltage of 80 V was applied until the tracking dye crossed the Stacking gel. Later the current was increased to 2 mA per well and voltage up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained Using coomaasie brilliant blue solution overnight and destained using a mixture of 227ml of methanol, 46ml of acetic acid and 227ml of distilled water until the bands were clearly visible.

Statistical Analysis

Based on the absence or presence of protein bands, the similarity index was planned for all potential pairs of polypeptide types. The score was 1 for the presence and 0 for the absence of protein bands. Depending upon the outcome of electrophoretic band spectra, similarity index (s) was considered for all conceivable sets of protein type electropherograms using the following formula [18]:

Similarity (S) = w/(a+b-w)

where w = number of bands of common mobility; a =
number of bands in protein type 'a' and b = number of
bands in protein type 'b'.

Results and Discussion

The analysis of total seed protein profiles of diverse maize genotypes using SDS-PAGE revealed a rich diversity of proteins. Each band represents a distinct protein or protein complex within the maize seeds.

The protein banding patterns observed on the SDS-PAGE gel exhibited both similarities and differences among the maize genotypes. While certain bands were consistently present across all genotypes, others were genotype-specific. Notably, some genotypes displayed unique protein bands that were not found in others, indicating significant genetic variation in seed protein composition.

To elucidate the relationships among maize genotypes based on their protein profiles, hierarchical clustering analysis was performed. The analysis revealed two major clusters: Cluster A and Cluster B.
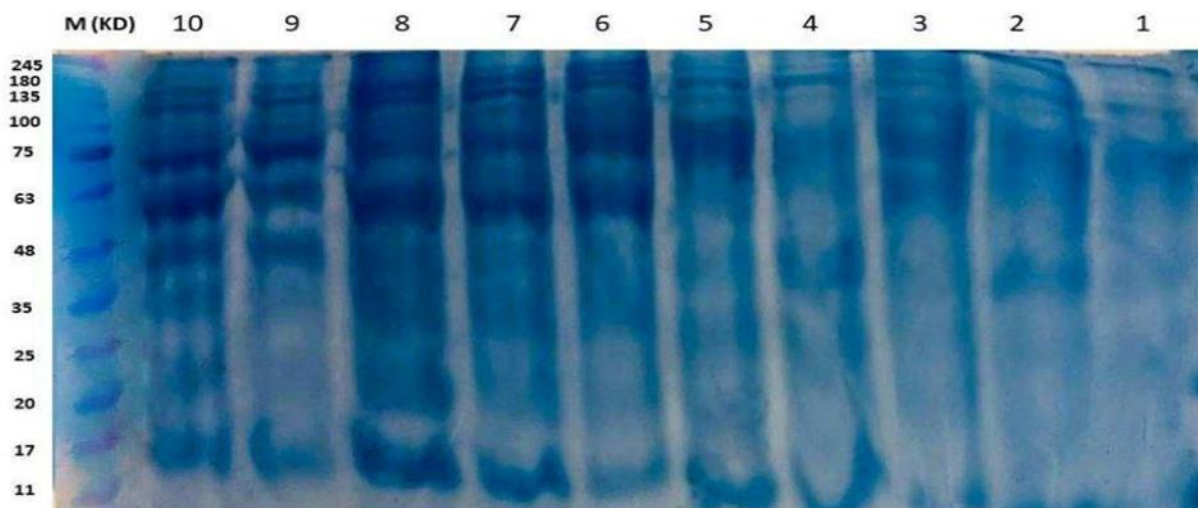

Figure (1): Total seed protein packages (KD -17 KDa 245) for ten structures acquired from corn regardless of the following structures for corn: 1. Sumer 2. Fajr 1 3. Almaha 4. Baghdad 3 5. Alnahrine 6. DKC 6777. ZP.glorya 7 PLOWEER 8. 9.KWS . 10. Syngenta Launcher Launch (1) and Program Absence (0) Relay on Acrylamide Gel.

Table (1): Total seed protein packages for ten genotypes of corn, according to the following order of genotypes. The appearance of the band (1) and the absence of the band (0).

No.	245 bp	200 bp	180 bp	135 bp	100 bp	75 bp	63 bp	48 bp	35 bp	25 bp	20 bp	17 bp
Somar	1	1	1	1	1	1	0	1	0	1	0	1
fajer1	1	1	1	1	1	1	0	1	0	1	0	1
Al maha	1	1	1	1	1	1	1	1	0	1	0	1
3 Baghdad	1	1	1	1	1	1	1	1	1	1	0	1
Al-nhren hajen	1	1	1	1	1	1	1	1	1	1	0	1
DKC 6777	1	1	1	1	1	1	1	1	1	1	1	1
ZP.glorya	1	1	1	1	1	1	1	1	1	1	1	1
PLOWEER	1	1	1	1	1	1	1	1	1	1	1	1
KWS	1	1	1	1	1	1	1	1	1	0	0	1
Syngenta	1	1	1	1	1	1	1	1	1	1	1	1

The results in Table (2) show that the patterns of the total protein gave a number of main bands amounting to 12 bands whose weights varied between 17 KDa and 245 KDa, and the total number of bands amounted to 108 bands, of which 8 were homologous and 6 were heterogeneous. The percentage of variation (difference) between the compositions was 32%, which indicates that the percentage of similarity (similarity) was 68%. The level of similarity is higher than the level of variation between the studied structures and is due to the geographical origin of the structures and their genetic material, or that the plant is the result of interspecific hybridization.

Table (2): Number of total seed protein bands migrated on polyacrylamide gel for maize plants.

Number of identified items	Covariance	Heterogeneous packages	Identical packages	Total number of packages	Number of main packages	Molecular sizes of bands in KDa
10	32	6	8	108	12	17-245

Cluster A comprises genotypes that share distinct protein profiles, suggesting genetic relatedness. These genotypes exhibit similar protein banding patterns, indicating conserved protein compositions. Notably, Cluster A includes both local and exotic genotypes, implying that genetic diversity can be found within specific geographical regions.

Cluster B encompasses genotypes with more divergent protein profiles. This cluster includes genotypes with unique protein bands not shared by others in the study. The presence of distinct protein markers in Cluster B genotypes underscores the genetic heterogeneity among maize genotypes, even within the same geographical region.

Principal Component Analysis (PCA) was employed to further explore the relationships among maize genotypes based on their protein profiles. PCA reduces the multi-dimensional protein data into a lower-dimensional space, making it easier to visualize genotype relationships.

The results of the clustering analysis, which appear in the form of a dendrogram (genetic relationship tree), were divided into two main genetic groups. The first group included structures 1 and 2, and the second group included the rest of the genotypes (corn). The genetic relationship tree showed that some structures were arranged within one group, and this is due to their origins. Common, as in the compositions Fajr 1 and Oryx, being local compositions, as shown in figure 2

When calculating the genetic similarity values using Jaccard for the 10 genotypes used in the study, The results showed the genetic tree of two groups, one of which included taxa 1 = Sumer and 2 = Al-Fajr, while the second group included taxa. 3= Almaha, 4= Baghdad 3, 5= Alnahrien, 6= DKC 6777, 7= ZP.glorya , 8= PLOWEER , 9 = KWS and 10 = Syngenta, as shown in Table 3 and Figure 2, where it was found that the highest similarity percentage was between 1 and 2 Sumer and 2 = Al-Fajr, respectively, amounting to (1), and the lowest similarity percentage was between 1 and 2 with the rest having a value of 6,7,8. . ,9.

Table (3): Results of a clustering analysis of similarity among ten maize genotypes.

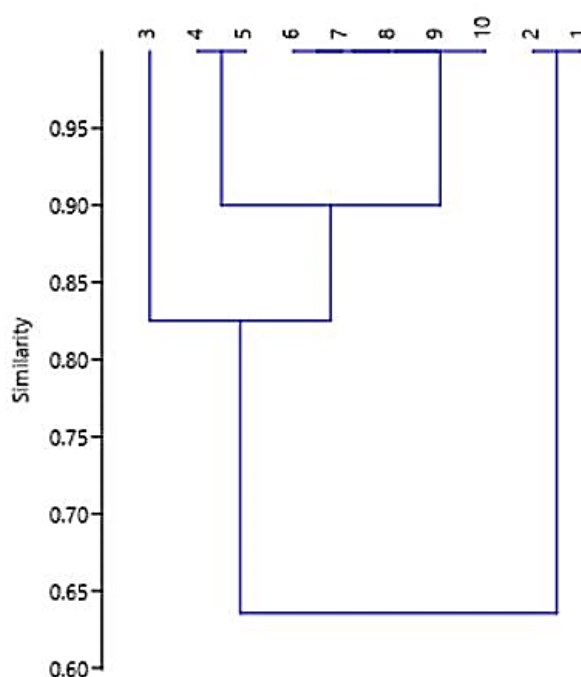|	1	2	3	4	5	6	7	8	9	10
1	1	1	0.75	0.66666667	0.66666667	0.6	0.6	0.6	0.6	0.6
2	1	1	0.75	0.66666667	0.66666667	0.6	0.6	0.6	0.6	0.6
3	0.75	0.75	1	0.88888889	0.88888889	0.8	0.8	0.8	0.8	0.8
4	0.66666667	0.66666667	0.88888889	1	1	0.9	0.9	0.9	0.9	0.9
5	0.66666667	0.66666667	0.88888889	1	1	0.9	0.9	0.9	0.9	0.9
6	0.6	0.6	0.8	0.9	0.9	1	1	1	1	1
7	0.6	0.6	0.8	0.9	0.9	1	1	1	1	1
8	0.6	0.6	0.8	0.9	0.9	1	1	1	1	1
9	0.6	0.6	0.8	0.9	0.9	1	1	1	1	1
10	0.6	0.6	0.8	0.9	0.9	1	1	1	1	1

Figure (2): Results of the clustering analysis of similarity between ten maize genotypes: 1 = Sumer and 2 = Al-Fajr, while the second group included taxa. 3= Almaha, 4= Baghdad 3, 5= Alnahrien, 6= DKC 6777, 7= ZP.glorya, 8= PLOWEER, 9= KWS and 10= Syngenta

The total seed protein profiles obtained through SDS-PAGE analysis highlight the remarkable biochemical diversity among maize genotypes. This diversity is likely attributed to both genetic and environmental factors [19]. The presence of unique protein bands in certain genotypes suggests that specific genotypes may possess distinctive protein markers associated with particular traits [20]. These protein markers could have implications for crop improvement programs, enabling the selection of genotypes with desirable protein profiles [21].

The clustering analysis revealed that genetic relatedness among maize genotypes does not always align with geographical origin [22] . Some local genotypes shared protein profiles with exotic genotypes, emphasizing the dynamic nature of maize genetics and the potential for genetic exchange and introgression [23]. The PCA results further emphasize the genetic distinctness of certain genotypes [24] . The separation of genotypes in the PCA plot underscores the potential for utilizing protein profiles as a means of genotype classification and selection [25].

Biochemical study of maize genotypes through analysis of the total seed protein has revealed a complex network of protein diversity. In addition, there is a similarity between Sumer and Afjar compared to the rest of the other eight genotypes. This research provides a basis for future investigations into the functional roles of these proteins and their contributions to maize traits. Understanding the biochemical basis of maize genotypes is critical to efforts to improve crops, enhance nutrition, and develop resilient maize varieties that are able to withstand environmental challenges.

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