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Full Length Research Paper

Genetic variation of durum wheat landraces using morphological and protein markers

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Genetic variations of cultivars are very interesting in reducing genetic vulnerability and lead to stable control of production. The aim of this research was to study genetic diversity among six durum wheat cultivars. For the first assay we evaluated seven morphological traits which are: spikelet per spike, spike length, spike width, beard length, plant height, width of truncation and barb length. The tested genotypes were classified in three groups according to the linkage distance analysis. The genetic variability was also evaluated for seed storage-proteins by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoregram allowed the estimation of the durum wheat genetic similarity (GS). This GS analysis based on Unweighted Pair Group Method with Arithmetic averages (UPGMA), permits to obtain the same genotypic clustering. No significant correlation was observed among the two methods tested. It is concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and genotypes classification, which can be used to improve the efficiency of wheat breeding programs.

Key words: Wheat genotypes, SDS-PAGE, genetic diversity, cluster analysis.

INTRODUCTION

Wheat genetic diversity has been well evaluated using morphological protein and molecular markers. Phenotypic identification based on morphological traits has been successfully used for genetic diversity analysis (Daâloul et al., 1998; Fakhfak et al., 1998). However, morphological traits have a number of limitations, including low polymorphism, low heritability, late expression, and may be controlled by epistatic and pleiotropic gene effects (Nakamura, 2001). While protein markers, like seed storage proteins, reflect with more accuracy the genotypes, independently from the environmental effects (Autran et al., 1995), protein markers are useful tools in identifying cultivar, registration of new varieties, classification of crop species and in studying genetic diversity, thereby improving the efficiency of wheat breeding programs in cultivar development (Gianibelli et al., 2001; Naghavi et al., 2009).

Proteins are grouped into four classes according to their solubility: albumins, globulins, prolamins and glutenins. According to the proteins quantity and quality, mainly gluten, wheat varieties are regrouped in different classes (Godon and Willm, 1991). Gluten, comprising roughly 78 - 85% of total wheat endosperm protein, is a very large complex composed mainly of polymerics and proteins known respectively as glutenins and gliadins (Mac Ritchie, 1994). Gliadins and glutenins have been extensively studied and the genetics and biochemistry are relatively well known (Magdalena et al., 2002; Starovicova et al., 2003; Picard et al., 2005). Glutenins confer elasticity to dough, whereas, gliadins are viscous and give extensibility to dough (Feillet, 2000).

The main objective of this study was to study genetic diversity in landraces of durum wheat using morphological data and seed storage proteins. This information will be useful to improve techniques for sampling wheat genetic variation which might increase efficiency of germplasm

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Abbreviations: MWM, Molecular weight marker; **LWM,** Low molecular weight; **HWM,** high molecular weight; **HWMGS,** high molecular weight glutenin subunits; **SDS-PAGE,** sodium dodecylsulphate polyacrylamide gel electrophoresis; **GS,** genetic similarity.

Table 1. Variance analysis (Mean squares and test F) for seven traits for six (6) durum wheat landraces.

Variation sources	df	Plant height	Spike length	Spike width	Barb length	Truncation width	Beard length	Spiklet per spike
Genotypes	5	2623.17**	9.24**	0.11**	15.52**	3.91**	1.73**	9.24**
CV (%)		18.8	9.2	18.4	20.8	18.4	16.7	25.3
R^2		0.87	0.50	0.73	0.64	0.78	0.91	0.90

Table 2. Mean values of different traits in six durum wheat genotypes.

Parameters	Hamira 1	Hamira 2	Hamira 3	Agili RC1	Agili RC2	Agili RP1
Plant height (cm)	84.60 b[1)]	126.00 c	120.00 a	135.80 d	131.40 cd	138.60 d
Spike length (cm)	8.50b	6.68 a	6.28 a	9.74 b	9.80 ab	9.20ab
Spike width (cm)	1.08bc	0.64a	0.60a	0.80d	0.88b	0.74cd
Barb length (cm)	10.00b	8.22a	8.04c	12.12b	11.40b	12.32ab
Beard length (mm)	2.50c	3.00d	2.90d	1.80a	2.00b	1.90b
Truncation width (mm)	5.40ab	6.20ab	6.00a	4.80c	4.90b	4.60c
Spikelet per spike	25.00c	19.80d	19.20d	23.40a	22.00b	22.00b

Values by same letters in each row are not significantly different at the 5% probability level by Duncan's multiple.

conservation.

MATERIALS AND METHODS

Plant materials

Six Tunisian landraces of durum wheat (*Triticum turgidum* L.var. durum (Desf.)) were used in this study (Hamira 1, Hamira 2, Hamira 3, Agili RC1, Agili RC 2 et Agili glabre RP1). These genotypes were evaluated with five replications at the experimental station of National Agronomic Institute. Each genotype was sown in a 1 m long row with 0.5 m row spacing. Morphological data on spikelet per spike, spike length, spike width, beard length, plant height, width of truncation and barb length were recorded from five plants which had been randomly chosen in each row and mean of quantitative data sets were used for analysis.

SDS PAGE electrophoresis

The seeds were crushed finely and the flour was mixed in an extraction buffer of 0.125 M Tris-Hcl (pH 6.8), 3% sodium dodecyl sulfate (SDS), 0.03% bromophenol blue and 5% 2-mercapto-ethanol. Samples were boiled for 2 min at 100°C and then centrifuged for 5 min at 10400 rpm before being fractionated by SDS-PAGE.

According to Lammeli (1970) method, a stacking gel containing 30% acrylamide, 1% Bis acrylamide, 0.4% SDS and 0.5 M Tris-Hcl (pH = 6.8), and separating gel containing 30% acrylamide, 1% Bis acrylamide, 0.4% SDS and 1.5 M Tris-Hcl (pH 8.8) were used. The two gels were polymerised in the presence of TEMED and 10% ammonium persulfate. Gels were stained overnight with 0.2% comassie Brilliant Blue and then distained overnight in water.

Estimates of genetic similarity

Genetics similarity (GS) between two genotypes A and B was calculated for each marker system and across marker systems according to the formula (Nei and Li, 1979):

$$GS_{AB} = 2N_{AB}/ (N_A+N_B)$$

where N_{AB} is the number of bands in common mobility, and N_A and N_B are the total number of bands in genotype A and B. Thus GS_{AB} reflects the proportion of bands in common between two genotypes and may range from 0 (no bands) to 1 (identical profiles of two lines).

Cluster analysis

The similarity matrices obtained with the two sets of data (morphological and biochemical level) were converted to dissimilarity matrix (d) using a formula d = 1 - GS and used to generate unweighted pair group method with arithmetic averages (UPGMA) dendrograms. All recorded data were analyzed for variance analysis (ANOVA) and to establish dendrograms using the computer software package SPSS and STATISTICA.

RESULTS

Morphological diversity evaluation

Analysis of variance showed a significant difference between the 6 durum wheat genotypes on the morphological level for the seven traits (Table 1).

Analysis of Dancun's multiple range tests showed that, plant height ranged between 84.6 cm for Hamira 1 and 138.6 cm for Agili RP1; landrace genotype was relatively taller in size (Table 2). Variation was observed between genotypes for spike length, spike width, barb length and spikelet per spike. Differences were also noted on

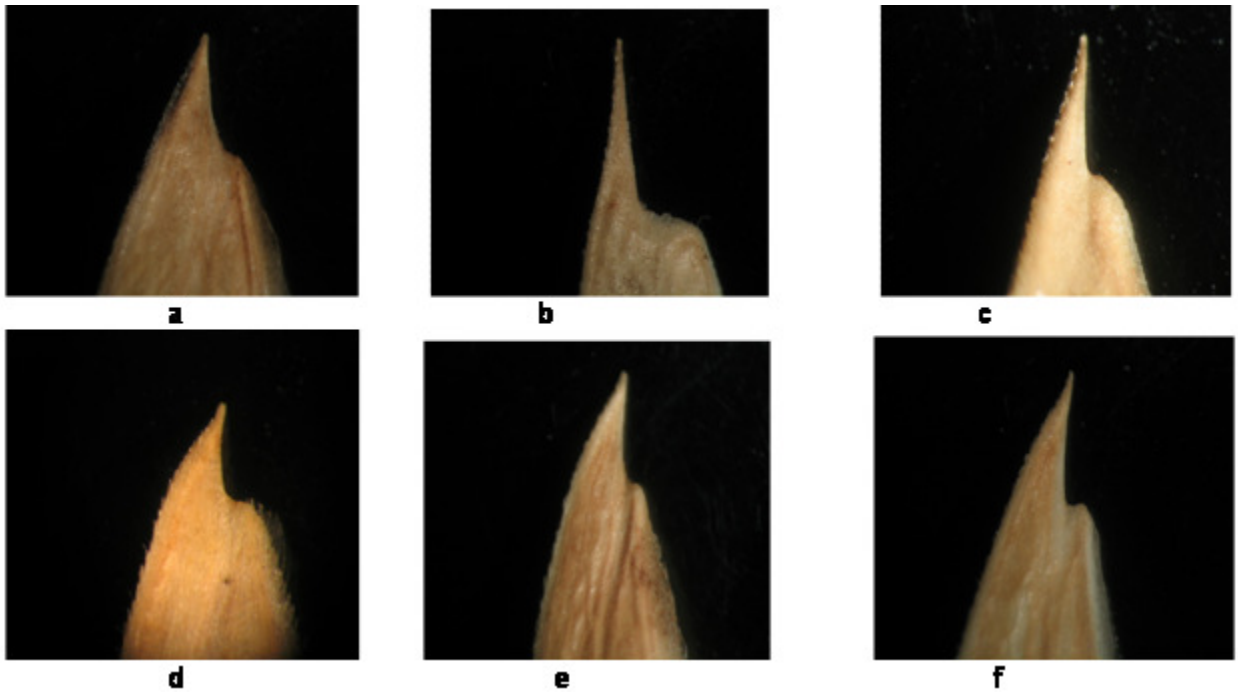

Figure1. Different shapes of truncation and bread observed for the six durum wheat genotype. (a) Hamira 1; (b) Hamira 2; (c) Hamira 3; (d) Agili RC2; (e) Agili RC1; and (f) Agili RP1.

Table 3. Coefficient matrix of six wheat varieties based on morphological traits.

Genotypes	Hamira 1	Hamira 2	Hamira 3	Agili RC1	Agili RC2	Agili RP1
Hamira 1	0.0					
Hamira 2	41.8	0.0				
Hamira 3	36.0	6.1	0.0			
Agili RC1	51.3	11.7	17.3	0.0		
Agili RC2	46.9	7.5	12.8	4.7	0.0	
Agili RP1	54.1	13.8	19.6	3.2	7.3	0.0

truncation width and beard length (Figure 1).

The dendrogram calculated from the similarity coefficient (Table 3) and unweighted pair group method with averages on the seven measured traits, is presented in Figure 2.

Genetic diversity evaluation

The six (6) durum wheat genotypes used in the present study showed various banding pattern using SDS-PAGE technique. In this study, SDS-PAGE of grain proteins was performed in order to investigate genetic diversity among wheat genotypes. Electrophoregram showing protein banding pattern of different wheat varieties are given in Figure 3. The presence or absence of bands is mentioned in Table 4.

The genetic similarity coefficient matrix (Table 5) of six durum wheat genotypes on the bases of linkage distances (Euclidian distances) was used to construct a dendrogram, to find the diversity among given durum wheat genotypes (Figure 4).

The study of correlation coefficient ($r = 0.30$) between morphological and biochemical dendrogram showed that no significant correlation was observed between the two dendrograms obtained.

DISCUSSION

Variation among six local durum wheat accessions was highly significant ($p < 0.01$) for all of the seven tested characters. In fact, high variability in Tunisian durum wheat was already noted by Gashaw et al. (2007) and Fakhfak et al. (1998). On the basis of morphological traits cluster analysis, placed the six durum wheat genotypes

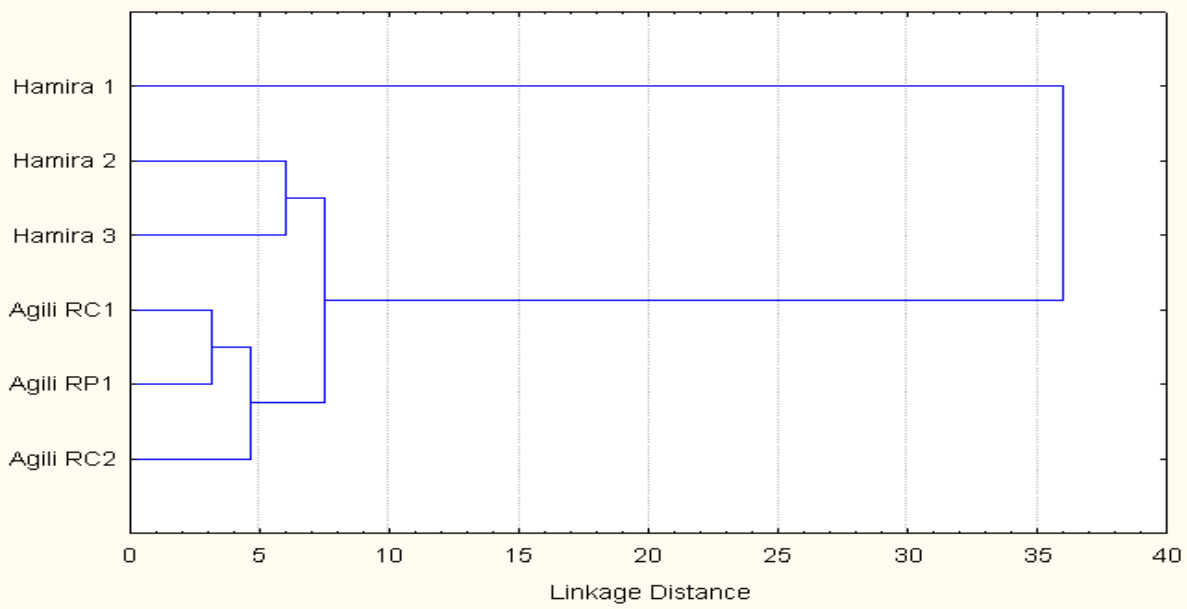

Figure 2. UPGMA cluster analysis showing the diversity and relationship among 6 durum wheat genotypes based on seven morphological traits.

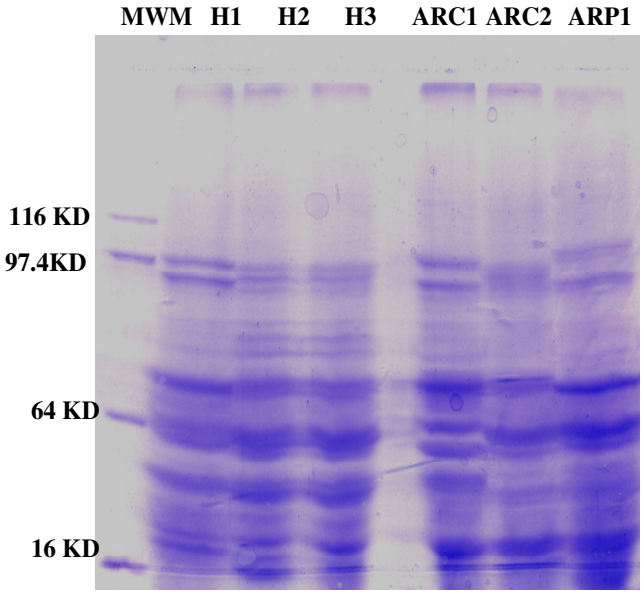

Figure 3. Electrophoregram showing protein banding pattern of different durum wheat genotypes (MWM: Molecular weight marker; H1: Hamira 1; H2: Hamira 2; H3: Hamira 3; ARC1: Agili RC1; ARC2: Agili RC2; APR1: Agili RP1).

into different groups. At Euclidean distance of 10, all genotypes showed similarity with one another and constitute two categories, one containing Hamira 1. The second formed by the rest of genotypes. At Euclidean distance of 5, the dendrogram revealed three main groups. The first contained Hamira 2, Hamira 3. The second was Agili RC1, Agili RC2, and Agili RP1. The genotype Hamira 1 was in another group. Same finding by Naghavi et al. (2009) based on morphological data (spiklet per spike, seed per spike, 100 grain weight, plant height, peduncle length and spike length) showed that the first two principal component scores separated Iranian landraces from cultivars showing that the studied cultivars are quite different from Iranian landraces.

According to the results of the SDS-PAGE, a total of 14 bands were obtained bands number 7, 9 and 13 are common in all genotypes but other bands showed variation. Agili RC1 is characterized by the presence of band 11. The genotype Agili RC2 and Agili glabre RP1 differed by the presence of bands 1 and 2 observed at Agili glabre RP1. Indeed, Hamira 1 was characterized by the presence of bands 3 and 14 but Hamira 3 presented bands 11 and 14. These bands did not exist at Hamira 1 which was characterized by the presence of the band 12. The genetic dissimilarity dendrogram calculated from similarity coefficient for LMW and HMW glutenin subunits bands revealed different groups. At linkage distance of 1.6, genotypes distributed into three groups. The first containing Hamira 2 and Hamira 3. The second was Agili RC1, Agili RC2, and Agili RP1. The genotype Hamira 1 was in another group. Thus, a large variation between genotype was noted. Several authors studied the polymorphism as well as proteins' different mobilities via the SDS-PAGE electrophoresis (Cherdouh et al., 2005; Carello et al., 2006). Indeed, Benmoussa et al. (2000) showed large variation among LMW and HMW glutenin subunits. It has been suggested that deletions and insertions within the repetitive regions are responsible for these variations in length. Moreover, Naghavi et al. (2009) reported that most of landraces showed different

Table 4. Matrix of presence or absence of bands of different durum wheat genotypes.

Band	Hamira 1	Hamira 2	Hamira 3	Agili RC1	Agili RC2	Agili RP1
Band 1	1	1	1	1	0	1
Band 2	1	1	1	1	0	1
Band 3	0	1	0	0	0	0
Band 4	1	1	1	0	0	0
Band 5	1	1	1	0	0	0
Band 6	1	1	1	0	0	0
Band 7	1	1	1	1	1	1
Band 8	0	0	0	1	1	1
Band 9	1	1	1	1	1	1
Band 10	1	1	1	0	0	0
Band 11	0	0	1	1	0	0
Band 12	1	0	0	0	0	0
Band 13	1	1	1	1	1	1
Band 14	0	1	1	1	1	1

Table 5. Coefficient matrix of six durum wheat genotypes based on SDS-PAGE using UPGMA method.

Genotypes	Hamira 1	Hamira 2	Hamira 3	Agili RC1	Agili RC2	Agili RP1
Hamira 1	0.00					
Hamira 2	1.73	0.00				
Hamira 3	1.73	1.41	0.00			
Agili RC1	2.83	2.65	2.24	0.00		
Agili RC2	3.00	2.83	2.83	1.73	0.00	
Agili RP1	2.65	2.45	2.45	1.00	1.41	0.00

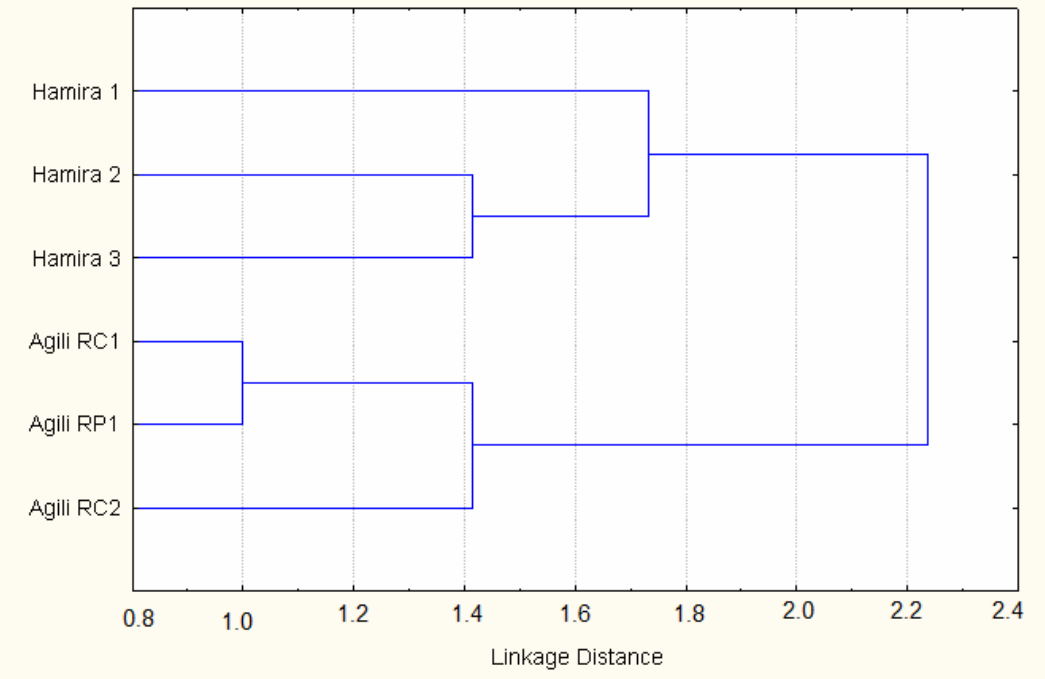

Figure 4. UPGMA cluster analysis showing the diversity and relationship among 6 durum wheat genotypes based on SDS-PAGE.

HMWGS compositions compared with cultivars. These differences may be due to the dissimilarity of materials and/or the fact that these cultivars are not originated from Iranian landraces. The variation in high molecular weight protein subunits could be the result of gene silencing in some varieties.

However, other studies showed low degree of hetero-geneity between wheat genotypes tested (Lawrence and Shephred, 1980; Mohd et al., 2007; Siddiqui and Naz, 2009). This low level of genetic diversity may be attributed to reduce number of varieties used for wheat cropping.

No significant correlation was observed in the present study between the morphological and biochemical dendrogram. This result was also obtained by using morphological traits and SDS-PAGE electro-phoresis on 30 ancestrals to modern hard red winter wheat (*Triticum aestivum* L.) cultivars. A large variation was observed in the morphological and biochemical level between durum wheat genotypes. These two methods could be used to study genetic diversity in different durum wheat genotypes. The choice of the method for genetic diversity estimation depends largely upon the tools available and how well it fits in breeding scheme. Both biochemical and agronomical traits will be useful to breeders to formulate crosses by choosing genotypes with appro-priate characters. SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm.

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