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**Response to drought and heat stress on wheat quality, with special emphasis on bread-making quality, in durum wheat**

Carlos Guzmán\*, Jorge Enrique Autrique, Suchismita Mondal, Ravi Prakash Singh, Velu Govindan, Anayeli Morales-Dorantes, Gabriel Posadas-Romano, Jose Crossa, Karim Ammar, Roberto Javier Peña

Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo Postal 6-641, Mexico DF.

\* Author for correspondence: Carlos Guzman (Phone: +52 (595) 952 1900 ext. 2280; Fax:+52 (555) 804 7558; Email: c.guzman@cgiar.org

For Field Crops Research

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**Abstract**

Durum wheat accounts for more than 50% of the total wheat-growing area in the Mediterranean region, where is used for the preparation of diverse food products, including pasta and bread. The effects of drought and heat stresses on grain morphology, grain composition (protein, iron and zinc micronutrients), processing and pasta and bread-making quality in durum wheat varieties were analyzed. The results revealed significant differences among the genotypes, as well as unique responses to the environmental stresses. Micronutrients concentration (iron and zinc), processing and pasta-making quality was favored by drought but not by heat stress. Overall, the durum wheat lines showed inferior values for bread volume compared to the bread-wheat checks. However, some durum genotypes in specific environment had almost the same performance. To develop durum wheat cultivars with similar bread-making quality to that of bread wheat, it is necessary to achieve a better balance of tenacity and extensibility. The development of durum lines with good bread-making quality could increase the commercial value of this crop.

**Keywords:** durum wheat; processing quality; pasta-making; bread-making; drought stress; heat stress.

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## 47 **1. Introduction**

48           Durum wheat (*Triticum turgidum* ssp. *durum* Desf. em. Husn.) accounts for around 6% of  
49 total wheat production (37.7 million tonnes in 2013; International Grain Council October, 2014),  
50 occupying approximately 20 million hectares worldwide. In the Mediterranean region durum  
51 wheat accounts for more than 50% (reaching 90% in some countries) of the total wheat-growing  
52 area, due to its role as the staple food in many countries. Durum grain is used for the preparation  
53 of diverse food products, including bread, couscous, frekeh, bulgur, and most importantly, pasta.  
54 Pasta is generally recognized worldwide as beneficial to a nutritionally balanced diet (Ames,  
55 Clarke, Marchylo, Dexter & Woods, 1999), and consumer demand is reflected in the upward  
56 trend in pasta production. Almost 9.3 million tons were purchased in 2001, and two years later in  
57 2003 almost 10.5 million tons were purchased. By 2012, approximately 13.5 million tons were  
58 produced (IPO, 2013), an increase, which is an important indicator of the increase in demand for  
59 durum wheat throughout the world.

60           At the International Maize and Wheat Improvement Center (CIMMYT) durum wheat  
61 breeding draws on a large, genetically wide, gene pool to develop germplasm, which is widely  
62 distributed among breeding programs of durum-producing countries. The priority of the wheat  
63 breeding program is to develop high-yielding, disease-resistant varieties that can tolerate  
64 drought, heat stresses, and produce high grain quality. The latter is essential in durum wheat  
65 varieties to be accepted by industry, local food manufacturers and consumers. The most  
66 important parameters affecting industrial quality for pasta-making from durum wheat grain are  
67 probably gluten quality (strength) and the yellow color of semolina. Although environmental and

68 processing conditions play a significant role in these two traits, research has shown that they are  
69 under strong genetic control. The favourable dough or gluten properties of durum lines have  
70 been associated mainly with the presence of specific glutenins coded by the *Glu-B1* and *Glu-B3*  
71 loci (Ammar, Kronstad & Morris, 2000; Boggini & Pogna, 1989; Boggini, Tusa & Pogna, 1995;  
72 Brites & Carrillo, 2001; Peña, Zarco-Hernandez, Amaya-Celis & Mujeeb-Kazi, 1994). Semolina  
73 color will depend to a great extent on the genes involved in pigment accumulation (enzymes  
74 involved in pigment biosynthesis as phytoene synthase and pigment degradation as lipoxygenase  
75 or polyphenol oxidase) (see Ficco et al., 2014 for a review). Apart from pasta, durum wheat is  
76 widely used to elaborate other non-baked goods as couscous and bulgur. In the former high  
77 yellow color is appreciated while in bulgur light yellow color is preferred (Belibagli et al. 2009).  
78 Both products also demand high protein content to avoid stickiness and to have high water  
79 absorption capacity (Bayram and Öner 2006; Ounane et al. 2006).

80         Although durum wheat generally exhibits inferior bread-making quality compared with  
81 bread wheat (*T. aestivum* L. ssp. *aestivum* .) in terms of loaf volume and crumb structure  
82 (Boggini et al., 1995; Peña et al., 1994), approximately 24% of the global durum wheat  
83 production, and up to 70-90% in some Middle East countries, is used for bread-making (Quaglia,  
84 1988). Speciality breads made with durum are common in the south of Italy (Boggini et al.,  
85 1995), and this popularity is spreading to other countries (Sissons, 2008). In the regions of West  
86 Asia and North Africa 50% of durum wheat is processed into single and two-layered flat breads  
87 and 35% is used for leavened breads (Liu, Shepherd & Rathjen, 1996). In those countries durum  
88 breads are very popular with consumers probably due to their sensory properties, particularly to  
89 their pleasant aroma (Rao, Pozniak, Hucl & Briggs, 2010) and special taste. They are slow to go  
90 stale (Pasqualone, Summo, Bilancia & Caponi, 2007) and consequently their longer shelf-life

91 due to the high water absorption of durum wheat flour related to increased content in damage  
92 starch, is another desirable characteristic of durum wheat breads (Boyacıoğlu & D'Appolonia,  
93 1994). Besides, although yellow bread wheat flours are typically undesirable for bread-making,  
94 in the case of durum wheat breads a distinctive yellow colour is an important factor influencing  
95 whether it is accepted by the consumer (Brescia et al., 2007; Pasqualone, Caponio & Simeone,  
96 2004).

97 Significant research has been done to identify the traits necessary to enhance durum  
98 bread-making quality, and in this process several durum genotypes with acceptable bread-  
99 making characteristics have been identified (Ammar et al., 2000; Edwards et al., 2007; Peña et  
100 al., 1994). Gluten strength (determined by glutenins composition) has generally been accepted as  
101 the main component that must be increased to improve baking performance of bread wheat, but  
102 gluten extensibility should also be improved (Ammar et al., 2000; Boggini et al., 1995; Edwards  
103 et al., 2007; Rao et al., 2010). However, very little information is available about the effect of the  
104 environment on the bread-making characteristics of durum wheat. In typical areas of durum  
105 wheat cultivation (Mediterranean and countries in western Asia), water deficiency and high  
106 temperatures during grain filling are major factors that define quality. The effect of those stresses  
107 in some durum quality traits (semolina milling and pasta making quality) has been previously  
108 studied (De Stefanis, Sgrulletta, De Vita & Pucciannati, 2002; Flagella, Giuliani, Giuzio, Volpi  
109 & Masci, 2010; Li, Wu, Hernandez-Espinosa & Peña 2013).

110 Besides processing and end-use quality, nutritional quality is also becoming an important  
111 priority in wheat breeding. Wheat is good source of diverse beneficial compounds including  
112 fibre, phytochemicals and micronutrients. Among different micronutrients, iron and zinc are  
113 deficiency in the diet of two billion people (WHO 2012), and because of this have become the

114 focus of micronutrient biofortification, which serves to enhance iron and zinc grain  
115 concentration. Modern wheat cultivars have been shown to be poor sources of these  
116 micronutrients for meeting daily requirements for humans (Cakmak, Pfeiffer & McClafferty,  
117 2010). Not much research has been carried out about durum wheat and iron and zinc content.  
118 According to Ficco et al. (2009), there is some genetic variation to breed for iron and zinc in  
119 durum wheat, although more studies screening for larger genetic variability and examining the  
120 environment effect are required.

121 The objective of this study was to analyse the effects of drought and heat stresses on  
122 different quality traits with special emphasis on bread-making quality in a set of durum wheat  
123 varieties, which are representative of CIMMYT durum germplasm.

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## 125 **2. Materials and methods**

### 126 *2.1 Plant Materials/Agronomic trials*

127 A trial consisting of six CIMMYT durum wheat cultivars (Mexicali C75, Yavaros C79,  
128 Altar C84, Atil C2000, Jupare C2001 and Cirno C2008) and two bread wheat ones (Kachu and  
129 Roelfs F2007), were sown in 2012-2013 and 2013-2014 crop seasons in Ciudad Obregon,  
130 Sonora, in northwestern Mexico. The trial was planted with two replicates in a randomized  
131 complete Block Design under six different environmental conditions: full drip irrigation  
132 (optimum conditions), full basin irrigation, reduced irrigation or medium drought stress, severe  
133 drought stress, medium heat stress, and severe heat stress. All the trials were planted in  
134 November except medium heat stress (planted in January) and heat stress (planted in February).  
135 All the trials had full irrigation (>500 mm) except medium drought stress (300 mm) and severe  
136 drought stress (180mm). Weed, diseases, and insects were all well controlled. In all the trials, N

137 was applied (pre-planting) at a rate of 50 kg of N/ha and at tillering 150 additional units of N  
138 were applied in all the trials except in severe drought stress (50 N units). At maturity whole  
139 plots were harvested and 1kg of seed from each of the wheat lines was used for analyzing the  
140 quality traits.

141 The meteorology data of the experimental station in Ciudad Obregon was characterized  
142 by almost no precipitation during the wheat growing season. Maximum temperatures were  
143 between 31 and 32 °C in March and April, the grain filling time for all treatments, except for  
144 plants under heat stress at temperatures between 35 to 36 °C during grain filling in May.  
145 Flowering time and physiological maturity in most of the cultivars used occur at similar times,  
146 due to the fact that these genotypes were bred for the same growing area. According to the  
147 general growing stages of durum wheat in Ciudad Obregon, drought stress was continuous from  
148 stem elongation to grain ripening in moderate and severe drought stress trials during stem  
149 elongation and flowering. In severe heat stress trial, higher temperatures than in the normal  
150 planting time started from shoot elongation and remained in the grain filling stage until ripening.  
151 Detailed temperature data is shown in ESM1.

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## 153 *2.2 Grain and flour parameters*

154 Grain morphological characteristics were evaluated with digital image system SeedCount  
155 SC5000 (Next Instruments, Australia). Thousand kernel weight (g) and test weight (kg/hl) were  
156 obtained. Grain iron and zinc content (mg/kg) were measured using a bench-top, non-destructive,  
157 energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme  
158 8000, Oxford Instruments Plc, Abingdon, UK), previously standardized for high throughput  
159 screening of iron (Fe) and zinc (Zn) in whole wheat grain (Paltridge et al., 2012). Grain protein

160 (%) and moisture content were determined by near-infrared spectroscopy (NIR Systems 6500,  
161 Foss Denmark) calibrated based on official AACC methods 39-10 and 46-11A (AACC, 2010).  
162 Grain samples previously conditioned at 16% of moisture were milled into flour using Brabender  
163 Quadrumat Jr (C. W. Brabender OHG, Germany). Whole-meal flour samples were also obtained  
164 with a UDY Cyclone mill carrying a 0.5 mm screen. The protein and moisture content in flour  
165 was estimated using near-infrared spectroscopy (NIR Systems 6500, Foss Denmark) calibrated  
166 based on the AACC methods commented above. Grain protein and flour protein content values  
167 were reported at 12.5% and 14% moisture basis, respectively. Flour yellowness and whole-meal  
168 flour yellowness were obtained as the b value of a Minolta color meter (Konica Minolta, Japan).

169 Glutenins subunits composition were identified by SDS-PAGE in polyacrylamide gels,  
170 according to the methodology of Peña et al. (2004), following the nomenclature of Payne and  
171 Lawrence (1983) for HMW glutenins and Nieto-Taladriz et al. (1997) for LMW glutenins.

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### 173 *2.3 Rheological tests*

174 Sodium dodecyl sulfate (SDS) sedimentation volume was carried out according to the  
175 modified protocol described in Peña et al. (1990) using 1g of flour or of whole-meal flour.  
176 Additionally, 35g flour samples were run in a mixograph (National Mfg. Co.) to obtain optimum  
177 dough mixing time and %Torque\*min according to AACC method 54-40A (AACC, 2010).  
178 Gluten extensibility (alveograph L), tenacity (alveograph P), elasticity or strength (alveograph  
179 W) and tenacity/extensibility ratio (alveograph P/L) were determined according to the  
180 Alveograph manufacturer's instructions (Chopin, France), using 60g flour samples and constant  
181 water absorption (55%). Higher water absorption than in the official methodology (50%) was  
182 used to compensate for the typically greater water absorption caused by high levels of starch



183 damage occurring during milling of the very hard durum wheat grain (Ammar et al., 2000;  
184 Dexter, Preston, Martin & Gander, 1994, Peña et al., 1994).

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#### 186 *2.4 Bread-making characteristics*

187 Bread was baked using the straight-dough baking test 10-09 of the AACCC (AACCC 2010)  
188 with some modifications. 100g of flour were mixed with 3g of shortening, 3g of nonfat dry milk,  
189 adjusting water absorption to 73%, in order to maintain uniform dough handling consistency as  
190 judged by the baker. Fermentation time was reduced to 90 minutes, according to the method used  
191 by Dexter et al. (1994) and Sapirstein et al. (2007), who showed short fermentation times  
192 resulted in better bread-making performance for durum wheat. Bread loaf volume was  
193 determined by colza (*Brassica* sp.) seed displacement using a volumeter. Crumb structure (gas-  
194 cell size and size distribution) was scored as very poor, poor, fair, good and very good.

195 All the phenotypic data recorded is shown in ESM2.

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#### 197 *2.5 Statistical Analysis*

198 Combined analyses of variance (ANOVA) across environments for GY and other quality  
199 traits were performed using procedure Proc Anova of the SAS statistical software (2010). The  
200 model is a complete fixed effect linear model with the main focus of estimate means of the  
201 durum wheat lines in specific and across environments during two years.

202 The basic conventional fixed effect linear model for describing the univariate mean  
203 response of genotypes in environments and years, and their interactions is  $y_{ijrk} = \mu + R(EY)_{ijk} +$   
204  $Y_k + G_i + E_j + (GY)_{ik} + (EY)_{jk} + (GE)_{ij} + (GEY)_{ijk} + \epsilon_{ijrk}$ , where  $y_{ijrk}$  is the response of the  $i^{\text{th}}$   
205 genotype ( $i=1,2,\dots,I$ ) in the  $r^{\text{th}}$  replicated ( $r=1,2,\dots,L$ ) of the  $j^{\text{th}}$  environment ( $j=1,2,\dots,J$ ) and the

206  $k^{\text{th}}$  year ( $k=1,2,\dots,M$ ),  $\mu$  is the grand mean over all genotypes, environments, and years,  $R(EY)_{rjk}$   
207 is the effect of the  $r^{\text{th}}$  replicate within the  $j^{\text{th}}$  environment and the  $k^{\text{th}}$  year,  $Y_k$  is the effect of the  
208  $k^{\text{th}}$  year,  $G_i$  is the effect of the  $i^{\text{th}}$  durum wheat line,  $E_j$  is the effect of the  $j^{\text{th}}$  environment,  $(GY)_{ik}$   
209 is the effect of the interaction between the  $i^{\text{th}}$  durum wheat line with the  $k^{\text{th}}$  year,  $(EY)_{jk}$  is the  
210 effect of the interaction between the  $j^{\text{th}}$  environment with the  $i^{\text{th}}$  durum wheat line,  $(GE)_{ij}$  is the  
211 effect of the interaction between the  $i^{\text{th}}$  durum wheat line with the  $k^{\text{th}}$  year,  $(GEY)_{ijk}$  is the effect  
212 of the triple interaction among the  $i^{\text{th}}$  durum wheat line with  $j^{\text{th}}$  environment and the  $k^{\text{th}}$  year, and  
213  $\epsilon_{ijrk}$  is the error assumed to be normally and independent distributed NID  $(0, \sigma^2)$ , where  $\sigma^2$  is the  
214 error variance, assumed to be constant.

215         Averages values in each environment (averaging years and genotypes), in each year  
216 (averaging environment and genotypes) and in each genotype (averaging environment and years)  
217 and least significant difference (LSD) between the different mean values were calculated using  
218 SAS.

219         Pearson correlation coefficients ( $r$ ) and significances for each comparison in the whole  
220 study were obtained using SAS.

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### 222 **3. Results**

#### 223 *3.1 Effects of genotype, environment, year and their interactions*

224         The results of the analysis of the variance (Table 1) showed that almost all the factors had  
225 a significant effect on the different traits. Genotype and environment were the most important  
226 factors explaining the variation found, followed by GxE and ExY interaction. The environment  
227 effect was particularly high in traits as test weight, thousand kernel weight, Zn content and  
228 alveograph P, which explained more than 47% of the variation. In the case of Fe content, flour

229 yellowness, SDS sedimentation volume, mixograph optimum dough mixing time and torque,  
230 alveograph L, the genotype effect was predominant and responsible for at least 25% of the  
231 variation, and in some cases as flour SDS sedimentation and bread loaf volume explained more  
232 than 55% of variation. For this latter trait the GxE effect was also particularly important. GxE  
233 effect also explained about 12% of the variation in traits Fe and Zn content, flour SDS  
234 sedimentation volume, mixograph optimum dough mixing time and torque, and alveograph P, L  
235 and P/L. The ExY effect was high for test weight (30%) and also important for thousand kernel  
236 weight, flour yellowness, flour SDS sedimentation volume and alveograph W. The triple  
237 interaction GxExY effect was significant for mixograph optimum dough development time and  
238 torque and alveograph W. The effects of the particular year and the GxY were not significant for  
239 all of the traits.

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Table 1. *Effects of genotype, environment, year and their interactions on quality traits: % of the total sum of squares from ANOVA analysis*

Source/Trait	FSDS														
	TW	TKW	FeC	ZnC	GPC	FY(b)	S	MixT	TQ	ALVP	ALVL	ALVW	ALVP/L	LV	
<b>Genotype</b>	17.7	8.7	29.3	5.4	6.4	36.3	55.9	26.2	34.0	16.3	44.0	26.2	33.8	56.0	
<b>Environment</b>	50.0	53.0	28.8	47.8	79.6	27.9	3.0	25.4	16.0	56.7	32.6	32.3	38.6	6.9	
<b>Year</b>	1.0	0*	0.8 <sup>†</sup>	3.1	0.9	1.0	0.3 <sup>‡</sup>	0*	0.9 <sup>†</sup>	0.4 <sup>‡</sup>	0.9	0.1*	0.6 <sup>‡</sup>	0.2 <sup>‡</sup>	
<b>GxE</b>	6.1	4.9	13.7	10.0	3.9	11.8	15.6	15.9	15.8	9.9	12.3	8.8	12.3	20.9	
<b>GxY</b>	0.8	1.8	3.7	6.1	0.6 <sup>‡</sup>	1.3	0.8 <sup>‡</sup>	0.5*	1.5	5.4	2.7	3.7	4.9	0.2 <sup>‡</sup>	
<b>ExY</b>	17.1	30.1	2.9 <sup>‡</sup>	7.2	4.5	15.8	15.1	7.2	6.2	3.0	2.1	12.2	1.5	9.5	
<b>GxExY</b>	5.0	3.3	12.0	15.5	2.6	3.4	6.5	15.9	16.0	5.8	3.5	13.4	5.9	4.3	

TW, Test Weight; TKW, Thousand Kernel Weight; FeC, Iron Content; ZnC, Zinc Content; GPC, Grain Protein Content; FY(b), Flour yellowness b value; FSDSS, Flour SDS Sedimentation; MixT, Mixograph Optimum Mixing Time; TQ, Mixograph Torque; ALVP, Alveograph Tenacity; ALVL, Alveograph Extensibility; ALVW, Alveograph Work; ALVP/L, Alveograph Tenacity/Extensibility Ratio; LV, Loaf Volume.

\*All the values were highly significant ( $p < 0.001$ ) except: \*, not significant; <sup>†</sup>,  $p < 0.05$ ; and <sup>‡</sup>,  $p < 0.01$ .

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259 Figure 1 shows the range of values for each quality trait with respect to genotype,  
260 environment, and year. The average value of each component and the letters to identify groups,  
261 based on the LSD test, are also shown. For grain test weight, most of the genotypes showed  
262 acceptable values in all the environments, except Mexicali grain density, which was severely  
263 affected in severe heat stress trial. This last environment together with severe drought stress were  
264 the ones reducing more test weight. For grain size (thousand kernel weight), the ranges of  
265 variation across genotypes were larger than for test weight, with more than 20g of difference in  
266 all the genotypes between the maximum value and the lowest one. As for test weight, severe  
267 drought stress and severe heat stress caused markedly negative effect on grain size, although in  
268 this case the reduction was much more prominent than for test weight. For both test weight and  
269 thousand kernel weight, severe heat stress affected more negatively, with average reductions of  
270 5% and 27%, respectively. In medium heat stress the range of variation was larger than in other  
271 environment, reflecting the different performance of the genotypes in this environment between  
272 both years of the study. In medium drought stress, genotypes showed higher test weight and  
273 thousand kernel weight values. The cultivar combining better acceptable test weight and large  
274 thousand kernel weight was Cirno.

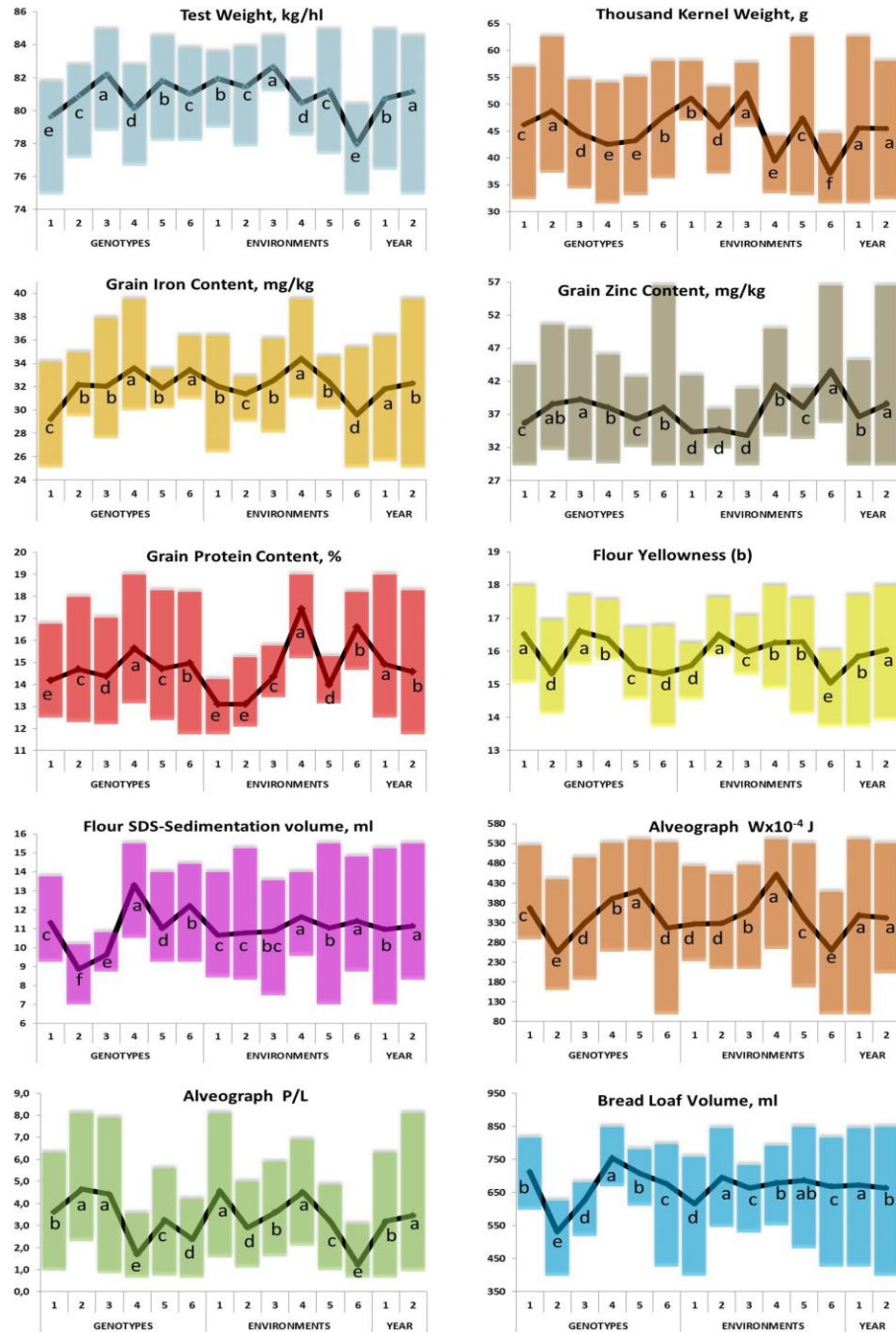
275 In traits traditionally related to grain size (grain protein, Fe and Zn content), the response  
276 of the genotypes was not consistent in all environments. For grain protein content, the  
277 environments with lower grain size (severe drought stress and severe heat stress) had  
278 significantly higher values. This did not happen in the case of Fe content, for which severe heat  
279 stress showed also the lowest value. On the contrary, for Zn content severe heat stress resulted in  
280 the highest value (56 mg/kg). In the case of severe drought stress, higher values than in the other  
281 environments were found for both micronutrients. All the genotypes showed large ranges for

282 grain protein content. Jupare did not show a wide range for Zn or Fe content; Yavaros and Cirno  
283 were also stable across environments for Fe content. Atil was the genotype, which had higher  
284 average grain protein and Fe content, while Altar was for Zn content.

285 In the case of flour yellowness, there were significant differences between the average  
286 values of the genotypes, Mexicali and Altar were the highest, while Yavaros and Cirno were the  
287 lowest. Additionally, severe heat stress caused the greatest adverse effect for this trait with no  
288 very large differences in the other environment.

289 The rheological tests showed significant differences in genotypes. For flour SDS  
290 sedimentation volume and alveograph P/L two groups could be distinguished: the first one with  
291 high flour SDS sedimentation volume and more balanced gluten (and lower alveograph P/L),  
292 composed by Mexicali, Atil, Jupare and Cirno; and the second with lower flour SDS  
293 sedimentation volume and more tenacious gluten in which Yavaros and Altar were included. For  
294 alveograph W Cirno showed low values, as Yavaros and Altar. The environment differences  
295 were smaller for flour SDS sedimentation volume, with, as expected, the stressed environments  
296 showing higher values, followed by medium-stressed environments, and then the fully irrigated  
297 environments. Similar responses were observed for alveograph W except for severe heat stress  
298 trial which had the lowest values. For alveograph P/L severe heat stress trial was again the one  
299 showing lower values, with large differences compared to the environment with higher values for  
300 this trait, full drip irrigation and severe drought stress.

301 Overall, the differences caused by the year in terms of average, maximum and minimum  
302 values were small for all the traits.



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304 **Figure 1-** Maximum, average and minimum values for different quality traits obtained by each

305 genotype in the whole trial, and in each environment and year by the six genotypes. The

306 range of values (maximum and minimum) is represented by bars. The average values in

307 each case are represented with a continuous dark line. Letters identify the different groups

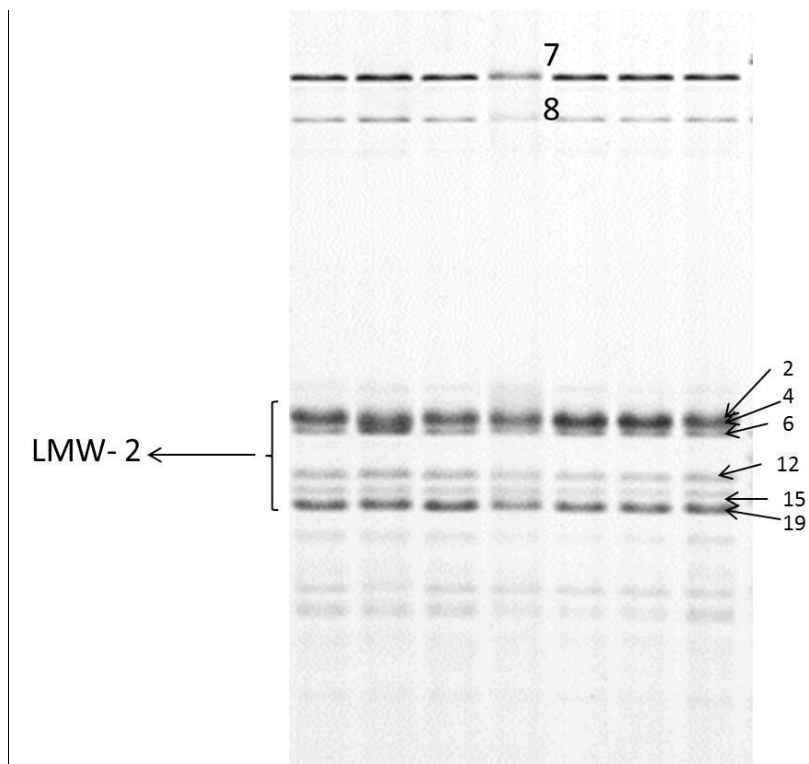
308 between genotypes, environments and years, based on LSD test. Genotypes are as follow: 1,

309 Mexicali; 2, Yavaros; 3, Altar; 4, Atil; 5, Jupare; 6, Cirno. Environments are as follow: 1,  
310 drip full irrigation; 2, basin full irrigation; 3, medium drought stress; 4, severe drought  
311 stress; 5, medium heat stress; 6, severe heat stress. Years are as follow: 1, cycle 2012-2013;  
312 2, cycle 2013-2014.

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### 314 3.3 Glutenins composition and bread-making analysis

315 All the cultivars resulted to have the same glutenins composition. For HMW the *Glu-B1*  
316 locus had the allele 7+8 and for LMW they were LMW-2 type (Fig. 2). The specific alleles for  
317 LMW were 6 for Glu-A3, 2+4+15+19 for Glu-B3, and 12 for Glu-B2 (aaa).



318

319 **Figure 2** – SDS-PAGE electrophoresis of HMW and LMW glutenins of durum wheat samples.

320 Lanes are as follow: 1, Mexicali C75; 2, Yavaros 79; 3, Altar 84; 4, Atil C2000; 5, Jupare C  
321 2001; 6, Cirno C 2008.



322 The bread-making analysis showed great differences between the different durum  
323 genotypes (Fig. 1, Table 2). Overall, Yavaros and Altar were not suitable to elaborate spongy  
324 pan bread (high loaf volume), while Mexicali, Atil and Jupare were at least satisfactory. The  
325 performance of Cirno was more dependent on the environment. In full drip irrigation trial the  
326 results for bread-making were in average the lowest.

327 Figure 3 shows the performance in bread-making of the durum wheat varieties compared  
328 to two popular bread wheat varieties (Kachu and Roelfs) grown in the same environment and  
329 used as checks, while Table 2 shows the loaf volume specific values of each genotype in each  
330 environment. Kachu is considered usually good for bread-making and Roelfs excellent, which  
331 could be confirmed with the data showed on Table 2. Altar and particularly Yavaros were far  
332 from the volumes obtained with the checks. Yavaros did not reach 70% of the volume in any  
333 environment, while Altar only in severe heat stress trial reached 80% of Kachu bread volume.  
334 Atil, Jupare and particularly Mexicali were positively affected by severe heat stress trial in  
335 bread-making compared to the checks. Mexicali reached in severe heat stress trial 99% and 93%  
336 of the volumes of Kachu and Roelfs, respectively. Atil, although more consistent across the  
337 environments, also reached 98% and 92% respect to Kachu and Roelfs under severe heat stress.  
338 Cirno was not able to perform satisfactorily for bread making across environments. In full basin  
339 irrigation environment most of the varieties also increased bread-making capacity compared to  
340 the checks. Medium drought stress also favored bread-making.

341 The bread crumb structure (ESM2) was in accordance with the loaf volume in most of the  
342 cases, showing very poor, poor or fair crumb structure in the breads with lower volume  
343 (Yavaros, Altar) and good or very good in those breads with higher volumes.

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**Figure 3 – Bread-making of durum wheat varieties.** Colored bars show the percentage of each bread loaf volume obtained with durum wheat cultivars respective to Kachu and Roelfs bread wheat checks. Each color represents a different environment as indicated in the legend.

Table 2. *Bread loaf volume (ml) of bread wheat checks and durum wheat varieties in six different environments.*

	Kachu	Roelfs	Altar	Atil	Cirno	Jupare	Mexicali	Yavaros
Full drip irrigation	813*	864	556	713	668	649	639	471
Full basin irrigation	838	881	629	803	749	711	695	583
Medium drought stress	810	881	615	730	714	695	689	538
Severe drought stress	901	935	673	705	685	703	748	559
Medium heat stress	866	903	628	775	741	730	693	553
Severe heat stress	816	866	661	801	499	758	808	480

\*Average values in each environment (averaging field reps and years)

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### 3.4 Relationships between quality parameters

359 Correlation coefficients were calculated for the whole trial (Table 3). Several significant  
 360 correlations were found between grain morphology parameters (test weight and thousand kernel

361 weight) and other quality traits. Grain protein and zinc content were inversely correlated with  
362 thousand kernel weight while Fe content was not. Some of the parameters related to gluten  
363 quality (flour SDS sedimentation volume, mixograph torque, alveograph L and W) were also  
364 correlated negatively with grain size and positively with grain protein content. The association  
365 between loaf volume and gluten strength (flour SDS sedimentation volume, mixograph torque,  
366 alveograph W) was high in all cases. As expected, gluten extensibility (alveograph L) was also  
367 highly correlated with loaf volume.

368           The correlations between whole-meal flour and refined flour were medium-high for  
369 yellowness (0.54) and very high for SDS sedimentation volume (0.91).

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Table 3. Correlation coefficients (*r*) among quality parameters obtained across the entire trial

	TW	TKW	FeC	ZnC	GPC	WMFY(b)	FY(b)	WMSDSS	FSDSS	MixT	TQ	ALVP	ALVL	ALVW	ALVP/L	LV
<b>TW</b>	1.00															
<b>TKW</b>	0.75	1.00														
<b>FeC</b>	0.29	NS*	1.00													
<b>ZnC</b>	-0.42	-0.44	0.21	1.00												
<b>GPC</b>	-0.58	-0.68	0.19	0.60	1.00											
<b>WMFY(b)</b>	-0.10	-0.35	NS	0.17	NS	1.00										
<b>FY(b)</b>	NS	-0.23	NS	-0.20	NS	0.54	1.00									
<b>WMSDSS</b>	-0.44	-0.45	0.18	NS	0.41	NS	0.19	1.00								
<b>FSDS</b>	-0.45	-0.48	NS	0.11	0.34	0.18	0.29	0.91	1.00							
<b>MixT</b>	NS	NS	NS	-0.23	-0.37	NS	0.34	0.19	0.25	1.00						
<b>TQ</b>	NS	-0.21	NS	NS	NS	0.22	0.41	0.36	0.46	0.90	1.00					
<b>ALVP</b>	0.39	NS	0.31	NS	NS	NS	0.33	NS	NS	0.27	0.37	1.00				
<b>ALVL</b>	-0.57	-0.51	NS	0.22	0.37	NS	NS	0.65	0.66	NS	NS	-0.67	1.00			
<b>ALVW</b>	NS	-0.31	0.30	NS	0.26	0.18	0.44	0.54	0.59	0.35	0.59	0.61	NS			
<b>ALVP/L</b>	0.44	0.33	0.19	NS	-0.22	NS	NS	-0.47	-0.44	NS	NS	0.86	-0.85			
<b>LV</b>	-0.30	-0.42	NS	NS	NS	0.20	0.46	0.78	0.81	0.36	0.52	NS	0.65			

TW, Test Weight; TKW, Thousand Kernel Weight; FeC, Iron Content; ZnC, Zinc Content; GPC, Grain Protein Content; WMFY(b), Whole-meal Flour Yellowness b value; FY(b), Flour Yellowness b value; WMSDSS, Whole-meal SDS Sedimentation; FSDSS, Flour SDS Sedimentation; MixT, Mixograph Optimum Mixing Time; TQ, Mixograph Torque; ALVP, Alveograph Tenacity; ALVL, Alveograph Extensibility; ALVW, Alveograph Work; ALVP/L, Alveograph Tenacity/Extensibility Ratio; LV, Loaf Volume.

\*Correlations not significant ( $P>0.05$ ).

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#### 384 4. Discussion

385 In this study, a set of six durum wheat varieties developed by the CIMMYT breeding  
386 program was analyzed for quality traits. In general, variation was observed in genotypes and the  
387 environments for each quality parameter. This was confirmed by the analysis of the variance,  
388 which showed that both genotype and environment contributed mostly to the variability found in  
389 the data. With similar environmental conditions in both years, no significant variation by year  
390 was observed in this study for most traits. The GxE, ExY and GxExY interactions were also  
391 important, but not GxY. Our results agree with some previous reports and disagree with others.  
392 Li et al. (2013) who carried out a similar experiment with drought and heat stress in the same  
393 location, found a predominant genotype effect for test weight, flour yellowness, flour SDS  
394 sedimentation volume and mixograph optimum dough mixing time and an environment effect for  
395 thousand kernel weight, grain protein content, which completely agrees with our results, with the  
396 exception of test weight that in our case was predominantly defined by the environment and  
397 other interactions. Ames et al. (1999) in an experiment with both dry and irrigated conditions,  
398 also found that rheological tests as flour SDS sedimentation volume, mixograph optimum dough  
399 mixing time or gluten index were largely controlled by the genotype. Pigment content or flour  
400 yellowness is another trait that consistently across studies has showed great genotype influence.  
401 Rharrabti et al. (2003), Li et al. (2013) and the current study found that genotype explained 53,  
402 87 and 36.3 % of the variation for that trait, respectively, being the most important factor in the  
403 three studies.

404 All the above results strengthen the well-established idea that some quality traits,  
405 particularly those related with processing and pasta-making quality have a very strong genetic  
406 control and therefore the genotypes respond consistently across environments for these

407 characteristics. Most of those traits (flour SDS sedimentation volume, mixograph optimum  
408 dough mixing time and torque, alveograph W) also showed consistent correlations between them  
409 in the different environments. In a previous study (Li et al., 2013) it was revealed that in general  
410 drought stress favored gluten strength and yellowness (processing and pasta-making quality),  
411 which was confirmed with our study. On the contrary, heat stress was found to reduce gluten  
412 strength by both studies. Li et al. 2013 found a significant increase in flour yellowness in heat  
413 stressed environments, probably due to a “concentration effect” of the pigment carotenoid. In our  
414 experiment, flour yellowness was reduced in severe heat stress trial, which disagree with Li et al.  
415 (2013). This discrepancy with this study carried out in the same location could be due to a  
416 different tolerance to heat stress of the genotypes used in both studies, or to other environmental  
417 changes. Temperatures in May, when grain filling for severe heat stress trial happened, were  
418 something higher in our study (maximum temperatures around 1.5 °C above), which could affect  
419 the carotenoid pigment synthesis.

420 In the case of Fe and Zn content had a different response in our study. For Fe content,  
421 both genotype and environment were equally important, followed by GxE and GxExY. For Zn  
422 content environment was much more determining factor. These results are different from the  
423 study of Ficco et al. (2009) carried out in the Mediterranean region. In that case GxE was the  
424 most important factor for both micronutrients, followed by genotype, with only a slight  
425 environmental impact. This is likely due to the differences in environmental conditions used for  
426 testing in both studies, which were very highly contrasted in this study. In the study, Zn content  
427 was favored by smaller grain (“concentration effect” in severe drought stress and severe heat  
428 stress), but Fe content only in the case of severe drought stress. In the case of severe heat stress,  
429 in spite of showing the smaller grain size values, Fe content decreased respect to optimum

430 condition trial (drip full irrigation). This may reflect differences in the passive iron transport  
431 through the soil-plant system compared to Zn. Azaz et al. (2012) found also the micronutrients  
432 “concentration effect” with water stress management. Joshi et al. (2010) reported that iron and  
433 zinc concentrations were also found to be influenced by higher temperature. It is also important  
434 to note that a significant trend in grain micronutrients concentration related to the release year of  
435 the varieties was not found (data not shown). Some studies have shown that modern breeding  
436 focused on grain yield has reduced the grain micronutrient concentrations in bread wheat (Fan,  
437 Zhao, Fairweather-Tait, Poulton, Dunham & McGrath, 2008; Garvin, Welch & Finley, 2006),  
438 which is in agreement with the results from Ficco et al. (2009). Additionally, with the limited  
439 number of genotypes in this study the variation for micronutrients could not be accurately  
440 assessed and further studies are required. At the same time, studies of other grain components  
441 related to the potential bioavailability of these micronutrients (i.e. phytic acid) are required.

442         The average value for both iron and zinc (32.1 and 37.6 mg/kg, respectively) revealed by  
443 this study does not seem adequate to meet daily requirements of humans in which durum wheat  
444 provides the main source of essential minerals (North African and West Asian countries where  
445 wheat contributes >50% to daily energy intake). A wheat-heavy diet consumed over lengthy  
446 periods can result in micronutrient malnutrition and related severe health complications (Cakmak  
447 et al., 2008). Therefore, durum wheat breeding programs should be more focused on improving  
448 micronutrients concentration, as is being done in bread wheat (Velu et al., 2011). The negative  
449 significant effect revealed by the study on Fe content under heat stress is another important  
450 concern, as high temperature is common in durum wheat cultivation areas.

451         Performing alveograph analysis in durum wheat, Ammar et al. (2000) found that  
452 alveograph P is more dependent on the environment than in the genotype, and the contrary for

453 alveograph L. Our studies led to the same conclusion, with 44% of dough extensibility being  
454 explained by the genotype. This is an important trait for improving bread-making in durum  
455 wheat. Ammar et al. (2000) found that alveograph W was also more dependent on the genotype,  
456 a finding that agrees with previous research by Mariani et al. (1995). Our study determined that  
457 alveograph W was more dependent on environment, probably because alveograph P was also.  
458 Due to the low alveograph L values showed by the durum wheat cultivars in our study,  
459 alveograph P is the main determinant of alveograph W. As discussed above, gluten strength was  
460 favored by drought stress conditions due to influence of grain size on the protein content  
461 (concentration effect), as revealed by the high inverse relationship between those parameters.  
462 This effect has also been shown in the work of Flagella et al. (2010) and Li et al. (2013).  
463 However, it is contrary to the findings in studies undertaken by Rharrabti et al. (2003), who  
464 found less gluten strength with increased grain protein content in Mediterranean conditions due  
465 to drier conditions. On the other hand, severe heat stress, in spite high grain protein content, had  
466 lower gluten strength (alveograph W) compared to any other environment. This effect has been  
467 associated with a change in the glutenin/gliadin ratio in bread wheat by Blumenthal et al. (1993),  
468 and was also found in durum wheat by Li et al. (2013) in some varieties. There were no changes  
469 in flour SDS sedimentation volume in severe heat stress indicating that this test gives  
470 information not only about gluten strength but also about gluten extensibility, as indicated by the  
471 coefficient correlations.

472 In the case of bread-making, our results showed that this ability depends largely on the  
473 genotype, even in this study in which all cultivars had the same composition of glutenins (Glu-  
474 B1 7+8 and LMW-2). This finding is in agreement with Rao et al. (2010) who showed that high  
475 bread-making quality is heritable in tetraploid wheat and that as a result, selection in durum



476 breeding programs for improved bread-making potential should be possible. Ammar et al. (2000)  
477 found also a strong genotypic effect, but their research showed the glutenin composition was  
478 heterogeneous among the lines tested. Glutenin composition has been said to be the key  
479 determinant of gluten strength in durum wheat, and also in bread-making. There is no clear  
480 consensus about the best *Glu-B1* allele for bread-making: Ammar et al. (2000) found 6+8 better  
481 than 7+8; Boggini & Pogna (1989) indicated 7+8 > 20 >6+8; Peña et al. (1994) found 7+8 > 6+8  
482 >20. In the case of LMW glutenins, it is well established that LMW-2 type confers higher gluten  
483 strength and consequently higher bread volumes. The lines used in our research had all the  
484 subunit 7+8 and LMW-2 types, so glutenin composition cannot be used in this case to explain  
485 the big differences found in dough properties and bread-making among the genotypes of this  
486 study. This indicates that there are other qualitative or quantitative grain factors that also play a  
487 role in determining dough viscoelastic properties, which should be identified in more extensive  
488 studies. Our data also strengthens the idea expressed by Ammar et al. (2000), who due to the  
489 conflicting conclusions about the best glutenins alleles for durum wheat bread-making pointed  
490 out that the use of glutenins as markers to select for improved bread-making in durum wheat  
491 might not be justified. The high relationship between bread loaf volume and SDS sedimentation  
492 volume (0.81), suggests that this test could be a much more efficient tool for rapid and low-cost  
493 identification of suitable durum wheat genotypes for bread-making. The efficiency of this  
494 methodology for this purpose has been already demonstrated with CIMMYT germplasm (Peña et  
495 al. 1994,  $r = 0.81$ ) and with cultivars of diverse origin (Ammar et al. 2000,  $r = 0.56$ ). We also  
496 showed the high efficiency to predict bread-making ( $r = 0.78$ ) of the same test run with whole-  
497 meal flour, which would allow, as in the case of yellowness determination, the save of time and  
498 resources in the selection process.

499 Overall, the durum wheat lines in this study showed inferior values for loaf volume and  
500 lower crumb structure quality compared to the bread-wheat checks. The less viscoelastic  
501 properties of the gluten and excessive starch damage could be the main reasons to explain this.  
502 However, some durum genotypes in specific environment as severe heat stress had almost the  
503 same performance that bread-wheat checks used in this study (Kachu and Roelfs), which are  
504 considered optimal for the bread-making process. This fact agrees with previous studies (Ammar  
505 et al. 2000; Peña et al. 1994), which have already proven that the long-established belief that  
506 durum wheat is not suitable for pan bread may be incorrect (Boggini et al. 1995). Based on the  
507 high correlations values obtained, gluten strength (alveograph W) and extensibility (alveograph  
508 L) were identified as key factors to enhance loaf volume, as well as the balance between gluten  
509 tenacity and extensibility (alveograph P/L). In previous studies, durum wheat flours were showed  
510 to a have high alveograph P/L values, leading to bread more compact (Brescia et al. 2007).  
511 Gluten extensibility with adequate levels of gluten strength is required in durum wheat for bread-  
512 making. Atil, the variety with higher extensibility by far, and Mexicali in severe heat stress trial  
513 (that have a great extensibility increase in this environment), were the ones showing better  
514 breads. This data supports the idea that to develop durum wheat cultivars with similar bread-  
515 making quality to those of bread wheat lines, it is necessary to achieve a better balance of  
516 tenacity and extensibility in conjunction with increased strength (Ammar et al. 2000; Dexter et  
517 al. 1994; Rao et al. 2010). For this purpose, the glutenin alleles or rheological tests traditionally  
518 used to enhance gluten strength for pasta-making quality may not be more suitable for bread-  
519 making, as durum wheat lines with strong gluten also tends to exhibit tenacious and inextensible  
520 gluten (Rao et al. 2001). Besides, the importance of gluten extensibility in bread-making is what  
521 confers to durum wheat in severe heat stress an increase in the bread volumes compared to bread

522 wheat, which showed their best performance in severe drought stress. Durum wheat samples lack  
523 extensibility in control and drought stressed environments while bread wheat cultivars had  
524 balanced gluten on those environments. Heat stress caused the increase of extensibility in both,  
525 favoring the bread-making quality of durum wheat lines but not that of bread wheat, whose more  
526 extensible gluten was not better for bread-making. For unknown reasons (no significant changes  
527 in grain protein content or other traits), extensibility was also higher in basin full irrigation trial  
528 with respect to drip full irrigation, which also led to better bread volumes in this environment.  
529 Thus, this fact should be also taken into account when growing durum wheat for bread-making.  
530 Severe drought stress, because increasing gluten strength but not extensibility, led in most of the  
531 cases to inferior bread volumes compared with the rest of the environments. Both drought and  
532 heat stresses are often present in the main areas where durum wheat is grown (Mediterranean  
533 countries and Middle East), where local bread accounts for half of the durum wheat  
534 consumption.

535         It is also significant that the results obtained in bread-making with durum wheat are not  
536 the consequence of a planned and designed breeding program for this purpose. Durum wheat  
537 breeding programs are focused on obtaining lines with pasta-making quality (high gluten  
538 strength), which means there is probably lot of room for improvement in durum bread-making if  
539 systematic selection is applied. Extensibility could be increased applying selection for this trait  
540 with tools not extensively used in durum wheat quality breeding as the alveograph. Introgression  
541 of *Glu-D1* or *Glu-D3* glutenins from bread wheat is other possibility (Pogna et al. 1996).The  
542 development of durum lines with good bread-making quality could increase the commercial  
543 value of this crop and open alternative markets in years of high production. Moreover, durum  
544 wheat flour is a derivate in semolina production, so its use in bread-making could also offset the

545 cost of semolina production. This is particularly interesting for those countries where durum has  
546 a superior yield performance compared to bread wheat. In addition to this, for CIMMYT bread  
547 wheat breeding program, the development of durum lines with enhanced bread-making quality it  
548 is also convenient, because durum lines are being used to transfer a variety of traits to bread  
549 wheat through intra-specific (bread x durum) and inter-specific (synthetics) hybridization. Using  
550 parental durum lines with good bread-making quality in those crosses would contribute to ensure  
551 desirable bread-making quality in the novel lines developed.

552

## 553 **5. Conclusions**

554 In this study, a set of durum wheat cultivars revealed differences in micronutrients (Fe  
555 and Zn), processing, pasta-making and bread-making quality. Additionally, their unique  
556 responses to the environmental stresses of drought and heat were measured. Processing and  
557 pasta-making quality was favored by drought but not by heat stress. Cvs. Mexicali and Jupare  
558 under heat stress and Atil in several environments showed bread volumes close to bread wheat  
559 checks. Gluten extensibility was identified as a key trait to be improved to enhance bread loaf  
560 volume in durum wheat.

561

## 562 **Acknowledgements**

563 We greatly appreciate financial support from the CRP-Wheat and HarvestPlus challenge  
564 program of CGIAR consortium and from Fondo Sectorial SAGARPA-CONACYT (No. 146788  
565 - Sistema de mejoramiento genético para generar variedades resistentes a royas, de alto  
566 rendimiento y alta calidad para una producción sustentable en México de trigo) of the Mexican  
567 government. We are also grateful to Julie Mollins for the editing of this manuscript.

568

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713 **Response to drought and heat stress on wheat quality, with special emphasis on bread-**  
714 **making quality, in durum wheat**

715 Carlos Guzmán\*, Jorge Enrique Autrique, Suchismita Mondal, Ravi Prakash Singh, Velu  
716 Govindan, Anayeli Morales-Dorantes, Gabriel Posadas-Romano, Jose Crossa, Karim Ammar,  
717 Roberto Javier Peña

718 Global Wheat Program, International Maize and Wheat Improvement Center (CIMMYT), Apdo  
719 Postal 6-641, Mexico DF.

720 \* Author for correspondence: Carlos Guzman (Phone: +52 (595) 952 1900 ext. 2280; Fax:+52  
721 (555) 804 7558; Email: c.guzman@cgiar.org

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723 For Field Crops Research

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**Abstract**

Durum wheat accounts for more than 50% of the total wheat-growing area in the Mediterranean region, where is used for the preparation of diverse food products, including pasta and bread. The effects of drought and heat stresses on grain morphology, grain composition (protein, iron and zinc micronutrients), processing and pasta and bread-making quality in durum wheat varieties were analyzed. The results revealed significant differences among the genotypes, as well as unique responses to the environmental stresses. Micronutrients concentration (iron and zinc), processing and pasta-making quality was favored by drought but not by heat stress. Overall, the durum wheat lines showed inferior values for bread volume compared to the bread-wheat checks. However, some durum genotypes in specific environment had almost the same performance. To develop durum wheat cultivars with similar bread-making quality to that of bread wheat, it is necessary to achieve a better balance of tenacity and extensibility. The development of durum lines with good bread-making quality could increase the commercial value of this crop.

**Keywords:** durum wheat; processing quality; pasta-making; bread-making; drought stress; heat stress.

757

758 **1. Introduction**

759 Durum wheat (*Triticum turgidum* ssp. *durum* Desf. em. Husn.) accounts for around 6% of  
760 total wheat production (37.7 million tonnes in 2013; International Grain Council October, 2014),  
761 occupying approximately 20 million hectares worldwide. In the Mediterranean region durum  
762 wheat accounts for more than 50% (reaching 90% in some countries) of the total wheat-growing  
763 area, due to its role as the staple food in many countries. Durum grain is used for the preparation  
764 of diverse food products, including bread, couscous, frekeh, bulgur, and most importantly, pasta.  
765 Pasta is generally recognized worldwide as beneficial to a nutritionally balanced diet (Ames,  
766 Clarke, Marchylo, Dexter & Woods, 1999), and consumer demand is reflected in the upward  
767 trend in pasta production. Almost 9.3 million tons were purchased in 2001, and two years later in  
768 2003 almost 10.5 million tons were purchased. By 2012, approximately 13.5 million tons were  
769 produced (IPO, 2013), an increase, which is an important indicator of the increase in demand for  
770 durum wheat throughout the world.

771 At the International Maize and Wheat Improvement Center (CIMMYT) durum wheat  
772 breeding draws on a large, genetically wide, gene pool to develop germplasm, which is widely  
773 distributed among breeding programs of durum-producing countries. The priority of the wheat  
774 breeding program is to develop high-yielding, disease-resistant varieties that can tolerate  
775 drought, heat stresses, and produce high grain quality. The latter is essential in durum wheat  
776 varieties to be accepted by industry, local food manufacturers and consumers. The most  
777 important parameters affecting industrial quality for pasta-making from durum wheat grain are  
778 probably gluten quality (strength) and the yellow color of semolina. Although environmental and  
779 processing conditions play a significant role in these two traits, research has shown that they are

780 under strong genetic control. The favourable dough or gluten properties of durum lines have  
781 been associated mainly with the presence of specific glutenins coded by the *Glu-B1* and *Glu-B3*  
782 loci (Ammar, Kronstad & Morris, 2000; Boggini & Pogna, 1989; Boggini, Tusa & Pogna, 1995;  
783 Brites & Carrillo, 2001; Peña, Zarco-Hernandez, Amaya-Celis & Mujeeb-Kazi, 1994). Semolina  
784 color will depend to a great extent on the genes involved in pigment accumulation (enzymes  
785 involved in pigment biosynthesis as phytoene synthase and pigment degradation as lipoxygenase  
786 or polyphenol oxidase) (see Ficco et al., 2014 for a review). Apart from pasta, durum wheat is  
787 widely used to elaborate other non-baked goods as couscous and bulgur. In the former high  
788 yellow color is appreciated while in bulgur light yellow color is preferred (Belibagli et al. 2009).  
789 Both products also demand high protein content to avoid stickiness and to have high water  
790 absorption capacity (Bayram and Öner 2006; Ounane et al. 2006).

791         Although durum wheat generally exhibits inferior bread-making quality compared with  
792 bread wheat (*T. aestivum* L. ssp. *aestivum* .) in terms of loaf volume and crumb structure  
793 (Boggini et al., 1995; Peña et al., 1994), approximately 24% of the global durum wheat  
794 production, and up to 70-90% in some Middle East countries, is used for bread-making (Quaglia,  
795 1988). Speciality breads made with durum are common in the south of Italy (Boggini et al.,  
796 1995), and this popularity is spreading to other countries (Sissons, 2008). In the regions of West  
797 Asia and North Africa 50% of durum wheat is processed into single and two-layered flat breads  
798 and 35% is used for leavened breads (Liu, Shepherd & Rathjen, 1996). In those countries durum  
799 breads are very popular with consumers probably due to their sensory properties, particularly to  
800 their pleasant aroma (Rao, Pozniak, Hucl & Briggs, 2010) and special taste. They are slow to go  
801 stale (Pasqualone, Summo, Bilancia & Caponi, 2007) and consequently their longer shelf-life  
802 due to the high water absorption of durum wheat flour related to increased content in damage

803 starch, is another desirable characteristic of durum wheat breads (Boyacıoğlu & D'Appolonia,  
804 1994). Besides, although yellow bread wheat flours are typically undesirable for bread-making,  
805 in the case of durum wheat breads a distinctive yellow colour is an important factor influencing  
806 whether it is accepted by the consumer (Brescia et al., 2007; Pasqualone, Caponio & Simeone,  
807 2004).

808         Significant research has been done to identify the traits necessary to enhance durum  
809 bread-making quality, and in this process several durum genotypes with acceptable bread-  
810 making characteristics have been identified (Ammar et al., 2000; Edwards et al., 2007; Peña et  
811 al., 1994). Gluten strength (determined by glutenins composition) has generally been accepted as  
812 the main component that must be increased to improve baking performance of bread wheat, but  
813 gluten extensibility should also be improved (Ammar et al., 2000; Boggini et al., 1995; Edwards  
814 et al., 2007; Rao et al., 2010). However, very little information is available about the effect of the  
815 environment on the bread-making characteristics of durum wheat. In typical areas of durum  
816 wheat cultivation (Mediterranean and countries in western Asia), water deficiency and high  
817 temperatures during grain filling are major factors that define quality. The effect of those stresses  
818 in some durum quality traits (semolina milling and pasta making quality) has been previously  
819 studied (De Stefanis, Sgrulletta, De Vita & Pucciannati, 2002; Flagella, Giuliani, Giuzio, Volpi  
820 & Masci, 2010; Li, Wu, Hernandez-Espinosa & Peña 2013).

821         Besides processing and end-use quality, nutritional quality is also becoming an important  
822 priority in wheat breeding. Wheat is good source of diverse beneficial compounds including  
823 fibre, phytochemicals and micronutrients. Among different micronutrients, iron and zinc are  
824 deficiency in the diet of two billion people (WHO 2012), and because of this have become the  
825 focus of micronutrient biofortification, which serves to enhance iron and zinc grain

826 concentration. Modern wheat cultivars have been shown to be poor sources of these  
827 micronutrients for meeting daily requirements for humans (Cakmak, Pfeiffer & McClafferty,  
828 2010). Not much research has been carried out about durum wheat and iron and zinc content.  
829 According to Ficco et al. (2009), there is some genetic variation to breed for iron and zinc in  
830 durum wheat, although more studies screening for larger genetic variability and examining the  
831 environment effect are required.

832 The objective of this study was to analyse the effects of drought and heat stresses on  
833 different quality traits with special emphasis on bread-making quality in a set of durum wheat  
834 varieties, which are representative of CIMMYT durum germplasm.

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## 836 **2. Materials and methods**

### 837 *2.1 Plant Materials/Agronomic trials*

838 A trial consisting of six CIMMYT durum wheat cultivars (Mexicali C75, Yavaros C79,  
839 Altar C84, Atil C2000, Jupare C2001 and Cirno C2008) and two bread wheat ones (Kachu and  
840 Roelfs F2007), were sown in 2012-2013 and 2013-2014 crop seasons in Ciudad Obregon,  
841 Sonora, in northwestern Mexico. The trial was planted with two replicates in a randomized  
842 complete Block Design under six different environmental conditions: full drip irrigation  
843 (optimum conditions), full basin irrigation, reduced irrigation or medium drought stress, severe  
844 drought stress, medium heat stress, and severe heat stress. All the trials were planted in  
845 November except medium heat stress (planted in January) and heat stress (planted in February).  
846 All the trials had full irrigation (>500 mm) except medium drought stress (300 mm) and severe  
847 drought stress (180mm). Weed, diseases, and insects were all well controlled. In all the trials, N  
848 was applied (pre-planting) at a rate of 50 kg of N/ha and at tillering 150 additional units of N



849 were applied in all the trials except in severe drought stress (50 N units). At maturity whole  
850 plots were harvested and 1kg of seed from each of the wheat lines was used for analyzing the  
851 quality traits.

852 The meteorology data of the experimental station in Ciudad Obregon was characterized  
853 by almost no precipitation during the wheat growing season. Maximum temperatures were  
854 between 31 and 32 °C in March and April, the grain filling time for all treatments, except for  
855 plants under heat stress at temperatures between 35 to 36 °C during grain filling in May.  
856 Flowering time and physiological maturity in most of the cultivars used occur at similar times,  
857 due to the fact that these genotypes were bred for the same growing area. According to the  
858 general growing stages of durum wheat in Ciudad Obregon, drought stress was continuous from  
859 stem elongation to grain ripening in moderate and severe drought stress trials during stem  
860 elongation and flowering. In severe heat stress trial, higher temperatures than in the normal  
861 planting time started from shoot elongation and remained in the grain filling stage until ripening.  
862 Detailed temperature data is shown in ESM1.

863

## 864 *2.2 Grain and flour parameters*

865 Grain morphological characteristics were evaluated with digital image system SeedCount  
866 SC5000 (Next Instruments, Australia). Thousand kernel weight (g) and test weight (kg/hl) were  
867 obtained. Grain iron and zinc content (mg/kg) were measured using a bench-top, non-destructive,  
868 energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme  
869 8000, Oxford Instruments Plc, Abingdon, UK), previously standardized for high throughput  
870 screening of iron (Fe) and zinc (Zn) in whole wheat grain (Paltridge et al., 2012). Grain protein  
871 (%) and moisture content were determined by near-infrared spectroscopy (NIR Systems 6500,

872 Foss Denmark) calibrated based on official AACC methods 39-10 and 46-11A (AACC, 2010).  
873 Grain samples previously conditioned at 16% of moisture were milled into flour using Brabender  
874 Quadrumat Jr (C. W. Brabender OHG, Germany). Whole-meal flour samples were also obtained  
875 with a UDY Cyclone mill carrying a 0.5 mm screen. The protein and moisture content in flour  
876 was estimated using near-infrared spectroscopy (NIR Systems 6500, Foss Denmark) calibrated  
877 based on the AACC methods commented above. Grain protein and flour protein content values  
878 were reported at 12.5% and 14% moisture basis, respectively. Flour yellowness and whole-meal  
879 flour yellowness were obtained as the b value of a Minolta color meter (Konica Minolta, Japan).

880 Glutenins subunits composition were identified by SDS-PAGE in polyacrylamide gels,  
881 according to the methodology of Peña et al. (2004), following the nomenclature of Payne and  
882 Lawrence (1983) for HMW glutenins and Nieto-Taladriz et al. (1997) for LMW glutenins.

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### 884 *2.3 Rheological tests*

885 Sodium dodecyl sulfate (SDS) sedimentation volume was carried out according to the  
886 modified protocol described in Peña et al. (1990) using 1g of flour or of whole-meal flour.  
887 Additionally, 35g flour samples were run in a mixograph (National Mfg. Co.) to obtain optimum  
888 dough mixing time and %Torque\*min according to AACC method 54-40A (AACC, 2010).  
889 Gluten extensibility (alveograph L), tenacity (alveograph P), elasticity or strength (alveograph  
890 W) and tenacity/extensibility ratio (alveograph P/L) were determined according to the  
891 Alveograph manufacturer's instructions (Chopin, France), using 60g flour samples and constant  
892 water absorption (55%). Higher water absorption than in the official methodology (50%) was  
893 used to compensate for the typically greater water absorption caused by high levels of starch

894 damage occurring during milling of the very hard durum wheat grain (Ammar et al., 2000;  
895 Dexter, Preston, Martin & Gander, 1994, Peña et al., 1994).

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#### 897 *2.4 Bread-making characteristics*

898 Bread was baked using the straight-dough baking test 10-09 of the AACCC (AACCC 2010)  
899 with some modifications. 100g of flour were mixed with 3g of shortening, 3g of nonfat dry milk,  
900 adjusting water absorption to 73%, in order to maintain uniform dough handling consistency as  
901 judged by the baker. Fermentation time was reduced to 90 minutes, according to the method used  
902 by Dexter et al. (1994) and Sapirstein et al. (2007), who showed short fermentation times  
903 resulted in better bread-making performance for durum wheat. Bread loaf volume was  
904 determined by colza (*Brassica* sp.) seed displacement using a volumeter. Crumb structure (gas-  
905 cell size and size distribution) was scored as very poor, poor, fair, good and very good.

906 All the phenotypic data recorded is shown in ESM2.

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#### 908 *2.5 Statistical Analysis*

909 Combined analyses of variance (ANOVA) across environments for GY and other quality  
910 traits were performed using procedure Proc Anova of the SAS statistical software (2010). The  
911 model is a complete fixed effect linear model with the main focus of estimate means of the  
912 durum wheat lines in specific and across environments during two years.

913 The basic conventional fixed effect linear model for describing the univariate mean  
914 response of genotypes in environments and years, and their interactions is  $y_{ijrk} = \mu + R(EY)_{ijk} +$   
915  $Y_k + G_i + E_j + (GY)_{ik} + (EY)_{jk} + (GE)_{ij} + (GEY)_{ijk} + \epsilon_{ijrk}$ , where  $y_{ijrk}$  is the response of the  $i^{\text{th}}$   
916 genotype ( $i=1,2,\dots,I$ ) in the  $r^{\text{th}}$  replicated ( $r=1,2,\dots,L$ ) of the  $j^{\text{th}}$  environment ( $j=1,2,\dots,J$ ) and the

917  $k^{\text{th}}$  year ( $k=1,2,\dots,M$ ),  $\mu$  is the grand mean over all genotypes, environments, and years,  $R(EY)_{rjk}$   
918 is the effect of the  $r^{\text{th}}$  replicate within the  $j^{\text{th}}$  environment and the  $k^{\text{th}}$  year,  $Y_k$  is the effect of the  
919  $k^{\text{th}}$  year,  $G_i$  is the effect of the  $i^{\text{th}}$  durum wheat line,  $E_j$  is the effect of the  $j^{\text{th}}$  environment,  $(GY)_{ik}$   
920 is the effect of the interaction between the  $i^{\text{th}}$  durum wheat line with the  $k^{\text{th}}$  year,  $(EY)_{jk}$  is the  
921 effect of the interaction between the  $j^{\text{th}}$  environment with the  $i^{\text{th}}$  durum wheat line,  $(GE)_{ij}$  is the  
922 effect of the interaction between the  $i^{\text{th}}$  durum wheat line with the  $k^{\text{th}}$  year,  $(GEY)_{ijk}$  is the effect  
923 of the triple interaction among the  $i^{\text{th}}$  durum wheat line with  $j^{\text{th}}$  environment and the  $k^{\text{th}}$  year, and  
924  $\epsilon_{ijrk}$  is the error assumed to be normally and independent distributed NID  $(0, \sigma^2)$ , where  $\sigma^2$  is the  
925 error variance, assumed to be constant.

926         Averages values in each environment (averaging years and genotypes), in each year  
927 (averaging environment and genotypes) and in each genotype (averaging environment and years)  
928 and least significant difference (LSD) between the different mean values were calculated using  
929 SAS.

930         Pearson correlation coefficients ( $r$ ) and significances for each comparison in the whole  
931 study were obtained using SAS.

932

### 933 **3. Results**

#### 934 *3.1 Effects of genotype, environment, year and their interactions*

935         The results of the analysis of the variance (Table 1) showed that almost all the factors had  
936 a significant effect on the different traits. Genotype and environment were the most important  
937 factors explaining the variation found, followed by GxE and ExY interaction. The environment  
938 effect was particularly high in traits as test weight, thousand kernel weight, Zn content and  
939 alveograph P, which explained more than 47% of the variation. In the case of Fe content, flour

940 yellowness, SDS sedimentation volume, mixograph optimum dough mixing time and torque,  
941 alveograph L, the genotype effect was predominant and responsible for at least 25% of the  
942 variation, and in some cases as flour SDS sedimentation and bread loaf volume explained more  
943 than 55% of variation. For this latter trait the GxE effect was also particularly important. GxE  
944 effect also explained about 12% of the variation in traits Fe and Zn content, flour SDS  
945 sedimentation volume, mixograph optimum dough mixing time and torque, and alveograph P, L  
946 and P/L. The ExY effect was high for test weight (30%) and also important for thousand kernel  
947 weight, flour yellowness, flour SDS sedimentation volume and alveograph W. The triple  
948 interaction GxExY effect was significant for mixograph optimum dough development time and  
949 torque and alveograph W. The effects of the particular year and the GxY were not significant for  
950 all of the traits.

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Table 1. *Effects of genotype, environment, year and their interactions on quality traits: % of the total sum of squares from ANOVA analysis*

Source/Trait	FSDS														
	TW	TKW	FeC	ZnC	GPC	FY(b)	S	MixT	TQ	ALVP	ALVL	ALVW	ALVP/L	LV	
<b>Genotype</b>	17.7	8.7	29.3	5.4	6.4	36.3	55.9	26.2	34.0	16.3	44.0	26.2	33.8	56.0	
<b>Environment</b>	50.0	53.0	28.8	47.8	79.6	27.9	3.0	25.4	16.0	56.7	32.6	32.3	38.6	6.9	
<b>Year</b>	1.0	0*	0.8 <sup>†</sup>	3.1	0.9	1.0	0.3 <sup>‡</sup>	0*	0.9 <sup>†</sup>	0.4 <sup>‡</sup>	0.9	0.1*	0.6 <sup>‡</sup>	0.2 <sup>‡</sup>	
<b>GxE</b>	6.1	4.9	13.7	10.0	3.9	11.8	15.6	15.9	15.8	9.9	12.3	8.8	12.3	20.9	
<b>GxY</b>	0.8	1.8	3.7	6.1	0.6 <sup>‡</sup>	1.3	0.8 <sup>‡</sup>	0.5*	1.5	5.4	2.7	3.7	4.9	0.2 <sup>‡</sup>	
<b>ExY</b>	17.1	30.1	2.9 <sup>‡</sup>	7.2	4.5	15.8	15.1	7.2	6.2	3.0	2.1	12.2	1.5	9.5	
<b>GxExY</b>	5.0	3.3	12.0	15.5	2.6	3.4	6.5	15.9	16.0	5.8	3.5	13.4	5.9	4.3	

TW, Test Weight; TKW, Thousand Kernel Weight; FeC, Iron Content; ZnC, Zinc Content; GPC, Grain Protein Content; FY(b), Flour yellowness b value; FSDSS, Flour SDS Sedimentation; MixT, Mixograph Optimum Mixing Time; TQ, Mixograph Torque; ALVP, Alveograph Tenacity; ALVL, Alveograph Extensibility; ALVW, Alveograph Work; ALVP/L, Alveograph Tenacity/Extensibility Ratio; LV, Loaf Volume.

\*All the values were highly significant ( $p < 0.001$ ) except: \*, not significant; <sup>†</sup>,  $p < 0.05$ ; and <sup>‡</sup>,  $p < 0.01$ .

970 Figure 1 shows the range of values for each quality trait with respect to genotype,  
971 environment, and year. The average value of each component and the letters to identify groups,  
972 based on the LSD test, are also shown. For grain test weight, most of the genotypes showed  
973 acceptable values in all the environments, except Mexicali grain density, which was severely  
974 affected in severe heat stress trial. This last environment together with severe drought stress were  
975 the ones reducing more test weight. For grain size (thousand kernel weight), the ranges of  
976 variation across genotypes were larger than for test weight, with more than 20g of difference in  
977 all the genotypes between the maximum value and the lowest one. As for test weight, severe  
978 drought stress and severe heat stress caused markedly negative effect on grain size, although in  
979 this case the reduction was much more prominent than for test weight. For both test weight and  
980 thousand kernel weight, severe heat stress affected more negatively, with average reductions of  
981 5% and 27%, respectively. In medium heat stress the range of variation was larger than in other  
982 environment, reflecting the different performance of the genotypes in this environment between  
983 both years of the study. In medium drought stress, genotypes showed higher test weight and  
984 thousand kernel weight values. The cultivar combining better acceptable test weight and large  
985 thousand kernel weight was Cirno.

986 In traits traditionally related to grain size (grain protein, Fe and Zn content), the response  
987 of the genotypes was not consistent in all environments. For grain protein content, the  
988 environments with lower grain size (severe drought stress and severe heat stress) had  
989 significantly higher values. This did not happen in the case of Fe content, for which severe heat  
990 stress showed also the lowest value. On the contrary, for Zn content severe heat stress resulted in  
991 the highest value (56 mg/kg). In the case of severe drought stress, higher values than in the other  
992 environments were found for both micronutrients. All the genotypes showed large ranges for

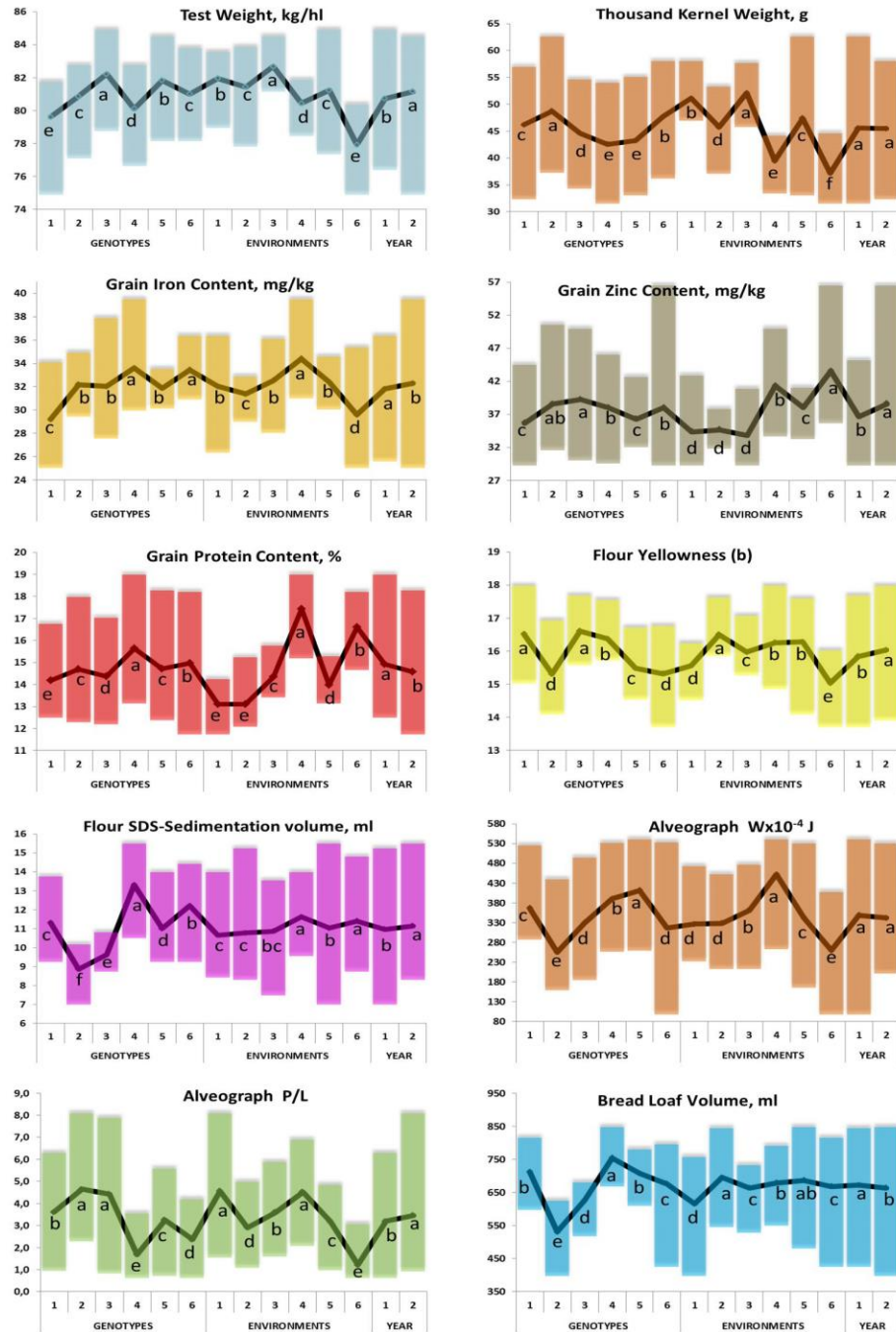
993 grain protein content. Jupare did not show a wide range for Zn or Fe content; Yavaros and Cirno  
994 were also stable across environments for Fe content. Atil was the genotype, which had higher  
995 average grain protein and Fe content, while Altar was for Zn content.

996 In the case of flour yellowness, there were significant differences between the average  
997 values of the genotypes, Mexicali and Altar were the highest, while Yavaros and Cirno were the  
998 lowest. Additionally, severe heat stress caused the greatest adverse effect for this trait with no  
999 very large differences in the other environment.

1000 The rheological tests showed significant differences in genotypes. For flour SDS  
1001 sedimentation volume and alveograph P/L two groups could be distinguished: the first one with  
1002 high flour SDS sedimentation volume and more balanced gluten (and lower alveograph P/L),  
1003 composed by Mexicali, Atil, Jupare and Cirno; and the second with lower flour SDS  
1004 sedimentation volume and more tenacious gluten in which Yavaros and Altar were included. For  
1005 alveograph W Cirno showed low values, as Yavaros and Altar. The environment differences  
1006 were smaller for flour SDS sedimentation volume, with, as expected, the stressed environments  
1007 showing higher values, followed by medium-stressed environments, and then the fully irrigated  
1008 environments. Similar responses were observed for alveograph W except for severe heat stress  
1009 trial which had the lowest values. For alveograph P/L severe heat stress trial was again the one  
1010 showing lower values, with large differences compared to the environment with higher values for  
1011 this trait, full drip irrigation and severe drought stress.

1012 Overall, the differences caused by the year in terms of average, maximum and minimum  
1013 values were small for all the traits.





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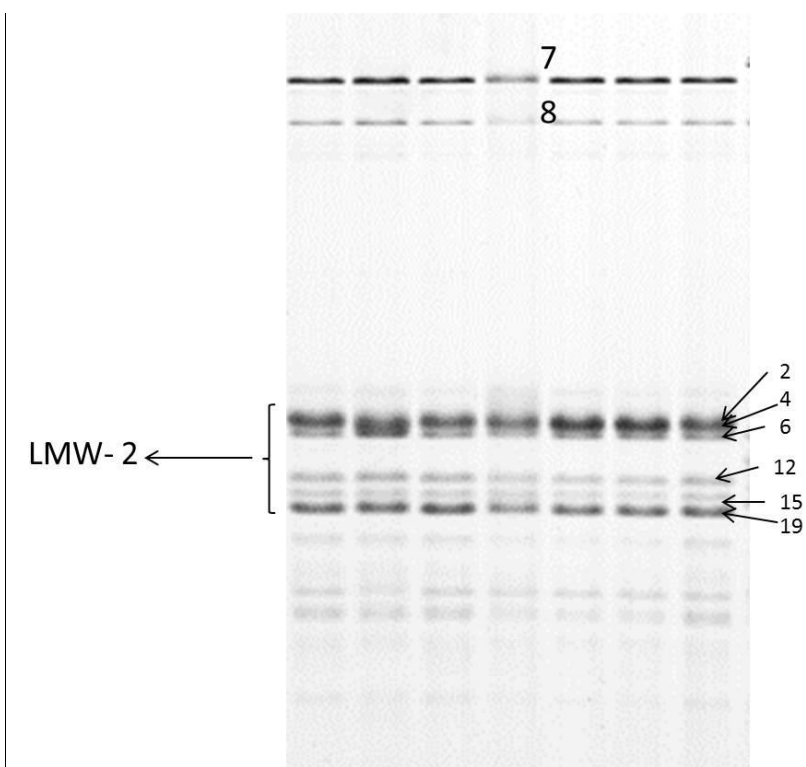
1015 **Figure 1-** Maximum, average and minimum values for different quality traits obtained by each  
 1016 genotype in the whole trial, and in each environment and year by the six genotypes. The  
 1017 range of values (maximum and minimum) is represented by bars. The average values in  
 1018 each case are represented with a continuous dark line. Letters identify the different groups  
 1019 between genotypes, environments and years, based on LSD test. Genotypes are as follow: 1,

1020 Mexicali; 2, Yavaros; 3, Altar; 4, Atil; 5, Jupare; 6, Cirno. Environments are as follow: 1,  
1021 drip full irrigation; 2, basin full irrigation; 3, medium drought stress; 4, severe drought  
1022 stress; 5, medium heat stress; 6, severe heat stress. Years are as follow: 1, cycle 2012-2013;  
1023 2, cycle 2013-2014.

1024

### 1025 3.2 *Glutenins composition and bread-making analysis*

1026 All the cultivars resulted to have the same glutenins composition. For HMW the *Glu-B1*  
1027 locus had the allele 7+8 and for LMW they were LMW-2 type (Fig. 2). The specific alleles for  
1028 LMW were 6 for Glu-A3, 2+4+15+19 for Glu-B3, and 12 for Glu-B2 (aaa).



1029

1030 **Figure 2** – SDS-PAGE electrophoresis of HMW and LMW glutenins of durum wheat samples.

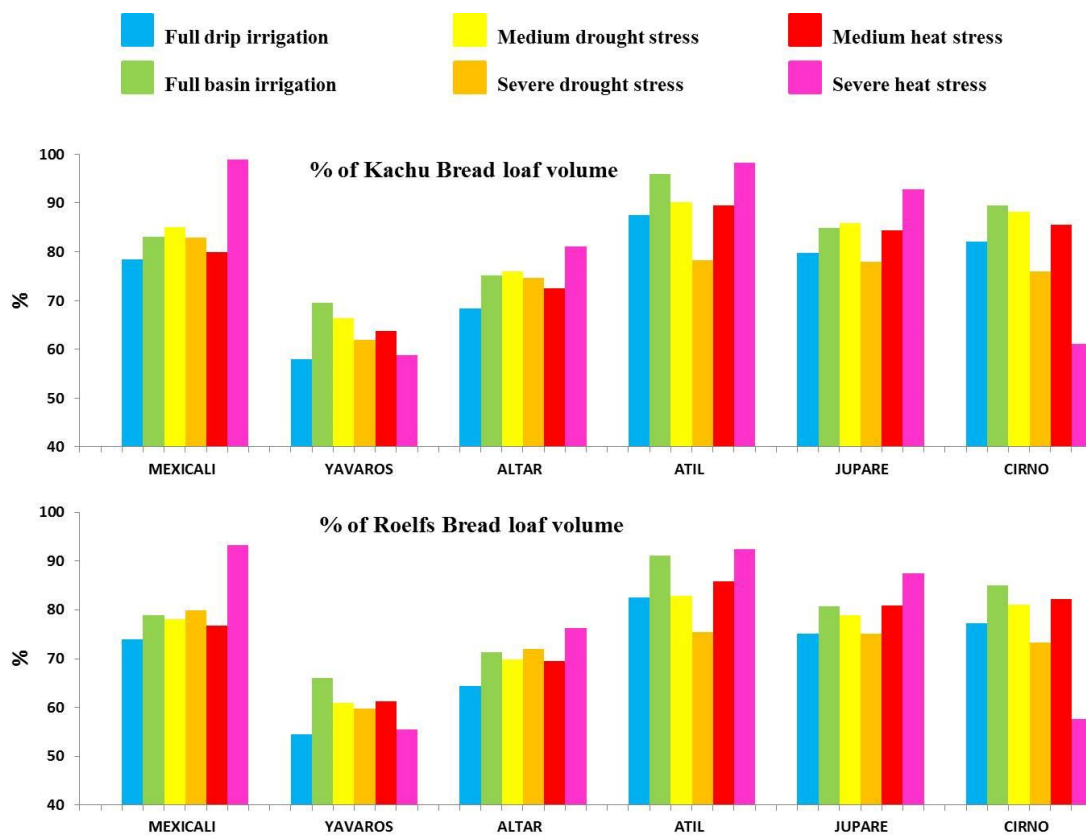
1031 Lanes are as follow: 1, Mexicali C75; 2, Yavaros 79; 3, Altar 84; 4, Atil C2000; 5, Jupare C  
1032 2001; 6, Cirno C 2008.

1033           The bread-making analysis showed great differences between the different durum  
1034 genotypes (Fig. 1, Table 2). Overall, Yavaros and Altar were not suitable to elaborate spongy  
1035 pan bread (high loaf volume), while Mexicali, Atil and Jupare were at least satisfactory. The  
1036 performance of Cirno was more dependent on the environment. In full drip irrigation trial the  
1037 results for bread-making were in average the lowest.

1038           Figure 3 shows the performance in bread-making of the durum wheat varieties compared  
1039 to two popular bread wheat varieties (Kachu and Roelfs) grown in the same environment and  
1040 used as checks, while Table 2 shows the loaf volume specific values of each genotype in each  
1041 environment. Kachu is considered usually good for bread-making and Roelfs excellent, which  
1042 could be confirmed with the data showed on Table 2. Altar and particularly Yavaros were far  
1043 from the volumes obtained with the checks. Yavaros did not reach 70% of the volume in any  
1044 environment, while Altar only in severe heat stress trial reached 80% of Kachu bread volume.  
1045 Atil, Jupare and particularly Mexicali were positively affected by severe heat stress trial in  
1046 bread-making compared to the checks. Mexicali reached in severe heat stress trial 99% and 93%  
1047 of the volumes of Kachu and Roelfs, respectively. Atil, although more consistent across the  
1048 environments, also reached 98% and 92% respect to Kachu and Roelfs under severe heat stress.  
1049 Cirno was not able to perform satisfactorily for bread making across environments. In full basin  
1050 irrigation environment most of the varieties also increased bread-making capacity compared to  
1051 the checks. Medium drought stress also favored bread-making.

1052           The bread crumb structure (ESM2) was in accordance with the loaf volume in most of the  
1053 cases, showing very poor, poor or fair crumb structure in the breads with lower volume  
1054 (Yavaros, Altar) and good or very good in those breads with higher volumes.

1055



1056

1057 **Figure 3 – Bread-making of durum wheat varieties.** Colored bars show the percentage of each  
 1058 bread loaf volume obtained with durum wheat cultivars respective to Kachu and Roelfs  
 1059 bread wheat checks. Each color represents a different environment as indicated in the  
 1060 legend.

1061

Table 2. Bread loaf volume (ml) of bread wheat checks and durum wheat varieties in six different environments.

	Kachu	Roelfs	Altar	Atil	Cirno	Jupare	Mexicali	Yavaros
Full drip irrigation	813*	864	556	713	668	649	639	471
Full basin irrigation	838	881	629	803	749	711	695	583
Medium drought stress	810	881	615	730	714	695	689	538
Severe drought stress	901	935	673	705	685	703	748	559
Medium heat stress	866	903	628	775	741	730	693	553

Severe heat stress	816	866	661	801	499	758	808	480
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\*Average values in each environment (averaging field reps and years)

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### 1064 *3.3 Relationships between quality parameters*

1065           Correlation coefficients were calculated for the whole trial (Table 3). Several significant  
1066 correlations were found between grain morphology parameters (test weight and thousand kernel  
1067 weight) and other quality traits. Grain protein and zinc content were inversely correlated with  
1068 thousand kernel weight while Fe content was not. Some of the parameters related to gluten  
1069 quality (flour SDS sedimentation volume, mixograph torque, alveograph L and W) were also  
1070 correlated negatively with grain size and positively with grain protein content. The association  
1071 between loaf volume and gluten strength (flour SDS sedimentation volume, mixograph torque,  
1072 alveograph W) was high in all cases. As expected, gluten extensibility (alveograph L) was also  
1073 highly correlated with loaf volume.

1074           The correlations between whole-meal flour and refined flour were medium-high for  
1075 yellowness (0.54) and very high for SDS sedimentation volume (0.91).

1076

Table 3. Correlation coefficients (*r*) among quality parameters obtained across the entire trial

	TW	TKW	FeC	ZnC	GPC	WMFY(b)	FY(b)	WMSDSS	FSDSS	MixT	TQ	ALVP	ALVL	ALVW	ALVP/L	LV
<b>TW</b>	1.00															
<b>TKW</b>	0.75	1.00														
<b>FeC</b>	0.29	NS*	1.00													
<b>ZnC</b>	-0.42	-0.44	0.21	1.00												
<b>GPC</b>	-0.58	-0.68	0.19	0.60	1.00											
<b>WMFY(b)</b>	-0.10	-0.35	NS	0.17	NS	1.00										
<b>FY(b)</b>	NS	-0.23	NS	-0.20	NS	0.54	1.00									
<b>WMSDSS</b>	-0.44	-0.45	0.18	NS	0.41	NS	0.19	1.00								
<b>FSDS</b>	-0.45	-0.48	NS	0.11	0.34	0.18	0.29	0.91	1.00							
<b>MixT</b>	NS	NS	NS	-0.23	-0.37	NS	0.34	0.19	0.25	1.00						
<b>TQ</b>	NS	-0.21	NS	NS	NS	0.22	0.41	0.36	0.46	0.90	1.00					
<b>ALVP</b>	0.39	NS	0.31	NS	NS	NS	0.33	NS	NS	0.27	0.37	1.00				
<b>ALVL</b>	-0.57	-0.51	NS	0.22	0.37	NS	NS	0.65	0.66	NS	NS	-0.67	1.00			
<b>ALVW</b>	NS	-0.31	0.30	NS	0.26	0.18	0.44	0.54	0.59	0.35	0.59	0.61	NS			
<b>ALVP/L</b>	0.44	0.33	0.19	NS	-0.22	NS	NS	-0.47	-0.44	NS	NS	0.86	-0.85			
<b>LV</b>	-0.30	-0.42	NS	NS	NS	0.20	0.46	0.78	0.81	0.36	0.52	NS	0.65			

TW, Test Weight; TKW, Thousand Kernel Weight; FeC, Iron Content; ZnC, Zinc Content; GPC, Grain Protein Content; WMFY(b), meal Flour Yellowness b value; FY(b), Flour Yellowness b value; WMSDSS, Whole-meal SDS Sedimentation; FSDSS, Flour Sedimentation; MixT, Mixograph Optimum Mixing Time; TQ, Mixograph Torque; ALVP, Alveograph Tenacity; ALVL, Alveograph Extensibility; ALVW, Alveograph Work; ALVP/L, Alveograph Tenacity/Extensibility Ratio; LV, Loaf Volume.

\*Correlations not significant ( $P>0.05$ ).

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#### 1090 4. Discussion

1091 In this study, a set of six durum wheat varieties developed by the CIMMYT breeding  
1092 program was analyzed for quality traits. In general, variation was observed in genotypes and the  
1093 environments for each quality parameter. This was confirmed by the analysis of the variance,  
1094 which showed that both genotype and environment contributed mostly to the variability found in  
1095 the data. With similar environmental conditions in both years, no significant variation by year  
1096 was observed in this study for most traits. The GxE, ExY and GxExY interactions were also  
1097 important, but not GxY. Our results agree with some previous reports and disagree with others.  
1098 Li et al. (2013) who carried out a similar experiment with drought and heat stress in the same  
1099 location, found a predominant genotype effect for test weight, flour yellowness, flour SDS  
1100 sedimentation volume and mixograph optimum dough mixing time and an environment effect for  
1101 thousand kernel weight, grain protein content, which completely agrees with our results, with the  
1102 exception of test weight that in our case was predominantly defined by the environment and  
1103 other interactions. Ames et al. (1999) in an experiment with both dry and irrigated conditions,  
1104 also found that rheological tests as flour SDS sedimentation volume, mixograph optimum dough  
1105 mixing time or gluten index were largely controlled by the genotype. Pigment content or flour  
1106 yellowness is another trait that consistently across studies has showed great genotype influence.  
1107 Rharrabti et al. (2003), Li et al. (2013) and the current study found that genotype explained 53,  
1108 87 and 36.3 % of the variation for that trait, respectively, being the most important factor in the  
1109 three studies.

1110 All the above results strengthen the well-established idea that some quality traits,  
1111 particularly those related with processing and pasta-making quality have a very strong genetic  
1112 control and therefore the genotypes respond consistently across environments for these

1113 characteristics. Most of those traits (flour SDS sedimentation volume, mixograph optimum  
1114 dough mixing time and torque, alveograph W) also showed consistent correlations between them  
1115 in the different environments. In a previous study (Li et al., 2013) it was revealed that in general  
1116 drought stress favored gluten strength and yellowness (processing and pasta-making quality),  
1117 which was confirmed with our study. On the contrary, heat stress was found to reduce gluten  
1118 strength by both studies. Li et al. 2013 found a significant increase in flour yellowness in heat  
1119 stressed environments, probably due to a “concentration effect” of the pigment carotenoid. In our  
1120 experiment, flour yellowness was reduced in severe heat stress trial, which disagree with Li et al.  
1121 (2013). This discrepancy with this study carried out in the same location could be due to a  
1122 different tolerance to heat stress of the genotypes used in both studies, or to other environmental  
1123 changes. Temperatures in May, when grain filling for severe heat stress trial happened, were  
1124 something higher in our study (maximum temperatures around 1.5 °C above), which could affect  
1125 the carotenoid pigment synthesis.

1126         In the case of Fe and Zn content had a different response in our study. For Fe content,  
1127 both genotype and environment were equally important, followed by GxE and GxExY. For Zn  
1128 content environment was much more determining factor. These results are different from the  
1129 study of Ficco et al. (2009) carried out in the Mediterranean region. In that case GxE was the  
1130 most important factor for both micronutrients, followed by genotype, with only a slight  
1131 environmental impact. This is likely due to the differences in environmental conditions used for  
1132 testing in both studies, which were very highly contrasted in this study. In the study, Zn content  
1133 was favored by smaller grain (“concentration effect” in severe drought stress and severe heat  
1134 stress), but Fe content only in the case of severe drought stress. In the case of severe heat stress,  
1135 in spite of showing the smaller grain size values, Fe content decreased respect to optimum



1136 condition trial (drip full irrigation). This may reflect differences in the passive iron transport  
1137 through the soil-plant system compared to Zn. Azaz et al. (2012) found also the micronutrients  
1138 “concentration effect” with water stress management. Joshi et al. (2010) reported that iron and  
1139 zinc concentrations were also found to be influenced by higher temperature. It is also important  
1140 to note that a significant trend in grain micronutrients concentration related to the release year of  
1141 the varieties was not found (data not shown). Some studies have shown that modern breeding  
1142 focused on grain yield has reduced the grain micronutrient concentrations in bread wheat (Fan,  
1143 Zhao, Fairweather-Tait, Poulton, Dunham & McGrath, 2008; Garvin, Welch & Finley, 2006),  
1144 which is in agreement with the results from Ficco et al. (2009). Additionally, with the limited  
1145 number of genotypes in this study the variation for micronutrients could not be accurately  
1146 assessed and further studies are required. At the same time, studies of other grain components  
1147 related to the potential bioavailability of these micronutrients (i.e. phytic acid) are required.

1148         The average value for both iron and zinc (32.1 and 37.6 mg/kg, respectively) revealed by  
1149 this study does not seem adequate to meet daily requirements of humans in which durum wheat  
1150 provides the main source of essential minerals (North African and West Asian countries where  
1151 wheat contributes >50% to daily energy intake). A wheat-heavy diet consumed over lengthy  
1152 periods can result in micronutrient malnutrition and related severe health complications (Cakmak  
1153 et al., 2008). Therefore, durum wheat breeding programs should be more focused on improving  
1154 micronutrients concentration, as is being done in bread wheat (Velu et al., 2011). The negative  
1155 significant effect revealed by the study on Fe content under heat stress is another important  
1156 concern, as high temperature is common in durum wheat cultivation areas.

1157         Performing alveograph analysis in durum wheat, Ammar et al. (2000) found that  
1158 alveograph P is more dependent on the environment than in the genotype, and the contrary for

1159 alveograph L. Our studies led to the same conclusion, with 44% of dough extensibility being  
1160 explained by the genotype. This is an important trait for improving bread-making in durum  
1161 wheat. Ammar et al. (2000) found that alveograph W was also more dependent on the genotype,  
1162 a finding that agrees with previous research by Mariani et al. (1995). Our study determined that  
1163 alveograph W was more dependent on environment, probably because alveograph P was also.  
1164 Due to the low alveograph L values showed by the durum wheat cultivars in our study,  
1165 alveograph P is the main determinant of alveograph W. As discussed above, gluten strength was  
1166 favored by drought stress conditions due to influence of grain size on the protein content  
1167 (concentration effect), as revealed by the high inverse relationship between those parameters.  
1168 This effect has also been shown in the work of Flagella et al. (2010) and Li et al. (2013).  
1169 However, it is contrary to the findings in studies undertaken by Rharrabti et al. (2003), who  
1170 found less gluten strength with increased grain protein content in Mediterranean conditions due  
1171 to drier conditions. On the other hand, severe heat stress, in spite high grain protein content, had  
1172 lower gluten strength (alveograph W) compared to any other environment. This effect has been  
1173 associated with a change in the glutenin/gliadin ratio in bread wheat by Blumenthal et al. (1993),  
1174 and was also found in durum wheat by Li et al. (2013) in some varieties. There were no changes  
1175 in flour SDS sedimentation volume in severe heat stress indicating that this test gives  
1176 information not only about gluten strength but also about gluten extensibility, as indicated by the  
1177 coefficient correlations.

1178         In the case of bread-making, our results showed that this ability depends largely on the  
1179 genotype, even in this study in which all cultivars had the same composition of glutenins (Glu-  
1180 B1 7+8 and LMW-2). This finding is in agreement with Rao et al. (2010) who showed that high  
1181 bread-making quality is heritable in tetraploid wheat and that as a result, selection in durum

1182 breeding programs for improved bread-making potential should be possible. Ammar et al. (2000)  
1183 found also a strong genotypic effect, but their research showed the glutenin composition was  
1184 heterogeneous among the lines tested. Glutenin composition has been said to be the key  
1185 determinant of gluten strength in durum wheat, and also in bread-making. There is no clear  
1186 consensus about the best *Glu-B1* allele for bread-making: Ammar et al. (2000) found 6+8 better  
1187 than 7+8; Boggini & Pogna (1989) indicated 7+8 > 20 >6+8; Peña et al. (1994) found 7+8 > 6+8  
1188 >20. In the case of LMW glutenins, it is well established that LMW-2 type confers higher gluten  
1189 strength and consequently higher bread volumes. The lines used in our research had all the  
1190 subunit 7+8 and LMW-2 types, so glutenin composition cannot be used in this case to explain  
1191 the big differences found in dough properties and bread-making among the genotypes of this  
1192 study. This indicates that there are other qualitative or quantitative grain factors that also play a  
1193 role in determining dough viscoelastic properties, which should be identified in more extensive  
1194 studies. Our data also strengthens the idea expressed by Ammar et al. (2000), who due to the  
1195 conflicting conclusions about the best glutenins alleles for durum wheat bread-making pointed  
1196 out that the use of glutenins as markers to select for improved bread-making in durum wheat  
1197 might not be justified. The high relationship between bread loaf volume and SDS sedimentation  
1198 volume (0.81), suggests that this test could be a much more efficient tool for rapid and low-cost  
1199 identification of suitable durum wheat genotypes for bread-making. The efficiency of this  
1200 methodology for this purpose has been already demonstrated with CIMMYT germplasm (Peña et  
1201 al. 1994,  $r = 0.81$ ) and with cultivars of diverse origin (Ammar et al. 2000,  $r = 0.56$ ). We also  
1202 showed the high efficiency to predict bread-making ( $r = 0.78$ ) of the same test run with whole-  
1203 meal flour, which would allow, as in the case of yellowness determination, the save of time and  
1204 resources in the selection process.

1205 Overall, the durum wheat lines in this study showed inferior values for loaf volume and  
1206 lower crumb structure quality compared to the bread-wheat checks. The less viscoelastic  
1207 properties of the gluten and excessive starch damage could be the main reasons to explain this.  
1208 However, some durum genotypes in specific environment as severe heat stress had almost the  
1209 same performance that bread-wheat checks used in this study (Kachu and Roelfs), which are  
1210 considered optimal for the bread-making process. This fact agrees with previous studies (Ammar  
1211 et al. 2000; Peña et al. 1994), which have already proven that the long-established belief that  
1212 durum wheat is not suitable for pan bread may be incorrect (Boggini et al. 1995). Based on the  
1213 high correlations values obtained, gluten strength (alveograph W) and extensibility (alveograph  
1214 L) were identified as key factors to enhance loaf volume, as well as the balance between gluten  
1215 tenacity and extensibility (alveograph P/L). In previous studies, durum wheat flours were showed  
1216 to a have high alveograph P/L values, leading to bread more compact (Brescia et al. 2007).  
1217 Gluten extensibility with adequate levels of gluten strength is required in durum wheat for bread-  
1218 making. Atil, the variety with higher extensibility by far, and Mexicali in severe heat stress trial  
1219 (that have a great extensibility increase in this environment), were the ones showing better  
1220 breads. This data supports the idea that to develop durum wheat cultivars with similar bread-  
1221 making quality to those of bread wheat lines, it is necessary to achieve a better balance of  
1222 tenacity and extensibility in conjunction with increased strength (Ammar et al. 2000; Dexter et  
1223 al. 1994; Rao et al. 2010). For this purpose, the glutenin alleles or rheological tests traditionally  
1224 used to enhance gluten strength for pasta-making quality may not be more suitable for bread-  
1225 making, as durum wheat lines with strong gluten also tends to exhibit tenacious and inextensible  
1226 gluten (Rao et al. 2001). Besides, the importance of gluten extensibility in bread-making is what  
1227 confers to durum wheat in severe heat stress an increase in the bread volumes compared to bread

1228 wheat, which showed their best performance in severe drought stress. Durum wheat samples lack  
1229 extensibility in control and drought stressed environments while bread wheat cultivars had  
1230 balanced gluten on those environments. Heat stress caused the increase of extensibility in both,  
1231 favoring the bread-making quality of durum wheat lines but not that of bread wheat, whose more  
1232 extensible gluten was not better for bread-making. For unknown reasons (no significant changes  
1233 in grain protein content or other traits), extensibility was also higher in basin full irrigation trial  
1234 with respect to drip full irrigation, which also led to better bread volumes in this environment.  
1235 Thus, this fact should be also taken into account when growing durum wheat for bread-making.  
1236 Severe drought stress, because increasing gluten strength but not extensibility, led in most of the  
1237 cases to inferior bread volumes compared with the rest of the environments. Both drought and  
1238 heat stresses are often present in the main areas where durum wheat is grown (Mediterranean  
1239 countries and Middle East), where local bread accounts for half of the durum wheat  
1240 consumption.

1241 It is also significant that the results obtained in bread-making with durum wheat are not  
1242 the consequence of a planned and designed breeding program for this purpose. Durum wheat  
1243 breeding programs are focused on obtaining lines with pasta-making quality (high gluten  
1244 strength), which means there is probably lot of room for improvement in durum bread-making if  
1245 systematic selection is applied. Extensibility could be increased applying selection for this trait  
1246 with tools not extensively used in durum wheat quality breeding as the alveograph. Introgression  
1247 of *Glu-D1* or *Glu-D3* glutenins from bread wheat is other possibility (Pogna et al. 1996).The  
1248 development of durum lines with good bread-making quality could increase the commercial  
1249 value of this crop and open alternative markets in years of high production. Moreover, durum  
1250 wheat flour is a derivate in semolina production, so its use in bread-making could also offset the

1251 cost of semolina production. This is particularly interesting for those countries where durum has  
1252 a superior yield performance compared to bread wheat. In addition to this, for CIMMYT bread  
1253 wheat breeding program, the development of durum lines with enhanced bread-making quality it  
1254 is also convenient, because durum lines are being used to transfer a variety of traits to bread  
1255 wheat through intra-specific (bread x durum) and inter-specific (synthetics) hybridization. Using  
1256 parental durum lines with good bread-making quality in those crosses would contribute to ensure  
1257 desirable bread-making quality in the novel lines developed.

1258

## 1259 **5. Conclusions**

1260 In this study, a set of durum wheat cultivars revealed differences in micronutrients (Fe  
1261 and Zn), processing, pasta-making and bread-making quality. Additionally, their unique  
1262 responses to the environmental stresses of drought and heat were measured. Processing and  
1263 pasta-making quality was favored by drought but not by heat stress. Cvs. Mexicali and Jupare  
1264 under heat stress and Atil in several environments showed bread volumes close to bread wheat  
1265 checks. Gluten extensibility was identified as a key trait to be improved to enhance bread loaf  
1266 volume in durum wheat.

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## 1268 **Acknowledgements**

1269 We greatly appreciate financial support from the CRP-Wheat and HarvestPlus challenge  
1270 program of CGIAR consortium and from Fondo Sectorial SAGARPA-CONACYT (No. 146788  
1271 - Sistema de mejoramiento genético para generar variedades resistentes a royas, de alto  
1272 rendimiento y alta calidad para una producción sustentable en México de trigo) of the Mexican  
1273 government. We are also grateful to Julie Mollins for the editing of this manuscript.

1274

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