

1 **Milling, processing and end-use quality traits of CIMMYT spring bread wheat**
2 **germplasm under drought and heat stress**

3 Nayelli Hernández-Espinosa¹, Suchismita Mondal¹, Enrique Autrique¹, Héctor
4 Gonzalez-Santoyo¹, José Crossa¹, Julio Huerta-Espino², Ravi Prakash Singh¹, Carlos
5 Guzmán*¹

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7 ¹Global Wheat Program, International Maize and Wheat Improvement Center
8 (CIMMYT), Apdo Postal 6-641, Mexico DF., Mexico.

9 ²Campo Experimental Valle de México, Instituto de Nacional de Investigaciones
10 Forestales, Agrícolas y Pecuarias, km. 13.5, Carr. Los Reyes- Texcoco, Coatlinchan,
11 56250, Edo. de México.

12 *Author for correspondence: c.cuzman@cgiar.org

13
14 For Field Crops Research

26 **Abstract**

27 The CIMMYT wheat breeding program aims to develop bread wheat (*Triticum*
28 *aestivum* L.) genotypes that have superior grain yields, disease resistance and stress
29 tolerance, along with appropriate quality to satisfy all stakeholders of the wheat value
30 chain. Grain quality for wheat consists of a combination of many defined parameters
31 including grain morphological characteristics, dough and final products properties, all
32 of which are defined by the genotype, the environment and their interactions. Our
33 current approach for improving grain quality is to study grain samples obtained under
34 high yield potential environments with optimum management. To assess the effect of
35 this strategy on quality under stressed environments, 54 genotypes were evaluated for
36 two years under six environmental conditions, including drought and heat stress. Grain
37 morphology (grain density and size), protein content and flour yield were severely
38 affected by the environment, as drought and heat stress had a strong negative effect on
39 all of these characteristics except protein content. Gluten quality (strength and
40 extensibility) was defined more by the genotype, although the environmental effects and
41 the interactions were also important, particularly for gluten extensibility. The current
42 selection strategy for quality traits carried out under optimum conditions was found to
43 be suitable to ensure high quality characteristics across several environments for most
44 of the parameters with an overall positive outcome.

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46 **Keywords:** wheat quality; wheat breeding; drought stress; heat stress

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51 **1. Introduction**

52 Wheat (*Triticum* spp.) was one of the first domesticated food crops, and for eight
53 thousand years, it has been the basic food staple of the major civilizations of Europe,
54 West Asia and North Africa. Currently, wheat exhibits large genetic diversity with over
55 25,000 types or cultivars, which are adapted to a wide range of temperate environments
56 (Feldman et al., 1995). FAO estimated that the world wheat production for 2015/2016
57 was approximately 735 million tons. Wheat grain can be processed into flour, semolina
58 and other products that form the basic ingredients of many foods worldwide (e.g. bread,
59 cookies, pastries, pasta, noodles, couscous, etc.). These foods provide about 20% of the
60 calories and protein source for a large portion of the world’s population. (FAOSTAT).
61 In densely populated countries, such as India or Pakistan, wheat is an important source
62 of calories and proteins, and its consumption will probably increase in other countries
63 such as Bangladesh due to the adoption of a “western lifestyle” (Shewry and Hey,
64 2015). Therefore, global wheat production needs to increase in the upcoming decades to
65 cover the rising demand for this grain.

66 The spring bread wheat (*T. aestivum* L.) breeding program of the International
67 Maize and Wheat Improvement Center (CIMMYT) breeds high yielding, disease
68 resistant and stress tolerant wheat germplasm and annually distributes it worldwide to
69 national partners mainly in four target areas (mega-environments): 1) Irrigated areas
70 (Northwestern India, Pakistan, Iran, Egypt, China, Mexico, etc.); 2) High rainfall areas
71 (West of Asia, Eastern Africa, highlands of Mexico, etc.); 3) Semi-arid areas (North
72 Africa, West Asia, South America, etc.); and 4) Warmer areas (Nepal, Bangladesh,
73 Eastern Gangetic Plains of India, Southern Pakistan, Sudan, etc.) (Rajaram and van
74 Ginkel, 1993). One key objective of the CIMMYT breeding program is to improve end-
75 use quality in conjunction with other relevant traits to satisfy all stakeholders of the

76 wheat value chain: farmers (bold and plump grain), millers (high test weight and high
77 flour yield), food manufacturers (processing quality) and consumers (end-use and
78 nutritional quality) (Guzmán et al., 2016a).

79 Wheat grain quality is determined by a combination of many defined
80 parameters. Multiple phenotypic traits of the grain, flour, dough, and final products
81 must be assessed to determine an overall quality and best end-use product (Battenfield
82 et al., 2016). Grain morphology, hardness, protein content, and dough handling
83 characteristics (or gluten properties) are some of the traits commonly assessed by
84 breeding programs focused on wheat quality. Generally, it is believed that the genotypic
85 make up of a cultivar is the most important factor when determining wheat quality (Li et
86 al., 2013; Souza et al., 1993); other authors (Blumenthal et al., 1995; Peterson et al.,
87 1998) propose that variation in rheological properties of dough are largely determined
88 by the genotype, however, the environment and its interaction with the genotype (GxE)
89 also play an important role in the expression of the grain quality of a cultivar.
90 Determining the magnitude of GxE is critical for the definition of the selection strategy
91 in a breeding program with a multi-environment focus such as CIMMYT, which aims to
92 develop cultivars that are able to maintain their quality in different environments,
93 including optimum, drought and heat stressed (mega-environments 1, 3 and 4,
94 respectively).

95 Several studies have focused on the effects of the environment and abiotic
96 stresses on the expression of wheat quality, some of which are already well identified
97 and understood. For example, heat stress has been associated with a dough weakening
98 effect (Blumenthal et al., 1993; Corbellini et al., 1997), whereas with drought stress,
99 there is an increase in the protein content and polymeric protein, which produces the
100 opposite effect (Guttieri et al., 2001, 2000). However, many of these studies are

101 characterized by having a limited number of genotypes (Corbellini et al., 1997; Guttieri
102 et al. 2000; Li et al., 2013; Rozbicki et al., 2015), and hence, the lack of diversity in the
103 responses of the cultivars could have led to the above conclusions. Williams et al.
104 (2008) reviewed the studies about environment and GxE effects on bread wheat quality
105 and recommended further research on this topic since our understanding of the
106 environmental influence and the presence of GxE on trait expression is incomplete.

107 The present study was conducted using a collection of 54 semi-dwarf, high
108 yielding spring bread wheat cultivars developed over the last 50 years to: (i) describe
109 the phenotypic variation for the main target traits that determine wheat quality in
110 CIMMYT-derived varieties, (ii) determine the effects of drought and heat stress and
111 GxE interactions on grain quality traits, and (iii) evaluate the selection strategy used at
112 CIMMYT breeding program to generate cultivars with suitable processing and end-use
113 quality in diverse target environments.

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115 **2. Materials and methods**

116 *2.1 Field Experiment*

117 A field trial consisting of 54 spring bread wheat cultivars developed by
118 CIMMYT and related breeding programs, including historical and modern breeding
119 lines (Table 2) were grown in Ciudad Obregón, Sonora, northwestern México, during
120 two crop seasons: 2012-2013 and 2013-2014. The trial was planted with three replicates
121 under six different environmental conditions: E1: optimum irrigation through the drip
122 method, planted in flat seedbeds (>500mm); E2: planted in flat seedbeds with full basin
123 irrigation (>500mm); E3: reduced (two) irrigation or medium drought stress (300mm);
124 E4: severe drought stress managed through drip irrigation (180mm); E5: medium heat
125 stress (500mm); and E6: severe heat stress (500mm). All trials were planted in

126 November, with the exception of medium heat stress (planted in January) and severe
127 heat stress (planted in February).

128 Except for E1, Nitrogen was applied (pre-planting) at a rate of 50 kg of N/ha,
129 and at tillering, 150 additional units of N were applied in all the trials. In E1, a total of
130 300kg N was applied, which included the pre-planting nitrogen application. Pesticides
131 and herbicides were used as needed to keep trials free from weeds, diseases and aphids.
132 At maturity, 1 kg of seed from each of the wheat lines of the two first field replicates
133 was used for analyzing the quality traits. The third field replicate could not be analyzed
134 due to the high cost and time required to perform the below mentioned grain quality
135 analysis for 648 more samples.

136 The meteorological data of the experimental station in Ciudad Obregon showed
137 almost no precipitation during the wheat growing season. Maximum temperatures
138 reached 31-32°C in March and April, the grain filling time for most of the treatments,
139 and for plants under heat stress, temperatures reached between 35 and 39°C during grain
140 filling in May-June (Electronic Supplementary Figure 1).

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142 *2.2 Grain parameters*

143 Thousand kernel weight (g) and test weight (kg/hl) were obtained using the
144 digital image system SeedCount SC5000 (Next Instruments, Australia). Grain protein
145 content (%), hardness (PSI, %) and moisture content were determined by near-infrared
146 spectroscopy (NIR Systems 6500, Foss Denmark) calibrated based on official AACC
147 methods 39–10, 55-30 and 46–11A, respectively (AACC, 2010).

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149 *2.3 Milling*

150 Grain samples were tempered by adding water levels for use in tempering hard,
151 medium-hard and soft wheat before milling, according to the official AACC method 26-
152 95 (AACC, 2010). All samples were milled into flour using a Brabender Quadrumat
153 Senior mill (Germany). Experimental flour yield (%) was recorded.

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155 *2.4 Flour parameters, rheological and baking test*

156 Flour protein (%) and moisture content (%) were determined by near-infrared
157 spectroscopy (NIR Systems 6500, Foss Denmark), calibrated as per official AACC
158 methods 46–11A and 39–11, respectively (AACC, 2010). Additionally, 35 g flour
159 samples were tested in a mixograph (National Mfg. Co.) to obtain optimum dough
160 mixing time and %Torque × min according to AACC method 54–40A (AACC, 2010).
161 Gluten extensibility (alveograph L), tenacity (alveograph P), elasticity or strength
162 (alveograph W) and tenacity/extensibility ratio (alveograph P/L) were determined
163 according to the Alveograph manufacturer's instructions (Chopin, France), using 60 g
164 flour samples according to AACC method 54-30A (AACC, 2010). The bread-making
165 process was carried out using the direct dough method with 100 g of flour (AACC
166 method 10-09). Bread loaf volume (LV) was determined by rapeseed displacement
167 using a volume-meter. The amounts of water added to the mixograph, alveograph and
168 baking were determined by near-infrared spectroscopy (NIR Systems 6500, Foss
169 Denmark), calibrated according to Guzmán et al. (2015).

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171 *2.5 Statistical analysis*

172 A fixed effect linear model combined across years, environments and their
173 interactions was performed for all 12 quality parameters using the PROC ANOVA
174 procedure of the SAS statistical software (2010). Additionally, averages, values and

175 least significant difference (LSD) of each quality trait for each environment were
176 calculated averaging across years and genotypes (Fig. 1). Furthermore, mean values and
177 least significant difference (LSD) for some quality traits and specific genotypes in three
178 environments (E1, E4 and E6) averaged across years were calculated.

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180 **3. Results**

181 *3.1 Effects of genotype, environment, year and their interactions*

182 We used data for 1, 296 grain samples of the 54 bread wheat genotypes grown in
183 six different environments for two cropping seasons and analyzed it for processing and
184 end-use quality traits. The results of the variance analysis (Table 1) show that all factors
185 were highly significant for the traits studied. The environmental and the genotypic
186 effects had the greatest influence on variability found for all studied quality parameters.
187 Particularly, environment was the most important factor affecting grain morphology
188 (test weight and thousand kernel weight), experimental flour yield and protein content,
189 whereas the genotypic effect was the most important for gluten strength (mixograph and
190 alveograph parameters) and extensibility (alveograph P/L), and bread-making quality
191 (loaf volume). The year had a minimal effect for most of the traits. The different
192 interactions between two or the three main factors (genotype, environment and year)
193 were variable depending on the trait. Gluten extensibility (alveograph P/L) was strongly
194 affected by interactions of the genotype with other factors (38.3% of its total variation
195 was explained by these interactions) as well as experimental flour yield and loaf volume
196 (17.5 and 18.7 %, respectively, of their variation explained by genotypic interactions
197 with other factors). For traits strongly influenced by the environment, the environment
198 by year interaction was also high (test weight and thousand kernel weight) or medium
199 (flour yield), although that was not the case for grain protein content.

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201 *3.2 Effect of environments on quality traits*

202 Fig. 1 gives the range of values for each quality trait across 54 genotypes for
203 each environment, which was broad for most of the traits. The mean values in each
204 environment (averaging genotypes and years) are shown with a continuous line, while
205 the letters show the significance of the differences among means based on the LSD test.
206 In the case of grain morphology traits, test weight and thousand kernel weight had the
207 highest values under medium drought stress, followed by the environments with full
208 irrigation. Severe drought stress seriously

209 affected both traits, but the most damage was observed under severe heat stress, where the lowest mean values were found (77.3 Kg/hL and 33.3
 210 g for test weight and thousand kernel weight, respectively), indicating grain shriveling.

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

Sources of variation	d.f.	TW	TKW	GRNHRD	GRNPRO	FLRYLD	MIXTIM	TQ	ALVW	ALVPL	LOFVOL
Environment (E)	5	43.0	45.9	22.1	66.8	33.9	9.3	15.0	33.2	6.4	22.7
Genotype (G)	53	22.8	31.3	40.8	14.0	28.4	68.0	67.3	51.4	48.0	55.0
Year (Y)	1	2.5	0.1	7.0	1.3	10.7	0.1	0.0	0.3	3.1	0.2
GxE	265	7.4	4.1	7.9	8.0	8.8	7.6	6.5	6.4	17.8	10.2
ExY	5	11.3	12.1	5.1	1.7	6.6	5.3	3.2	2.6	1.8	1.0
GxY	53	3.1	2.3	4.4	1.2	1.9	2.4	2.1	1.4	7.1	2.5
GxExY	265	7.5	2.5	7.3	4.4	6.8	5.6	4.5	3.7	13.6	6.0
Error	633	2.3	1.5	5.1	2.3	2.8	1.5	1.2	1.1	2.1	2.3

d.f.= degrees of freedom; TW, test weight; TKW, thousand kernel weight; GRNHRD, grain hardness; FLRYLD, experimental flour yield; MIXTIM, mixograph optimum mixing time; TQ, mixograph torque; ALVW, alveograph work; ALVP/L, alveograph tenacity/extensibility ratio; LOFVOL, bread loaf volume.

All the values were highly significant ($p < 0.001$)

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213 Mild heat stress also had negative effects on the both traits. These results are linked to those found for protein content and experimental
 214 flour yield: severe drought and heat stressed environments had the highest protein content (15.1 and 15%, respectively) and the lowest
 215 experimental flour yield (65.8 and 65.9%, respectively), whereas the full irrigation (drip and basin) environments had the lowest values for
 216 protein content (12.4 and 12.1%,

217 respectively) but the highest for experimental flour yield (70.5 and 70.4%, respectively).
218 Moderate drought and heat stressed environments gave intermediate values for these
219 traits.

220 Dough rheological parameters related to gluten strength (mixograph optimum
221 mixing time and torque, and alveograph W) were highest in the severe drought
222 environment, followed by the severe heat stress environment. The other environments
223 showed lower gluten strength, particularly in mild heat stress. In terms of gluten
224 extensibility, heat stress environments showed the highest values (lowest alveograph
225 P/L ratio) (0.8 and 0.7 for mild and severe heat, respectively), and in general drought
226 stressed environments had more tenacious gluten.

227 Finally, significant differences among environments were identified for bread-
228 making quality too. The highest values for bread loaf volume were found in severe
229 drought and heat stress environments (901 and 887 mL, respectively), whereas full
230 irrigation and mild heat stress showed the lowest performance for this trait (816 and 838
231 mL, respectively).

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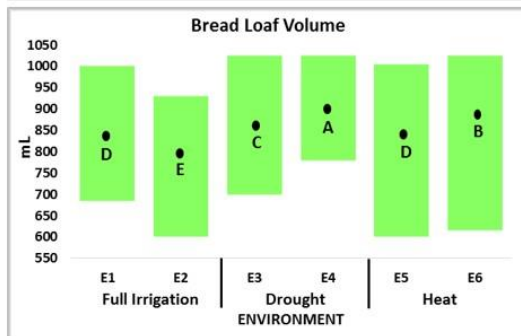
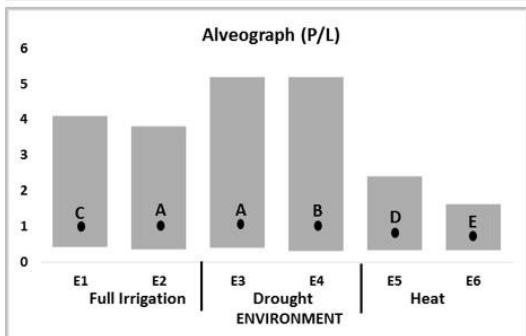
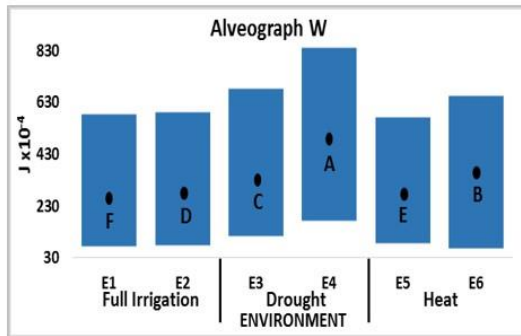
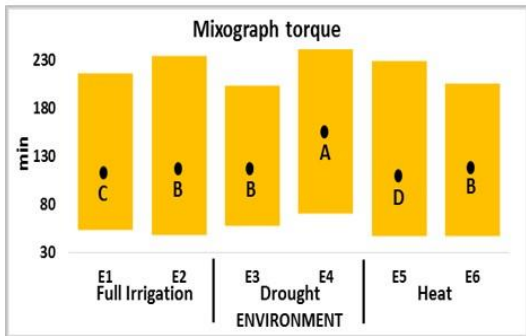
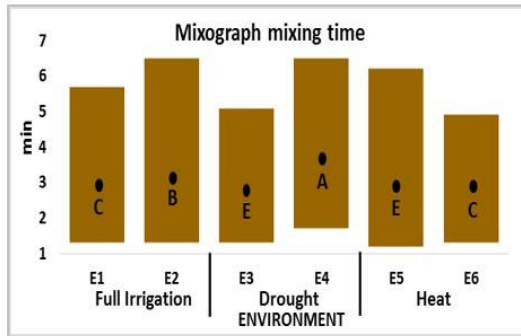
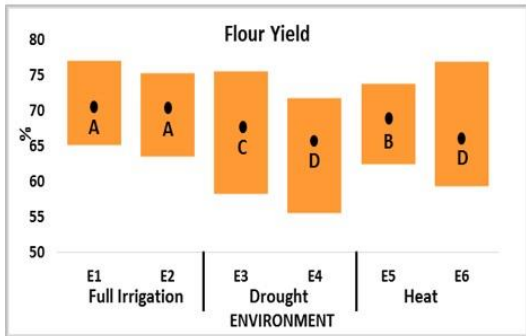
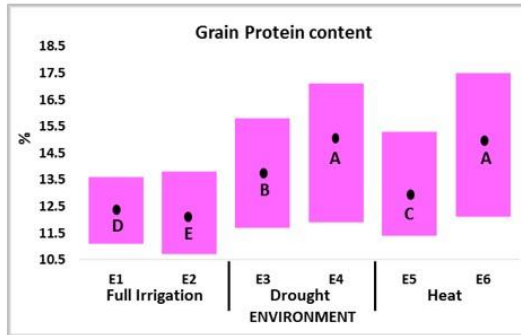
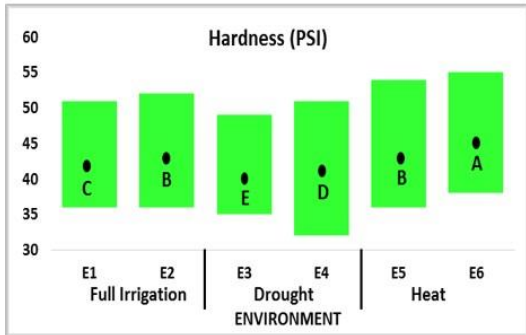
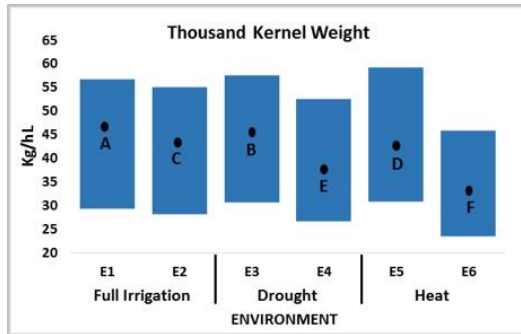
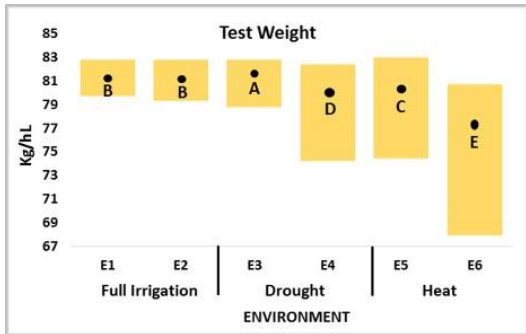
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252 **Figure 1.** Maximum, average and minimum values for ten grain quality traits of 54
253 genotypes in each environment averaged over two years. The ranges (maximum and
254 minimum values) are represented by bars and the average values by a dots inside the
255 bars. Letters show different groups across environments based on LSD test.
256 Environments were: (E1) full drip irrigation; (E2) full basin irrigation; (E3) medium
257 drought stress; (E4) severe drought stress; (E5) medium heat stress and (E6) severe heat
258 stress.



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3.3 Differences in genotypic response to the environment

262 In addition to the general responses for each quality trait in each environment,
263 we also examined the specific response of each genotype in different environments. In
264 Electronic Supplementary Table 1, all the quality traits data of each genotype in the six
265 environments (averaging field replicates and year) is given. In order to avoid an
266 excessively lengthy document, we mostly focused the interpretation and associated
267 discussion of the data on the specific genotypic responses in only three representative
268 quality traits: alveograph W (gluten strength), alveograph P/L (gluten
269 tenacity/extensibility balance) and loaf volume (bread-making quality), which are
270 considered the most important traits at the CIMMYT breeding program for selection
271 purposes apart from grain hardness (a trait that did not show large genetic variability in
272 the current study); and in three environments with more contrasting conditions: full drip
273 irrigation (optimum), severe drought stress, and severe heat stress (E1, E4 and E6,
274 respectively).

275 Table 2 shows the mean values over two years for the 54 genotypes observed for
276 the three quality parameters mentioned above in the three environments. Based on these
277 mean values, a rank relative to the rest of the genotypes was also assigned to each
278 genotype (from 1 to 54). The rank of each genotype is also given for the severe drought
279 and heat stressed environments and in brackets, the number of positions the genotype
280 moves up or down in relation to the ranking that each genotype had in the optimum
281 environment. For example, ‘Sonalika’ had the mean value for alveograph W of 137
282 J*10-4 being placed in the 50th position in the optimum environment. This cultivar was
283 on the 52nd position in the severe drought environment (two positions lower in ranking
284 with respect to E1), and on the 49th position in the severe heat stress environment (one
285 position higher in ranking with respect to E1). This analysis of ranking approach was
286 used to distinguish genotypes that were affected differently by the environmental stress,

287 since overall, all genotypes had an increase in gluten strength and extensibility and loaf
288 volume in stressed environments (Fig. 1). For the ranking of alveograph P/L, lower
289 values (extensible gluten) were considered better than higher values (tenacious gluten).

290 Based on Table 2, the 54 genotypes can be classified in two overall groups:
291 stable and unstable, based on rank shifts in different environments. Stability in this case
292 means they do not show a big change in the ranking between the reference environment
293 (full irrigation) and the stressed environments (severe drought and heat). It is important
294 to mention that some genotypes showing medium values (center of the distribution)
295 could seem less stable using this system than the genotypes showing the highest/lowest
296 values simply because only a small change in value is required to considerably change
297 ranked position. Some examples of cultivars that were stable in their rankings for the
298 three traits across the three environments were ‘Attila’, ‘Super 152’ and ‘Misr 1’. On
299 the other hand, ‘Baj#1’ or ‘Inqalab’ were good examples of unstable genotypes for these
300 quality traits across the stressed environments. Other genotypes, such as ‘Vorobey’,
301 were not stable for gluten quality traits (alveograph W and P/L) but were stable for
302 bread-making quality in both stressed environments, or some genotypes were unstable
303 for a specific quality trait, e.g. ‘Lok1’ for alveograph P/L but were stable for the other
304 two traits. There were many different cases, but overall, a large proportion of the
305 genotypes did not show an important change in the position in their ranking across the
306 examined environments, particularly for alveograph W (only six and thirteen of the 54
307 genotypes had a change larger than ten positions in the ranking in severe drought and
308 heat stress environments, respectively). For loaf volume, 17 genotypes had a change
309 larger than ten positions in the ranking in both stressed environments, whereas for
310 alveograph extensibility 15 and 25 genotypes had a change larger than ten positions in
311 the ranking in drought and heat stressed environments, respectively.

312 In addition to this, scatter plots showing the correlation between the position in
313 the ranking of the genotypes in the optimum environment versus the position in the
314 ranking of the genotypes in the severe drought and heat stress environment were also
315 developed (Electronic Supplementary Table 2). The correlations were higher for
316 alveograph W than for loaf volume and particularly higher than for alveograph P/L,
317 which means that more genotypes keep a similar position in the ranking for alveograph
318 W across environments. With this type of plot it was possible to identify how accurate
319 the selection process was when performing only in the optimum environment. For
320 example, for alveograph W, we can select the best 27 genotypes (50% of the
321 population) for this trait. Of those, only two genotypes would not be among the best 27
322 in the severe drought stress environment, and five of them would not be among the best
323 27 in the severe heat stress environment, although that fact does not mean that these
324 genotypes are necessarily losing gluten strength in the stressed environments. For
325 alveograph P/L and loaf volume a very similar situation was found.

326 The same exercise was done with a portion of the rest of the recorded traits: test
327 weight, thousand kernel weight, grain protein content and flour yield (Electronic
328 Supplementary Table 3). Overall, these traits showed less stability than the above-
329 mentioned traits related with gluten characteristics and end-use quality. For test weight,
330 25 and 24 genotypes had a change larger than ten positions in drought and heat stressed
331 environments, respectively; for thousand kernel weight, 16 and 22 genotypes; for grain
332 protein content, 26 and 25 genotypes; and for flour yield, 21 and 18 genotypes had a
333 change in the rank larger than ten positions in drought and heat stressed environments.
334 The scatter plots showing the correlation between the positions in the ranking of the
335 genotypes (Electronic Supplementary Table 2 – sheet 2) also showed that for these
336 traits, more genotypes changed their position in the ranking. In this case, for example, if

337 we select the best 27 genotypes for test weight under the optimum environment, of
338 those genotypes, eight would not be among the best 27 in the severe drought stress
339 environment and ten of them would not be among the best 27 in the severe heat stress
340 environment. A very similar situation was found for thousand kernel weight and for
341 grain protein content, but not for flour yield, where the correlations were higher.

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348 **Table 2.** Variability for three quality traits of 54 bread wheat genotypes across three environments (full drip irrigation or optimum, severe
349 drought and severe heat). Average values were calculated averaging field replicates and two cropping years. The position in the ranking of each
350 genotype is indicated. For severe drought and heat stress environment, the positions won or lost in the ranking for each genotype with respect to
351 the optimum environment is indicated between brackets..

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Genotype name/cross	Alveograph W (J*10 ⁻⁴)						Alveograph P/L						Bread loaf volumen (mL)					
	Optimum		Drought		Heat		Optimum		Drought		Heat		Optimum		Drought		Heat	
	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)
Sonalika	137	50	233	52 (-2)	185	49 (+1)	1	34	2.5	52 (-18)	0.6	21 (13)	768	47	794	54 (-7)	853	41 (+6)
Siete Cerros 66	253	29	483	31 (-3)	294	39 (-10)	2.6	54	3.6	54 (0)	0.9	48 (6)	758	48	834	48 (0)	795	49 (-1)
Pavon F76	301	15	610	9 (+6)	475	8 (+7)	1	30	1.0	36 (-6)	0.8	40 (10)	876	15	928	20 (-5)	894	27 (-12)
Opata M85	222	38	391	43 (-5)	306	38 (0)	0.6	6	0.6	12 (-6)	0.7	36 (-30)	878	14	928	21 (-7)	839	42 (-28)
Seri M82	190	44	449	37 (+7)	450	13 (+31)	1	31	0.8	25 (+6)	0.8	42 (-11)	858	19	939	16 (+3)	931	15 (+4)
WL 711	203	42	362	47 (-5)	174	51 (-9)	1.4	51	1.3	47 (+4)	0.7	35 (16)	738	51	824	49 (+2)	765	52 (-1)
Attila	134	51	347	48 (+3)	256	45 (+6)	0.5	2	0.4	1 (+1)	0.4	1 (1)	853	20	943	14 (+6)	904	20 (0)
PBW343	152	48	257	50 (-2)	184	50 (-2)	1.3	44	1.1	42 (+2)	1.0	50 (-6)	801	41	865	39 (+2)	751	53 (-12)
Inqalab 91	291	18	442	39 (-21)	380	26 (-8)	1	33	1.5	49 (-16)	1.4	54 (-21)	828	30	848	45 (-15)	808	47 (-17)
Seher 06	345	10	525	21 (-11)	387	25 (-15)	0.9	26	0.8	22 (+4)	0.5	13 (13)	824	34	865	40 (-6)	883	30 (+4)
Norteña F2007	390	5	669	5 (0)	417	20 (-15)	1.2	40	0.9	30 (+10)	0.6	23 (17)	848	22	930	18 (+4)	900	23 (-1)
Kachu#1	400	3	648	6 (-3)	482	7 (-4)	0.9	23	1.1	41 (-18)	0.7	34 (-11)	921	4	941	15 (-11)	971	8 (-4)
Tacupeto M2001	257	28	490	30 (-2)	433	15 (+13)	0.7	14	0.7	14 (0)	0.8	43 (-29)	840	23	904	30 (-7)	900	24 (-1)
Baviacora T92	288	20	559	18 (+2)	417	18 (+2)	1.1	37	0.9	31 (+6)	0.8	39 (-2)	806	40	946	11 (+29)	918	18 (+22)
Roelfs F2007	308	14	572	16 (-2)	530	4 (+10)	0.7	13	1.0	35 (-22)	0.7	29 (-16)	865	17	951	10 (+7)	975	7 (+10)

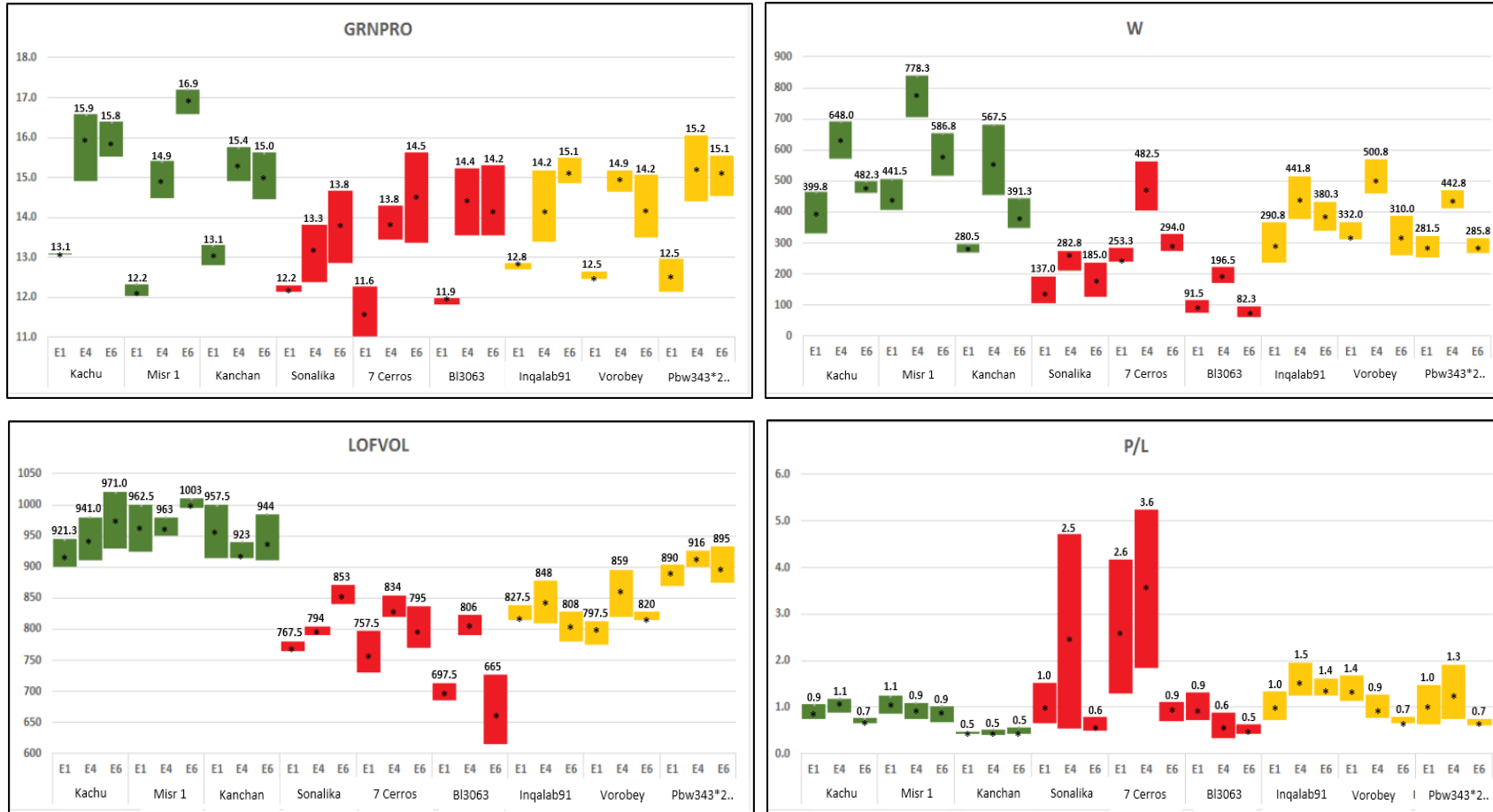
Misr 1	442	2	778	1 (+1)	587	1 (+1)	1.1	38	0.9	32 (+6)	0.9	47 (-9)	963	1	963	5 (-4)	1003	2 (-1)
Lasani 08	358	8	706	4 (+4)	531	3 (+5)	1.4	49	1.9	51 (-2)	1.3	52 (-3)	828	31	891	31 (0)	859	36 (-5)
Faisalabad 2008	391	4	755	2 (+2)	485	6 (-2)	1.3	47	1.1	43 (+4)	0.7	26 (21)	829	29	956	6 (+23)	874	34 (-5)
Munal#1	234	33	494	29 (+4)	421	17 (+16)	0.6	10	0.6	5 (+5)	0.5	11 (-1)	875	16	973	3 (+13)	953	11 (+5)
Súper 152	273	24	546	19 (+5)	392	23 (+1)	0.9	29	0.9	28 (+1)	0.7	31 (-2)	824	35	887	35 (0)	859	37 (-2)
Quaiu#1	232	34	437	41 (-7)	275	43 (-9)	0.7	20	0.6	8 (+12)	0.7	25 (-5)	819	36	884	36 (0)	857	38 (-2)
Danphe#1	243	32	590	13 (+19)	373	27 (+5)	0.6	8	0.6	6 (+2)	0.4	6 (2)	950	3	1014	1 (+2)	985	5 (-2)
Cachanilla F2000	353	9	586	14 (-5)	439	14 (-5)	1.3	45	0.9	33 (+12)	0.5	15 (30)	833	27	944	13 (+14)	888	29 (-2)
Super Seri#1	253	30	518	22 (+8)	520	5 (+25)	0.6	7	0.6	4 (+3)	0.6	22 (-15)	894	9	954	8 (+1)	985	6 (+3)
Vorobey	332	11	501	24 (-13)	310	36 (-25)	1.4	50	0.9	34 (+16)	0.7	33 (17)	798	42	859	42 (0)	820	45 (-3)
Weebill#1	300	16	593	12 (+4)	475	9 (+5)	1.2	41	0.8	26 (+15)	0.7	37 (4)	811	39	936	17 (+22)	904	21 (+18)
Babax/Lr42//Babax...	229	35	455	34 (+1)	360	29 (+6)	0.8	22	0.7	16 (+6)	0.5	8 (14)	840	24	915	26 (-2)	989	4 (+20)
Grackle	244	31	463	32 (-1)	357	30 (+1)	0.6	4	0.5	3 (+1)	0.4	3 (1)	863	18	956	7 (+11)	1018	1 (+18)
Elvira/5/Cndo/R143...	365	7	640	8 (-1)	473	10 (-3)	1.7	52	1.3	48 (+4)	1.1	51 (1)	754	50	889	32 (+18)	814	46 (+4)
Trch*2/3/C80.1/3*...	322	12	583	15 (-3)	454	12 (0)	0.7	18	0.7	18 (0)	0.6	20 (-2)	836	26	946	12 (+14)	901	22 (+4)
Whear/Kronstad	509	1	750	3 (-2)	545	2 (-1)	1.3	46	1.0	39 (+7)	0.9	49 (-3)	838	25	930	19 (+6)	825	43 (-18)
Cndo/R143//Ente...	193	43	456	33 (+10)	322	34 (+9)	0.7	16	0.7	13 (+3)	0.5	16 (0)	825	33	910	28 (+5)	930	16 (+17)
SW89.5277//Borl95...	180	47	438	40 (+7)	351	31 (+16)	1.7	53	1.3	45 (+8)	0.8	46 (7)	730	53	870	37 (+16)	871	35 (+18)
Navojoa M2007	204	41	454	35 (+6)	274	44 (-3)	0.6	3	0.6	11 (-8)	0.4	2 (1)	851	21	908	29 (-8)	914	19 (+2)
Baj#1	290	19	501	26 (-7)	321	35 (-16)	0.7	19	0.9	27 (-8)	0.4	5 (14)	898	6	919	24 (-18)	934	14 (-8)
Borlaug100 F2014	314	13	597	10 (+3)	417	19 (-6)	0.9	24	0.8	23 (+1)	0.5	17 (7)	883	12	913	27 (-15)	958	10 (+2)
Becard/Quaiu	261	27	499	27 (0)	343	32 (-5)	0.9	25	0.7	19 (+6)	0.7	38 (-13)	828	32	888	34 (-2)	855	40 (-8)
Babax/Lr42//Babax..	227	37	452	36 (+1)	310	37 (0)	1.3	48	1.1	44 (+4)	0.8	45 (3)	774	45	865	41 (+4)	823	44 (+1)
BL3063	92	54	197	54 (0)	82	54 (0)	0.9	27	0.6	9 (+18)	0.5	14 (13)	698	54	806	53 (+1)	665	54 (0)
Lok 1	145	49	322	49 (0)	191	48 (+1)	0.7	17	2.5	53 (-36)	1.3	53 (-36)	731	52	813	51 (+1)	786	51 (+1)
HD 2687	106	53	232	53 (0)	172	53 (0)	1.2	42	0.7	20 (+22)	0.7	28 (14)	779	44	838	47 (-3)	878	32 (+12)
HUW 234	210	39	378	45 (-6)	228	47 (-8)	0.8	21	0.7	17 (+4)	0.5	9 (12)	794	43	843	46 (-3)	856	39 (+4)
Kanchan	281	23	568	17 (+6)	391	24 (-1)	0.5	1	0.5	2 (-1)	0.5	12 (-11)	958	2	923	22 (-20)	944	13 (-11)
Francolin #1	227	36	496	28 (+6)	277	42 (-6)	0.6	5	0.9	29 (-24)	0.4	4 (1)	895	8	920	23 (-15)	900	25 (-17)
HUW234+Lr34/Prinia..	189	45	404	42 (+3)	277	41 (+4)	0.6	11	1.0	38 (-27)	0.6	19 (-8)	813	38	855	43 (-5)	891	28 (+10)
Sha7/Vee#5/5/Vee...	119	52	253	51 (+1)	174	52 (0)	1.2	43	1.9	50 (-7)	0.8	41 (2)	758	49	809	52 (-3)	790	50 (-1)
Neloki	287	21	647	7 (+14)	422	16 (+5)	1	35	1.0	37 (-2)	0.6	18 (17)	885	11	996	2 (+9)	990	3 (+8)
Whear/Sokoll	263	26	501	25 (+1)	463	11 (+15)	0.7	15	0.6	10 (+5)	0.7	27 (-12)	880	13	889	33 (-20)	929	17 (-4)
Kachu/Saua	390	6	597	11 (-5)	415	21 (-15)	1.1	36	1.0	40 (-4)	0.6	24 (12)	896	7	968	4 (+3)	969	9 (-2)
Fret2*2/4/Sni/Trap...	187	46	376	46 (0)	327	33 (+13)	0.9	28	0.8	21 (+7)	0.7	30 (-2)	769	46	821	50 (-4)	878	33 (+13)
Attila*2/PBW65/..	272	25	544	20 (+5)	370	28 (-3)	0.7	12	0.7	15 (-3)	0.5	10 (2)	908	5	953	9 (-4)	945	12 (-7)

PBW343*2/Kukuna/...	282	22	443	38 (-16)	286	40 (-18)	1	32	1.3	46 (-14)	0.7	32 (0)	890	10	916	25 (-15)	895	26 (-16)
Kachu/Wblll*2/...	291	17	511	23 (-6)	406	22 (-5)	1.1	39	0.8	24 (+15)	0.8	44 (-5)	830	28	868	38 (-10)	881	31 (-3)
Super 152/Baj#1	207	40	388	44 (-4)	228	46 (-6)	0.6	9	0.6	7 (+2)	0.5	7 (2)	819	37	854	44 (-7)	804	48 (-11)

355 Based on the results shown in Table 2, a set of nine genotypes were selected for
356 a more detailed analysis of their performance across the same three environments (E1,
357 E2 and E3). Figure 2 shows the minimum and maximum mean across years and mean
358 values for nine genotypes in the three environments for the traits used in Table 2, and
359 also for grain protein content. These genotypes can be placed in three groups: 1)
360 excellent quality across all environments ('Kachu#1', 'Misr1' and 'Kanchan'); 2) low
361 quality across all environments ('Sonalika', 'Siete Cerros' and 'BL3063'); and 3)
362 variable quality across environments ('Inqalab 91', 'Vorobey' and
363 'PBW343*2/Kukuna//Parus/3/PBW343*2/Kukuna'). The genotypes in the first group
364 had high values for the quality traits in the full irrigation environments, which either
365 improved or remained stable in the stressed environments while protein content
366 increased. These genotypes had strong gluten and good extensibility, which led to high
367 loaf volume values. Based on these results and according to Guzmán et al. (2016a),
368 these genotypes produce high grain quality suitable for the mechanized baking industry
369 in all the environments. The cultivars in the second group show, in general, the worst
370 performance with weak and/or tenacious gluten and bread-making quality. The protein
371 content increase in the stressed environments did not lead to higher values on the other
372 quality traits in all cases or the increase was not large enough to lead to a qualitative
373 change in the potential end-use type of these genotypes. These genotypes remained in
374 potential end-use types of handmade baking and utility wheat across all environments.
375 Finally, genotypes in the third group represented cultivars that had different quality in
376 the full irrigation environment, and the increased protein content in stressed
377 environments was linked to great and diverse changes in gluten and bread-making
378 qualities (positives or negatives) that led to qualitative changes in the potential end-use
379 of those cultivars.

380

381 **Figure 2.** Mean (averaged 2 years), maximum and minimum values for four grain quality traits of nine bread wheat cultivars in three
 382 environments (E1: full drip irrigation; E2: severe drought stress; E3: severe heat stress). The number on the top of the bars indicates the average
 383 value of each genotype.



384

385 **4. Discussion**

386 The current approach of the CIMMYT spring bread wheat breeding program to
387 improve grain quality is to analyze genotypes grown under optimum conditions for
388 grain yield potential. These conditions in Ciudad Obregon (main CIMMYT wheat
389 breeding and yield testing site) are full irrigation with sowing time in November,
390 heading around February-March and harvesting at the end of April- early May, which
391 match the conditions of important target areas for the CIMMYT breeding program such
392 as Northwestern India, most of Pakistan or Egypt (Mega-environment 1). The grain
393 yield of elite lines under these optimum conditions in Ciudad Obregon is usually around
394 8-9 tons/ha, very high for such a short season. This leads to medium-low grain protein
395 levels (around 11-13%) in most of the lines. The lines grown in Ciudad Obregon are
396 analyzed for diverse quality traits (see Guzmán et al., 2016a for a better description) to
397 guarantee good gluten quality (diverse levels of gluten strength combined with good
398 extensibility) in semi-hard or hard grains. This set of quality parameters is preferred for
399 most products in developing countries, where more than 70% of the varieties grown
400 have CIMMYT origin (Lantican et al., 2016). Although the CIMMYT breeding
401 program also targets other regions where drought and heat stress are present (mega-
402 environments 3 and 4), the selection process for grain quality traits is currently done
403 only with materials grown under optimum conditions, for our past results indicated that
404 the highest discrimination of advanced lines for quality traits occurred when grains from
405 high yielding environments were analyzed. This is due to a reduction in protein content
406 caused by high yields. Lines identified to have good quality traits under this condition
407 are expected to show better or even excellent quality when they are grown under
408 drought or heat stress conditions, causing a significant reduction in grain yield but an
409 increase in protein content. To check this hypothesis, a large trial with diverse

410 CIMMYT-derived cultivars/ new breeding lines were evaluated for quality traits under
411 six different environments in Ciudad Obregon (where irrigation can be adjusted to
412 simulate drought stress and sowing time can be delayed to generate heat stress). At the
413 same time that our breeding-for-quality strategy was being evaluated, a large amount of
414 data related to abiotic stress effects on grain quality traits was also generated, which
415 contributes to the understanding of genetic and environmental effects on quality traits.
416 Such understanding facilitates both effective selection for quality in breeding programs,
417 and strategies to establish more uniform and consistent plots of commercial wheat that
418 are better suited to the needs of the value chain (Williams et al., 2008).

419 Large ranges of values were found for most of the traits, something that does not
420 happen in all breeding programs. The ANOVA showed strong and main environmental
421 effects on traits related to grain filling and morphology (grain density and size) and
422 directly affected by them (protein content and experimental flour yield). This agrees
423 with previous studies on spring bread wheat (Mikhaylenko et al., 2000; Rozbicki et al.,
424 2015; Studnicki et al., 2016), winter bread wheat (Bilgin et al. 2016) and durum wheat
425 (Guzmán et al. 2016b; Rharrabti et al., 2003). The effects of severe drought, and
426 particularly severe heat stress, on grain morphology traits (test weight and thousand
427 kernel weight) were negative, leading to a much higher protein content level in these
428 environments compared to the non-stressed, fully irrigated environments, which was
429 probably due to a concentration effect (Guttieri et al., 2000; Saint-Pierre et al., 2008).
430 The mild drought and heat stress environments also showed higher levels of protein
431 content, although in these cases, the effects on grain density and size were moderate,
432 which agrees with Guttieri et al. (2001) in the case of mild drought stress. These results
433 from the severely stressed environments were expected, as drought and heat stress
434 during grain filling are known to be responsible for shortening the grain growth period

435 and improper grain filling, affecting the overall grain yield of the crop (Guttieri et al.,
436 2001; Ramya et al., 2015; Rane et al., 2007). Reduced activity of the soluble starch
437 synthase enzyme at high temperatures in the range 30-40 °C leads to a lower conversion
438 of sucrose to starch (Jenner, 1994), which is another possible reason that explains the
439 shriveling of grains under severe heat stress environment. Besides affecting protein
440 content, the loss of grain plumpness under stress is the main reason for the lower flour
441 milling yields (Guttieri et al., 2001; Spiertz et al., 2006). The different genotype by
442 environment interactions (GxE, GxY, and GxExY, as shown in Table 1) also had a
443 significant effect on flour yield (18% of the variation explained by those interactions),
444 along with other grain characteristics commented above such as grain size (9%) and
445 particularly test weight (18%). This also agrees with the stability showed by these
446 genotypes in the exercise done in Electronic Supplementary Table 2, in which close to
447 the half of the genotypes of the study had a significant change in their rank with respect
448 to the other genotypes. These results do not support the strategy of selecting for these
449 traits only under the optimum environment. Fortunately, CIMMYT runs elite and
450 advanced yield trials under six different environments (including drought and heat), and
451 the grains produced in these trials are evaluated by the breeders for grain size and good
452 grain morphology (round shape, not deep crease), which probably helps to select for test
453 weight and indirectly for flour yield under those stressed environment. Grain hardness
454 is another parameter affecting milling quality . Compared to the full irrigation optimum
455 environments, harder and softer grains were found in the drought and heat stress
456 environments, respectively. Based on the literature, grains are probably harder in
457 drought stress due to higher protein content (Peterson et al., 1992) than to smaller grain
458 size (Gazza et al., 2008). But this does not provide a completely clear explanation as to
459 why softer grains under heat stress were found, where protein content was higher and

460 the grain was smaller compared to drought stress. In transgenic wheats overexpressing
461 the HMW glutenin gene 1Dx5 Rakzsegi et al. (2005) found harder grains with a lower
462 ratio of glutenins/gliadins than in the control. This could lead to a change in the network
463 structure of the glutenin proteins that alters the efficiency of deposition affecting the
464 overall seed development and finally grain hardness. In our case, the heat stress
465 probably led to a lower ratio of glutenins/gliadins (Blumenthal et al., 1998; Li et al.,
466 2013), opposing the mutated line described above to softer grains.

467 The genotypic effect was considerable and the main effect for all the traits
468 related to gluten quality (gluten strength and extensibility, and bread loaf volume). The
469 strong genetic control of these traits has been already reported by several authors (Souza
470 et al. 1993; Yong et al., 2004). These results were positive in relation to the efficiency
471 of the breeding program, as strong genetic control will make the selection process more
472 efficient and will obtain genetic gains faster for the targeted traits in several
473 environments. Besides, the environment effect was also significant for these traits. Both
474 abiotic stresses led to higher gluten strength, probably due to the higher protein content
475 levels. Particularly, in the severe drought stress environment, most of the samples had
476 very strong gluten and, in general, somewhat more tenacious (higher alveograph P/L)
477 gluten than in the optimum environment. This is explained by the higher protein content
478 and probably by a higher proportion of polymeric glutenin too, as it has been shown
479 before for bread (Panozzo et al., 2001) and durum wheat (Flagella et al., 2010). For
480 severe heat stress, although protein content was higher than in severe drought stress
481 environment, the gluten strength was not as high as in severe drought stress, and gluten
482 extensibility (lower alveograph P/L) was higher than in any other environment. This is
483 related to the findings of Blumenthal et al. (1995) and Corbellini et al. (1997), who
484 reported a decrease in glutenin/gliadin ratio and in the percentage of very large glutenin

485 polymers during grain-filling under heat stress conditions. Therefore, these differential
486 changes in the amount of polymeric protein are likely the main factors that explain why
487 gluten strength did not increase as much as under severe drought stress, and instead
488 gluten extensibility was increased. Our results for extensibility fully agree with previous
489 findings (Wrigley et al., 1994; Blumenthal et al., 1995; Li et al., 2013), although we did
490 not detect the absolute weakening dough effect in severe heat stress usually reported in
491 Australian studies, probably due to the high grain protein level reached in our severe
492 heat stressed trials. The weakening dough effect was found in the mild heat stressed
493 trial. Finally bread-making quality was, in general, favored by the abiotic stresses,
494 probably due to the higher gluten strength in most of the genotypes (Mikhaylenko et al.,
495 2000), and a more balanced or extensible gluten in others (Peterson et al. 1998).

496 To assess the efficiency of our breeding approach (selection done under
497 optimum conditions), the importance of genotype by environment interactions
498 (including interaction with year and triple interaction of genotype, environment and
499 year) cannot be ignored, particularly for alveograph P/L, for which the variation
500 explained by those interactions reach the 38% of the total variation. Gluten extensibility
501 represented by alveograph P/L is an important trait within the CIMMYT breeding
502 program for selection, as it is required for all bread wheat products, and it is used for
503 internal end-use type classification (Guzmán et al., 2016a). Having a high level of
504 genotype by environment interactions indicates that the response to the environment of
505 the genotypes is not homogeneous and, therefore, carrying the selection in only one
506 location would not lead to genetic gains for a specific trait across different
507 environments. The analysis of the ranking of the genotypes for this trait showed a
508 considerable part of the genotypes having a change in their ranking position in severe
509 and drought stress environments with respect to the optimum environment. This is

510 probably not a big concern for the severe heat environment because although the
511 ranking of genotypes was quite different compared to the optimum environment, a large
512 majority of the genotypes gained gluten extensibility under those conditions, which is in
513 most cases, a desirable effect. This means that genotypes selected for good gluten
514 extensibility under optimum conditions will likely have good or even better extensibility
515 under heat stress environment too, as it was shown in Fig. 2 (all genotypes except
516 Inqalab91). On the other hand, this will probably not always be the case for drought
517 stress conditions, where there is a slight trend to increase gluten tenacity, and some
518 genotypes that show balanced gluten in optimum environment changed to tenacious
519 gluten under drought stress. The genotypes Inqalab91, PBW343*2/KUKUNA//PARUS
520 /3/PBW343*2/KUKUNA or to a lesser extent Kachu (Fig. 2) are good examples of this.
521 This is not a desirable effect and, although there is a small percentage of genotypes that
522 show the change, an evaluation process under the two environments could be beneficial
523 for the selection of genotypes with good extensibility for semi-arid environments. It is
524 also necessary to understand the high and low molecular weight glutenins that are
525 present in stably performing genotypes, which can also predict the stability. Bread-
526 making quality was also more affected than other traits by genotype by environment
527 interactions (18.7 % of the total variation). In this case, drought environment enhanced
528 loaf volume in all genotypes except one, and most genotypes showed high values.
529 Therefore, selecting for bread-making under drought stress will not add much value to
530 the breeding process. However, in the severe heat stress environment, while most of the
531 genotypes had a considerable increase in loaf volume, seven of the total 54 genotypes
532 showed lower loaf volumes compared to the optimum environment (B13063 or Inqalab
533 91 in Fig.2), and around 31% of them (17 genotypes) had a significant change in their
534 rankings. This may indicate that for a more accurate selection for this specific product

535 (pan bread, mechanized bread industry), genotypes targeted for mega-environment 5
536 (heat stressed irrigated areas) could also be analyzed for bread-making quality under
537 those conditions. Due to the lack of capacity to perform many more quality analysis in
538 the same period of time (currently around 2,400 breeding lines are analyzed in four
539 months at CIMMYT), using rapid tests to predict quality traits (Guzmán et al., 2016c)
540 or new breeding technologies such as genomic selection (Battenfield et al., 2016) could
541 provide good possibilities for the prediction of quality traits for several environments. It
542 is also important to mention that CIMMYT provides the wheat germplasm developed in
543 its breeding program to national partners worldwide, who will test the materials under
544 their specific environmental conditions. The materials that show promising agronomic
545 performance will be tested for grain quality too, and, therefore, the selection process
546 continues out of Mexico, before a wheat genotype is released as a variety.

547

548 **5. Conclusions**

549 The research shows the importance of the genotype and the environment to
550 explain the diversity for quality traits. It is evident that the breeding program at
551 CIMMYT has developed spring bread wheat cultivars with diverse levels of gluten
552 strength combined with good extensibility, and in most cases, their desirable gluten
553 characteristics and end-use quality when they are grown under abiotic stress conditions
554 (drought and heat) are maintained. The current selection strategy for quality traits
555 carried out under optimum conditions seems to be suitable for obtaining acceptable
556 quality traits across several environments for most of the traits. Under optimum
557 conditions, a good range of variability is found for most important quality traits, which
558 allows selecting good genotypes and discarding the bad ones. Under drought and heat
559 stressed environments, most of the genotypes improve their quality traits, which makes

560 selection process more difficult. Selection for gluten extensibility under drought stress
561 and for bread-making quality under heat stress could be recommended when resources
562 are available to ensure an optimum performance of the genotypes across diverse
563 environments.

564

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683 **Milling, processing and end-use quality traits of CIMMYT spring bread wheat**
684 **germplasm under drought and heat stress**

685 Nayelli Hernández-Espinosa¹, Suchismita Mondal¹, Enrique Autrique¹, Héctor
686 Gonzalez-Santoyo¹, José Crossa¹, Julio Huerta-Espino², Ravi Prakash Singh¹, Carlos
687 Guzmán*¹

688

689 ¹Global Wheat Program, International Maize and Wheat Improvement Center
690 (CIMMYT), Apdo Postal 6-641, Mexico DF., Mexico.

691 ²Campo Experimental Valle de México, Instituto de Nacional de Investigaciones
692 Forestales, Agrícolas y Pecuarias, km. 13.5, Carr. Los Reyes- Texcoco, Coatlinchan,
693 56250, Edo. de México.

694 *Author for correspondence: c.cuzman@cgiar.org

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696 For Field Crops Research

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708 **Abstract**

709 The CIMMYT wheat breeding program aims to develop bread wheat (*Triticum*
710 *aestivum* L.) genotypes that have superior grain yields, disease resistance and stress
711 tolerance, along with appropriate quality to satisfy all stakeholders of the wheat value
712 chain. Grain quality for wheat consists of a combination of many defined parameters
713 including grain morphological characteristics, dough and final products properties, all
714 of which are defined by the genotype, the environment and their interactions. Our
715 current approach for improving grain quality is to study grain samples obtained under
716 high yield potential environments with optimum management. To assess the effect of
717 this strategy on quality under stressed environments, 54 genotypes were evaluated for
718 two years under six environmental conditions, including drought and heat stress. Grain
719 morphology (grain density and size), protein content and flour yield were severely
720 affected by the environment, as drought and heat stress had a strong negative effect on
721 all of these characteristics except protein content. Gluten quality (strength and
722 extensibility) was defined more by the genotype, although the environmental effects and
723 the interactions were also important, particularly for gluten extensibility. The current
724 selection strategy for quality traits carried out under optimum conditions was found to
725 be suitable to ensure high quality characteristics across several environments for most
726 of the parameters with an overall positive outcome.

727

728 **Keywords:** wheat quality; wheat breeding; drought stress; heat stress

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733 1. Introduction

734 Wheat (*Triticum* spp.) was one of the first domesticated food crops, and for eight
735 thousand years, it has been the basic food staple of the major civilizations of Europe,
736 West Asia and North Africa. Currently, wheat exhibits large genetic diversity with over
737 25,000 types or cultivars, which are adapted to a wide range of temperate environments
738 (Feldman et al., 1995). FAO estimated that the world wheat production for 2015/2016
739 was approximately 735 million tons. Wheat grain can be processed into flour, semolina
740 and other products that form the basic ingredients of many foods worldwide (e.g. bread,
741 cookies, pastries, pasta, noodles, couscous, etc.). These foods provide about 20% of the
742 calories and protein source for a large portion of the world's population (FAOSTAT). In
743 densely populated countries, such as India or Pakistan, wheat is an important source of
744 calories and proteins, and its consumption will probably increase in other countries such
745 as Bangladesh due to the adoption of a "western lifestyle" (Shewry and Hey, 2015).
746 Therefore, global wheat production needs to increase in the upcoming decades to cover
747 the rising demand for this grain.

748 The spring bread wheat (*T. aestivum* L.) breeding program of the International
749 Maize and Wheat Improvement Center (CIMMYT) breeds high yielding, disease
750 resistant and stress tolerant wheat germplasm and annually distributes it worldwide to
751 national partners mainly in four target areas (mega-environments): 1) Irrigated areas
752 (Northwestern India, Pakistan, Iran, Egypt, China, Mexico, etc.); 2) High rainfall areas
753 (West of Asia, Eastern Africa, highlands of Mexico, etc.); 3) Semi-arid areas (North
754 Africa, West Asia, South America, etc.); and 4) Warmer areas (Nepal, Bangladesh,
755 Eastern Gangetic Plains of India, Southern Pakistan, Sudan, etc.) (Rajaram and van
756 Ginkel, 1993). One key objective of the CIMMYT breeding program is to improve end-
757 use quality in conjunction with other relevant traits to satisfy all stakeholders of the

758 wheat value chain: farmers (bold and plump grain), millers (high test weight and high
759 flour yield), food manufacturers (processing quality) and consumers (end-use and
760 nutritional quality) (Guzmán et al., 2016a).

761 Wheat grain quality is determined by a combination of many defined
762 parameters. Multiple phenotypic traits of the grain, flour, dough, and final products
763 must be assessed to determine an overall quality and best end-use product (Battenfield
764 et al., 2016). Grain morphology, hardness, protein content, and dough handling
765 characteristics (or gluten properties) are some of the traits commonly assessed by
766 breeding programs focused on wheat quality. Generally, it is believed that the genotypic
767 make up of a cultivar is the most important factor when determining wheat quality (Li et
768 al., 2013; Souza et al., 1993); other authors (Blumenthal et al., 1995; Peterson et al.,
769 1998) propose that variation in rheological properties of dough are largely determined
770 by the genotype, however, the environment and its interaction with the genotype (GxE)
771 also play an important role in the expression of the grain quality of a cultivar.
772 Determining the magnitude of GxE is critical for the definition of the selection strategy
773 in a breeding program with a multi-environment focus such as CIMMYT, which aims to
774 develop cultivars that are able to maintain their quality in different environments,
775 including optimum, drought and heat stressed (mega-environments 1, 3 and 4,
776 respectively).

777 Several studies have focused on the effects of the environment and abiotic
778 stresses on the expression of wheat quality, some of which are already well identified
779 and understood. For example, heat stress has been associated with a dough weakening
780 effect (Blumenthal et al., 1993; Corbellini et al., 1997), whereas with drought stress,
781 there is an increase in the protein content and polymeric protein, which produces the
782 opposite effect (Guttieri et al., 2001, 2000). However, many of these studies are

783 characterized by having a limited number of genotypes (Corbellini et al., 1997; Guttieri
784 et al. 2000; Li et al., 2013; Rozbicki et al., 2015), and hence, the lack of diversity in the
785 responses of the cultivars could have led to the above conclusions. Williams et al.
786 (2008) reviewed the studies about environment and GxE effects on bread wheat quality
787 and recommended further research on this topic since our understanding of the
788 environmental influence and the presence of GxE on trait expression is incomplete.

789 The present study was conducted using a collection of 54 semi-dwarf, high
790 yielding spring bread wheat cultivars developed over the last 50 years to: (i) describe
791 the phenotypic variation for the main target traits that determine wheat quality in
792 CIMMYT-derived varieties, (ii) determine the effects of drought and heat stress and
793 GxE interactions on grain quality traits, and (iii) evaluate the selection strategy used at
794 CIMMYT breeding program to generate cultivars with suitable processing and end-use
795 quality in diverse target environments.

796

797 **2. Materials and methods**

798 *2.1 Field Experiment*

799 A field trial consisting of 54 spring bread wheat cultivars developed by
800 CIMMYT and related breeding programs, including historical and modern breeding
801 lines (Table 2) were grown in Ciudad Obregón, Sonora, northwestern México, during
802 two crop seasons: 2012-2013 and 2013-2014. The trial was planted with three replicates
803 under six different environmental conditions: E1: optimum irrigation through the drip
804 method, planted in flat seedbeds (>500mm); E2: planted in flat seedbeds with full basin
805 irrigation (>500mm); E3: reduced (two) irrigation or medium drought stress (300mm);
806 E4: severe drought stress managed through drip irrigation (180mm); E5: medium heat
807 stress (500mm); and E6: severe heat stress (500mm). All trials were planted in

808 November, with the exception of medium heat stress (planted in January) and severe
809 heat stress (planted in February).

810 Except for E1, Nitrogen was applied (pre-planting) at a rate of 50 kg of N/ha,
811 and at tillering, 150 additional units of N were applied in all the trials. In E1, a total of
812 300kg N was applied, which included the pre-planting nitrogen application. Pesticides
813 and herbicides were used as needed to keep trials free from weeds, diseases and aphids.
814 At maturity, 1 kg of seed from each of the wheat lines of the two first field replicates
815 was used for analyzing the quality traits. The third field replicate could not be analyzed
816 due to the high cost and time required to perform the below mentioned grain quality
817 analysis for 648 more samples.

818 The meteorological data of the experimental station in Ciudad Obregon showed
819 almost no precipitation during the wheat growing season. Maximum temperatures
820 reached 31-32°C in March and April, the grain filling time for most of the treatments,
821 and for plants under heat stress, temperatures reached between 35 and 39°C during grain
822 filling in May-June (Electronic Supplementary Figure 1).

823

824 *2.2 Grain parameters*

825 Thousand kernel weight (g) and test weight (kg/hl) were obtained using the
826 digital image system SeedCount SC5000 (Next Instruments, Australia). Grain protein
827 content (%), hardness (PSI, %) and moisture content were determined by near-infrared
828 spectroscopy (NIR Systems 6500, Foss Denmark) calibrated based on official AACC
829 methods 39–10, 55-30 and 46–11A, respectively (AACC, 2010).

830

831 *2.3 Milling*

832 Grain samples were tempered by adding water levels for use in tempering hard,
833 medium-hard and soft wheat before milling, according to the official AACC method 26-
834 95 (AACC, 2010). All samples were milled into flour using a Brabender Quadrumat
835 Senior mill (Germany). Experimental flour yield (%) was recorded.

836

837 *2.4 Flour parameters, rheological and baking test*

838 Flour protein (%) and moisture content (%) were determined by near-infrared
839 spectroscopy (NIR Systems 6500, Foss Denmark), calibrated as per official AACC
840 methods 46–11A and 39–11, respectively (AACC, 2010). Additionally, 35 g flour
841 samples were tested in a mixograph (National Mfg. Co.) to obtain optimum dough
842 mixing time and %Torque × min according to AACC method 54–40A (AACC, 2010).
843 Gluten extensibility (alveograph L), tenacity (alveograph P), elasticity or strength
844 (alveograph W) and tenacity/extensibility ratio (alveograph P/L) were determined
845 according to the Alveograph manufacturer's instructions (Chopin, France), using 60 g
846 flour samples according to AACC method 54-30A (AACC, 2010). The bread-making
847 process was carried out using the direct dough method with 100 g of flour (AACC
848 method 10-09). Bread loaf volume (LV) was determined by rapeseed displacement
849 using a volume-meter. The amounts of water added to the mixograph, alveograph and
850 baking were determined by near-infrared spectroscopy (NIR Systems 6500, Foss
851 Denmark), calibrated according to Guzmán et al. (2015).

852

853 *2.5 Statistical analysis*

854 A fixed effect linear model combined across years, environments and their
855 interactions was performed for all 12 quality parameters using the PROC ANOVA
856 procedure of the SAS statistical software (2010). Additionally, averages, values and

857 least significant difference (LSD) of each quality trait for each environment were
858 calculated averaging across years and genotypes (Fig. 1). Furthermore, mean values and
859 least significant difference (LSD) for some quality traits and specific genotypes in three
860 environments (E1, E4 and E6) averaged across years were calculated.

861

862 **3. Results**

863 *3.1 Effects of genotype, environment, year and their interactions*

864 We used data for 1, 296 grain samples of the 54 bread wheat genotypes grown in
865 six different environments for two cropping seasons and analyzed it for processing and
866 end-use quality traits. The results of the variance analysis (Table 1) show that all factors
867 were highly significant for the traits studied. The environmental and the genotypic
868 effects had the greatest influence on variability found for all studied quality parameters.
869 Particularly, environment was the most important factor affecting grain morphology
870 (test weight and thousand kernel weight), experimental flour yield and protein content,
871 whereas the genotypic effect was the most important for gluten strength (mixograph and
872 alveograph parameters) and extensibility (alveograph P/L), and bread-making quality
873 (loaf volume). The year had a minimal effect for most of the traits. The different
874 interactions between two or the three main factors (genotype, environment and year)
875 were variable depending on the trait. Gluten extensibility (alveograph P/L) was strongly
876 affected by interactions of the genotype with other factors (38.3% of its total variation
877 was explained by these interactions) as well as experimental flour yield and loaf volume
878 (17.5 and 18.7 %, respectively, of their variation explained by genotypic interactions
879 with other factors). For traits strongly influenced by the environment, the environment
880 by year interaction was also high (test weight and thousand kernel weight) or medium
881 (flour yield), although that was not the case for grain protein content.

882

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

Sources of variation	d.f.	TW	TKW	GRNHRD	GRNPRO	FLRYLD	MIXTIM	TQ	ALVW	ALVPL	LOFVOL
Environment (E)	5	43.0	45.9	22.1	66.8	33.9	9.3	15.0	33.2	6.4	22.7
Genotype (G)	53	22.8	31.3	40.8	14.0	28.4	68.0	67.3	51.4	48.0	55.0
Year (Y)	1	2.5	0.1	7.0	1.3	10.7	0.1	0.0	0.3	3.1	0.2
GxE	265	7.4	4.1	7.9	8.0	8.8	7.6	6.5	6.4	17.8	10.2
ExY	5	11.3	12.1	5.1	1.7	6.6	5.3	3.2	2.6	1.8	1.0
GxY	53	3.1	2.3	4.4	1.2	1.9	2.4	2.1	1.4	7.1	2.5
GxExY	265	7.5	2.5	7.3	4.4	6.8	5.6	4.5	3.7	13.6	6.0
Error	633	2.3	1.5	5.1	2.3	2.8	1.5	1.2	1.1	2.1	2.3

d.f.= degrees of freedom; TW, test weight; TKW, thousand kernel weight; GRNHRD, grain hardness; FLRYLD, experimental flour yield; MIXTIM, mixograph optimum mixing time; TQ, mixograph torque; ALVW, alveograph work; ALVP/L, alveograph tenacity/extensibility ratio; LOFVOL, bread loaf volume.

All the values were highly significant ($p < 0.001$)

887 3.2 Effect of environments on quality traits

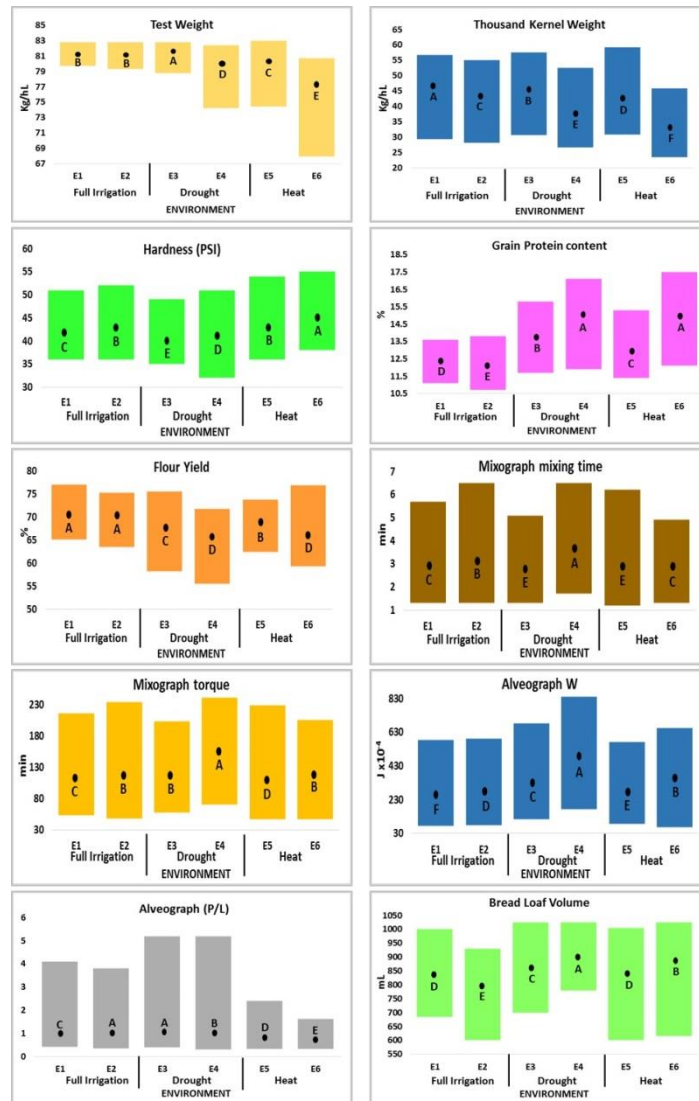
888 Fig. 1 gives the range of values for each quality trait across 54 genotypes for
889 each environment, which was broad for most of the traits. The mean values in each
890 environment (averaging genotypes and years) are shown with a continuous line, while
891 the letters show the significance of the differences among means based on the LSD test.
892 In the case of grain morphology traits, test weight and thousand kernel weight had the
893 highest values under medium drought stress, followed by the environments with full
894 irrigation. Severe drought stress seriously affected both traits, but the most damage was
895 observed under severe heat stress, where the lowest mean values were found (77.3
896 Kg/hL and 33.3 g for test weight and thousand kernel weight, respectively), indicating
897 grain shriveling. Mild heat stress also had negative effects on the both traits. These
898 results are linked to those found for protein content and experimental flour yield: severe
899 drought and heat stressed environments had the highest protein content (15.1 and 15%,
900 respectively) and the lowest experimental flour yield (65.8 and 65.9%, respectively),
901 whereas the full irrigation (drip and basin) environments had the lowest values for
902 protein content (12.4 and 12.1%, respectively) but the highest for experimental flour
903 yield (70.5 and 70.4%, respectively). Moderate drought and heat stressed environments
904 gave intermediate values for these traits.

905 Dough rheological parameters related to gluten strength (mixograph optimum
906 mixing time and torque, and alveograph W) were highest in the severe drought
907 environment, followed by the severe heat stress environment. The other environments
908 showed lower gluten strength, particularly in mild heat stress. In terms of gluten
909 extensibility, heat stress environments showed the highest values (lowest alveograph
910 P/L ratio) (0.8 and 0.7 for mild and severe heat, respectively), and in general drought
911 stressed environments had more tenacious gluten.

912 Finally, significant differences among environments were identified for bread-
 913 making quality too. The highest values for bread loaf volume were found in severe
 914 drought and heat stress environments (901 and 887 mL, respectively), whereas full
 915 irrigation and mild heat stress showed the lowest performance for this trait (816 and 838
 916 mL, respectively).

917
 918

919 **Figure 1.** Maximum, average and minimum values for ten grain quality traits of 54
 920 genotypes in each environment averaged over two years. The ranges (maximum and
 921 minimum values) are represented by bars and the average values by a dots inside the
 922 bars. Letters show different groups across environments based on LSD test.
 923 Environments were: (E1) full drip irrigation; (E2) full basin irrigation; (E3) medium
 924 drought stress; (E4) severe drought stress; (E5) medium heat stress and (E6) severe heat
 925 stress.



926

927 *3.3 Differences in genotypic response to the environment*

928 In addition to the general responses for each quality trait in each environment,
929 we also examined the specific response of each genotype in different environments. In
930 Electronic Supplementary Table 1, all the quality traits data of each genotype in the six
931 environments (averaging field replicates and year) is given. In order to avoid an
932 excessively lengthy document, we mostly focused the interpretation and associated
933 discussion of the data on the specific genotypic responses in only three representative
934 quality traits: alveograph W (gluten strength), alveograph P/L (gluten
935 tenacity/extensibility balance) and loaf volume (bread-making quality), which are
936 considered the most important traits at the CIMMYT breeding program for selection
937 purposes apart from grain hardness (a trait that did not show large genetic variability in
938 the current study); and in three environments with more contrasting conditions: full drip
939 irrigation (optimum), severe drought stress, and severe heat stress (E1, E4 and E6,
940 respectively).

941 Table 2 shows the mean values over two years for the 54 genotypes observed for
942 the three quality parameters mentioned above in the three environments. Based on these
943 mean values, a rank relative to the rest of the genotypes was also assigned to each
944 genotype (from 1 to 54). The rank of each genotype is also given for the severe drought
945 and heat stressed environments and in brackets, the number of positions the genotype
946 moves up or down in relation to the ranking that each genotype had in the optimum
947 environment. For example, 'Sonalika' had the mean value for alveograph W of 137
948 J*10-4 being placed in the 50th position in the optimum environment. This cultivar was
949 on the 52nd position in the severe drought environment (two positions lower in ranking
950 with respect to E1), and on the 49th position in the severe heat stress environment (one
951 position higher in ranking with respect to E1). This analysis of ranking approach was

952 used to distinguish genotypes that were affected differently by the environmental stress,
953 since overall, all genotypes had an increase in gluten strength and extensibility and loaf
954 volume in stressed environments (Fig. 1). For the ranking of alveograph P/L, lower
955 values (extensible gluten) were considered better than higher values (tenacious gluten).

956 Based on Table 2, the 54 genotypes can be classified in two overall groups:
957 stable and unstable, based on rank shifts in different environments. Stability in this case
958 means they do not show a big change in the ranking between the reference environment
959 (full irrigation) and the stressed environments (severe drought and heat). It is important
960 to mention that some genotypes showing medium values (center of the distribution)
961 could seem less stable using this system than the genotypes showing the highest/lowest
962 values simply because only a small change in value is required to considerably change
963 ranked position. Some examples of cultivars that were stable in their rankings for the
964 three traits across the three environments were ‘Attila’, ‘Super 152’ and ‘Misr 1’. On
965 the other hand, ‘Baj#1’ or ‘Inqalab’ were good examples of unstable genotypes for these
966 quality traits across the stressed environments. Other genotypes, such as ‘Vorobey’,
967 were not stable for gluten quality traits (alveograph W and P/L) but were stable for
968 bread-making quality in both stressed environments, or some genotypes were unstable
969 for a specific quality trait, e.g. ‘Lok1’ for alveograph P/L but were stable for the other
970 two traits. There were many different cases, but overall, a large proportion of the
971 genotypes did not show an important change in the position in their ranking across the
972 examined environments, particularly for alveograph W (only six and thirteen of the 54
973 genotypes had a change larger than ten positions in the ranking in severe drought and
974 heat stress environments, respectively). For loaf volume, 17 genotypes had a change
975 larger than ten positions in the ranking in both stressed environments, whereas for

976 alveograph extensibility 15 and 25 genotypes had a change larger than ten positions in
977 the ranking in drought and heat stressed environments, respectively.

978 In addition to this, scatter plots showing the correlation between the position in
979 the ranking of the genotypes in the optimum environment versus the position in the
980 ranking of the genotypes in the severe drought and heat stress environment were also
981 developed (Electronic Supplementary Table 2). The correlations were higher for
982 alveograph W than for loaf volume and particularly higher than for alveograph P/L,
983 which means that more genotypes keep a similar position in the ranking for alveograph
984 W across environments. With this type of plot it was possible to identify how accurate
985 the selection process was when performing only in the optimum environment. For
986 example, for alveograph W, we can select the best 27 genotypes (50% of the
987 population) for this trait. Of those, only two genotypes would not be among the best 27
988 in the severe drought stress environment, and five of them would not be among the best
989 27 in the severe heat stress environment, although that fact does not mean that these
990 genotypes are necessarily losing gluten strength in the stressed environments. For
991 alveograph P/L and loaf volume a very similar situation was found.

992 The same exercise was done with a portion of the rest of the recorded traits: test
993 weight, thousand kernel weight, grain protein content and flour yield (Electronic
994 Supplementary Table 3). Overall, these traits showed less stability than the above-
995 mentioned traits related with gluten characteristics and end-use quality. For test weight,
996 25 and 24 genotypes had a change larger than ten positions in drought and heat stressed
997 environments, respectively; for thousand kernel weight, 16 and 22 genotypes; for grain
998 protein content, 26 and 25 genotypes; and for flour yield, 21 and 18 genotypes had a
999 change in the rank larger than ten positions in drought and heat stressed environments.
1000 The scatter plots showing the correlation between the positions in the ranking of the

1001 genotypes (Electronic Supplementary Table 2 – sheet 2) also showed that for these
1002 traits, more genotypes changed their position in the ranking. In this case, for example, if
1003 we select the best 27 genotypes for test weight under the optimum environment, of
1004 those genotypes, eight would not be among the best 27 in the severe drought stress
1005 environment and ten of them would not be among the best 27 in the severe heat stress
1006 environment. A very similar situation was found for thousand kernel weight and for
1007 grain protein content, but not for flour yield, where the correlations were higher.

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1014 **Table 2.** Variability for three quality traits of 54 bread wheat genotypes across three environments (full drip irrigation or optimum, severe
1015 drought and severe heat). Average values were calculated averaging field replicates and two cropping years. The position in the ranking of each
1016 genotype is indicated. For severe drought and heat stress environment, the positions won or lost in the ranking for each genotype with respect to
1017 the optimum environment is indicated between brackets.

Genotype name/cross	Alveograph W (J*10 ⁻⁴)						Alveograph P/L						Bread loaf volumen (mL)					
	Optimum		Drought		Heat		Optimum		Drought		Heat		Optimum		Drought		Heat	
	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)
Sonalika	137	50	233	52 (-2)	185	49 (+1)	1	34	2.5	52 (-18)	0.6	21 (13)	768	47	794	54 (-7)	853	41 (+6)
Siete Cerros 66	253	29	483	31 (-3)	294	39 (-10)	2.6	54	3.6	54 (0)	0.9	48 (6)	758	48	834	48 (0)	795	49 (-1)
Pavon F76	301	15	610	9 (+6)	475	8 (+7)	1	30	1.0	36 (-6)	0.8	40 (10)	876	15	928	20 (-5)	894	27 (-12)
Opata M85	222	38	391	43 (-5)	306	38 (0)	0.6	6	0.6	12 (-6)	0.7	36 (-30)	878	14	928	21 (-7)	839	42 (-28)
Seri M82	190	44	449	37 (+7)	450	13 (+31)	1	31	0.8	25 (+6)	0.8	42 (-11)	858	19	939	16 (+3)	931	15 (+4)
WL 711	203	42	362	47 (-5)	174	51 (-9)	1.4	51	1.3	47 (+4)	0.7	35 (16)	738	51	824	49 (+2)	765	52 (-1)
Attila	134	51	347	48 (+3)	256	45 (+6)	0.5	2	0.4	1 (+1)	0.4	1 (1)	853	20	943	14 (+6)	904	20 (0)
PBW343	152	48	257	50 (-2)	184	50 (-2)	1.3	44	1.1	42 (+2)	1.0	50 (-6)	801	41	865	39 (+2)	751	53 (-12)
Inqalab 91	291	18	442	39 (-21)	380	26 (-8)	1	33	1.5	49 (-16)	1.4	54 (-21)	828	30	848	45 (-15)	808	47 (-17)
Seher 06	345	10	525	21 (-11)	387	25 (-15)	0.9	26	0.8	22 (+4)	0.5	13 (13)	824	34	865	40 (-6)	883	30 (+4)
Norteña F2007	390	5	669	5 (0)	417	20 (-15)	1.2	40	0.9	30 (+10)	0.6	23 (17)	848	22	930	18 (+4)	900	23 (-1)
Kachu#1	400	3	648	6 (-3)	482	7 (-4)	0.9	23	1.1	41 (-18)	0.7	34 (-11)	921	4	941	15 (-11)	971	8 (-4)
Tacupeto M2001	257	28	490	30 (-2)	433	15 (+13)	0.7	14	0.7	14 (0)	0.8	43 (-29)	840	23	904	30 (-7)	900	24 (-1)
Baviacora T92	288	20	559	18 (+2)	417	18 (+2)	1.1	37	0.9	31 (+6)	0.8	39 (-2)	806	40	946	11 (+29)	918	18 (+22)
Roelfs F2007	308	14	572	16 (-2)	530	4 (+10)	0.7	13	1.0	35 (-22)	0.7	29 (-16)	865	17	951	10 (+7)	975	7 (+10)
Misr 1	442	2	778	1 (+1)	587	1 (+1)	1.1	38	0.9	32 (+6)	0.9	47 (-9)	963	1	963	5 (-4)	1003	2 (-1)
Lasani 08	358	8	706	4 (+4)	531	3 (+5)	1.4	49	1.9	51 (-2)	1.3	52 (-3)	828	31	891	31 (0)	859	36 (-5)
Faisalabad 2008	391	4	755	2 (+2)	485	6 (-2)	1.3	47	1.1	43 (+4)	0.7	26 (21)	829	29	956	6 (+23)	874	34 (-5)
Munal#1	234	33	494	29 (+4)	421	17 (+16)	0.6	10	0.6	5 (+5)	0.5	11 (-1)	875	16	973	3 (+13)	953	11 (+5)
Súper 152	273	24	546	19 (+5)	392	23 (+1)	0.9	29	0.9	28 (+1)	0.7	31 (-2)	824	35	887	35 (0)	859	37 (-2)
Quaiu#1	232	34	437	41 (-7)	275	43 (-9)	0.7	20	0.6	8 (+12)	0.7	25 (-5)	819	36	884	36 (0)	857	38 (-2)
Danphe#1	243	32	590	13 (+19)	373	27 (+5)	0.6	8	0.6	6 (+2)	0.4	6 (2)	950	3	1014	1 (+2)	985	5 (-2)
Cachanilla F2000	353	9	586	14 (-5)	439	14 (-5)	1.3	45	0.9	33 (+12)	0.5	15 (30)	833	27	944	13 (+14)	888	29 (-2)
Super Seri#1	253	30	518	22 (+8)	520	5 (+25)	0.6	7	0.6	4 (+3)	0.6	22 (-15)	894	9	954	8 (+1)	985	6 (+3)
Vorobey	332	11	501	24 (-13)	310	36 (-25)	1.4	50	0.9	34 (+16)	0.7	33 (17)	798	42	859	42 (0)	820	45 (-3)

Weebill#1	300	16	593	12 (+4)	475	9 (+5)	1.2	41	0.8	26 (+15)	0.7	37 (4)	811	39	936	17 (+22)	904	21 (+18)
Babax/Lr42//Babax...	229	35	455	34 (+1)	360	29 (+6)	0.8	22	0.7	16 (+6)	0.5	8 (14)	840	24	915	26 (-2)	989	4 (+20)
Grackle	244	31	463	32 (-1)	357	30 (+1)	0.6	4	0.5	3 (+1)	0.4	3 (1)	863	18	956	7 (+11)	1018	1 (+18)
Elvira/5/Cndo/R143...	365	7	640	8 (-1)	473	10 (-3)	1.7	52	1.3	48 (+4)	1.1	51 (1)	754	50	889	32 (+18)	814	46 (+4)
Trch*2/3/C80.1/3*...	322	12	583	15 (-3)	454	12 (0)	0.7	18	0.7	18 (0)	0.6	20 (-2)	836	26	946	12 (+14)	901	22 (+4)
Whear/Kronstad	509	1	750	3 (-2)	545	2 (-1)	1.3	46	1.0	39 (+7)	0.9	49 (-3)	838	25	930	19 (+6)	825	43 (-18)
Cndo/R143//Ente...	193	43	456	33 (+10)	322	34 (+9)	0.7	16	0.7	13 (+3)	0.5	16 (0)	825	33	910	28 (+5)	930	16 (+17)
SW89.5277/Borl95...	180	47	438	40 (+7)	351	31 (+16)	1.7	53	1.3	45 (+8)	0.8	46 (7)	730	53	870	37 (+16)	871	35 (+18)
Navojoa M2007	204	41	454	35 (+6)	274	44 (-3)	0.6	3	0.6	11 (-8)	0.4	2 (1)	851	21	908	29 (-8)	914	19 (+2)
Baj#1	290	19	501	26 (-7)	321	35 (-16)	0.7	19	0.9	27 (-8)	0.4	5 (14)	898	6	919	24 (-18)	934	14 (-8)
Borlaug100 F2014	314	13	597	10 (+3)	417	19 (-6)	0.9	24	0.8	23 (+1)	0.5	17 (7)	883	12	913	27 (-15)	958	10 (+2)
Becard/Quaiu	261	27	499	27 (0)	343	32 (-5)	0.9	25	0.7	19 (+6)	0.7	38 (-13)	828	32	888	34 (-2)	855	40 (-8)
Babax/Lr42//Babax..	227	37	452	36 (+1)	310	37 (0)	1.3	48	1.1	44 (+4)	0.8	45 (3)	774	45	865	41 (+4)	823	44 (+1)
BL3063	92	54	197	54 (0)	82	54 (0)	0.9	27	0.6	9 (+18)	0.5	14 (13)	698	54	806	53 (+1)	665	54 (0)
Lok 1	145	49	322	49 (0)	191	48 (+1)	0.7	17	2.5	53 (-36)	1.3	53 (-36)	731	52	813	51 (+1)	786	51 (+1)
HD 2687	106	53	232	53 (0)	172	53 (0)	1.2	42	0.7	20 (+22)	0.7	28 (14)	779	44	838	47 (-3)	878	32 (+12)
HUW 234	210	39	378	45 (-6)	228	47 (-8)	0.8	21	0.7	17 (+4)	0.5	9 (12)	794	43	843	46 (-3)	856	39 (+4)
Kanchan	281	23	568	17 (+6)	391	24 (-1)	0.5	1	0.5	2 (-1)	0.5	12 (-11)	958	2	923	22 (-20)	944	13 (-11)
Francolin #1	227	36	496	28 (+6)	277	42 (-6)	0.6	5	0.9	29 (-24)	0.4	4 (1)	895	8	920	23 (-15)	900	25 (-17)
HUW234+Lr34/Prinia..	189	45	404	42 (+3)	277	41 (+4)	0.6	11	1.0	38 (-27)	0.6	19 (-8)	813	38	855	43 (-5)	891	28 (+10)
Sha7/Vee#5/5/Vee...	119	52	253	51 (+1)	174	52 (0)	1.2	43	1.9	50 (-7)	0.8	41 (2)	758	49	809	52 (-3)	790	50 (-1)
Neloki	287	21	647	7 (+14)	422	16 (+5)	1	35	1.0	37 (-2)	0.6	18 (17)	885	11	996	2 (+9)	990	3 (+8)
Whear/Sokoll	263	26	501	25 (+1)	463	11 (+15)	0.7	15	0.6	10 (+5)	0.7	27 (-12)	880	13	889	33 (-20)	929	17 (-4)
Kachu/Saua	390	6	597	11 (-5)	415	21 (-15)	1.1	36	1.0	40 (-4)	0.6	24 (12)	896	7	968	4 (+3)	969	9 (-2)
Fret2*2/4/Sni/Trap...	187	46	376	46 (0)	327	33 (+13)	0.9	28	0.8	21 (+7)	0.7	30 (-2)	769	46	821	50 (-4)	878	33 (+13)
Attila*2/PBW65/..	272	25	544	20 (+5)	370	28 (-3)	0.7	12	0.7	15 (-3)	0.5	10 (2)	908	5	953	9 (-4)	945	12 (-7)
PBW343*2/Kukuna/...	282	22	443	38 (-16)	286	40 (-18)	1	32	1.3	46 (-14)	0.7	32 (0)	890	10	916	25 (-15)	895	26 (-16)
Kachu//Wbl11*2/...	291	17	511	23 (-6)	406	22 (-5)	1.1	39	0.8	24 (+15)	0.8	44 (-5)	830	28	868	38 (-10)	881	31 (-3)
Super 152/Baj#1	207	40	388	44 (-4)	228	46 (-6)	0.6	9	0.6	7 (+2)	0.5	7 (2)	819	37	854	44 (-7)	804	48 (-11)

1018 Based on the results shown in Table 2, a set of nine genotypes were selected for
1019 a more detailed analysis of their performance across the same three environments (E1,
1020 E2 and E3). Figure 2 shows the minimum and maximum mean across years and mean
1021 values for nine genotypes in the three environments for the traits used in Table 2, and
1022 also for grain protein content. These genotypes can be placed in three groups: 1)
1023 excellent quality across all environments ('Kachu#1', 'Misr1' and 'Kanchan'); 2) low
1024 quality across all environments ('Sonalika', 'Siete Cerros' and 'BL3063'); and 3)
1025 variable quality across environments ('Inqalab 91', 'Vorobey' and
1026 'PBW343*2/Kukuna//Parus/3/PBW343*2/Kukuna'). The genotypes in the first group
1027 had high values for the quality traits in the full irrigation environments, which either
1028 improved or remained stable in the stressed environments while protein content
1029 increased. These genotypes had strong gluten and good extensibility, which led to high
1030 loaf volume values. Based on these results and according to Guzmán et al. (2016a),
1031 these genotypes produce high grain quality suitable for the mechanized baking industry
1032 in all the environments. The cultivars in the second group show, in general, the worst
1033 performance with weak and/or tenacious gluten and bread-making quality. The protein
1034 content increase in the stressed environments did not lead to higher values on the other
1035 quality traits in all cases or the increase was not large enough to lead to a qualitative
1036 change in the potential end-use type of these genotypes. These genotypes remained in
1037 potential end-use types of handmade baking and utility wheat across all environments.
1038 Finally, genotypes in the third group represented cultivars that had different quality in
1039 the full irrigation environment, and the increased protein content in stressed
1040 environments was linked to great and diverse changes in gluten and bread-making
1041 qualities (positives or negatives) that led to qualitative changes in the potential end-use
1042 of those cultivars.

1043 **Figure 2.** Mean (averaged 2 years), maximum and minimum values for four grain quality traits of nine bread wheat cultivars in three
 1044 environments (E1: full drip irrigation; E2: severe drought stress; E3: severe heat stress). The number on the top of the bars indicates the average
 1045 value of each genotype.



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1047 **4. Discussion**

1048 The current approach of the CIMMYT spring bread wheat breeding program to
1049 improve grain quality is to analyze genotypes grown under optimum conditions for
1050 grain yield potential. These conditions in Ciudad Obregon (main CIMMYT wheat
1051 breeding and yield testing site) are full irrigation with sowing time in November,
1052 heading around February-March and harvesting at the end of April- early May, which
1053 match the conditions of important target areas for the CIMMYT breeding program such
1054 as Northwestern India, most of Pakistan or Egypt (Mega-environment 1). The grain
1055 yield of elite lines under these optimum conditions in Ciudad Obregon is usually around
1056 8-9 tons/ha, very high for such a short season. This leads to medium-low grain protein
1057 levels (around 11-13%) in most of the lines. The lines grown in Ciudad Obregon are
1058 analyzed for diverse quality traits (see Guzmán et al., 2016a for a better description) to
1059 guarantee good gluten quality (diverse levels of gluten strength combined with good
1060 extensibility) in semi-hard or hard grains. This set of quality parameters is preferred for
1061 most products in developing countries, where more than 70% of the varieties grown
1062 have CIMMYT origin (Lantican et al., 2016). Although the CIMMYT breeding
1063 program also targets other regions where drought and heat stress are present (mega-
1064 environments 3 and 4), the selection process for grain quality traits is currently done
1065 only with materials grown under optimum conditions, for our past results indicated that
1066 the highest discrimination of advanced lines for quality traits occurred when grains from
1067 high yielding environments were analyzed. This is due to a reduction in protein content
1068 caused by high yields. Lines identified to have good quality traits under this condition
1069 are expected to show better or even excellent quality when they are grown under
1070 drought or heat stress conditions, causing a significant reduction in grain yield but an
1071 increase in protein content. To check this hypothesis, a large trial with diverse

1072 CIMMYT-derived cultivars/ new breeding lines were evaluated for quality traits under
1073 six different environments in Ciudad Obregon (where irrigation can be adjusted to
1074 simulate drought stress and sowing time can be delayed to generate heat stress). At the
1075 same time that our breeding-for-quality strategy was being evaluated, a large amount of
1076 data related to abiotic stress effects on grain quality traits was also generated, which
1077 contributes to the understanding of genetic and environmental effects on quality traits.
1078 Such understanding facilitates both effective selection for quality in breeding programs,
1079 and strategies to establish more uniform and consistent plots of commercial wheat that
1080 are better suited to the needs of the value chain (Williams et al., 2008).

1081 Large ranges of values were found for most of the traits, something that does not
1082 happen in all breeding programs. The ANOVA showed strong and main environmental
1083 effects on traits related to grain filling and morphology (grain density and size) and
1084 directly affected by them (protein content and experimental flour yield). This agrees
1085 with previous studies on spring bread wheat (Mikhaylenko et al., 2000; Rozbicki et al.,
1086 2015; Studnicki et al., 2016), winter bread wheat (Bilgin et al. 2016) and durum wheat
1087 (Guzmán et al. 2016b; Rharrabti et al., 2003). The effects of severe drought, and
1088 particularly severe heat stress, on grain morphology traits (test weight and thousand
1089 kernel weight) were negative, leading to a much higher protein content level in these
1090 environments compared to the non-stressed, fully irrigated environments, which was
1091 probably due to a concentration effect (Guttieri et al., 2000; Saint-Pierre et al., 2008).
1092 The mild drought and heat stress environments also showed higher levels of protein
1093 content, although in these cases, the effects on grain density and size were moderate,
1094 which agrees with Guttieri et al. (2001) in the case of mild drought stress. These results
1095 from the severely stressed environments were expected, as drought and heat stress
1096 during grain filling are known to be responsible for shortening the grain growth period

1097 and improper grain filling, affecting the overall grain yield of the crop (Guttieri et al.,
1098 2001; Ramya et al., 2015; Rane et al., 2007). Reduced activity of the soluble starch
1099 synthase enzyme at high temperatures in the range 30-40 °C leads to a lower conversion
1100 of sucrose to starch (Jenner, 1994), which is another possible reason that explains the
1101 shriveling of grains under severe heat stress environment. Besides affecting protein
1102 content, the loss of grain plumpness under stress is the main reason for the lower flour
1103 milling yields (Guttieri et al., 2001; Spiertz et al., 2006). The different genotype by
1104 environment interactions (GxE, GxY, and GxExY, as shown in Table 1) also had a
1105 significant effect on flour yield (18% of the variation explained by those interactions),
1106 along with other grain characteristics commented above such as grain size (9%) and
1107 particularly test weight (18%). This also agrees with the stability showed by these
1108 genotypes in the exercise done in Electronic Supplementary Table 2, in which close to
1109 the half of the genotypes of the study had a significant change in their rank with respect
1110 to the other genotypes. These results do not support the strategy of selecting for these
1111 traits only under the optimum environment. Fortunately, CIMMYT runs elite and
1112 advanced yield trials under six different environments (including drought and heat), and
1113 the grains produced in these trials are evaluated by the breeders for grain size and good
1114 grain morphology (round shape, not deep crease), which probably helps to select for test
1115 weight and indirectly for flour yield under those stressed environment. Grain hardness
1116 is another parameter affecting milling quality. Compared to the full irrigation optimum
1117 environments, harder and softer grains were found in the drought and heat stress
1118 environments, respectively. Based on the literature, grains are probably harder in
1119 drought stress due to higher protein content (Peterson et al., 1992) than to smaller grain
1120 size (Gazza et al., 2008). But this does not provide a completely clear explanation as to
1121 why softer grains under heat stress were found, where protein content was higher and

1122 the grain was smaller compared to drought stress. In transgenic wheats overexpressing
1123 the HMW glutenin gene 1Dx5 Rakzsegi et al. (2005) found harder grains with a lower
1124 ratio of glutenins/gliadins than in the control. This could lead to a change in the network
1125 structure of the glutenin proteins that alters the efficiency of deposition affecting the
1126 overall seed development and finally grain hardness. In our case, the heat stress
1127 probably led to a lower ratio of glutenins/gliadins (Blumenthal et al., 1998; Li et al.,
1128 2013), opposing the mutated line described above to softer grains.

1129 The genotypic effect was considerable and the main effect for all the traits
1130 related to gluten quality (gluten strength and extensibility, and bread loaf volume). The
1131 strong genetic control of these traits has been already reported by several authors (Souza
1132 et al. 1993; Yong et al., 2004). These results were positive in relation to the efficiency
1133 of the breeding program, as strong genetic control will make the selection process more
1134 efficient and will obtain genetic gains faster for the targeted traits in several
1135 environments. Besides, the environment effect was also significant for these traits. Both
1136 abiotic stresses led to higher gluten strength, probably due to the higher protein content
1137 levels. Particularly, in the severe drought stress environment, most of the samples had
1138 very strong gluten and, in general, somewhat more tenacious (higher alveograph P/L)
1139 gluten than in the optimum environment. This is explained by the higher protein content
1140 and probably by a higher proportion of polymeric glutenin too, as it has been shown
1141 before for bread (Panozzo et al., 2001) and durum wheat (Flagella et al., 2010). For
1142 severe heat stress, although protein content was higher than in severe drought stress
1143 environment, the gluten strength was not as high as in severe drought stress, and gluten
1144 extensibility (lower alveograph P/L) was higher than in any other environment. This is
1145 related to the findings of Blumenthal et al. (1995) and Corbellini et al. (1997), who
1146 reported a decrease in glutenin/gliadin ratio and in the percentage of very large glutenin

1147 polymers during grain-filling under heat stress conditions. Therefore, these differential
1148 changes in the amount of polymeric protein are likely the main factors that explain why
1149 gluten strength did not increase as much as under severe drought stress, and instead
1150 gluten extensibility was increased. Our results for extensibility fully agree with previous
1151 findings (Wrigley et al., 1994; Blumenthal et al., 1995; Li et al., 2013), although we did
1152 not detect the absolute weakening dough effect in severe heat stress usually reported in
1153 Australian studies, probably due to the high grain protein level reached in our severe
1154 heat stressed trials. The weakening dough effect was found in the mild heat stressed
1155 trial. Finally bread-making quality was, in general, favored by the abiotic stresses,
1156 probably due to the higher gluten strength in most of the genotypes (Mikhaylenko et al.,
1157 2000), and a more balanced or extensible gluten in others (Peterson et al. 1998).

1158 To assess the efficiency of our breeding approach (selection done under
1159 optimum conditions), the importance of genotype by environment interactions
1160 (including interaction with year and triple interaction of genotype, environment and
1161 year) cannot be ignored, particularly for alveograph P/L, for which the variation
1162 explained by those interactions reach the 38% of the total variation. Gluten extensibility
1163 represented by alveograph P/L is an important trait within the CIMMYT breeding
1164 program for selection, as it is required for all bread wheat products, and it is used for
1165 internal end-use type classification (Guzmán et al., 2016a). Having a high level of
1166 genotype by environment interactions indicates that the response to the environment of
1167 the genotypes is not homogeneous and, therefore, carrying the selection in only one
1168 location would not lead to genetic gains for a specific trait across different
1169 environments. The analysis of the ranking of the genotypes for this trait showed a
1170 considerable part of the genotypes having a change in their ranking position in severe
1171 and drought stress environments with respect to the optimum environment. This is

1172 probably not a big concern for the severe heat environment because although the
1173 ranking of genotypes was quite different compared to the optimum environment, a large
1174 majority of the genotypes gained gluten extensibility under those conditions, which is in
1175 most cases, a desirable effect. This means that genotypes selected for good gluten
1176 extensibility under optimum conditions will likely have good or even better extensibility
1177 under heat stress environment too, as it was shown in Fig. 2 (all genotypes except
1178 Inqalab91). On the other hand, this will probably not always be the case for drought
1179 stress conditions, where there is a slight trend to increase gluten tenacity, and some
1180 genotypes that show balanced gluten in optimum environment changed to tenacious
1181 gluten under drought stress. The genotypes Inqalab91, PBW343*2/KUKUNA//PARUS
1182 /3/PBW343*2/KUKUNA or to a lesser extent Kachu (Fig. 2) are good examples of this.
1183 This is not a desirable effect and, although there is a small percentage of genotypes that
1184 show the change, an evaluation process under the two environments could be beneficial
1185 for the selection of genotypes with good extensibility for semi-arid environments. It is
1186 also necessary to understand the high and low molecular weight glutenins that are
1187 present in stably performing genotypes, which can also predict the stability. Bread-
1188 making quality was also more affected than other traits by genotype by environment
1189 interactions (18.7 % of the total variation). In this case, drought environment enhanced
1190 loaf volume in all genotypes except one, and most genotypes showed high values.
1191 Therefore, selecting for bread-making under drought stress will not add much value to
1192 the breeding process. However, in the severe heat stress environment, while most of the
1193 genotypes had a considerable increase in loaf volume, seven of the total 54 genotypes
1194 showed lower loaf volumes compared to the optimum environment (BI3063 or Inqalab
1195 91 in Fig.2), and around 31% of them (17 genotypes) had a significant change in their
1196 rankings. This may indicate that for a more accurate selection for this specific product

1197 (pan bread, mechanized bread industry), genotypes targeted for mega-environment 5
1198 (heat stressed irrigated areas) could also be analyzed for bread-making quality under
1199 those conditions. Due to the lack of capacity to perform many more quality analysis in
1200 the same period of time (currently around 2,400 breeding lines are analyzed in four
1201 months at CIMMYT), using rapid tests to predict quality traits (Guzmán et al., 2016c)
1202 or new breeding technologies such as genomic selection (Battenfield et al., 2016) could
1203 provide good possibilities for the prediction of quality traits for several environments. It
1204 is also important to mention that CIMMYT provides the wheat germplasm developed in
1205 its breeding program to national partners worldwide, who will test the materials under
1206 their specific environmental conditions. The materials that show promising agronomic
1207 performance will be tested for grain quality too, and, therefore, the selection process
1208 continues out of Mexico, before a wheat genotype is released as a variety.

1209

1210 **5. Conclusions**

1211 The research shows the importance of the genotype and the environment to
1212 explain the diversity for quality traits. It is evident that the breeding program at
1213 CIMMYT has developed spring bread wheat cultivars with diverse levels of gluten
1214 strength combined with good extensibility, and in most cases, their desirable gluten
1215 characteristics and end-use quality when they are grown under abiotic stress conditions
1216 (drought and heat) are maintained. The current selection strategy for quality traits
1217 carried out under optimum conditions seems to be suitable for obtaining acceptable
1218 quality traits across several environments for most of the traits. Under optimum
1219 conditions, a good range of variability is found for most important quality traits, which
1220 allows selecting good genotypes and discarding the bad ones. Under drought and heat
1221 stressed environments, most of the genotypes improve their quality traits, which makes

1222 selection process more difficult. Selection for gluten extensibility under drought stress
1223 and for bread-making quality under heat stress could be recommended when resources
1224 are available to ensure an optimum performance of the genotypes across diverse
1225 environments.

1226

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1230

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