



HAL
open science

Molecular characterization of storage proteins for selected durum wheat varieties grown in different environments

Thierry Aussenac, Larbi Rhazi, Olfa Daaloul Bouacha, Salah Rezgui, Sadok Nouaigui

► To cite this version:

Thierry Aussenac, Larbi Rhazi, Olfa Daaloul Bouacha, Salah Rezgui, Sadok Nouaigui. Molecular characterization of storage proteins for selected durum wheat varieties grown in different environments. *Journal of Cereal Science*, 2015, 61, pp.97-104. 10.1016/j.jcs.2014.09.005 . hal-04559676

HAL Id: hal-04559676

<https://hal.science/hal-04559676>

Submitted on 25 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Molecular characterization of storage proteins for selected durum wheat**
2 **varieties grown in different environments**

3 **Olfa Daaloul Bouacha^{1,*}, Larbi Rhazi², Thierry Aussenac², Salah Rezgui¹,**
4 **Sadok Nouaigui³**

5 ¹ National Agronomic Institute of Tunisia, 43, Avenue Charles Nicolle 1082, Tunis
6 Mahrajène, Tunisia

7 ² Institut Polytechnique LaSalle Beauvais, 19 rue Pierre Waguet, 60026 Beauvais, France.

8 ³ High School of Food Industries, 58 Avenue Alain Savary, C Khadra, Tunis, Tunisia
9

10 *Corresponding author: Tel.: 0021627535313; Fax; 0021671799391

11 *E-mail address:* olfadaaloul@hotmail.fr

12 *Keywords:* HMW-GS_y to HMW-GS_x ratio, HMW-GS to LMW-GS ratio, Protein variability,
13 ExGxF interaction.

14 *Abbreviations:* CE, capillary electrophoresis; CIMMYT, International Maize and Wheat
15 Improvement Center; E, environment; F, fertilizer; G, genotype; HMW-GS, high molecular weight
16 glutenin subunit; ICARDA, International Center for Agricultural Research in the Dry Areas; INRAT,
17 Institut National de Recherches Agronomiques de Tunis; K, potassium; LMW-GS, low molecular
18 weight glutenin subunit; N, nitrogen; P, protein content; SA, semi-arid; SDS-PAGE, sodium dodecyl
19 sulfate polyacrylamide gel electrophoresis; SH, sub-humid.

20

21 **ABSTRACT**

22 The variations of the amounts of individual high molecular weight glutenin subunits (HMW-
23 GS), of the ratios HMW-GS_y to HMW-GS_x and HMW-GS to low molecular weight glutenin
24 subunits (LMW-GS) and of protein content were evaluated for eight durum wheat cultivars in
25 two regions using four fertilizer combinations during two successive years. All measured
26 parameters showed significant variation with genotypes (G), environments (E) and fertilizers
27 (F). The interaction ExGxF was highly significant for glutenin amount variation. Amongst
28 cultivars possessing HMW-GS 20, landraces seem to better valorize the N-fertilizer use for
29 the accumulation of HMW-GS_y than high yielding cultivars. Both HMW-GS_y to HMW-GS_x
30 and HMW-GS to LMW-GS ratios were found to be positively correlated ($p < 0.05$) with total
31 protein content.

32

33 **Introduction**

34 The main storage proteins of cereals are gliadins and glutenins. Glutenins are polymeric
35 fractions composed of high molecular weight and low molecular weight glutenin subunits
36 (HMW-GS and LMW-GS) linked with intermolecular disulfide bonds. The HMW subunits
37 are classified into two types on the basis of their molecular values and sequences: *x*-type
38 which migrates more slowly on SDS-PAGE and *y*-type which migrates faster (Shewry and
39 Tatham, 1990). The HMW-GS are the most studied protein fractions because of their
40 relationship to gluten strength and bread-making quality (MacRitchie et al., 1990). The ratios
41 between the different components of proteins have been shown to reflect the qualitative
42 changes in durum wheat quality (Flagella et al., 2010; Triboï et al., 2000). A high HMW-GS
43 to LMW-GS ratio has been associated with a better technological performance (Flagella et al.,
44 2010). Fertilization and water availability are considered the most important environmental
45 factors affecting protein content and composition. Nitrogen-supply has been shown
46 accompanied with an increase of grain protein content, gliadin and glutenin content and the
47 gliadin to glutenin ratio (Triboï et al., 2000). A water deficit through the growing season was
48 shown accompanied with an increase in protein content and in HMW-GS to LMW-GS ratio
49 (Flagella et al., 2010).

50 The aim of this paper was to assess the glutenin variation of eight durum wheat cultivars in
51 different environments using the ratios HMW-GS_y to HMW-GS_x and HMW-GS to LMW-
52 GS.

53 **1. Material and Methods**

54 **1.1. Cultivars and experimental conditions**

55 The experiment was laid out as a randomized 3 block design with a factorial set of treatments
56 that included 8 genotypes and 4 fertilizer treatments of nitrogen and potassium combinations
57 (N₀K₀, N₀K₁, N₁K₀ and N₁K₁), each block included 32 experimental units. The eight cultivars

58 were composed of ‘Chili’, ‘Biskri’, ‘Mahmoudi’, ‘INRAT69’ ‘Karim’, ‘Razzak’, ‘Khiar’ and
59 ‘Omrabiaa’. The experiment was repeated in two regions of Tunisia: semi-arid (Kef) and sub-
60 humid (Bousalem) and during two growing seasons (2007 and 2008).

61 **1.2. Glutenin extraction**

62 A glutenin extraction was carried out on 3 samples from each variety as described by Fu and
63 Kovacs (1999) and Rhazi et al. (2009) with some modifications. Samples of 30 mg of flour
64 were stirred for 15 min at room temperature with 1ml of 0.08 M Tris-HCl buffer (pH=7.5)
65 containing 50% propan-1-ol, then they were centrifuged at 15,900 g for 10 min at 15°C. The
66 supernatant was discarded (eliminating albumins, globulins and gliadins). The pellet was
67 added to 600 µl of the buffer containing Tris –HCl 0.2 M (pH=9.2), 2% (w/v) of SDS and 1%
68 of DDT, and dispersed by sonication with amplitude of 40 % for 40 s using a stepped microtip
69 probe of 3 mm diameter (ultrasonic Processor, Sonics, model 75038) then the mixture was
70 maintained at a temperature of 60°C for 20 min and centrifuged at 12,500 g for 10 min at
71 20°C.

72 **1.3. Separation of glutenin subunits by CE LabChip 90®**

73 Analysis of the glutenin subunits was carried out on capillary electrophoresis (CE) LabChip
74 90® platform as described by Rhazi et al. (2009). The 384 samples (8 genotypes x 4
75 fertilizer treatments x 4 environments x 3 blocks) were used for quantitation of HMW-GS and
76 estimation of HMW-GS to LMW-GS ratio and HMW-GS_y to HMW-GS_x ratio. Results were
77 expressed as percentage of the time corrected area of individual HMW-GS to the total HMW-
78 GS area. The cultivar set was analyzed 3 times using multiple Chips.

79 **1.4. Protein content evaluation**

80 Grain protein content (P) was determined using the NIRS system (near infrared
81 spectroscopy using a Perten-Inframatic-8600).

82 **1.5. Statistical methods**

83 Analysis of variance was carried out using proc anova and option proc mixed of SAS
84 (version 9.1).When first or second order interaction were significant, the option PDMix800
85 was used to compare means. Proc corr was used to evaluate relationships between protein
86 content and ratios between the different protein components.

87 **2. Results and discussion**

88 **2.1. Wheat properties**

89 The durum wheat genotypes used in this study were composed of four landraces ‘Chili’,
90 ‘Biskri’, ‘Mahmoudi’ and ‘INRAT69’ and four high yielding varieties ‘Karim’, ‘Razzak’,
91 ‘Khiar’ and ‘Omrabiaa’. Landraces and high yielding varieties are known for their
92 contrasting qualities (Daaloul Bouacha et al., 2014). Using CE Lab-On-Chip technique,
93 different Glu-B1 HMW-GS patterns were identified: 6+8, 7+8 and 20 (Fig.1). The high
94 yielding varieties ‘Karim’, ‘Razzak’ and ‘Khiar’ possessed the subunits 7+8. ‘Biskri’
95 possessed the 6+8 composition and ‘Chili’, ‘Mahmoudi’, ‘INRAT69’ and ‘Omrabiaa’
96 possessed the HMW-GS 20.

97

98 **2.2. Impact of Genotypes (G), Environments (E), Fertilizers (F) and their** 99 **interactions on total protein content and quantitative parameters of glutenins**

100 In this study a combination of one region (sub-humid or semi-arid) and one cropping season
101 (2007 or 2008) was considered as a separate environment (E). HMW-GS_y and HMW-GS_x are
102 complementary and their distribution followed an inverse order of classification amongst
103 cultivars and they were found to be negatively correlated. The strong relationship between
104 these two fractions is due to the fact that they are very tightly linked genes on each locus.
105 Therefore, only the results from HMW-GS_y were reported.

106 **2.2.1 Genotypic impact**

107 Genotype was a significant source of variation for individual HMW-GS amounts, for HMW-
108 GS to LMW-GS ratios and for HMW-GS_y to HMW-GS_x ratios (Table 1). This is in
109 accordance with Luo et al. (2000) indicating that the content of HMW-GS and LMW-GS was
110 genetically controlled. Furthermore, Fuertes-Mendizábal et al. (2010) found that the relative
111 proportions of HMW-GS subunits were strongly conserved by genetic determination.

112 HMW-GS to LMW-GS ratios showed significant ($p < 0.05$) inter-genotype variations. Karim
113 showed the highest ratio with a mean of 25.10 followed by Biskri and Khiar with respectively
114 23.3 and 23.29 (Table 2). Cultivar Chili showed intermediate ratio with a mean of 19.75.
115 Mahmoudi and INRAT69 were comparable to Chili with similar values ($p > 0.05$). The lowest
116 HMW-GS to LMW-GS ratio was observed with Omrabiaa. These variations were attributed
117 to genotype factor which was considered the only significant source for the quantitative
118 variation of HMW-GS and LMW-GS (Luo et al., 2000).

119 The HMW-GS relative amounts varied significantly with genotypes (Table 2). Cultivar Biskri
120 showed the highest amounts of HMW-GS_y with a mean of 38.04 %, followed by Mahmoudi,
121 Karim, Razzak and Khiar with similar values ranging from 21.60 % to 22.14 %. Omrabiaa
122 showed the lowest amount of HMW-GS_y with a mean of 17.22%.

123 Changes in the relative quantity of HMW-GS were also reflected in the variation of HMW-
124 GS_y to HMW-GS_x ratios. Comparison amongst the HMW-GS_y to HMW-GS_x ratio means of
125 the eight cultivars according to their HMW-GS composition showed that cultivars possessing
126 the 7+8 HMW-GS composition showed similar HMW-GS ratios ranging from 0.27 to 0.28
127 (Table 2). Similarities in the expression of HMW-GS between high yielding cultivars Karim,
128 Razzak and Khiar could be explained by the fact that these three cultivars are lines selected
129 from CIMMYT crosses and could have the same origin. On the other hand, cultivars with
130 HMW-GS 20 showed different ($p < 0.05$) HMW-GS ratios ranging from 0.20 for cultivar
131 Omrabiaa, which is a line selected in ICARDA, to 0.28 for cultivar Mahmoudi, which is a

132 pure line selected from a land variety in INRAT. These results could indicate that possessing
133 the same HMW-GS composition did not translate systematically into the same variability in
134 both HMW-GS types. The observed variability could be explained by the degree of subunit's
135 expression which would be specific for each cultivar.

136 Protein content varied significantly amongst genotypes from 15.29 % to 18.70%. Table 2
137 showed that the highest overall protein content mean was found for Mahmoudi, Chili and
138 Biskri, landrace genotypes, while Khiar, high yielding genotype, was the cultivar with the
139 lowest overall protein content mean. These results showed that genotypes with high grain
140 yield were associated with low protein content; this is in accordance with Li et al. (2013).

141 The present study showed statistically significant variations among genotypes in relation to
142 total protein content and relative quantitative composition of glutenin fraction. It is well
143 known that protein content and protein composition are affected by genotypic (G) factors. The
144 G factors control the protein level via a number of major genes and quantitative trait loci
145 (QTLs) (Bogard et al., 2011; Uauy et al., 2006). Protein content might be directly genetically
146 controlled by the presence of certain genes in specific cultivar. For example, it was
147 established that NAM gene is an important element to enhance grain protein contents (Hu et
148 al., 2013). In addition, a deletion or a non-functional copy of certain NAM gene is related to
149 the low protein amount of wheat (Uauy et al., 2006). However, genes implicated mostly in the
150 protein content are associated with other physiological function of the plant (Habash et al.,
151 2007). The grain protein content gene is an early regulator of a complex regulatory network
152 that contains hundreds of genes influencing both monocarpic senescence and nutrient
153 remobilization from the leaves to the grain (Cantu et al., 2011).

154 Variation in relative quantitative glutenin subunits could be due to the variation in the control
155 of the transcription and or the expression of HMW-GS. These differences suggest that HMW

156 glutenin genes did not share the same regulatory elements. However, Kolster et al. (1993)
157 suggested that glutenin loci share regulatory mechanism which implies common *cis* and *trans*
158 regulators may control the expression of glutenin alleles, regardless of the genotype. In
159 addition, Storlie et al. (2009) suggested that HMW glutenin regulation occurs at various
160 points between gene transcription and protein function; the HMW glutenin probably undergo
161 translational and post-translation control. Therefore, these suggest that regulatory systems did
162 not affect expression of glutenin genes in the same way. More investigation would be
163 necessary to elucidate the mechanism of regulation.

164 **2.2.2. Fertilizer effect**

165 The application of N and K fertilizers showed significant effects on protein content and
166 HMW-GS, while it was of smaller contribution in the variation of HMW-GS/LMW-GS ratios
167 (Table 1). Protein content varied among fertilizer combinations from a mean of 15.67 % to
168 18.20 % (Table 3). Results showed that protein content increased significantly with nitrogen
169 application in both regions, in accordance with previous results (Li et al., 2011; Malik et al.,
170 2013). In addition, potassium application appeared to enhance the assimilation and
171 remobilization of other elements like nitrogen, since Zörb et al. (2014) reported that
172 potassium deficiency may restrict the ability to use N.

173 Comparison amongst overall means of fertilizer treatments showed significant differences in
174 HMW-GS ($p < 0.05$) (Fig.2. A₂). K_0N_1 and K_1N_1 treatments showed significantly higher
175 means of HMW-GS_y amounts (23.49 % and 23.94 %, respectively) than K_0N_0 and K_1N_0
176 combinations (22.24 % and 22.38 %, respectively). Thus nitrogen fertilizer, used alone or in
177 combination with potassium, appeared to affect positively the HMW-GS_y amounts. This
178 result was in accordance with previous findings showing that the protein subunits contents
179 increased with the supply of nitrogen (Malik et al., 2013). Nitrogen application appeared to

180 enhance the y-type HMW-GS expression rather than the x-type HMW-GS (Fig. 2. B₂). This
181 could enhance technological parameters since previous results showed that the accumulation
182 of HMW-GS_y was related to quality parameters. The HMW-GS to LMW-GS ratio was
183 significantly ($p < 0.05$) higher for the K₁N₁ treatment. An overall mean of 21.44 was scored for
184 this application, while, this ratio showed similar values for K₀N₀, K₁N₀ and K₀N₁
185 combinations ranging from 19.91 to 20.24 ($p > 0.05$) (Fig. 2. C₂). Changes observed in this
186 study suggest that fertilizer treatments affect storage protein distribution. In a previous report
187 applying N fertilizer significantly increased the proportions of LMW-GS and gliadins and
188 decreased the proportions of albumins and globulins but had not significant impact on HMW-
189 GS (Kindred et al., 2008). However, Tea et al. (2004) showed that monomers, LMW-GS and
190 HMW-GS levels increased significantly; and HMW-GS was the most affected. In addition,
191 other researchers showed that nitrogen impact was influenced by application levels (Fuertes-
192 Mendizábal et al., 2010), application mode (Luo et al., 2000) and application stage (Yue et al.,
193 2007).

194 The source regulation of the accumulation of storage proteins is also in good agreement with
195 the presence of two regulatory elements in the promoter region of the genes of several grain
196 storage proteins. It is known that the “endosperm motif” acts as a positive element under
197 high- N conditions; and the “GNC4-like motif” acts as a negative element under low-N
198 conditions for grains of barley (Müller and Knudsen, 1993). All of these modifications could
199 not be achieved without the direct implication of other plant mechanisms such as assimilation,
200 transport, amino acid biosynthesis and remobilization. However, knowledge on the
201 biochemical and molecular mechanisms affected by nitrogen fertilization is still fragmentary.

202 **2.2.3. Environmental effect**

203 Substantial differences in protein content were found among the four environments,
204 characterized by different soil and climatic conditions including rainfall rate and temperature.

205 Protein content was consistently higher in semi-arid areas than sub-humid areas averaging
206 18.32% versus 15.81%. In addition, a significant year effect was noted; protein contents were
207 higher during 2008 as compared to 2007, in both regions. Differences observed appeared to
208 be due to rainfall distribution during the grain filling period. Excessive rainfall was scored
209 (129.4 mm in semi-arid area and 108 mm in sub-humid area) in 2007, while during 2008 the
210 amount of available water was limited in both regions (40.8 mm in semi-arid and 53 mm in
211 sub-humid regions).

212 The HMW-GS_y amounts varied also significantly with environments ($p < 0.05$) from a mean of
213 22.19 % in the sub-humid region (during 2008) to a mean of 24.08 % in the semi-arid during
214 the same cropping season (Fig. 2. A₁). HMW-GS_y to HMW-GS_x ratio varied significantly
215 from 0.29 in the sub-humid areas during 2008 to 0.32 in the semi-arid areas during the same
216 year (Fig. 2. B₁). Regarding the HMW-GS to LMW-GS ratio, it varied significantly from
217 18.75 during the sub-humid areas in 2007 to 22.73 in the semi-arid areas during 2008 (Fig. 2.
218 C₁). HMW-GS to LMW-GS ratios seemed to be increased in water limitation conditions of
219 semi-arid areas during a normal rainfall distribution. However, this ratio appeared to decrease
220 with the water availability conditions of the sub-humid areas during a year of excessive
221 rainfall.

222 In this study, we showed that protein contents were affected by environmental conditions.
223 These variations attributed to environment might be due to differential effects of high
224 temperature and low precipitation on protein and starch deposition, and was accompanied
225 with differences in protein distribution. Malik et al. (2013) showed that temperature before
226 anthesis was found to affect grain protein concentration substantially, while temperature after
227 anthesis was of less importance. This could be explained by the fact that increased
228 temperature reduces the vegetative cycle of plant and thus leads to lower assimilation of
229 carbohydrates (Bertholdsson, 1999). As a consequence, plants accumulate less starch and

230 thereby have a higher protein content (Savin and Nicolas, 1996). Increase of protein content
231 was also observed in case of water limitation. When water stress occurred throughout the
232 growing season an increase in grain protein concentration was found (Flagella et al., 2010).
233 However, when high rainfall or irrigation occurred during grain filling an increase of protein
234 content was observed due to an increase of nitrogen uptake (Gooding et al., 2003). In
235 addition, our investigation showed that environment influenced glutenin distribution. In
236 general, it was found that drought promoted HMW-GS accumulation in the early grain filling
237 (Dai et al., 2013). Zhang et al. (2013) showed that concentration of HMW-GS in grains were
238 up-regulated by the single early drought, the early drought combined with late heat and the
239 double drought stress treatments, while it was found to be down-regulated by early heat and
240 double heat stress events. In literature, water regime showed significant increase of glutenin,
241 HMW-GS, LMW-GS and HMW-GS to LMW-GS ratio (Flagella et al., 2010). Variations in
242 protein fraction composition due to temperature factor were observed (Triboï et al., 2003).
243 Changes in glutenin and glutenin subunits were associated to general increases in total protein
244 content (Flagella et al., 2010). Qualitative and quantitative protein regulation might be related
245 to mechanisms involved in reserves remobilization during abiotic stress.

246 **2.2.4. Effect of interactions between Environment, Fertilizer and Genotype**

247 Interactions E x G, G x F, E x F and E x G x F were highly significant for all parameters
248 except for GxF and ExGxF interactions which did not have a significant effect on protein
249 content (Table 1).

250 Regarding the interaction between environments and fertilizers, protein content showed a
251 better expression in the semi-arid areas with low input of fertilizers. However, similar values
252 were obtained in sub-humid areas with high fertilizer input while in the semi-arid region,
253 means of protein content of 15.29 and 18.84 % during 2007 and 2008 respectively, using

254 K₀N₀ were noted. In addition, in the sub-humid region similar protein values were obtained
255 under N and NK fertilizer treatments (Table 3).

256 Comparison of protein means according to genotype x environment interaction showed that
257 protein content was expressed differently amongst cultivars in the four considered
258 environments; for example, cultivars Chili, Biskri and Omrabiaa showed significant
259 differences in protein values between the sub-humid and the semi-arid areas, however cultivar
260 Karim showed similar protein values in SH07, SH08 and SA07 and a significantly different
261 protein mean in SA08. On the other hand, we can conclude that cultivars known for their
262 contrasting quality could present similar protein contents in different environments; cultivars
263 Razzak, Karim and Khiar in SA08 showed similar ($p>0.05$) protein contents to cultivar
264 Mahmoudi in SH07 and cultivar INRAT69 in SH08. These results stipulated that the degree
265 of protein accumulation in the grain was strongly influenced by genotype x environment
266 interaction

267 According to E x F x G interaction, HMW-GS relative quantity varied significantly ($p<0.05$)
268 intra-genotypes. When comparing the overall means of HMW-GS, the behavior of each
269 cultivar changed from one environment to another. For some varieties, nitrogen fertilization
270 increased the HMW-GS_y amounts whereas for others quite the contrary. Differences between
271 environments and some cultivars could be due to differences in rainfall distribution between
272 2007 and 2008, especially since Dai et al. (2013) found that irrigated and rainfed conditions
273 had different influences on the glutenin subunits.

274 In water availability conditions, the nitrogen fertilization sometimes led to increase the
275 HMW-GS amounts. Concerning the group of cultivars carrying the HMW-GS 20, nitrogen
276 fertilization appeared to increase the HMW-GS_y amount for landraces Chili, INRAT69 and
277 Mahmoudi in sub-humid and semi-arid regions in 2007 and in semi-arid regions during 2008,
278 but not for the high yielding cultivar Omrabiaa in the same environments. These differences

279 could be explained by a better adaptation of landraces to valorize nitrogen during excessive
280 rainfall conditions than high yielding cultivar (Omrabiaa). The stability of HMW-GS
281 expression appeared to be a function of the genotypic effect. Such a result was found by
282 Triboï et al. (2000) for bread wheat cultivars.

283 Variations in the HMW-GS to LMW-GS ratio for each genotype according to ExGxF
284 interaction, was influenced by fertilizer treatments under specific conditions. Cultivar Biskri
285 showed similar values ($p>0.05$) of HMW-GS to LMW-GS ratio in the sub-humid region in a
286 year of excessive rainfall with all fertilizer treatments, while in semi-arid region the nitrogen
287 fertilizer increased this ratio in 2007. For Chili and INRAT69 cultivars, the sub-humid areas
288 allowed the nitrogen and potassium fertilizer to increase the HMW-GS to LMW-GS ratio.
289 These two cultivars appeared to be more adapted to excessive rainfall distribution and better
290 valorized the use of fertilizers in sub-humid areas. Regarding Karim cultivar, this ratio was
291 increased only with nitrogen fertilizer and especially in sub-humid area. However, HMW-GS
292 to LMW-GS ratio for Razzak cultivar was increased with fertilizer treatment in all
293 environments. Fertilizer use and environmental condition combinations did not affect the
294 HMW-GS to LMW-GS ratio for Omrabiaa cultivar.

295 Our investigation showed that individual factors (G, E and F) alone presented a larger impact
296 on protein characteristics which is in agreement with previous studies (Li et al., 2011).
297 However, Malik et al. (2013) reported that interactions such as variation in temperature before
298 and after anthesis, N amount and timing, soil and starter fertilizer showed a higher influence
299 than factors studied separately. In this study, we were interested in the evaluation of impact of
300 various factors on total protein content, HMW-GS, LMW-GS, HMW-GS/LMW-GS ratio. In
301 the previous study (Malik et al., 2013), mainly formation of glutenin polymers were
302 evaluated. Therefore, changes observed herein due to different factors and their interactions
303 could certainly influence gluten protein polymer structure. Malik et al. (2013) found that

304 glutenin proteins extractable and unextractable in SDS were affected differently depending on
305 factors and their interactions.

306 Much more work on molecular and biochemical regulation of grain protein accumulation
307 remains to be done. The roles of hormones and other growth regulating compounds in
308 endosperm development are still uncovered. The implications of signal transduction pathways
309 that coordinate the response to the environment are also to elucidate.

310 **2.3. Relationship between protein content and glutenins composition**

311 Variations in HMW-GS_y to HMW-GS_x ratio were related to protein content. A highly
312 significant positive correlation was found between total protein content and HMW-GS_y to
313 HMW-GS_x ratio ($r=0.30^{**}$). This is in agreement with previous report (Triboï et al., 2000).
314 This relationship was evaluated for the four environments and in each fertilizer combination
315 by class of cultivars according to their HMW-GS composition (Fig.3). Concerning the 20x-
316 20y composition, a positive significant correlation was observed in sub-humid region during
317 the two seasons with both fertilizer treatments. Nitrogen and potassium fertilizers could affect
318 positively the relationship between HMW-GS_y to HMW-GS_x ratio and total protein content (r
319 $=0.86^{**}$ and 0.66^{*} with K_0N_1 and $r = 0.67^{**}$ and 0.93^{**} with K_1N_0 during 2007 and 2008,
320 respectively). Whereas, association of this ratio to protein content was only significant in the
321 semi-arid area during 2007 ($r =0.77^{**}$ for K_0N_1 and $r= 0.69^{*}$ for K_1N_1 fertilizer treatments).
322 During 2008, the semi-arid area showed that the use of fertilizers did not affect the
323 relationship between HMW-GS_y to HMW-GS_x ratio and protein content. This result could be
324 explained by the high value of protein content obtained without fertilizer treatment, when
325 compared to the other environments (sub-humid 2007 and 2008) (Table 3). For the group of
326 6-8 composition, including only one cultivar in this study, no significant variation was
327 observed for HMW-GS_y to HMW-GS_x ratio in relation to protein content, except, in semi-
328 arid region during 2007 where a slightly significant negative correlation was observed with

329 nitrogen-fertilizer application. Regarding the 7+8 composition class, HMW-GS_y to HMW-
330 GS_x ratio variation with protein content was restricted to some specific conditions. A positive
331 association was established in the semi-arid region during 2008 with K₁N₁ fertilizer
332 combination ($r = 0.72^*$) and a highly significant negative correlation was found in the same
333 region during 2007 without fertilizer application ($r = - 0.86^{**}$).

334 The sub-humid area and the semi-arid area in a year of excessive rainfall during grain filling
335 period showed an increase in protein content accompanied with an increase in the HMW-GS_y
336 to HMW-GS_x ratio, when using fertilizers. In conditions of water availability during the grain
337 filling period, nitrogen and potassium fertilizers affected positively the relationship between
338 HMW-GS_y to HMW-GS_x ratio and total protein content. However, in cases of water shortage
339 (in semi-arid region during 2008 a year of conventional rainfall conditions) the fertilizer use
340 did not affect the relationship between protein content and HMW-GS_y to HMW-GS_x ratio.
341 These results stipulate that increased protein content did not translate systematically a
342 remaining gluten quality. Therefore, increased protein quality could be associated with higher
343 amounts of *x*-type or *y*-type, which could reflect different gluten qualities. It was shown that
344 increasing HMW-GS *y*-type could contribute in ameliorating flour quality thanks to the
345 greater potential polymer resulting from inter and intra- molecular disulfide bonds, since it
346 was established that *y*-type contains more cysteine residues than *x*-type.

347 In addition, variation in HMW-GS to LMW-GS ratio was related to protein content. A
348 significant positive correlation was found between these two parameters ($r=0.12^*$). This
349 relationship was considered a function of the class of genotype according to their HMW-GS
350 composition. For genotype with 6+8 composition, the correlation between HMW-GS to
351 LMW-GS ratio and total protein content was significant at $p<0.05$ ($r=0.30^*$). Whereas, for
352 cultivars with HMW-GS 20 and 7+8, this correlation was significant at $p<0.01$ ($r = 0.30^{**}$
353 for both classes).

354 Association between HMW-GS to LMW-GS ratio and total protein content was
355 compared for overall fertilizers use. Using K_0N_0 , protein content and HMW-GS to LMW-GS
356 ratio were positively associated ($r = 0.31^{**}$), whereas, no such association was established
357 ($p > 0.05$) when nitrogen or potassium fertilizer combinations were used.

358 **Conclusions**

359 Our study has permitted to confirm that environment, fertilizer and genotypes were very
360 important source of variation for protein content and composition. Significant differences
361 were observed between landraces and high yielding cultivars for the expression of protein
362 content. The identification of HMW-GS amongst the eight genotypes showed three different
363 patterns. Consistently, the group of varieties carrying the 7+8 subunits, showed the same
364 relative percentages of HMW-GS_y and HMW-GS_x distribution while the group of varieties
365 carrying the HMW-GS 20 showed a much larger variability in the individual HMW-GS
366 amounts. HMW-GS_y to HMW-GS_x ratio could be considered as an indicator of the variation
367 of HMW-GS amounts amongst cultivars. Comparison between landraces and high yielding
368 cultivars possessing the same HMW-GS composition (20 showed different behaviors when N-
369 fertilizer was applied; landraces seemed to be more adapted to valorize N-fertilizer's use for
370 the accumulation of HMW-GS_y than high yielding cultivars (Omrabiaa). This could support a
371 better ability of these landrace cultivars to be adapted to pasta manufacturing. Such a
372 relationship between glutenin subunits and technological quality would be important to
373 elucidate.

374 **References**

375

376 Bertholdsson, N.O., 1999. Characterization of malting barley cultivars with more or less stable grain
377 protein content under varying environmental conditions. *European Journal of Agronomy* 10(1), 1-
378 8.

379 Bogard, M., Jourdan, M., Allard, V., Martre, P., Perretant, M.R., Ravel, C., Heumez, E., Orford, S.,
380 Snape, J., Griffiths, S., Gaju, O., Foulkes, J., Le Gouis, J., 2011. Anthesis date mainly explained
381 correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in
382 a winter wheat population segregating for flowering time QTLs. *Journal of Experimental Botany*
383 62, 3621-3636.

384 Cantu, D., Pearce, S.P., Distelfeld, A., Christiansen, M.W., Uauy, C., Akhunov, E., Fahima, T.,
385 Dubcovsky, J., 2011. Effect of down-regulation of the high grain protein content (GPC) genes on
386 the wheat transcriptome during monocarpic senescence. *BioMed Central Genomics* 12(492).
387 doi:10.1186/1471-2164-12-492

388 Daaloul Bouacha, O., Nouaigui, S., Rezgui, S., 2014. Effects of N and K fertilizers on durum wheat
389 quality in different environments. *Journal of Cereal Science* 59, 9-14.

390 Dai, Z., Yin, Y., Li, Y., Cao, L., Wang, Z., 2013. Variation of high-molecular-weight glutenin subunits
391 and glutenin macropolymer particle distribution in wheat grains produced under different water
392 regimes. *The Crop Journal* 1(1), 84-89.

393 Flagella, Z., Giuliani, M.M., Giuzio, L., Volpi, C., Masci, S., 2010. Influence of water deficit on durum
394 wheat storage protein composition and technological quality. *European Journal of Agronomy* 33,
395 197-207.

396 Fu, B.X., Kovacs, M.I.P., 1999. Rapid Single Step procedure for isolating total glutenin proteins of
397 wheat flour. *Journal of Cereal Science* 29, 113-116.

398 Fuertes-Mendizábal, T., Aizpurua, A., González-Moro, M.B., Estavillo, J.M., 2010. Improving wheat
399 breadmaking quality by splitting the N fertilizer rate. *European Journal of Agronomy* 33(1), 52-
400 61.

401 Gooding, M.J., Ellis, R.H., Shewry, P.R., Schofield, J.D., 2003. Effects of restricted water availability
402 and increased temperature on the grain filling, drying and quality of winter wheat. *Journal of*
403 *Cereal Science* 37, 295-309.

404 Habash, D., Bernard, S., Schondelmaier, J., Weyen, J., Quarrie, S., 2007. The genetics of nitrogen use in
405 hexaploid wheat: N utilisation, development and yield. *Theoretical and Applied Genetics* 114(3),
406 403-419.

407 Hu, X.G., Wu, B.H., Liu, D.C., Wei, Y.M., Gao, S.B., Zheng, Y.L., 2013. Variation and relationship of
408 NAM-G1 gene and grain protein content in *Triticum timopheevii* Zhuk. *Journal of Plant*
409 *Physiology* 170, 330-337.

410 Kindred, D.R., Verhoeven, T.M.O., Weightman, R.M., Swanston, J.S., Agu, R.C., Brosnan, J.M.,
411 Sylvester-Bradley, R., 2008. Effects of variety and fertilizer nitrogen on alcohol yield, grain yield,
412 starch and protein content, and protein composition of winter wheat. *Journal of*
413 *Cereal Science* 48, 46-57.

414 Kolster, P., Krechting, C.F., Van Gelder, W.M.J., 1993. Expression of individual HMW glutenin subunit
415 genes of wheat (*Triticum aestivum* L.) in relation to differences in the number and type of
416 homoeologous subunits and differences in genetic background. *Theoretical and Applied Genetics*
417 87, 209-216.

418 Li, Y., Yin, Y.P., Zhao, Q., Wang, Z.L., 2011. Changes of glutenin subunits due to water –nitrogen
419 interaction influence size and distribution of glutenin macropolymer particles and flour quality.
420 *Crop Science* 51, 2809-2819.

421 Li, Y.F., Wu, Y., Hernandez-Espinosa, N., Pena, R.J., 2013. Heat and drought stress on durum wheat :
422 responses of genotypes, yield and quality parameters. *Journal of Cereal Science* 57(3), 398-404.

423 Luo, C., Branlard, G., Griffin, W.B., McNei, D.L., 2000. The Effect of Nitrogen and Sulphur
424 Fertilisation and their Interaction with Genotype on Wheat Glutenins and Quality Parameters.
425 *Journal of Cereal Science* 31, 185-194.

426 MacRitchie, F., DuCros, D.L., Wrigley, C.W., 1990. Flour polypeptides related to wheat quality. In:
427 Pomeranz, Y. (Eds.), *Advances in Cereal Science and technology*. American Association of
428 Cereal Chemists, St. Paul, pp. 79-145.

429 Malik, A.H., Kuktaite, R., Johansson, E., 2013. Combined effect of genetic and environmental factors
430 on the accumulation of proteins in the wheat grain and their relationship to bread –making quality.
431 *Journal of Cereal Science* 57 (2), 170-174.

432 Müller, M., Knudsen, S., 1993. The nitrogen response of a barley C-hordein promoter is controlled by
433 positive and negative regulation of the GCN4 and endosperm box. *The Plant Journal* 4, 343-355.

434 Rhazi, L., Bodard, A.L., Fathollahi, B., Aussenac, T., 2009. High throughput microchip-based
435 separation and quantitation of high-molecular-weight-glutenin subunits. *Journal of Cereal Science*
436 49, 272-277.

437 Savin, R., Nicolas, M., 1996. Effects of short periods of drought and high temperature on grain growth
438 and starch accumulation of two malting barley cultivars. *Functional Plant Biology* 23(2), 201-210.

439 Shewry, P.R., Tatham, A.S., 1990. The prolamin storage proteins of cereals seeds: structure and
440 evolution. *Biochemical Journal* 267, 1-12.

441 Storlie, E.W., Ihry, R.J., Baehr, L.M., Tieszen, K.A., Engbers, J.H., Anderson-Daniels, J.M., Davis,
442 E.M., Gilbertson, A.G., Harden, N.R., Harris, K.A., Johnson, A.J., Kerkvleit, A.M., Moldan,
443 M.M., Bell, M.E., Wanous, M.K., 2009. Genomic regions influencing gene expression of the
444 HMW glutenins in wheat. *Theoretical and Applied Genetics* 118, 295-303.

445 Tea, I., Genter, T., Naulet, N., Boyer, V., Lummerzheim, M., Kleiber, D., 2004. Effect of foliar sulfur
446 and nitrogen fertilization on wheat storage protein composition and dough mixing properties.
447 *Cereal Chemistry* 81, 759-766.

448 Triboi, E., Abad, A., Michelena, A., Lloveras, J., Ollier, J.L., Daniel, C., 2000. Environmental effects on
449 the quality of two wheat genotypes: 1. quantitative and qualitative variation of storage
450 proteins. *European Journal of Agronomy* 13, 47-64.

451 Triboi, E., Martre, P., Triboi-Blondel, A.M., 2003. Environmental -induced changes in protein
452 composition in developing grains of wheat are related to changes in total protein content. *Journal*
453 *of Experimental Botany* 54(388), 1731-1742.

454 Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J., 2006. A NAC gene regulating
455 senescence improves grain protein, zinc, and iron content in wheat. *Science* 314, 1298-1301.

456 Yue, H., Jiang, D., Dai, T., Qin, X., Jing, Q., Cao, W., 2007. Effect of nitrogen application rate on
457 content of glutenin macropolymer and high molecular weight glutenin subunits in grains of two
458 winter wheat cultivars. *Journal of Cereal Science* 45, 248-256.

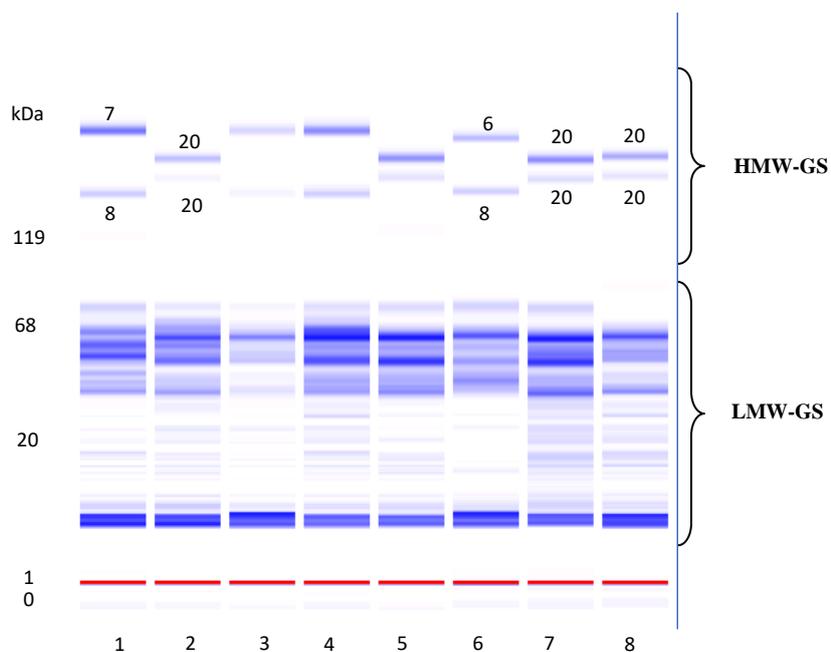
459 Zhang, X., Cai, J., Wollenweber, B., Liu, F., Dai, T., Cao, W., Jiang, D., 2013. Multiple heat and
460 drought events affect grain yield and accumulations of high molecular weight glutenin subunits
461 and glutenin macropolymers in wheat. *Journal of Cereal Science* 57 (1), 134-140.

462 Zörb, C., Senbayram, M., Peiter, E., 2014. Potassium in agriculture status and perspectives. Journal of
463 Plant Physiology 171(9), 656-669.

464 Revised Figures

465

466



467

468 **Fig.1.** Gel image overlay of glutenin subunits from cultivars Karim, Omrabiaa, Razzak, Khiar, Chili,
469 Biskri, Mahmoudi and INRAT69, respectively: 1, 2, 3, 4, 5, 6, 7 and 8, separated by EC LabChip 90.

470

471

472

473

474

475

476

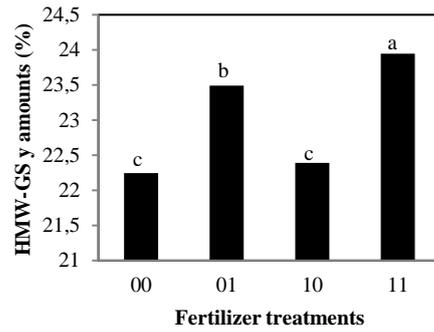
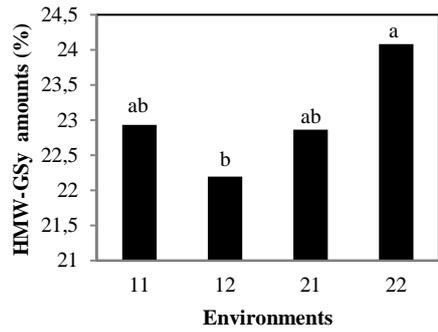
477

478

479

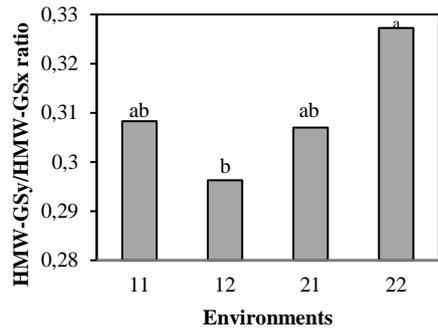
A₁

A₂

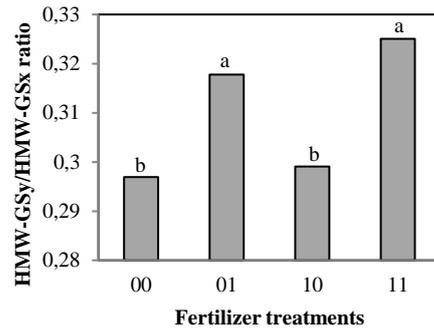


480

B₁

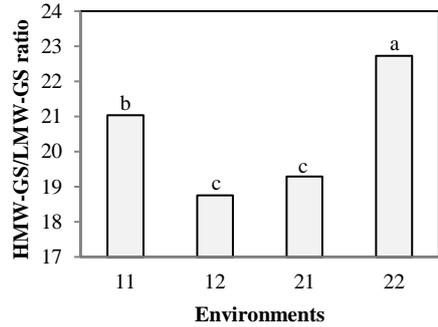


B₂

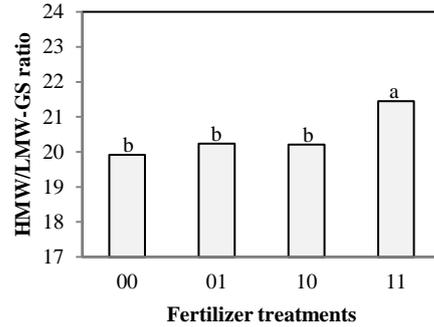


481

C₁



C₂



482

483

484

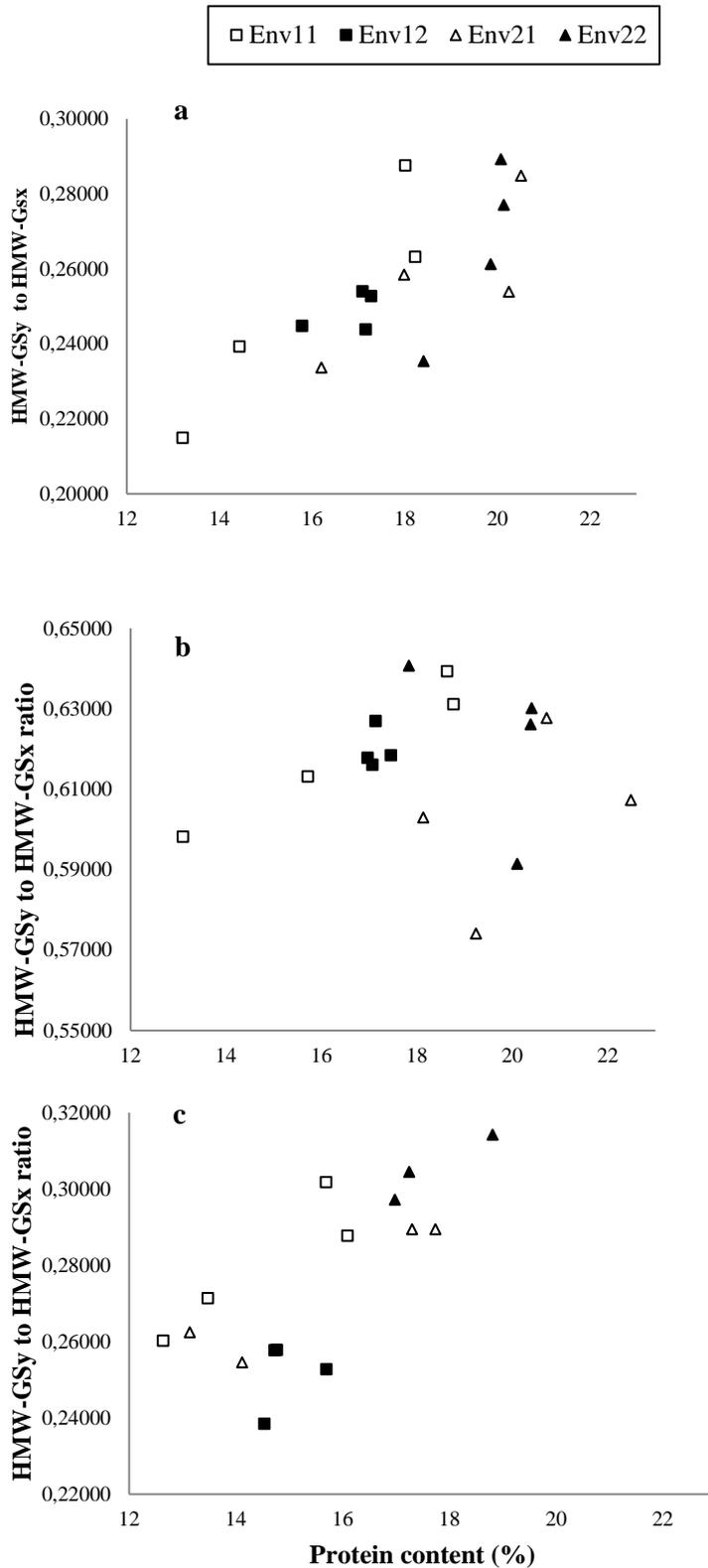
485

486

487

488

Fig.2. Variability of HMW-GS, HMW-GSy to HMW-GSx ratio and HMW-GS to LMW-GS ratio respectively with Environments (**A₁**, **B₁** and **C₁**) (11: SH07; 12: SH08; 21: SA07; 22: SA08) and with fertilizer treatments (**A₂**, **B₂** and **C₂**) (00: K₀N₀; 01: K₀N₁; 10: K₁N₀; 11: K₁N₁). Means with the same letter do not differ significantly ($p < 0.05$).



489

490

491

492 **Fig.3.** Relationship between HMW-GSx to HMW-GSy ratio and total protein content in the four
 493 environments (Env11; SH07, Env 12; SH08 , Env 21; SA07 and Env 22; SA08) with the four fertilizer

494 combination for the 3 classes of cultivars according to their HMW-GS composition at Glu-B1:**20, 6+8**
495 and **7+8 (a, b and c, respectively)**.

496