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Genetic Discrimination among 21 Corn (*Zeay mays* L.) Varieties through Total Soluble Seed Protein Profile

التمييز الوراثي بين 21 صنف من الذرة(*Zea mays* L.) من خلال أنماط البروتين الذائب الكلي للبذور

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Abstract:

This study was conducted to investigate the ability of total soluble seed protein to discriminate among 21 of maize genotypes through using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). According to SDS analysis , a total of 118 amplified bands were obtained ranging in their molecular size 18-86KDa. Five out of main nine bands were polymorphic and four monomorphic with an average of polymorphism reaches 55.5%.

Phylogenetic tree divided 21 of corn genotypes between two major groups each of them divided in to two subgroups .The first main group included 13 genotypes , while the second main group included eight genotypes .

The ability of protein profile to give a distinctive pattern for a particular genotype could serve in future of studying corn seed response to different abiotic stress and studying genetic diversity .

Key words: cluster analysis, SDS-analysis, Biochemical Markers, corn.

الخلاصة:

نظمت الدراسة الحالية للتحري عن قابلية بروتين البذور الذائب الكلي للتمييز بين 21 من الانماط الوراثية للذرة من خلال الترحيل الكهربائي على هلام البولي اكريل امايد حيث اظهرت نتائج تحليل الترحيل الكهربائي 118 من الحزم المتضاعفة الكلية تراوحت في اوزانها الجزيئية بين 18 -86 و كانت خمس من الحزم الرئيسة متعددة الاشكال واربعة منها متماثلة الاشكال

قسمت شجرة العلاقة الوراثية الانماط الوراثية ال 21 من الذرة الى اثنين من المجاميع الرئيسة قسم كل منها بدوره الى اثنين من المجاميع الفرعية حيث ضمت المجموعة الرئيسة الاولى13 والثانية ثمانية من الانماط الوراثية

ان قابلية انماط البروتين على اعطاء نمط معين لكل نمط وراثي يمكن ان يخدم مستقبلا في دراسة استجابة بذور الذرة لمختلف الاجهادات غير الحيوية ودراسة التنوع الوراثي

Introduction:

Zea mays (corn) belongs to (Poaceae) family , it is a member of the most world's successful family of agricultural crops , including wheat , rice , oats , sorghum , barley , and sugarcane . It belongs to the genus Zea , a group of annual and perennial grasses native to Mexico and Central America . (1).

Maize is the most world's widely grown crop with an annual global production of 817 million tons in 2009. (2).

Maize is also an important source of cooking oil , biofuel , and animal feed . (3). A side from being economically important , maize has long been used as a model organism for eukaryotic biology , transmission genetics , biochemical genetics , plant biology , and more recently , genome evolution . (4).

More recently, maize had also became a prominent model for investigation of leaf and floral development, evolution, genetic diversity, and plant domestication (5).

Despite its value as a model genetic organism, maize had always presented a challenge for genome analysis because the large genome contains duplicated chromosomal segments and has high content of retro elements (6).

Characterization of crop genetic diversity based on morphological traits had advantages, such as being easy to detect and measure, and their relevance to germplasm users and breeders (7).

The main disadvantages of these markers are complex genetic control of many morphological traits and that they could be influenced strongly by environmental conditions (7), however, these traits were useful especially when used in conjunction with other markers from other sources, especially DNA markers (8).

Despite that phenotypic variation is positively associated with genetic diversity, but is also dependent on environmental factors, as well as, on the interaction between genotypes and environment (9)

Biochemical markers are markers that reveal polymorphisms at the protein level. They are proteins produced as a result of gene expression and detect variant at the gene product level (10).

The mechanism of identification of these markers depend on fact that when a mutation in the DNA results in an amino acid being replaced this lead to change migration rate and confirmation of protein and this detected by electrophoresis, usually two, or sometimes even more loci can be distinguished for an enzyme and these are termed isoloci (11).

Biochemical marker is effectively concern with differentiation of hybrids and wild varieties (12),cultivars fingerprinting(13), phylogenetic diversity , relationships of varieties (14) and varieties identification (15).

There is another application for seed protein electrophoresis pattern in revealing accelerated aged seed exposed to RH and 40C° for 0 for nine days ,this study shows that total soluble seed protein banding pattern of different aged has been decline in band intensity, band numbers or loss of some bands as period of ageing advanced (16).

In maize electrophoresis analysis of total soluble protein (2-D PAGE or SDS-PAGE) and isozymes profiles were used to evaluate the response to salt stress in maize genotype.

SDS-PAGE analysis has reveled that plant grown under NaCl showed induction or repression in the synthesis of few polypeptide in shoots and roots(17).

Studying the effects of drought stress depending on soluble proteins in two maize varieties revealed that there was no relationship between protein changes and drought tolerance (18).

Hydro priming which is a simple technique to improve seed germination and seedling in maize could be studied at molecular level analysis of the priming-induced changes in maize embryo proteome and to identify priming-associated proteins through 2-DE_two-dimensional electrophoresis of seed protein (19).

SDS-PAGE analysis could reveal plant response to UV-B stress by electrophoresis for leaves protein after UV-B stress was given to the seeds of maise at two different time intervals (30 and 60 min).

Stressed seeds were grown under normal environment condition when compared with control plants, increased numbers of protein bands were observed in UV-B treated plants.

This refer to that the plant synthesis new proteins under UV treatment for the adaptation to the environmental conditions. These stressed proteins could be used as biomarkers for identification of stressed plant. Identification of quantitative trait loci for UV stress resistance may well be an effective analytical tool (20).

Studying of SDS-PAGE analysis in corn germplasm provide an excellent tool in evaluation of biodiversity and building of new varieties (21) .

Materials and Methods:

Samples Collection and Protein Extraction:

A collection of 21 corn varieties (**1.**Buhooth 106,**2.** IPA 2052,**3.** IPA 5015,**4.** IPA 5012, **5.**IPA 5018,**6.** IPA 5011,**7.** IPA 5026, **8.**Sarah,**9.** Al-Maha,**10.** SNH 8605,**11.** SNH 5610,**12.** Kr 640,**13.** 3078,**14.** Pio 3751,**15.** DKC 6120, **16.**DKC 5783,**17.** 89 May 70,**18.** Biotech Bag, **19.**Manlcet, **20**.DKC 6418, **21.**1017) were used for characterization of total seed protein.

Two sprouted (3days old) seeds were grounded in centrifuge tube by using micro pestle and 200µl Tris HCl extraction buffer (25mM, pH 8.8) was added. The mixture was agitated thoroughly and kept at 8C° for overnight for protein extraction. Then the mixture was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected. This protein extract was dissolved in an equal volume of working buffer (0.06 M Tris-HCl,pH 6.8,2% SDS,10% glycerol,0.025% bromophenol blue)and incubated at 60-70ºC for 10 minutes, cooled immediately for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. The supernatant was used for loading on to the gel (15)

(22) method was applied for preparation of SDS-PAGE (sodium dodecyl sulfate-polyacryl amide gel) of total soluble seed proteins was carried out by using 12.5 per cent gels prepared as following(Table 1):

1. Stock solutions :

Solution A 30% acrylamide solution (29.2g acrylamide and 0.8g bis acrylamide, water was added to make up 100 ml).

Solution B 1.5 M Tris buffer, pH 8.8 (dissolves 18.17g of Tris and 0.4g of SDS in water, adjusted for pH 8.8 with HCL, and make up to 100ml).

Solution C 0.5 M Tris buffer, pH 8.8(dissolves 6.06g of Tris and 0.4g of SDS in water, adjusted for pH 6.8 with HCL, and make up to 100 ml).

Solution D 10% ammonium persulfate (add 1ml of water to 0.1g of ammonium persulfate, prepared just prior to use).

2.Gel solution composition (quantity; in ml)(% ; gel concentration):

Preparation of two slab gels at concentration of 12.5% as in table

1. Mix sol.A, sol.B (or sol. C) and water, add TEMED and sol D, and gently mix; immediately cast a gel (s).

2. Stock solution of 10% SDS solution:

The stock solution is convenient for preparation of electrophoresis buffer (dissolve 10g of SDS into water, to make up to 100 ml).

Table 1: Gel solution composition for slab gels at concentration of 12.5%.

Solutions	Separation gel 12.5%	Stacking gel 4.5%
Sol. A	7.5 ml	0.9 ml
Sol. B	4.5 ml	-
Sol. C	-	1.5 ml
Sol. D	0.07 ml	0.018 ml
Temed	0.01 ml	0.01 ml
Water	1 ml	3.6 ml

4. Sample preparation:

(1% SDS, 1% 2-mercaptoethanol, 10 mM of Tris buffer, pH 6.8, 20% glycerin) extracted protein was added to sample buffer at a ratio of 1:4 and heated at 100 C◦ in water path for 1-2 minutes, and load in wells.

5. Electrophoresis buffer:

Ten ml of 10% SDS solution was added, to 3g of Tris and 14.4 g of glycine, and make up to 1000 ml with water. 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.

6. Staining and destaining:

a-Staining solution : (water was added to 2.5g of Coomassi Brilliant Blue**,** 500 ml of methanol and 100 ml of acetic acid, to make up to 1000 ml) .

b-De staining solution : (water was added to 250 ml of methanol and 70 ml of acetic acid, to make up to 1000 ml).

Staining using coomaasie brilliant blue solution continue for about overnight while destaining continued till bands clearly visualized in white fluorescent light. The size of SDS-PAGE products were estimated by comparing with pre-stained protein standards (**10 KDa, 17 KDa, 28 KDa, 35 KDa, , 48 KDa, 63 KDa,75 KDa, 100 KDa, 130 KDa , and 180 KDa**). For loading samples, from each variety, 20 µL of the extracted protein (4:16) and standard ladder (2:8).

Results and Discussion:

The photograph from stained polyacrylamid gel was used to score the data for protein analysis starting from the higher fragment size product to lower fragment size product.Presence of a product was identified as (1) and absence was identified as (0) as shown in Table 2. (23).

Electrophoretic analysis of proteins exposed a total of 118 bands ranging in their molecular size from 18 to 86 KDa , five out of nine bands were polymorphic result in an average of polymorphism reaches 55.5% .Protein pattern resulted in fingerprinting six out of 21 corn genotypes as shown in table 3, figure 1 and figure 2.

Identical protein pattern (high similarity) could be result from conservative nature of the seed protein (24**)** ,these profile could be used as a general biochemical fingerprint for the studied genotypes (13) .

Table 2: Total seed protein patterns result from SDS electrophoresis of 21 Corn genotypes : the presence of band (+), or absence (-) and their molecular size in KDa

M.S of fragment in KDa	Corn genotypes																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
86	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
69	0	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0
60	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
49	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
31	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0
29	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0
25	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0
23	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Fingerprinted genotypes																					

Table 3 : Detailed results of protein electrophoresis profile of 21 corn varieties .

Fragment size range (KDa)	No. of main bands	No. of amplified bands	No. of monomorphic bands	No. of polymorphic bands	No. of unique bands	Polymorphism (%)	No.of identified genotypes
18-86	9	118	4	5	0	55.5	6

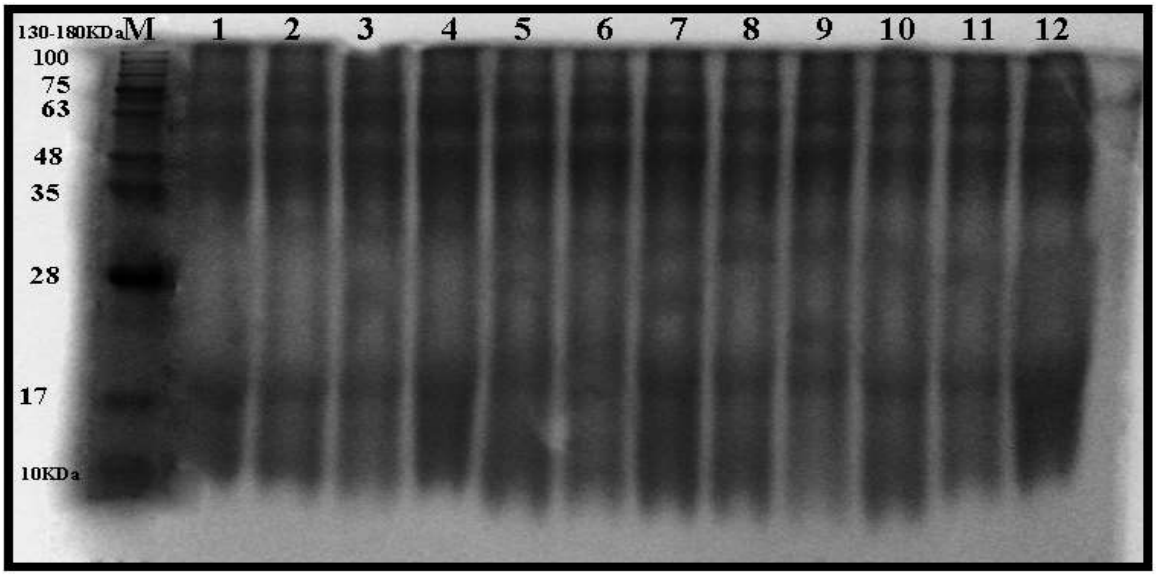

Figure 1. SDS-PAGE analysis of corn seed proteins numbered from 1to 12 : **1.**Buhooth 106 **2.** IPA 2052 **3.** IPA 5015 **4.** IPA 5012 **5.**IPA 5018 **6.** IPA 5011 **7.** IPA 5026 **8.**Sarah **9.** Al-Maha **10.** SNH 8605 **11.** SNH 5610 **12.** Kr 640

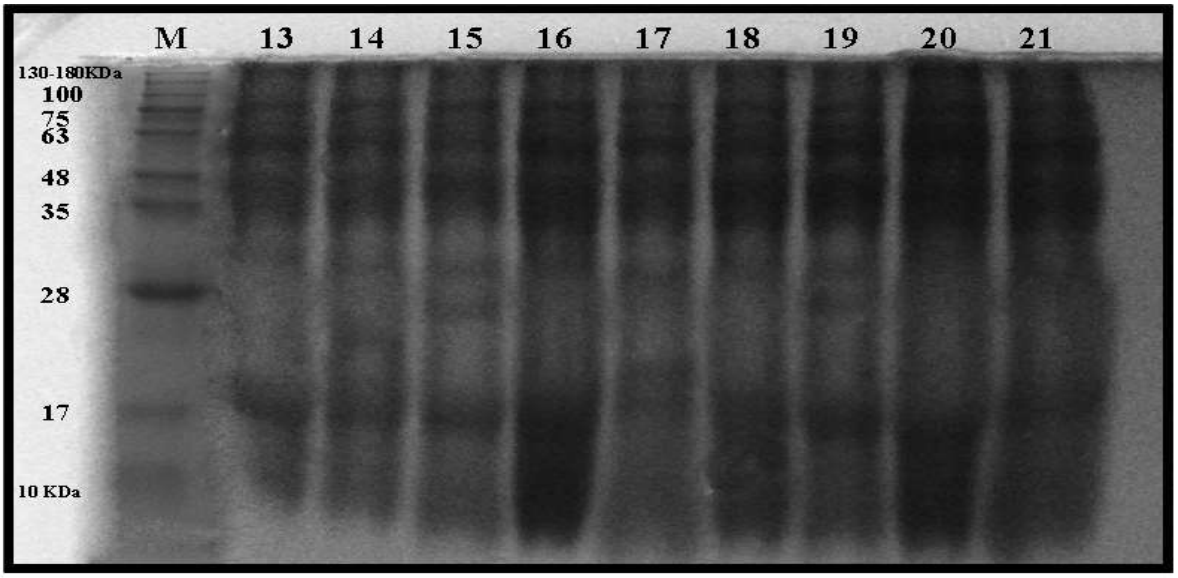

Figure 2. SDS-PAGE analysis of corn seed proteins numbered from 13to 21 : **13.** 3078 **14.** Pio 3751 **15.** DKC 6120 **16.**DKC 5783 **17.** 89 May 70 **18.** Biotech Bag **19.**Manlcet, **20.**DKC 6418, **21. 1017**

The phylogenetic tree was created by the un weighted pair-group method arithmetic (UPGMA) average cluster analysis (25and 26).

Figure 3 shows that phyllogram divided 21 of corn genotypes between two major groups each of them in turn divided in to two subgroups .The first main group include 13 genotypes , the first small subgroup include three genotypes(17,7 and 6) while the other large subgroup include 10 genotypes(8,10,11,12,18,16,1,20,21 and 9).

The second main group include eight genotypes , the first small subgroup included one genotype (14) , while the other large subgroup include seven genotypes (13,2,15,19,6,3 and 4)

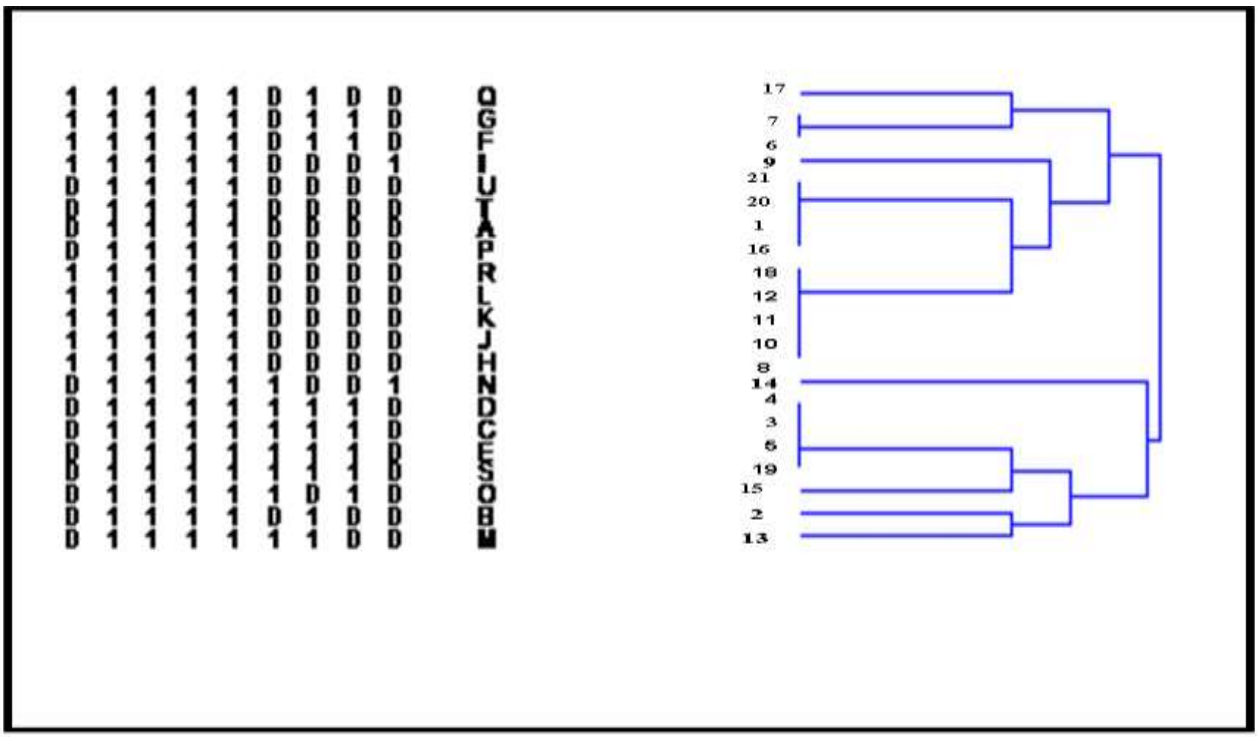

Figure 3.UPGMA dendrogram illustrating the trees of genetic relationship among 21 corn genotypes using protein electrophoresis pattern.

The main value for creating a dendrogram is to understand genetic relationships among studied genotypes which help plant breeders in future to prevent gene erosion within varieties by selecting a large number of different clones of each variety (27).

Studying corn seed storage protein profile had an advantage in evaluation of seed response to abiotic stresses as salt tolerance (17),drought stress(18) and UV- B radiation (20).

Protein pattern of corn could reveal genetic diversity among genotypes (9 and 21).This investigation is a step in future studies of maize genotypes seed protein in Iraq for response to abiotic stress .

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