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RESEARCH ARTICLE

Comparative Quality Analysis of Gluten Strength and the Relationship with High Molecular Weight Glutenin Subunits of 6 Tunisian Durum Wheat Genotypes

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Abstract Six Tunisian durum wheat genotypes (4 landraces and 2 improved) were evaluated for protein content, gluten strength, rheological characteristics, and HMW-GS patterns using a LabChip system. Variance analysis identified genotypic variation. The landraces Azizi, Mahmoudi, Chili, and Arbi exhibited the highest protein concentrations and gluten contents, and best dough tenacity and extensibility values. The Mahmoudi and Chili varieties had the highest protein contents (17.06 and 17.32% dry mass, respectively). Arbi and Chili had the highest gluten contents (60.88 and 60.59%, respectively). Azizi, Mahmoudi, and Chili were characterized by higher dough tenacity, lower dough extensibility, and a greater alveograph configuration ratio P/L. The high molecular weight glutenin subunits 6+8 (Azizi and Mahmoudi) and 7+15 (Chili), coded by the *Glu-B1* locus, improved gluten strength and viscoelastic dough properties. Calculated HMW to LMW-GS ratios were within a narrow range of 0.17-0.29. Some genotypes have potential to be used as parents in breeding programs.

Keywords: alveograph, durum wheat, genotype, high-molecular-weight glutenin subunit (HMW-GS), LabChip

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Introduction

The end-use value of durum wheat (*Triticum turgidum* L. var. *durum*) depends on the quality and quantity of stored endosperm protein. The polymeric composition of gluten, particularly the glutenin fraction, is responsible for the main differences in bread-making qualities among durum wheat genotypes (1). Native glutenin is a macropolymer composed of high molecular weight (Mw) glutenin subunits (HMW-GS) and low Mw glutenin subunits (LMW-GS) joined together by intermolecular disulfide bonds (2). The HMW-GS fraction is the most intensively studied protein fraction because of its critical influences of type, amount, and size distribution on end-product quality (1,3). Based on worldwide observations related to correlations between HMW-GS patterns and wheat quality, Payne *et al.* (4) proposed use of the locus "*Glu-1* quality score" to predict the baking quality of wheat varieties.

Several methods have been developed and adapted to identify typical durum genotypes with high qualitative performance. Alveograph testing, usually used for rheological analysis of bread dough strength (5), has been adapted for assessment of durum semolina dough (6-8). SDS-PAGE,

typically used to analyze the physical properties of bread wheat dough (9), was recently adapted for use with durum wheat for analysis of HMW-GS polymorphism (6,10). The method is laborious, slow, and not reproducible. LabChip technology, which emerged in the early 1990's, has overcome these difficulties and has demonstrated great potential for identification of HMW-GS, as well as for relative quantification of the ratio of HMW-GS to LMW-GS (11). This approach would be useful for parent selection of desirable glutenin alleles in relation to a high gluten strength potential.

The aim of this investigation was to evaluate the protein concentration, gluten content, rheological properties, and biochemical characteristics (HMW-GS composition) of 4 durum wheat landraces (Azizi, Mahmoudi, Chili, and Arbi) compared to 2 improved genotypes (Karim and Razzek). HMW-GS patterns were then associated with gluten strength.

Materials and Methods

Durum wheat samples Grains of 6 Tunisian durum wheat genotypes, including the 4 landraces Azizi, Mahmoudi, Chili, and Arbi, and the 2 improved varieties of Karim and Razzek, were used.

Landraces were chosen on the basis of quality differences. The genotypes Karim and Razzek, even though improved for increased yield and despite being recommended for irrigated areas, are susceptible to diseases (12). All studied genotypes were provided by the National Gene Bank of Tunisia and were grown under the same field conditions during the 2009-2010 crop year at the National Institute of Field Crops (INFC), northwest of Tunisia.

Sample preparation for quality testing All durum wheat samples were manually cleaned by elimination of damaged grains, organic or non organic elements other than grains of durum wheat. The initial moisture of each sample was determined in advance (13). Each sample of durum wheat was placed in a hermetically sealed bottle and was conditionnated by adding a quantity of water determined by the following formula in order to bring the final moisture to 16.5%. The conditionnement is done 48 h before milling at ambient temperature. Water is added, with agitation by hand, in 2 steps with episodes of rest of 6 h to ensure a better penetration of water into kernel, and an easier separation of the bran from the endosperm (14). After that, samples were milled into semolina using an ISO Brabender mill (Quadrumat junior; CWBrabender, South Hackensack, NJ, USA) for doing analysis for protein, for gluten, and for alveograph testing. Then, the moisture content of semolina was determined following an ISO-approved method (13). All these latter analyzes were performed at the Laboratory of Plant Origin Products, Central Analysis and Testing Laboratory of the Ministry of Industry and Technology of Tunisia.

Quality evaluation

***Protein content*:** The protein content (Prot) was determined following the standard Kjeldhal method (N×5.7) (15). This method consists of mineralization of the samples of semolina with sulfuric acid in the presence of catalyst on the automatic block digester (K432; Büchi, Switzerland), alacalinization of the products of the reaction and distillation (Distillation unit K350; Büchi, Switzerland). Results were reported on dry matter (DM) basis.

***Wet and dry gluten contents*:** The semolina wet gluten (WG) (16) and dry gluten (DG) (17) contents were determined following the classical methods in which gluten was washed manually according to the ISO methods. The excess of wash solution adhering to the gluten ball was removed from the remaining gluten by taking this one between fingers, then two layers of cloths, application of three short compressions and weighed by an electronic balance of 0.01 g resolution (Gram Precision BH-600; Galilo Equipment, Spain). The obtained ball of wet gluten was then baked in a drying oven with forced convection (Deltalabo, France) at 130ºC for 6 h.

***Rheological properties*:** The rheological properties of dough were analyzed using an alveograph ISO (18) (Chopin MA 82; Tripette and Renaud, Villeneuve-la-Garenne, France). Alveograph testing was performed for durum wheat samples with 55-59% water content (15% moisture basis), rather than 50% for the flour of common wheat. This modification was used to compensate for greater water absorption typically caused by high levels of starch damage that occur during milling of hard durum wheat grains (19). A 2.5% sodium chloride solution, by dissolving 25 g of sodium chloride in 1 L of distillated water, was used to hydrate a 250 g sample of each semolina to the level specified in the ISO method 5530/4-1991 (15% moisture) (18). Samples were mixed separately at 24ºC for 7 min. A relaxing time of 18 min and a second mixing time of 4 min were required before cutting the dough. Then, 5 pieces of dough were cut from the extruded dough of each sample and allowed to stand for 20 min at 25ºC. At the end of the resting period, each sample was inserted into the alveograph. (Chopin MA 82). A bubble was blown and the resulting air pressure profile was recorded on the recording manometer of the alveograph. Resulting alveograms were used to determine over-pressure (P, mm) as an indicator of dough tenacity or resistance to deformation. The abscissa (mm) at the point of bubble rupture was used as a measure of dough extensibility (L), and the deformation energy (W, 10-4 J). The configuration ratio P/L was calculated as an

indicator of the rheological balance of the dough. The alveograph analysis was performed at the Professional Training Center in Food of Tunisia.

Protein extraction and characterization using labChip analysis

***Extraction of HMW-GS*:** Glutenins were extracted and purified based on the protocol of Uthayakumaran *et al.* (20). An amount of 20 mg of whole meal or flour sample was extracted once using 1 mL of dimethyl sulfoxide (DMSO; Cambridge Isotope Laboratories, Tewksbury, MA, USA) and twice with 1 mL of 50% propan-1-ol (Alfa Aesar GmbH, Germany) mixed each time on a vortex (S48; SKS Sience, Watervliet, NY, USA) for 10 s and centrifuged for 10 min at 16,000×*g* using a Hermle Z300K centrifuge (55070111; Hermle Labortechnik GmbH, Wehingen, Germany). The supernatant containing monomeric proteins (albumins, globulins, and gliadins) was discarded. Glutenin subunits were then extracted at 65ºC for 30 min using 300 μL of 1% SDS solution (IMPAG GmbH, Germany) containing 1% dithiothreitol (DTT; BioRad, Hercules, CA, USA) followed by centrifugation (16,000×*g*, Hermle Z300K centrifuge; Hermle Labortechnik GmbH) for 10 min. Each clarified extract (4 μL) was mixed with 2 μL of Agilent sample buffer (Agilent, Santa Clara, CA, USA) and 84 μL of deionized water. This mixture was applied to one of the 10 sample wells on a LabChip (Experion Pro260; Bio-Rad).

***Biochemical analysis of samples*:** Extracted proteins were analyzed using an Experion Pro260 (BioRad) automated electrophoresis system that uses LabChip microfluidic technology to automate protein (10-260 kDa with an equivalent of upper and lower marker of 1.2 to 260 kDa) electrophoresis under denaturing conditions. Electrophoresis was run under manufacturer recommended conditions.

Statistical analysis All tests were performed in triplicate with the exception of alveograph testing, which was performed on 5 dough sheets prepared from the same dough. Analysis of variance (ANOVA), applied for each quality parameter, was performed using SPSS (SPSS13 software; IBM, Armonk, NY, USA) using the mean values of 3 replicates with a probability threshold of $p<0.05$. The coefficient of variation (CV) was calculated as a measure of relative variability. The multiple correlation coefficient R^2 was calculated to measure the strength of association between genotypes and different testing results.

Results and Discussion

There were highly significant differences ($p<0.001$) between durum wheat genotypes for all tested parameters (Table 1). This genotypic variability indicated that each variety had distinct nutritional and technological properties (breadmaking potential related to the gluten strength).

Protein concentration There were highly significant differences ($p<0.001$) in the protein content between all studied genotypes (Table 1). These differences can be explained by the fact that proteins result from the expression of many genes, and each genotype has a wide allelic variability in each loci (21). Gate (22) attributed this difference to the variable ability of genotypes to absorb and remobilize nitrogen. Generally, durum wheat is useful only when the protein content is greater than 13% DM. Wheat with a protein content of at least 13% give excellent products because of the consistent relationship between grain protein and gluten pasta. In fact, wheat varieties with protein contents below 11% produce lower quality products (23). In this study, this was the case of all studied durum wheat genotypes. Protein concentrations ranged from 16.19% DM in the Arbi to 17.32% DM in the Chili variety. Both values were higher than in the improved

Table 1. Analysis of variance for protein concentration, gluten content and alveograph parameters for six Tunisian durum wheat genotypes[1)]

Source of variation	DF	Pr	WG	DG	P	L	W	P/L
Genotype	5	21.48**	298.71**	37.5**	1352.1**	48.8**	1400.1**	11.29**
Error	12	0.18	0.14	0.16	160.83	18.5	395.33	0.80
CV (%)		16.57	18.24	19.77	12.95	24.51	15.97	24.08
R^2		0.98	0.99	0.99	0.77	0.52	0.59	0.85

[1)]Pr, protein concentration (%DM); WG, wet gluten content (%); DG, dry gluten content (%); P, overpressure (mm); L, length (mm); W, energy of deformation (10^{-4} J); CV, coefficient of variation; R^2, multiple correlation coefficient; significant at *p=0.05 or **p=0.01 (F-test)

Table 2. Means of semolina protein for six Tunisian durum wheat genotypes[1)]

Sample	Azizi	Mahmoudi	Chili	Arbi	Razzek	Karim
Semolina protein (% DM)	16.48	17.06	17.32	16.98	12.54	11.2

[1)]Mean values represent the average of 3 replicates; DM, dry matter

Table 3. Means of dry gluten (DG) and wet gluten (WG) content for six Tunisian durum wheat genotypes[1)] (unit: %)

Sample	Azizi	Mahmoudi	Chili	Arbi	Razzek	Karim
WG	49.67	57.34	60.59	60.88	42.84	36.84
DG	16.17	18.75	20.63	20.04	13.17	12.33
DG/WG	32.55	32.7	34.04	32.9	30.74	33.47

[1)]Mean values represent average of three replicates; WG, wet gluten content (%); DG, dry gluten content (%)

Table 4. Means and dough strength measurements for six Tunisian durum wheat genotypes[1)]

	Azizi	Mahmoudi	Chili	Arbi	Razzek	Karim
Alveograph P, mm	194	204	177	150	157	165
Alveograph L, mm	19	19	16	23	24	27
Alveograph W, 10^{-4}	180	177	179	157	170	124
P/L	9.97	10.6	9.13	6.52	6.53	5.7

[1)]Mean values represent average of three replicates; P, overpressure (mm); L, length (mm); W, energy of deformation (10^{-4} J); P/L, configuration ratio

genotypes, whose values did not exceed 12.5% DM (Table 2). This superiority of local landraces can be explained by lower kernel production for local genotypes (24) and a negative correlation between grain yield and protein concentration (24,25). As a result of this generally inverse relationship, available nitrogen in older cultivars is distributed into fewer kernels than in high yield modern cultivars (26). However, no clear relationships were identified among the protein yield, concentration and glutenin composition (27).

Wet and dry gluten contents Wet and dry gluten contents differed between the 6 genotypes. Wet and dry gluten contents were consistently higher in landraces than in improved genotypes (Table 3). Wet gluten values ranged from 49.67% in the Azizi to 60.88% in the Arbi variety, while dry gluten values ranged from 16.17% in the Azizi to 20.63% in the Chili variety. Therefore, varietal choice is the most important factor affecting the gluten content. Ames *et al.* (28) also found a strong dependence of gluten strength on genotype. In this study, the Arbi and Chili varieties had the highest wet and dry gluten contents, respectively. According to Triccoli *et al.* (29), dry gluten is an important trait for pasta quality, and genotypes having high protein and gluten contents are preferred for selection during quality breeding. In addition, the water absorption capacity (DG/WG ratio) of the dough was, on average, 30% for all tested genotypes (Table 3). Liu *et al.* (30) also found water absorption of pasta dough to be 31-35%, compared to 60% for bread dough. Landraces overall exhibited the best protein concentrations, gluten contents, and dough tenacity and extensibility values. The Mahmoudi and Chili varieties had higher protein contents (17.06 and 17.32% DM, respectively). The Arbi and Chili varieties had the highest wet and dry gluten contents (60.88 and 20.63%, respectively).

Alveograph testing An alveograph was used to measure the gluten strength (W), which showed a wide range of values for the 6 durum wheat genotypes (Table 4). The alveograph W value is the best parameter for determining the durum wheat gluten strength. Weaker gluten characteristics exhibited by durum wheat semolina were evidenced by lower alveograph deformation energy (W) values, which generally increased with an increased dough/gluten strength (31,32). This was partly due to a reduced ability of glutenin subunits to form large polymers, possibly because of a reduced intermolecular disulfide bond formation capacity, as indicated by the lower proportion of SDS-unextractable polymeric proteins. This was not the case for durum wheat. In fact, the gluten strength values of all studied genotypes were higher (124 to 180 10-4 J) than for results reported for other durum wheat genotypes by Cubada *et al.* (33) (48 to 120 10-4 J) and Mohammed *et al.* (34) (64.3 to 187.6 10-4 J). Dough from the Azizi, Mahmoudi, and Chili varieties exhibited higher P and W values and lower L values of all varieties tested. Conversely, dough from the Arbi, Karim, and Razzek varieties had lower P and W values of all tested varieties (Table 4). It was possible to classify the studied genotypes into 2 groups based on their rheological behavior. Group I, consisting of the Azizi, Mahmoudi, and Chili varieties, was characterized by a high dough tenacity, a low extensibility value, and a greater configuration ratio (P/L) value. This group typified the technological characteristics of durum wheat.

Greater dough strength resistance values and lower extensibility values could not be attributed to differences in the protein quantity as durum wheat was characterized by a higher protein concentration (6) than all other varieties. These values were probably associated with an increase in the quality of gluten protein as alveograph testing measures dough viscoelastic properties and, thereby, the gluten strength, which can be considered as a synonym for quality

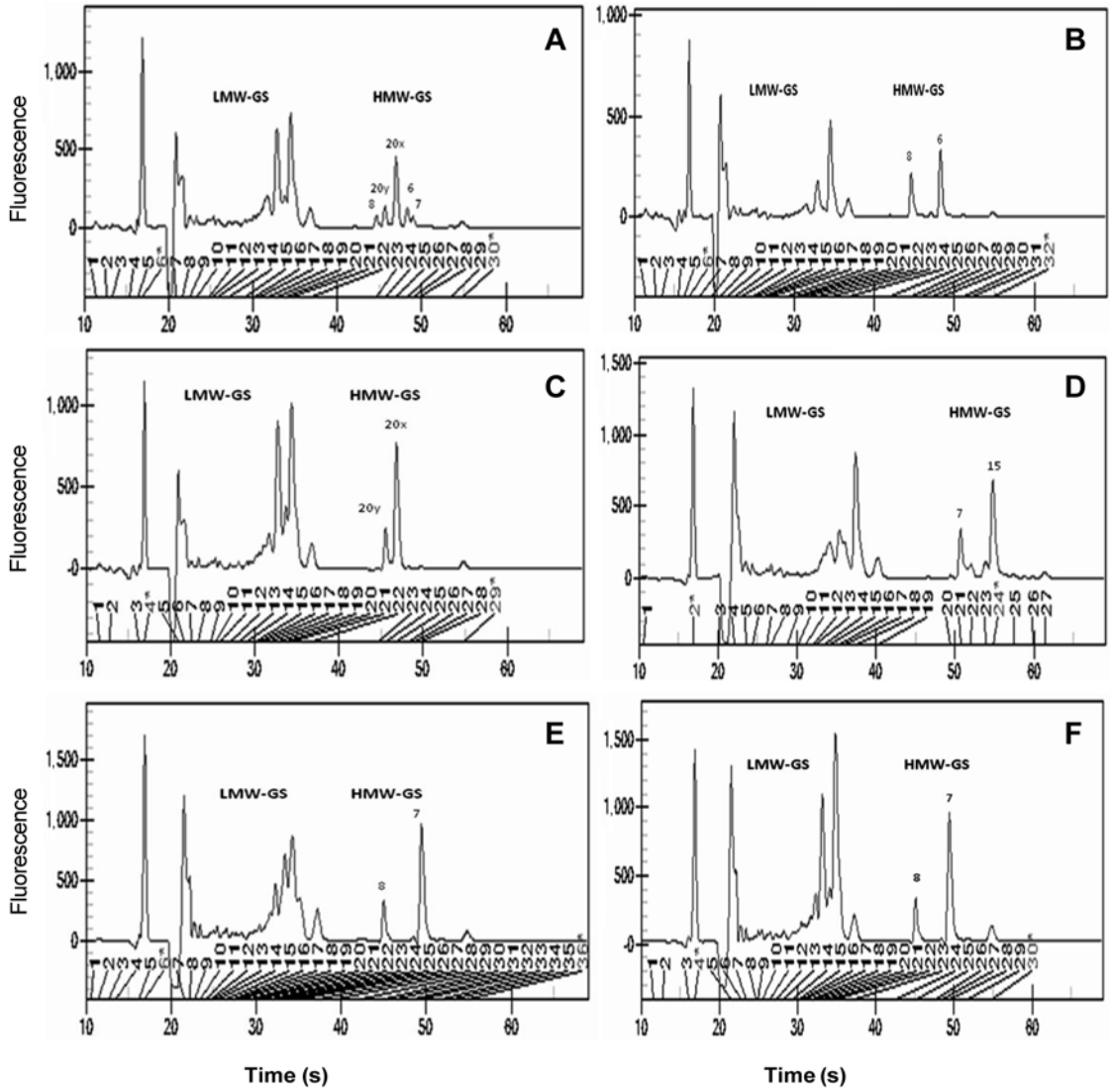

Fig. 1. Separation by LabChip of high and low molecular weight glutenin subunits of durum wheat genotypes. The HMW subunits of glutenin are labeled by subunit numbers. (A) Azizi; (B) Mahmoudi; (C) Arbi; (D) Chili; (E) Karim; (F) Razzek

aptitude (35). Group II, consisting of the landrace Arbi and the 2 improved genotypes of Karim and Razzak, was characterized by higher dough extensibility and mean configuration ratio P/L values that was closest to bread wheat values.

Separation and quantification of high molecular weight glutenin subunits using LabChip LabChip was used to assess grain storage proteins based on analysis of HMW subunit Mw values and investigations of genetic diversity (polymorphism) among different wheat genotypes. The HMW subunits of glutenin, because of their relatively large size, are well separated from other low Mw polypeptides in the HMW-GS region of the profiles (right end) shown in Fig. 1. Electropherograms of purified and reduced separation of wheat glutenins of all genotypes are shown in Fig. 2. The relative proportion of HMW-GS for each variety, used as an indicator of dough strength and baking performance (35), showed dependence on the genotype (Table 5). Flagella *et al*. (36) suggested a large influence of genotype on grain protein composition but not on grain protein content. In particular, among the 2 durum wheat cultivars, Ofanto and Simeto studied during 3 growing seasons (2000-2001, 2001-2002, and 2002-2003), Simeto showed better technological performance (high gluten strength) checked by SDS test and gluten index, and higher glutenin content and HMW-GS/LMW-GS ratio values, and a higher percentage of unextractable polymeric proteins for all experimental years (2001, 2002, and 2003). The quantification of HMW-GS can be useful because it can be used to detect an over-expression of certain subunits. Indeed, the over-expression of subunit 7 was encountered in some French origin varieties (11). The ratio of HMW to LMW-GS ranged from 0.17 in the Razzek to 0.29 in the Chili

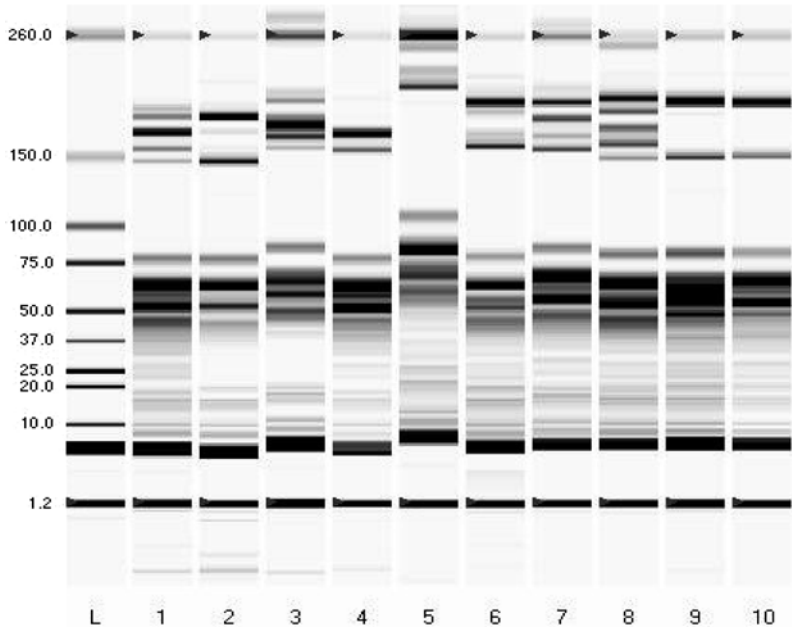

Fig. 2. Electropherograms of the purified and reduced separates of wheat glutenins of all genotypes. Lanes: L, ladder; 1, Azizi; 2, Mahmoudi; 3, unstudied genotype; 4, Arbi; 5, Chili; 6, unstudied genotype; 7, unstudied genotype; 8, unstudied genotype; 9, Karim; 10, Razzek.

varieties (Table 5). Edwards *et al.* (8) reported a range from 0.13 to 0.61. The Chili and Mahmoudi varieties had higher HMW/LMW-GS ratio values (0.24-0.29) (Table 5) and better alveograph P values (177-204 mm) and W (179-177 10-4 J) values, respectively (Table 4). The Karim and Razzek varieties exhibited lower HMW/LMW-GS ratios (0.17-0.19), and P (157-165 mm) and W (170-124 10-4 J) values, respectively. According to the narrow range of HMW/LMW ratio values (0.17-0.29) (Table 5) and the wide variation in dough strength values across this range (Table 4), variation in this ratio probably affected the dough rheological characteristics.

Better quality dough for bread-making is obtained with higher HMW-GS to LMW-GS ratio values than with lower values (2). Southan and MacRithie (37) reported that changes in this ratio result in modification of the Mw distribution of the polymeric proteins in glutenins, thus affecting the gluten strength. In this study, the dough strength increased with an increase in the proportion of HMW-GS (Table 4, 5). These findings are similar to a report of Sissons *et al.* (38) but are in contrast with a report of Edwards *et al.* (8). There was a significant (p<0.01) negative correlation between the HMW-GS/LMW-GS ratio and alveograph parameters. The formation of a well developed network for durum wheat would preferably involve LMW-GS over HMW-GS as the primary contributor to greater gluten strength by formation of an associative polymer type structure, (8,10,39).

Relationship between quality characteristics and the HMW-GS composition In this study, the Azizi and Mahmoudi varieties expressed HMW-GS 6+8 while the Chili variety expressed HMW-GS 7+15. HMW-GS is characterized by a greater gluten strength (a greater dough tenacity, a significant P/L configuration ratio and a higher overall bread-making quality) than varieties expressing the HMW-GS 7+8 (Karim and Razzek) and HMW-GS 20 (Arbi) alleles at locus *Glu-B1*. In addition, the deformation energy W was, on average, greater for genotypes expressing HMW-GS 6+8 and 7+15 than for genotypes expressing HMW-GS 7+8 and 20. Differences in the semolina protein concentration (Table 2) and the wet and dry gluten contents (Table 3) between HMW-GS genotypes were also highly significant (p<0.01). Based on the results of this study, HMW-GS 20 (Arbi) is associated with poor quality. This concord with Brites *et al.* (7), Edwards *et al.* (8), and Sissons *et al.* (10) findings, whereas HMW-GS 6+8 is associated with better quality (7), better even than 7+8. In contrast, Tahir (40) found 6+8 to be inferior to 7+8. According to Sissons *et al*. (10), 6+8 and 7+8 are not different, but both have a significantly higher gluten index than subunit 20 (6+8= 7+8>20). A positive effect for the HMW-GS 7+15 subunit at the *Glu B1* locus on gluten strength was identified in this study. This pair of HMW-GS subunits 7+15, previously described by Carmona *et al.* (41) in Khorassan wheat (an ancient good quality tetraploid wheat (42) grown in the Mediterranean region), has a

Table 5. Quantification of individual HMW-GS subunits and determination of HMW-GS to LMW-GS for the six durum wheat genotypes

Sample	HMW-GS[1)]	HMW/LMW[2)]	Composition of HMW-GS (kDa)	Relative percentage of HMW-GS (%)
Azizi	6+8	0.18	8 (146.61)	8.86
	7+8		20y (156.89)	16.62
	20		20x (171.46)	54
			6 (186.59)	13.52
			7 (193.87)	7
Mahmoudi	6+8	0.29	8 (146.29)	41.86
			6 (186.12)	58.14
Chili	7+15	0.24	15 (213.86)	73.44
			7 (250.25)	26.56
Arbi	20	0.18	20y (155.71)	23.5
			20x (169.98)	76.5
Razzek	7+8	0.17	8 (150.64)	24
			7 (199.25)	76
Karim	7+8	0.19	8 (149.58)	23.27
			7 (199.5)	76.73

[1)]High molecular weight glutenin subunits designations according to Payne *et al.* (4)
[2)]HMW/LMW, ratio of HMW to LMW subunits

greater SDS-sedimentation volume and quality index than subunits 6+8.

The landraces Azizi (6+8), Mahmoudi (6+8), and Chili (7+15) were good sources of favorable glutenin subunits that would be desirable in breeding programs for improving pasta quality. The Karim and Razzek (7+8) varieties were not good sources. The wide variation in gluten strength suggests that the presence of a particular allelic pattern is an indicator of quality performance. Variations in gluten HMW-GS provide markers of gluten strength, with the exception of HMW-GS 20, in breeding programs, but does not guarantee the level of performance. Rheological testing would be required for strength (W) screening. The quantity of HMW-GS directly impacts dough strength; however, further work in identifying patterns (HMW and LMW) is certainly required to provide additional information about interesting alleles and allele combinations related to a high gluten strength (43). Selection of varieties with improved qualities and development of lines when breeding for quality is, thus, possible.

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