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IDENTIFICATION STUDY OF SOME LOCAL BARLEY VARIETIES BY ELECTROPHORESIS USING SDS METHOD

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ABSTRACT

The study aimed to use the SDS method with the electrophoresis technology to distinguish the seeds of different varieties of local barley and included (Warka, Hader, Bawadi, Rayhan, I baa 99, Ibaa 265, Sameer, Nomar, Buhooth 244, Shieae, Buraq, Amel). By extracting the total barley protein from one grain of each variety, and when the results showed their difference in the number of bundles as well as their molecular weights, it was possible to obtain different electrical transfer patterns for the studied barley varieties using the single grain method, which can use the migration results alone as a proven method of differentiation for the identity of these varieties and to verify the authenticity The variety, in addition to its use in the detection of adulteration of barley and its mixtures, but this did not prevent the study of the phenotypic characteristics to determine the characteristics of the variety. As the studied varieties showed 16 patterns, when using the cluster analysis, the varieties showed the extent of convergence between them, as they were similar in the first four types of all the varieties and the difference in the following patterns began to appear. A clear difference from the varieties, Warka, Hader, Bawadi and Rayhan, while the Nomar and Buhooth244 classes were similar, although they differed in appearance.

***Keywords*:** Barley Varieties – Electrophoresis-SDS Method.

Introduction

Barley is one of the staple foods in some Asian regions because of its ability to grow and give it good yields in plants that are characterized by drought and high temperatures (Jacobs, 2016). Rain, which is one of the determining factors of agricultural production (Pourkheirandish and Komatsuda 2007).

Barley grains contain a percentage of proteins ranging between 10-20%. Prolamine and gluten proteins are the main storage proteins in barley grains, as they make up the majority of the protein found in the endosperm and constitute 52% and 23% respectively, while albumin and globulin proteins are present in small proportions. It is considered one of the secondary storage proteins as it constitutes the lowest (Houde *et al*., 2018). Prolamine proteins are characterized by their high content of amino acids, clotinine and proline, but they are low in basic amino acids with a positive charge (His, Iys, Arg), so they are not glycosylated proteins (Goug *et al*., 1992), which are among the proteins capable of Involution and binding to other protein and non-protein molecules by hydroxyl group or functional group (Drickamer, 2006).

Horden proteins are classified into A, B, C and D depending on the molecular weight and the nature of the amino acids, with molecular weights ranging from 12 to 100 kDa (Sullivan *et al*., 2013). Strelec *et al*. (2011) stated that barley proteins have a wide range of neutralization points. The electrophoresis ranges between (3.5-9.5), while Mohamed *et al*. (2007) stated that the best acidity number for the precipitation of barley proteins is at a pH of 4.5. Barley can be divided according to the presence of the outer shells of the barley grain and its thickness, and it can also be classified depending on the length of the plant and the length of the kernel, or depending on the color of the grain (colorless, white, black, purple, blue or red), and the last three colors are the result of the presence of a pigment. The anthocyanins in the shell of the pill give the red or purple color to the pills, and when the pigments are concentrated in the iron layer, they give the blue color (Yunis *et al.,* 1987).

Materials and Methods

Barley seeds were obtained from the Variety Discrimination Laboratory of the Seed Inspection and Certification Department, with one seed for each variety. The laboratory experiment was carried out in the Genetic Footprint Laboratory / Laboratories Department of the Seed Inspection and Certification Department to distinguish 12 varieties of local barley.

Protein extraction

One seed was crushed in a ceramic mortar for each barley variety and the powder was placed in a test tube with a volume of 1 ml and the extraction solution (Tris- HCl pH7.5, 0.1M) containing SDS was added to it and mixed with the Italian electric shaker Filp twice every 15 minutes and left with a degree At room temperature for 24 hours, separation was carried out in a US centrifuge at a speed of 10,000 rpm.

Preparation of Gel

The separation gel consists of two layers and is as shown below :

Separating gel pH = 6.8(110ml D.W+ 10% SDS+ 12.12 gm Tras base)	
29.6 ml	D.W
23.5 ml	Acrylamide 30%+ Bisacrylamide 0.8%
17.6 ml	4xStacking Tris solution
Stacking gel pH= 8.8(110ml D.W+ 10% SDS+ 36.4 gm Tras base)	
16.8 ml	D.W
2.6 ml	Acrylamide 30% + Bisacrylamide 0.8%
6.4 ml	4xStacking Tris solution

After mixing the ingredients, the volume is supplemented to 100 ml with Dari gel solution and 0.1 ml of (10%) ammonium per sulfate solution and 0.3 ml of dilution are added to the gel solution immediately before pouring.

Preparing the gel

Electrophoresis was carried out on a Polyacrylamide gel using a vertical migration method in a device manufactured from BDH in the presence of SDS teratogenic agents according to the method reported by Leammli (1979). By mixing the separating gel components and pouring between two glass plates, a period of hardening was left, adding the stacking gel and placing the comb designed to form holes in the gel and leaving the gel to complete to harden after which the comb was carefully lifted so that the gel was ready for the modeling process.

Separation

20 µL of the model was placed in the gel hole, and the device tank was filled with a circulating solution, and the current was passed at a rate of 2 mA per model and the current was stopped when the dye reached the end of the gel, after which the gel was extracted from the glass slide by injecting distilled water between the sides of the gel and the inner surface of the glass by mediation Medical syringe.

Staining

Dyeing was carried out using a dyeing solution by placing the gel in the dye solution for two hours and then removing the dye from the gel using the depigmenting solution until the firmness appears clearly.

Results and Discussion

The results of the study showed that there was a difference between the patterns of the electrically separated proteins in terms of locations, number of bundles and their molecular weights, as well as in the thickness of the bundle. As it was clearly observed 15 bundles in protein varieties Ray and Research 244 and Bawadi and 10 bundles in Hader, Buraq, Nomar and Ibaa 265 and 12 bundles in Samirwaba classed 99 and Rayhan and 10 bundles in the Hadramilat classes. The figure shows the dimensions of the beams that clearly distinguish them from the point of origin and the presence of a difference between the types of electrically separated proteins in terms of number of bundles and locations, their molecular weights and beam thickness. A clear difference can be seen between the migration patterns of cultivar proteins, as this trait is considered an accurate differentiation method for each class, as the number of packages and their locations differed accordingly It varies according to the variety. Ladizinsky and Hymowitz (1979) stated that the change in the density of the bundles that appear on the gel is the predominant feature of showing differences between cultivars. The Warka, Hader, Bawadi and Ibaa 265 cultivars gave more clear transmissions with discrete and uncombined beams, ranging from high concentration of low molecular weights beams to low concentrations of high molecular weights, while the Ibaa 99, Sameer, Nomar, and Research 244, Ray and Shiny, and hope for the largest number of beams and their density, being the density. Discriminatory adjective. Figure (2) shows the cluster diagram of the convergence ratios between the studied varieties. It shows that the patterns of electrophoresis gave a more comprehensive and accurate description for identifying between the items and fixing their identity, while none of the physical or chemical methods could give a fixed identity to the class alone.

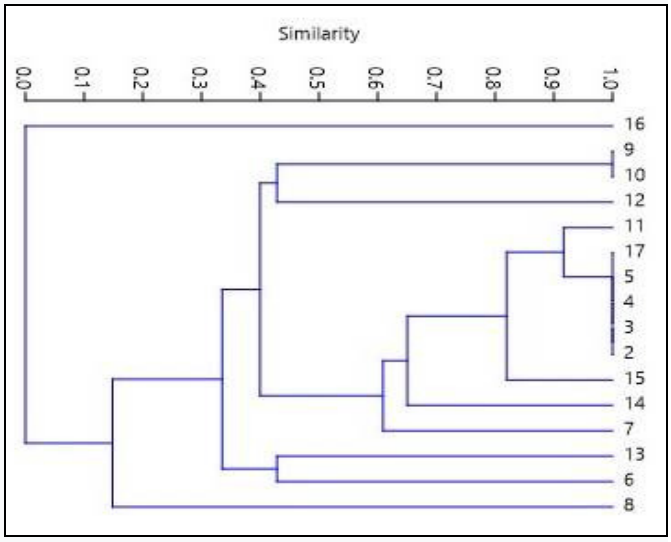

Fig. 1 : Shows the electrical migration of the local barley varieties

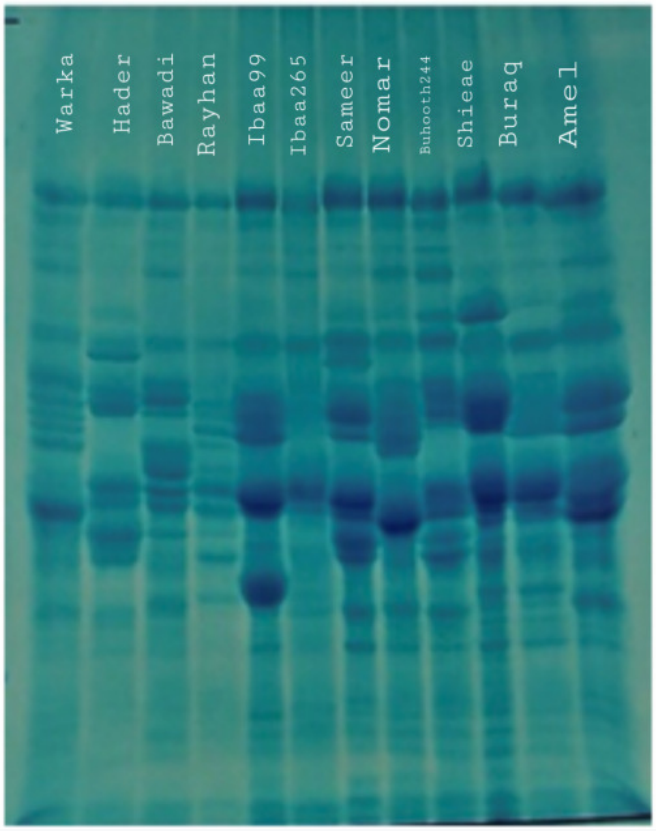

Fig. 2 : Cluster analysis of local barley varieties

Conclusions

The studied barley varieties differed according to the different packages and molecular weights, and the possibility of using the method of electrical transfer to determine the identity of the varieties. Therefore, we recommend conducting studies on a large scale using this technique to determine the identity of the different varieties cultivated in different regions and countries and to find similarities, differences and convergences between them.

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