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Protein patterns of wheat grains with phylogenetic inferences

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ABSTRACT

A comparative study of total grain protein was carried out by sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to characterise the differences between and within two species of wheat *(Triticum aestivum* and *Triticum durum*) represented by 12 cultivars. The densitometric profile data of the polypeptides showed an obvious variation in the number and position of bands from one cultivar to another. There were some bands in common between both species (103, 85, 70, 50, 46.9, 45, 40, 38, 34.1, 33, 31, 29, 28, 21, 20, 18, 14KD), while there were also some bands which characterise each species. Cladistic analysis showed that the four cultivars belonging to species *T. aestivum* separated earlier in evolutionary history, and can be recognized from the rest of the cultivars which belong to species *T. durum*.

KEYWORDS: *Triticum aestivum, Triticum durum*, protein polymorphism, electrophoresis, SDS-PAGE, cladistic analysis.

INTRODUCTION

Protein is commonly used as a genetic marker. It has been used to characterise cultivars of such diverse species as Festuca (Aiken & Gardiner 1991), Timoth (Cai &Bullen 1992), Lentil (Kraic *et al.* 1995), Buckwheat (Rogal & Javornik 1996), Peas (El-Akkad & El-Araby 1998) and Festulolium (Hahn & Schoberlien 1999).

El-Akkad (1998) used SDS-PAGE to determine the genetic variability of two wheat cultivars from Egypt (Giza 164 and Sakha 8). The two cultivars showed distinct variation in protein patterns. This variation was confirmed by using DNA analysis with the RAPD technique (Randomly Amplified Polymorphic DNA). It could be concluded that any of these markers was enough to distinguish between wheat cultivars. These results encouraged the author to use the protein pattern technique to carry out further work in this connection. Therefore the aim of this study is to investigate the seed protein variation between and within two species of wheat (*Triticum aestivum* and *Triticum durum)*, also to obtain information about the phylogenetic relationship between and within those wheat species and their twelve cultivars: four devoted to *T. aestivum* and eight to *T. durum*.

MATERIALS AND METHODS

Grains: twelve cultivars belonging to two species of wheat [*T*riticum *aestivum* and *T*riticum *durum*] were obtained as follows: Sakha 69, Giza 164 and Seds 1 were obtained from the Ministry of Agriculture, Giza, Egypt. Ucororgwa and ACSAD 65, 297, 299, 323, 357, 363, 737, 1031 were kindly provided by the Faculty of Agriculture, King Abd Al-Aziz University, Saudia Arabia. Symbol A will be given to ACSAD cultivars during this work.*T. aestivum* is represented by Sakha 69, Giza 164, Seds 1 and Ucororgwa, while *T. durum* is represented by ACSAD 65, 297, 299, 323, 357, 363, 737, 1031.

Protein Extraction: Half gram grains was used with eash cultivar. Grains were crushed to a fine powder using liquid nitrogen and mixed with 1½ml of extraction buffer [10g sucrose, 5ml 2-mercaptoethanol, 2.0g SDS and 2.422g Trizma base. The mixture (pH adjusted to 8.5) made up to 100 ml with distilled water, then left for 4 to 6 hours at 4°C. The slurry was then centrifuged for 20 minutes at 5000rpm. The supernatant was decanted to another fresh tube and used as the total protein extract.

Protein Electrophoresis: the SDS-PAGE method for the analysis of total wheat grain protein, was first optimised in order to obtain the most discriminatory separation of proteins, which enabled the detection of all potential differences among analysed samples. Elecotrophoresis was performed according to Laemmli (1970), using 15% polyacrylamide gels. Standard proteins of known molecular weights (20.2, 28.5, 34.1, 46.9, 81 and 103 KD) were run on a corresponding gel and used for characterisation and determination of molecular mass of wheat polypeptides. The staining of protein was done overnight at room temperature with a mixture of 0.25g Comassie blue R-250, 90 ml water : methanol (1:1 v/v), and 10ml of glacial acetic acid. After staining the gel was transferred for destain to a mixture of glacial acetic acid : methanol : water (3:17:20 v/v/v).

Data Analysis: once fingerprint patterns have been generated and scored, the bands are assigned to specific positions in lanes to be compared. Different strategies might be followed to quantify the pairwise similarity of the polypeptides represented in the different lanes. Evaluation of protein profiles was done visually and the gel was also scanned in LKB Recording Laser Densitometer equipped with LKB Recording Integrator.

Cladistic analysis: the characters used are the bands obtained from the biochemical analysis of the seed protein in the electrophoresis unit. Each number at a node or branching represents a hypothetical ancestor and the number on the lines correspond to the characters tabulated in table 1 (data matrix). The resulting character table was analysed using the PAUP package (Swofford 1985). For inferring phylogeny under the principle of maximum parsimony, the following options were used: OPT=DELTRAN, MULPARS, ALLTREE (when n<9), BANDB (9< n<16), Hold = 5.

Table 1: Data matrix for cladistic analysis of *Triticum aestivum* and *Triticum durum* wheat cultivars

Species and cultivars	Characters (seed protein patterns)
Triticum aestivum cultivars	
Sakha 69	00100100101000010010011001000110111010011111
Giza 164	00100100000011000101001101010010110000011111
Seds 1	01100000001001000101001101010011111010011111
Ucororgwa	01000010001010000001001101110011111010011111
Triticum durum cultivars	
ACSAD 65	00010000010100100000101110111011111001101101
ACSAD 297	00100000010100100000101110110011111001101101
ACSAD 299	00001001000010001000011110010011111100101101
ACSAD 323	00001001000010101000011110010011111100101101
ACSAD 357	10100000010000100010001100010011111000101101
ACSAD 363	00001001010000101000001100010011111000101101
ACSAD 737	00011001010000101000001100010011111100101101
ACSAD 1031	00101000010000101000001100010011111000101101

RESULTS AND DISCUSSION

The seed proteins of the 12 cultivars of wheat were analysed by SDS-PAGE and the electrophoretic pattern of the different cultivars is shown in figure 1. In total, forty five polymorphic bands were detected with molecular weight ranging from 112±2 to 14±2 KD, that are distributed along the gel in a range of R_f: 0.05-0.98.

As shown in data matrix (Table 1), there is an obvious variation in the number and position of bands from one cultivar to another. Both species share bands with molecular weights of 103, 85, 70, 50, 46.9, 45, 40, 38, 34.1, 33, 31, 29, 28, 21, 20.2, 18, 14 KD.
Many bands were found to be specific either to *Triticum aestivum* or *T. durum*. The former (*T. aestivum*) was characterised by the presence of bands of molecular weights 108, 99, 97, 91, 87, 83, 81, 75, 65, 41, 35, 25, 22, 16 KD, while *Triticum durum* was characterised by the presence of bands of molecular weights 112, 102, 101, 93, 89, 86, 82, 79, 62, 43, 46, 27, 24, 23 KD.

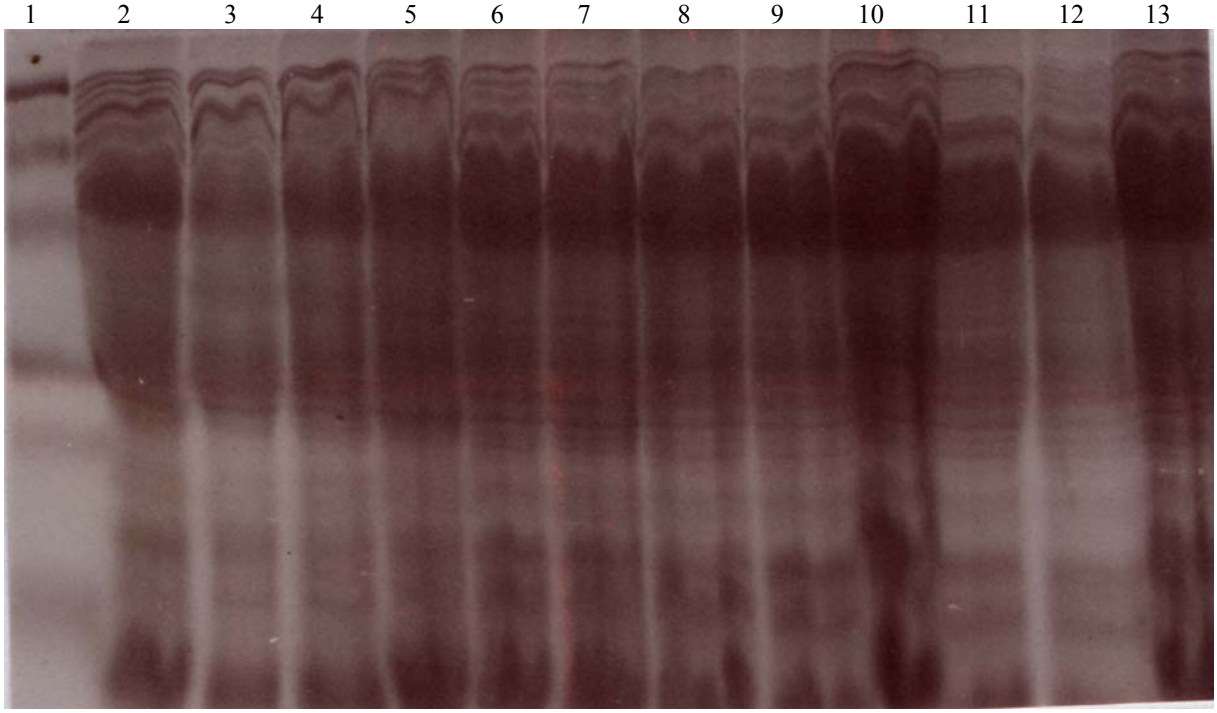

Figure 1: Electrophoresis protein pattern of the grins of different cultivars of two wheat species (*Triticum aestivum* and *Triticum durum*).
1: High molecular weight standard (from top to bottom), 103, 81, 46, 34, 28 and 20 KD.
2-5: *Triticum aestivum* cultivars (2: Sakha 69; 3: Giza 64; 4: Seds 1; 5: Ucororgwa);
6-13: *Triticum durum* cultivars (6: ACSAD 65; 7: ACSAD 297; 8: ACSAD 299; 9: ACSAD 323;
10: ACSAD 357; 11: ACSAD 363; 12: ACSAD 737; 13: ACSAD 1031).

For detection of relationships among the 12 cultivars of *T. aestivum* and *T. durum*, the *variability* of the bands detected with SDS-PAGE was used. Variations in electrophoretic pattern of proteins of the plant has been used successfully to identify mutants of some plant cultivars (Sammour 1994; Rabbani *et al.* 2001). Cladistic analysis showed obvious differences between the two species of wheat. The tree obtained (Figure 2) showed that in the first clade both Giza 64 and Seds 1 cultivars seem to have evolved early (on the basis of the bands of molecular weights, 103, 83, 75, 65, 41, 22, 16 KD), that both cultivars are belong to *T. aestivum*, and that both are Egyptian cultivars. Cultivar Ukororgwa joined with the rest of the wheat cultivars at the next clade at node 20 and then subsequently separated (bands with molecular weights 108, 97, 87, 85, 65, 41, 40, 25, 21, 16 KD). At node 19, cultivar Sakha 69 joined with the rest of the cultivars (the band with molecular weight 23 KD), and then subsequently separated (bands with molecular weights 99, 91, 87, 81, 70, 50, 35, 25, 22, 16 KD).

On the bases 0f the above mentioned results, it is clear that cultivars Ukororgwa and Sakha 69 separated early in the second clade from the rest of cultivars, and that both (along

with cultivars Giza 64 and Seds 1) belong to *T. aestivum*. Sakha 69, Giza 64, Seds 1 and Ukororgwa (*T. aestivum*) therefore probably evolved earlier than the rest of the cultivars of species *T. durum.*

All cultivars at node 18 belong to species *T. durum* (cultivars ACSAD), and they share the band of molecular weight 82 KD. These cultivars divide at the same node into three groups. The first group contains cultivar A357 (due to the presence of the bands with molecular weights 112, 65, 103, 89 KD). In the second group, the cultivars A65 and A297 are joined together (bands of molecular weights 89, 86, 62, 43, 40, 24 KD) at node 16. Cultivar A65 has a specific band pattern characterising it from its sister cultivar A297 (bands of molecular weights 102 and 36 KD, while A297 was characterised by the band of molecular weight 103 KD). The third group contains cultivars which joined together at node 17 (bands of molecular weights 101 and 79 KD). In this group, cultivar A1033 was separated and characterised by the bands of molecular weights 103 and 89 KD from the rest of its group. A363 was joined with the last three cultivars (A737, A299, A323) by the band of molecular weight 93 KD and separated from them by the presence of the band of molecular weight 89 KD and absence of that with molecular weight 27 KD. The last three cultivars (A737, A299, A323) were joined together by the band of molecular weight 27 KD and then cultivar A737 was separated by the presence of bands of molecular weights 102 and 89 KD. The other two cultivars (A299 and A323) are closely related to each other based on sharing the bands with molecular weights 85, 50 and 43 KD. The band of molecular weight 82KD characterises cultivar A323 from A299.

Thus, it might be concluded that protein SDS-PAGE is a reliable tool for identification and characterisation between and within the two species of wheat used in this investigation. It is clear from the cladogram that the four cultivars belonging to *T. aestivum,* (Sakha 69, Giza 164, Seds 1 and Ucororgwa), were separated earlier and could be recognised from the rest of the cultivars which belong to *T. durum*. Also, each cultivar has its unique bands which characterises its protein pattern from any of the other cultivars under investigation.

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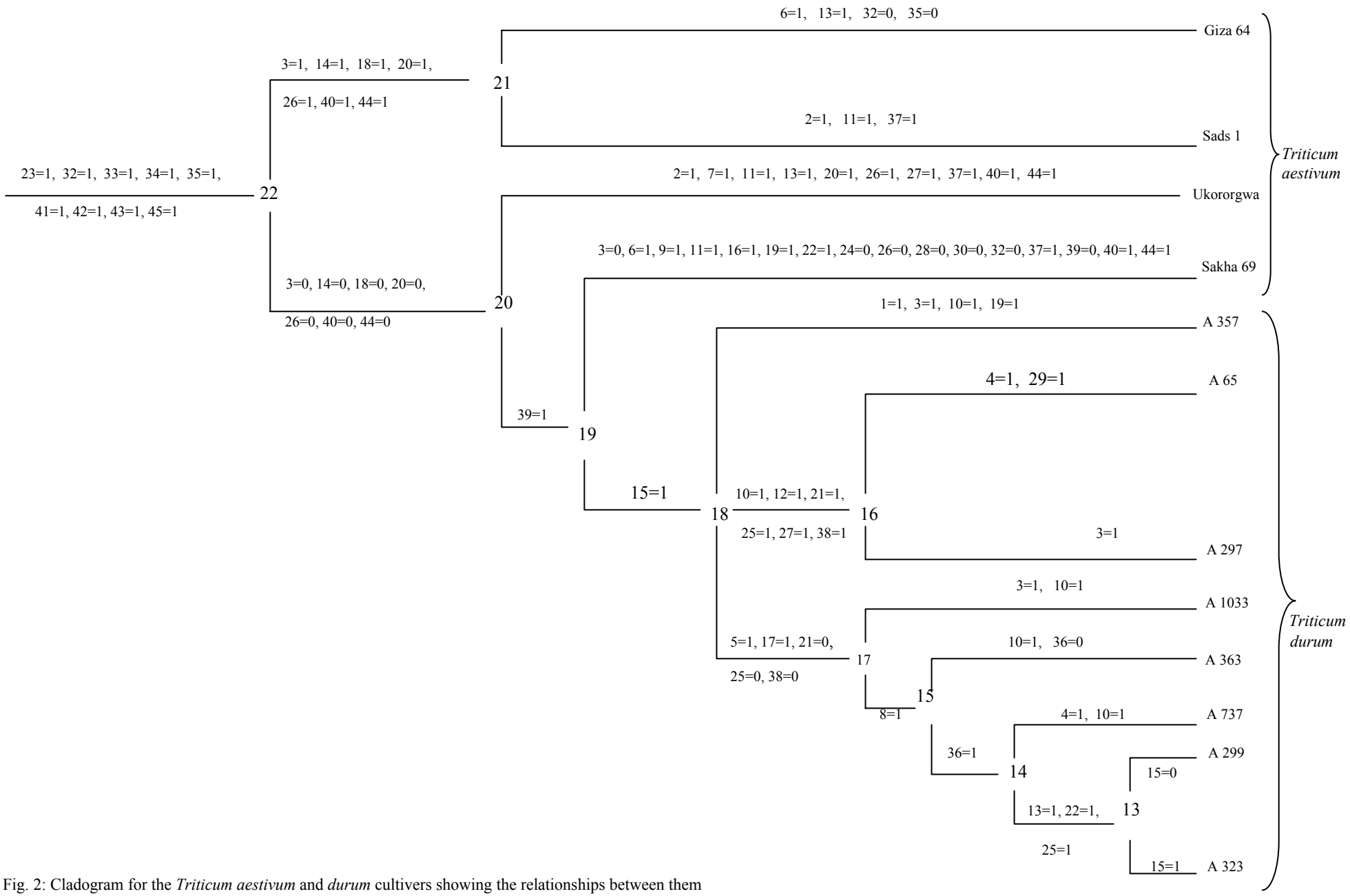

Fig. 2: Cladogram for the *Triticum aestivum* and *durum* cultivers showing the relationships between them based on seed protein patterns (length = 68, ci= 62). A= ACSAD

الملخص العربى

الأنماط البروتينية لحبوب القمح ودلالاتها التطورية الوراثية

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تمت دراسة الأنماط البروتينية لحبوب بعض السلالات الزراعية لنوعين من القمح (*تريتيكم ساتيفيم وتريتيكم ديورم*) وذلك بإستخدام طريقة التفريد الكهربى للبروتين. شملت الدراسة أثنتا عشرة سلالة من السلالات الشائعة فى مصر والسعودية وكان توزيعها كالتالى:-
1- أربعة سلالات تابعة للنوع *تريتيكم ساتيفيم* وهى: سخا 69 – جيزة 164 – سدس 1 - أكورورجوا
2- ثمانية سلالات تابعة للنوع *تريتيكم ديورم* وهى: أكساد 65 و297 و299 و323 و357 و363 و737 و1031
لقد أوضحت الدراسة أن البروتينات تختلف من حيث الكم (العدد) والكيف (الوزن الجزيئى) من سلالة إلى أخرى. وقد أشتركت جميع السلالات فى عدد من البروتينات ذات الأوزان الجزيئية التالية:
103, 85, 70, 50, 46, 45, 40, 38, 34, 33, 31, 29, 28, 21, 20, 18, 14 KD.
بينما تميزت حبوب سلالات النوع *تريتيكم ساتيفيم* بوجود البروتينات ذات الأوزان الجزيئية التالية:
108, 99, 97, 91, 87, 83, 81, 75, 65, 41, 35, 25, 22, 16 KD.
وتميزت حبوب سلالات النوع *تريتيكم ديورم* بوجود البروتينات ذات الأوزان الجزيئية التالية:
112, 102, 101, 93, 89, 86, 82, 79, 62, 43, 46, 27, 24, 23 KD.
وقد تم ربط ظهور وغياب تلك البروتينات فى النوعين محل الدراسة وأيضا فى السلالات المختلفة لبيان علاقتها التطورية-الوراثية بإستخدام برنامج رسم العلاقات التطورية (بوب) على جهاز الحاسب الألى.
أوضحت النتائج أن السلالات المختلفة لنوع *تريتيكم ساتيفيم* قد ظهرت مبكراً عن باقى سلالات النوع *تريتيكم ديورم* ، وأن السلالة جيزة 64 وسدس 1 هما أقدم الأنواع بناء على وجود مركبات البروتين ذات الأوزان الجزيئية المتباينة: 103, 83, 75, 65, 41, 22, 16 KD، بينما تعتبر سلالات النوع *تريتيكم ديورم* هى الأحدث تطوراً حيث أرتبطت جميعها مع بعضها البعض وتقاربت من حيث التركيب البروتينى.