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## GLIADINS POLYMORPHISM AND CLUSTER ANALYSES OF SYRIAN GROWN DURUM WHEAT

### ABSTRACT

A total of 187 *Triticum durum* (*Desf.*) genotypes were studied. These included 102 mutants, 15 local genotypes, 22 lines from ACSAD and 48 lines from ICARDA. Polyacrylamide gel electrophoresis under acidic conditions of pH 3.1 (A-PAGE) was used to separate gliadins groups of storage proteins for the identification and the classification of the genotypes under study. Results showed that the region of  $\omega$ -gliadins had a wider range for the number of bands than all other regions of gliadins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins). Cluster analyses using the Unweighted Pair Group Mean Average (UPGMA) method put the genotypes of all groups in trees on the basis of the gliadin bands distribution. Three categories were obtained: 1<sup>st</sup> – complete correspondence of the pedigrees and the trees, reflecting the importance of the gliadins as a decisive factor for the genotype position in the cluster; 2<sup>nd</sup> – the presence of genotypes with similar banding patterns but were unrelated in their pedigrees; 3<sup>rd</sup> – the genotypes originate from the same cross but are unrelated in the tree. It was concluded that tree clustering based on gliadin electrophoregrams may be used as an additional tool in revealing genetical relations among genotypes. However, one should keep in mind that several factors may influence the resulting tree. These include heterogeneity, incorrect band designation and uncertain or false pedigrees.

*Key words:* cluster analysis, Durum wheat, Gliadins, APAGE.

### INTRODUCTION

Gliadins are the wheat storage proteins that are thought to endow gluten viscosity to the dough (Payne *et al.* 1984). They are separated according to Bushuk and Zillman's (1978) system of Polyacrylamide Gel Electrophoresis under acidic conditions (A-PAGE). Their genetics is well documented with six major gliadin loci located on the short arms of chromosome groups 1 (*Gli-1*) and 6 (*Gli-2*) in addition to several minor loci reported by Metakovsky *et al.* (1997) and Pogna *et al.* (1993). Gliadins are useful markers for studies of wheat genetic resources (Redaelli *et al.* 1997), and for wheat variety identification due to their high amount of heterogeneity among the genotypes (Metakovsky and

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Branlard 1998). They are also used to detect heterogeneity within wheat entries and to determine whether this heterogeneity is due to genetic or mechanical admixing (MirAli 2000).

The usefulness of bread wheat gliadin bands resultant from A-PAGE for the assessment of genetic similarities was tested by Hegde and Singhal (2000). They used the Unweighted Pair Group Mean Average (UPGMA) to cluster 38 Indian varieties based on the presence or absence of their gliadin bands. This study showed that varieties with similar gliadin electrophoretic profiles could be grouped together and proposed this method to measure the magnitude of genetic similarity among varieties and varietal groups. More recently, MirAli (2002) used the same strategy on 96 bread wheat genotypes and found that harmony between the pedigrees of the genotypes and their relationship in the tree was not always achieved.

The aim of this study was to characterize a set of durum wheat (*Triticum durum* Desf.) grown in Syria using A-PAGE and to discuss the possibility of using cluster analysis based on their gliadin bands to determine the relationships among these genotypes.

## MATERIALS AND METHODS

### Plant material

A total of 187 durum wheat genotypes were used (Table 1). These include 102 mutant lines originally obtained from a mutation breeding program (MirAli 1987, 1991), 15 local and introduced varieties, 22 lines from the Arab Center for the Studies of Arid and Dry lands (ACSAD) (kindly provided by S.Basal), and 48 lines from the International Center for Agriculture Research in Dry Areas (ICARDA) (kindly supplied by M. Nachit). All genotypes were grown in 1998 under the same normal cultivation practices at an experimental site near Damascus.

### Analytical methods

A-PAGE of Bushuk and Zillman (1978) for gliadin separation was used with minor modification. Gliadins were separated in 6% polyacrylamide gels (160 × 180 × 1.5 mm) at 50 mA for 3 h 30'. Each gel contained 13 genotypes in addition to the Canadian variety Marquis as a control. The middle dens band of Marquis was given a mobility of 50 (Bushuk and Zillman 1978) and the relative mobility (RM) of all bands in each gel was computed accordingly. Band's presence (1) and absence (0) were recorded for all genotypes and the data were subjected to tree clustering using the Unweighted Pair-Group Mean Average and percent disagreement of the STATISTICA computer package.

**The studied genotypes and their available pedigrees**

Table 1

Lines	Characteristics of lines	ICARDA symbol
Group 1: Mutant lines		
L1–L34	Hamari type mutant	—
L35–L53	Semi–dwarf mutant	—
L54–L68	Hamari type mutant	—
L69–L73	<i>T.aestivum</i> × <i>T.durum</i>	—
L74–L89	<i>T.durum</i> × <i>T.dicoccoides</i>	—
Group 2: Local and introduced varieties		
Cham3	Released variety	—
Cham5	Released variety	—
Buhuth1	Released variety	—
Buhuth5	Released variety	—
OmRa5	Introduced line	—
OmRa9	Introduced line	—
OmRa3	Released variety	—
Gdzbate	Introduced line	—
Gdzbar	Introduced line	—
Daki	Introduced line	—
D15149	Introduced line	—
D12690	Introduced line	—
D11526	Introduced line	—
H300	Introduced line	—
Jez17	Released variety	—

Table 1

The studied genotypes and their available pedigrees (continued)		
Lines	Characteristics of lines	ICARDA symbol
Group 3: ACSAD genotypes		
A65	Stork × Gdavz469-AA-Stork	—
A297	Gediz-BAR/EgeXRuff-FG	—
A299	(MZA × 21563') × (GTA × 21563)	—
A323	CD14432 × CD10521	—
A357	DA 6 × OVI/CP//FG'S/12884-5L	—
A363	A 65 × G-VZ-469-CRS	—
A737	GERARDO 574 × Sahil 1/102	—
A1031	A 299 × SEB...7126/14	—
A1033	A 299 × SEBO...7126/13	—
A1037	(IZ.S 45 × A 71) × SEBO	—
A1073	A 65 × ROHO'S' 7177/84	—
A1075	A 65 × MEXI 75 - 7178/109	—
A1077	A 65 × MEXI 75 - 7178/113	—
A1085	A 71 × SEBO-7187/9	—
A1087	A 71 × SEBO-7187/11	—
A1095	A 71 × dwarf-15-/Cr/3///CII/RD	—
A1099	MEXI 75 × 300H	—
A1101	CHAM 1 × HAZAR	—
A1103	Bel × CD 26820-.7284/11	—
A1105	Bel × CD 26820-.7284/12	—
A1107	Bel × CD 26820-.7284/13	—
A1109	IZ'S'-103/3-140/2-1	—
Group 4: ICARDA genotypes		
LR1	Walmez-1	ICD86-0838-ABL-0TR-13AP-0TR-7AP-0TR
LR2	Mrb5 (Check)	—
LR3	Khbl/4/Mrb3	ICD85-0145-ABL-11AP-0TR-2AP-0TR
LR4	Stojocri-3	ICD83-0050-3AP-4AP-TR-1AP-0TR
LR5	Genil-3	ICD86-0615-ABL-0TR-2AP-0TR-8AP-0TR
LR6	Aw12/Bit	ICD84-0322-ABL-7AP-TR-AP-20AP-TR-1AP-0T ?
LR7	Stork (Check)	—
LR8	Genil-5	ICD86-0615-ABL-0TR-2AP-0TR-9AP-0TR
LR9	Omguer-6	ICD85-0988-15AP-TR-2AP-0TR
LR10	Guerou 1	ICD79-1463-1AP-2AP-4AP-0AP
LR11	Korifla(Check)	—

**The studied genotypes and their available pedigrees (continued)**

Table 1

Lines	Characteristics of lines	ICARDA symbol
Group 4: ICARDA genotypes (continued)		
LR12	Mrb3/Heider	ICD86-1601-ABL-0TR-15AP-0TR-9AP-0TR
LR13	Heider//Mt	ICD86-0414-ABL-0TR-2AP-0TR-8AP-0TR
LR14	Geruma-1	ICD86-0348-ABL-0TR-1AP-0TR-1AP-0TR
LR15	Genil-2	ICD86-0615-ABL-0TR-2AP-0TR-12AP-0TR
LR16	Waha(Check)	—
LR17	Omlahn-4	ICD85-0642-ABL-11AP-0TR-2AP-0TR-4AP-0TR
LR18	Genil-1	ICD86-0615-ABL-0TR-4AP-0TR
LR19	Omruf-1	ICD86-0436-ABL-0TR-9AP-0TR-1AP-0TR
LR20	Omsnima-1	ICD85-0538-ABL-TR-9AP-0TR
LR21	GdoVZ 512	ICD86-0759-ABL-0TR-2AP-0TR-2AP-0TR
LR22	Horani(Check)	—
LR23	Mrb3	L0589-4L-2AP-3AP-0AP
LR24	Mrb16/Ru	ICD85-1505-ABL-4AP-0TR
MR25	Stojorci-3	ICD83-0050-3Ap-4AP-TR-1AP-0TR
MR26	Mrb5 (Check)	—
MR27	Aw 12/Bit	ICD84-0322-ABL-7AP-TR-AP-21AP-0TR
MR28	Omruf-2	ICD86-0436-ABL-0TR-9AP-0TR-4AP-0TR
MR29	Awalbit-8	ICD84-0322-ABL-7AP-TR-AP-20AP-0TR
MR30	Stojorci-6	ICD83-0050-4AP-14AP-TR-3AP-0TR
MR31	Horani(Check)	—
MR32	Genil-4	ICD86-0615-ABL-0TR-2AP-0TR-11AP-0TR
MR33	Omruf-3	ICD86-0436-ABL-0TR-3AP-0TR
MR34	Awalbit-6	ICD84-0322-ABL-5AP-TR-AP-15AP-0TR
MR35	Korifla(Check)	—
MR36	Genil-1	ICD86-0615-ABL-0TR-4AP-0TR
MR37	Stojorci-7	ICD83-0050-4AP-14AP-TR-8AP-0TR
MR38	Stojorci-5	ICD83-0050-4AP-14AP-TR-2AP-0TR
MR39	Walmez-6	ICD86-0838-ABL-0TR-13AP-0TR-11AP-0TR
MR40	Waha(Check)	—
MR41	Mrb6	L0589-3L-1AP-2AP-1AP-0SH
MR42	Walmez-2	ICD86-0838-ABL-0TR-4AP-0TR
MR43	Belikh 2	L 92-6AP-1AP-1AP-0AP
MR44	Stojorci-9	ICD83-0050-3AP-6AP-0TR
MR45	Walmez-5	ICD86-0838-ABL-0TR-13AP-0TR-8AP-0TR
MR46	Stork(Check)	—
MR47	Mrb3	L0589-4L-2AP-3AP-0AP
MR48	Heider	ICD86-0414-ABL-0TR-4AP-0TR-14AP-0TR

## RESULTS

A-PAGE allowed gliadin separation into four major groups depending on their relative mobility (RM):  $\omega$ -gliadins which migrate up to RM=39,  $\gamma$ -gliadins (RM=40–56),  $\beta$ -gliadins (RM= 57–68) and  $\alpha$ -gliadins (RM=69–80). Table 2 shows the number of genotypes, range of the number of bands and the range of the number of bands for each gliadins group within each of the four groups of durum wheat genotypes.

Table 2  
Range of band number for each gliadin group within the studied four groups of durum wheat genotypes

Studied groups of genotypes	n	Range of band no.	Gliadin groups			
			$\alpha$	$\beta$	$\gamma$	$\omega$
1 - Mutants	102	15–25	3–6	3–4	3–5	3 – 11
2 - Local varieties	15	12–26	2–7	2–6	4–7	3 – 8
3 - ACSAD	22	12–17	1–2	2–4	4–5	3 – 9
4 - ICARDA	48	12–20	1–2	2–5	3–6	4 – 9

## Mutant lines

Fig. 1 shows the gliadin patterns of some mutant lines. Except for some lines between L71 and L89 which carried sometimes bands at RM=12, all other mutant lines had their first band appearing at RM=20. The number of bands ranged between 15 (L6) and 25 (L71–5).  $\omega$ -gliadins had the widest range (3–11 bands) compared to  $\alpha$ - (3–6 bands),  $\beta$ - (3–4 bands), and  $\gamma$ - gliadins (3–5 bands). Based on the gliadin profiles of these genotypes,

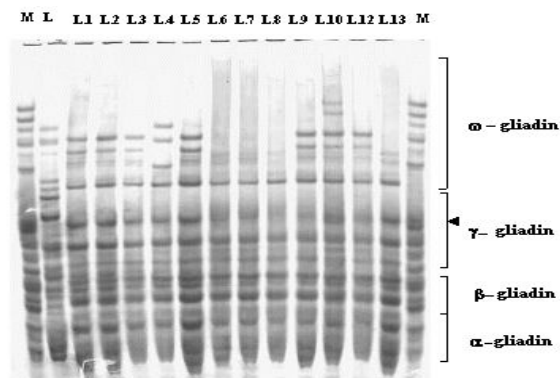


Fig. 1 A 6% polyacrylamide gel showing the gliadin patterns of some mutants where  $\omega$ -gliadins are shown as the most variable group. M (Marquis), L (Langdon) and the rest are mutant lines from a Syrian durum variety Hamari (see text)

the Unweighted Pair-Group Mean Average cluster analysis separated three major clusters (Fig. 2): The first cluster contained the common type of the mother line Hamari (L1–L34). The second cluster consisted of

a more closely related group that evolved from a semi dwarf *M2* mutant line from Hamari (L35–L53). The third cluster contained a largely heterogeneous set of lines that included offspring from a cross between Hamari and a hexaploid (Falsheto × Mexicani × Mahmoudi) (L69–L73) and offspring from a cross between Hamari and the wild tetraploid *Triticum dicoccoides* (L74–L87), (MirAli 1991).

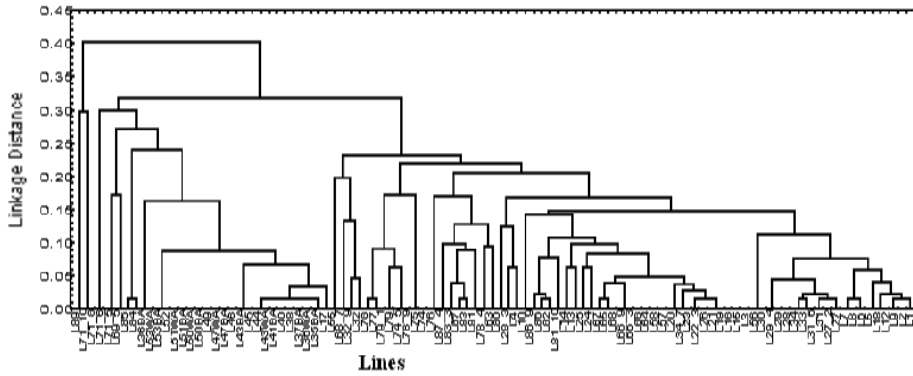


Fig. 2 Dendrogram of 102 durum mutants and their hybrids, Unweighted Pair Group Mean Average (UPGMA), Percent of disagreement:

$$\left\{ \begin{array}{l} \text{distance}(x, y) = \frac{\text{No of } x_i \neq y_i}{i} \end{array} \right\}$$

#### Local and introduced varieties

This group gave the highest range in band number, i.e. from 12 (OmRabi5) to 26 (Jezira17). Moreover, the range in band number within gliadin regions was not apparently shifted towards any particular region. (Table 2). Cluster analysis of the genotypes of this group showed very close relations among OmRabi series and D11526. Also, a close relation was obtained among Gdzbate, D15149 and D12690, all three were related to Gdzbar. Likewise, a close relation appeared between Buhuth1 and H300 and both were related to a lesser degree with Daki. Cham3 and Cham5 had 25% disagreement, whereas Buhuth5 was the most distant from all genotypes in this group with a 40% disagreement (Fig. 3).

#### ACSAD genotypes

This group was distinguished by having the lowest range in band number (12–17).  $\omega$ -gliadins region had the widest range (3–9 bands) compared to the  $\alpha$ - (1–2 bands), the  $\beta$ - (2–4 bands), and the  $\gamma$ -gliadins region (4–5 bands). UPGMA revealed that this group was the most homogeneous (the largest percent disagreement among the genotypes was 15%) (Fig. 4). A363 and A737 were almost indistinguishable and were found to have a common parent (Gerardo). Similarly, A1075 and A1077 resulted from the same cross A65 × Mexi 75 and were almost identical in their electrophoregrams. On the other hand, A65 and A297 were very close but had no common parents in their pedigrees. A1085, A1087, and

A1095 were relatively more distant despite having a common parent A71. Also, A1031 and A1033, which resulted from the same cross (A299 × Sib0), were not very closely related in the tree.

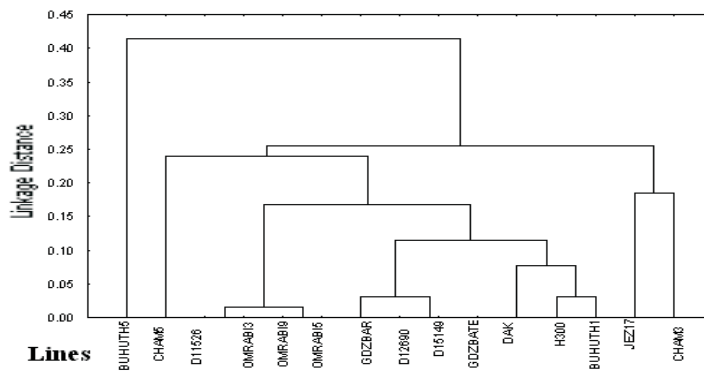


Fig. 3 Dendrogram of 15 local introduced durum genotypes, Unweighted Pair Group Mean Average (UPGMA), Percent of disagreement:  $\left\{ \begin{array}{l} \text{distance}(x,y) = \frac{\text{No of } x_i \neq y_i}{i} \end{array} \right\}$

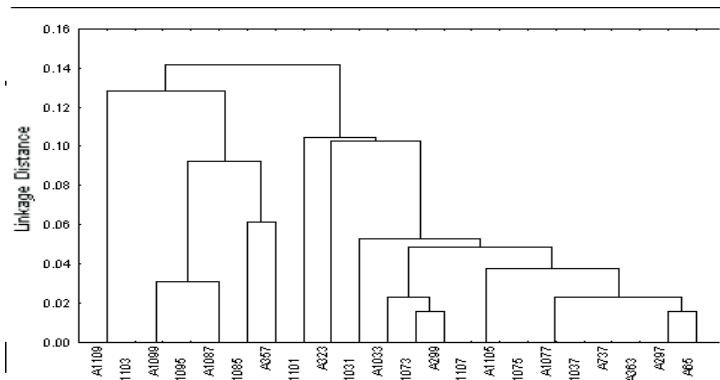


Fig. 4 Dendrogram of 22 ACSAD durum genotypes, Unweighted Pair Group Mean Average (UPGMA), Percent of disagreement:  $\left\{ \begin{array}{l} \text{distance}(x,y) = \frac{\text{No of } x_i \neq y_i}{i} \end{array} \right\}$

### ICARDA genotypes

Band number ranged between 12 and 20. The widest range occurred in the  $\omega$ -gliadins region (4–9 bands). The  $\beta$ - and the  $\gamma$ -gliadins regions were similar in their range for the number of bands. The range of the  $\alpha$ -gliadin region was very narrow (1–2 bands) (Table 2).

UPGMA analysis indicated a large variation among the studied genotypes (Fig. 5). It showed two main clusters that had about 50% disagreement; one of them had 33 genotypes and the other had 15 genotypes. There were genotypes having a very close relation in the cluster. These



were found to belong to the same series, for instance, MR37 (Stojorci-7) and MR38 (Stojorci -5) and also MR39 (Wadalmez-6) and MR45 (Wadalmez-5). A similar situation was found for MR41 (Omrabi-6) and MR47 (Omrabi-3). On the other hand, MR36 (Genil-1) and MR42 (Wadalmez-2) had complete accordance. These were found to have five common parents out of the six included in their pedigree. The other Genil lines, however, belonged to the second main cluster and were located further apart from each other (LR5= Genil3, LR15= Genil2, and MR32=Genil4).

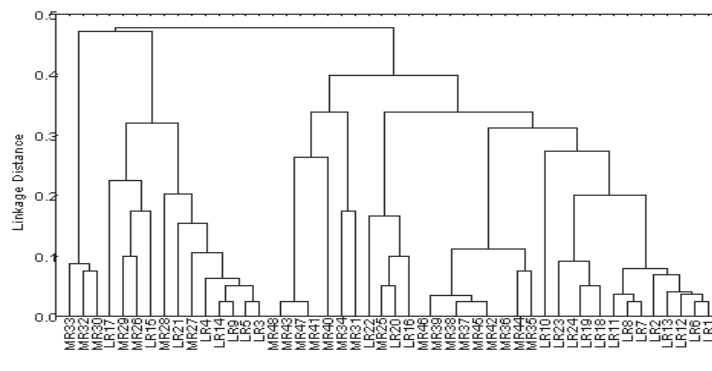


Fig. 5 Dendrogram of 48 ICARDA durum genotypes, Unweighted Pair Group Mean Average (UPGMA), Percent of disagreement:  $\left\{ \begin{matrix} \text{dis} \\ \tan \\ ce(x,y) = \frac{\text{No of } x_i \neq y_i}{i} \end{matrix} \right\}$

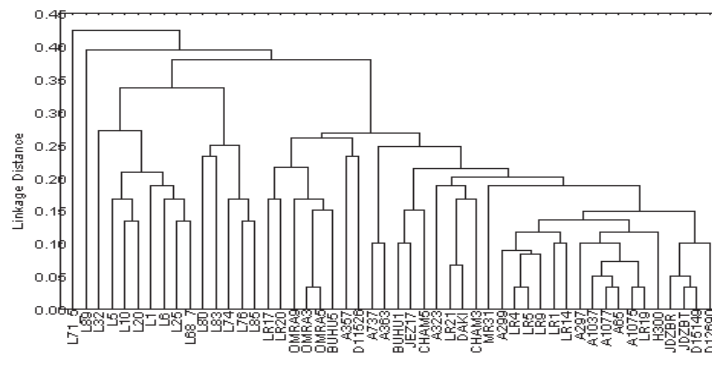


Fig. 6 Dendrogram of 50 genotypes, representing four studied groups. Unweighted Pair Group Mean Average (UPGMA), Percent of disagreement:  $\left\{ \begin{matrix} \text{dis} \\ \tan \\ ce(x,y) = \frac{\text{No of } x_i \neq y_i}{i} \end{matrix} \right\}$

Fifty genotypes representing all four groups were subjected to UPGMA cluster analysis to get an idea about the legitimacy of the grouping presented here. The results are shown in Fig. 6. The analysis splitted the genotypes into two main clusters (with a 37% disagreement)

with two genotypes (L71-5 and L89) are most distant. The first main cluster combined six small clusters: One contained four introduced lines (Jdzbr, Jdzbt, D15149 and D12690), the second and the third clusters contained mostly ACSAD and ICARDA lines respectively. The other three small clusters were not as closely related as the first three. They contained genotypes that represent all three categories. The second main cluster had two small clusters: One contained mutant lines crossed to either *T.dicoccoides* or to *T.aestivum* and the other contained mutant lines from the local durum variety Hamari.

#### DISCUSSION

Durum wheats are distinguished by having their first band appearing at  $RM>20$  (Sapirstein and Bushuk 1985). In the first group, which included mutants and crosses between these mutants and either bread wheat or the wild tetraploid wheat *T. dicoccoides*, all durum mutants followed this rule. However, some lines resultant from these crosses carried bands with  $RM=12$ . It is not unexpected for lines resulting from crosses with bread wheat to carry these lower mobility bands, since some of the segregants may carry bands from the D-genome of their maternal parent. However, the observation that segregants from the crosses with *T.dicoccoides* carried these lower mobility bands implies that these bands are not restricted to the D-genome and must have originated from either the A- or the B-genome of *T. dicoccoides*. Although no durum wheat variety carried these lower mobility bands, it seems that the progenitor of durum carries several dens bands in this  $\omega$ -gliadins region (MirAli, 1987). The high dense band numbers in the wild wheats may be due to its high protein content. Uthayakumaran *et al.* (1999) found that the gliadin/glutenin ratio was higher at high protein levels than at lower protein levels. This was accompanied with a negative effect on gluten strength.

Among all groups, the  $\omega$ -gliadins region had the widest range number of bands. Since this region was characterized by having many bands in the wild wheat, it seems plausible to consider the null alleles in durum varieties as being an evolutionary index. In this context, Lafiandra *et al.* (1987) found some genotypes carrying the null allele in  $\omega$ -gliadins. They suggested that it might be a result of partial deletion in the sequence or a result of gene silencing. It should be noted that  $\omega$ -gliadins differ from  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins which share a similar terminal N in their sequence (Kasarda 1980). They are considered to be sulfur rich (Shewry, *et al.*, 1997). The lack of sulfur in  $\omega$ -gliadins causes this group to be affected by hydration (Wellner *et al.*, 1996). Fido *et al.* (1997) found this gliadin group to have the most negative effect on dough strength followed by  $\alpha$ -gliadins and  $\beta$ -gliadins and with  $\gamma$ -gliadins having the least negative effect.

This study attempted a different approach to study the legitimacy of grouping durum wheat genotypes on the basis of the source of the material or on that of their pedigrees. Our results showed three categories regarding the conformity between the obtained pedigrees and the position of the genotypes in the tree:

- 1). The ideal situation of having complete correspondence between the pedigrees and the trees, reflecting the importance of the gliadins composition as being a decisive factor for the genotype position in the cluster.
- 2). The presence of genotypes with similar banding patterns (and hence being related in the tree) but being unrelated in their pedigrees. There are two possibilities for this case: either the genotypes in question are heterogeneous for the alleles responsible for these proteins, or the genotypes have false pedigrees. In this context, Metakovsky and Branlard (1998) considered it to be improbable to have two unrelated varieties with the same allelic composition. Due to the large allelic number in each locus they suggested the pedigrees to be false.
- 3). Genotypes originate from the same cross but are unrelated in the tree. This may be due to the fact that these genotypes were selected to have different morphological characteristics. In a previous study, high molecular weight glutenin subunits (HMW-GS) content of some of these genotypes revealed different allelic composition; for instance both A1031 and A1033 resulted from a cross between A299 X SEBO, however, A1031 contained subunits 6+8 and A1033 contained the allelic subunits 7+8 (MirAli *et al.* 1995).

The application of electrophoresis of gliadins using A-PAGE has been used mainly for cultivar "fingerprinting" (Bean and Lookhart 2000). These protein markers offer an easy, cheap and powerful tool to identify wheat varieties. They can be supplemented with the other protein markers, the glutenines, using SDS-PAGE to have the complete picture of wheat storage proteins. Using this technique, durum wheat varieties grown in Syria were all found to carry the null allele of *Glu-A1* (MirAli *et al.*, 1999).

It is concluded that the use of tree clustering based on gliadin electrophoregrams may be used as an additional tool in revealing genetical relations among the genotypes. This is particularly important since pedigree information is not always sufficient enough to determine the degree of relatedness among durum wheat varieties. However, care should be taken when drawing any conclusion since many factors influence the resulting tree. These factors include heterogeneity, incorrect band designation, and uncertain or false pedigrees.

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