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

Nayelli Hernández-Espinosa[1], Suchismita Mondal[1], Enrique Autrique[1], Héctor Gonzalez-Santoyo[1], José Crossa[1], Julio Huerta-Espino[2], Ravi Prakash Singh[1], Carlos Guzmán*[1]

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

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

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

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Abstract

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

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

1. Introduction

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

The spring bread wheat (*T. aestivum* L.) breeding program of the International Maize and Wheat Improvement Center (CIMMYT) breeds high yielding, disease resistant and stress tolerant wheat germplasm and annually distributes it worldwide to national partners mainly in four target areas (mega-environments): 1) Irrigated areas (Northwestern India, Pakistan, Iran, Egypt, China, Mexico, etc.); 2) High rainfall areas (West of Asia, Eastern Africa, highlands of Mexico, etc.); 3) Semi-arid areas (North Africa, West Asia, South America, etc.); and 4) Warmer areas (Nepal, Bangladesh, Eastern Gangetic Plains of India, Southern Pakistan, Sudan, etc.) (Rajaram and van Ginkel, 1993). One key objective of the CIMMYT breeding program is to improve end-use quality in conjunction with other relevant traits to satisfy all stakeholders of the wheat value chain: farmers (bold and plump grain), millers (high test weight and high flour yield), food manufacturers (processing quality) and consumers (end-use and nutritional quality) (Guzmán et al., 2016a).

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

Several studies have focused on the effects of the environment and abiotic stresses on the expression of wheat quality, some of which are already well identified and understood. For example, heat stress has been associated with a dough weakening effect (Blumenthal et al., 1993; Corbellini et al., 1997), whereas with drought stress, there is an increase in the protein content and polymeric protein, which produces the opposite effect (Guttieri et al., 2001, 2000). However, many of these studies are characterized by having a limited number of genotypes (Corbellini et al., 1997; Guttieri et al. 2000; Li et al., 2013; Rozbicki et al., 2015), and hence, the lack of diversity in the responses of the cultivars could have led to the above conclusions. Williams et al. (2008) reviewed the studies about environment and GxE effects on bread wheat quality and recommended further research on this topic since our understanding of the environmental influence and the presence of GxE on trait expression is incomplete.

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

2. Materials and methods

2.1Field Experiment

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

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

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

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

2.2Grain parameters

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

2.3Milling

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

2.4Flour parameters, rheological and baking test

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

2.5Statistical analysis

A fixed effect linear model combined across years, environments and their interactions was performed for all 12 quality parameters using the PROC ANOVA procedure of the SAS statistical software (2010). Additionally, averages, values and least significant difference (LSD) of each quality trait for each environment were calculated averaging across years and genotypes (Fig. 1). Furthermore, mean values and least significant difference (LSD) for some quality traits and specific genotypes in three environments (E1, E4 and E6) averaged across years were calculated.

3. Results

3.1Effects of genotype, environment, year and their interactions

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

3.2Effect of environments on quality traits

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

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.

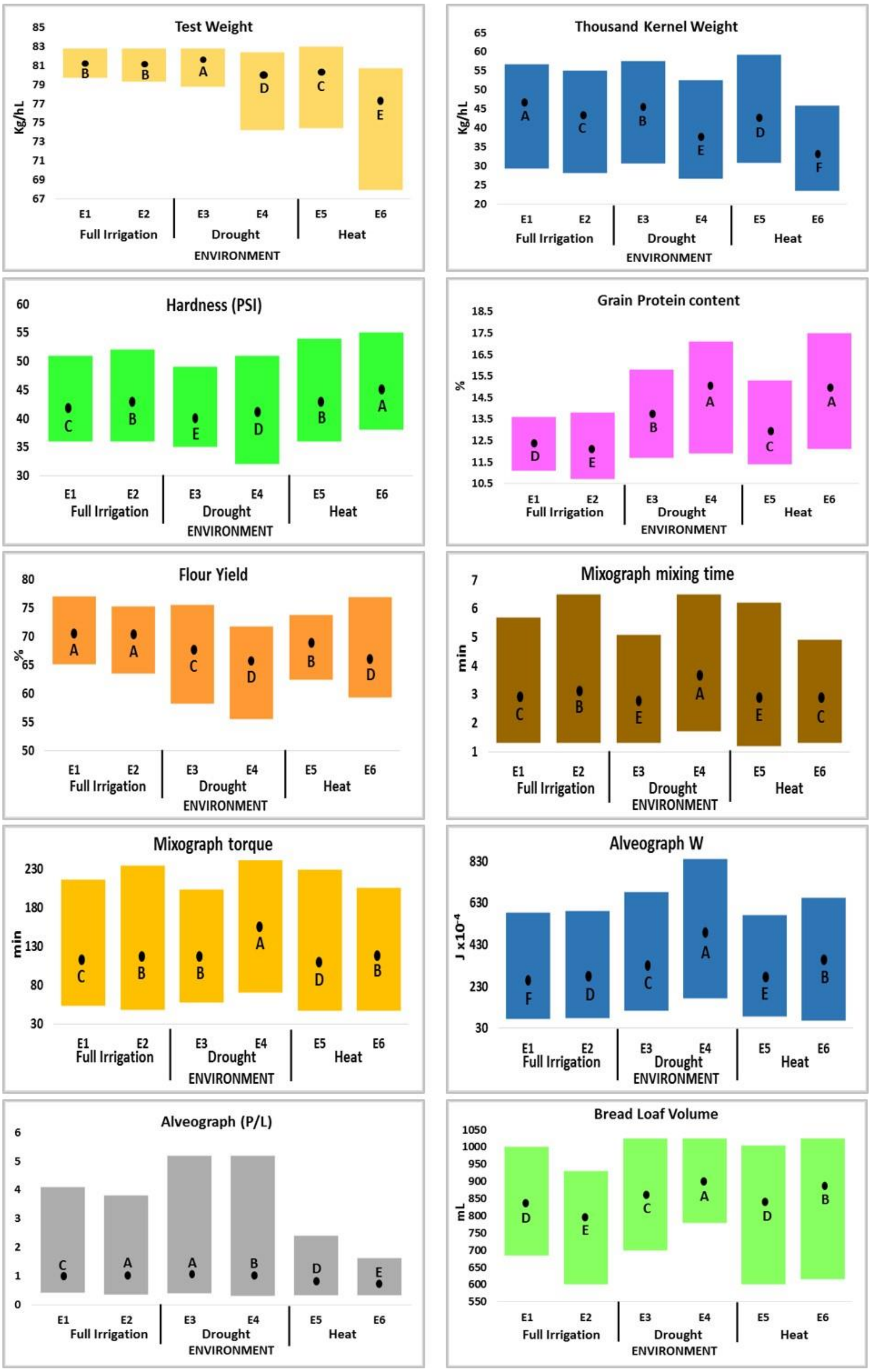
All the values were highly significant ($p<0.001$)

Mild heat stress also had negative effects on the both traits. These results are linked to those found for protein content and experimental flour yield: severe drought and heat stressed environments had the highest protein content (15.1 and 15%, respectively) and the lowest experimental flour yield (65.8 and 65.9%, respectively), whereas the full irrigation (drip and basin) environments had the lowest values for protein content (12.4 and 12.1%, respectively) but the highest for experimental flour yield (70.5 and 70.4%, respectively). Moderate drought and heat stressed environments gave intermediate values for these traits.

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

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

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



3.3Differences in genotypic response to the environment

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

Table 2 shows the mean values over two years for the 54 genotypes observed for the three quality parameters mentioned above in the three environments. Based on these mean values, a rank relative to the rest of the genotypes was also assigned to each genotype (from 1 to 54). The rank of each genotype is also given for the severe drought and heat stressed environments and in brackets, the number of positions the genotype moves up or down in relation to the ranking that each genotype had in the optimum environment. For example, ‘Sonalika’ had the mean value for alveograph W of 137 J*10-4 being placed in the 50th position in the optimum environment. This cultivar was on the 52nd position in the severe drought environment (two positions lower in ranking with respect to E1), and on the 49th position in the severe heat stress environment (one position higher in ranking with respect to E1). This analysis of ranking approach was used to distinguish genotypes that were affected differently by the environmental stress, since overall, all genotypes had an increase in gluten strength and extensibility and loaf volume in stressed environments (Fig. 1). For the ranking of alveograph P/L, lower values (extensible gluten) were considered better than higher values (tenacious gluten).

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

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

The same exercise was done with a portion of the rest of the recorded traits: test weight, thousand kernel weight, grain protein content and flour yield (Electronic Supplementary Table 3). Overall, these traits showed less stability than the above-mentioned traits related with gluten characteristics and end-use quality. For test weight, 25 and 24 genotypes had a change larger than ten positions in drought and heat stressed environments, respectively; for thousand kernel weight, 16 and 22 genotypes; for grain protein content, 26 and 25 genotypes; and for flour yield, 21 and 18 genotypes had a change in the rank larger than ten positions in drought and heat stressed environments. The scatter plots showing the correlation between the positions in the ranking of the genotypes (Electronic Supplementary Table 2 – sheet 2) also showed that for these traits, more genotypes changed their position in the ranking. In this case, for example, if we select the best 27 genotypes for test weight under the optimum environment, of those genotypes, eight would not be among the best 27 in the severe drought stress environment and ten of them would not be among the best 27 in the severe heat stress environment. A very similar situation was found for thousand kernel weight and for grain protein content, but not for flour yield, where the correlations were higher.

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

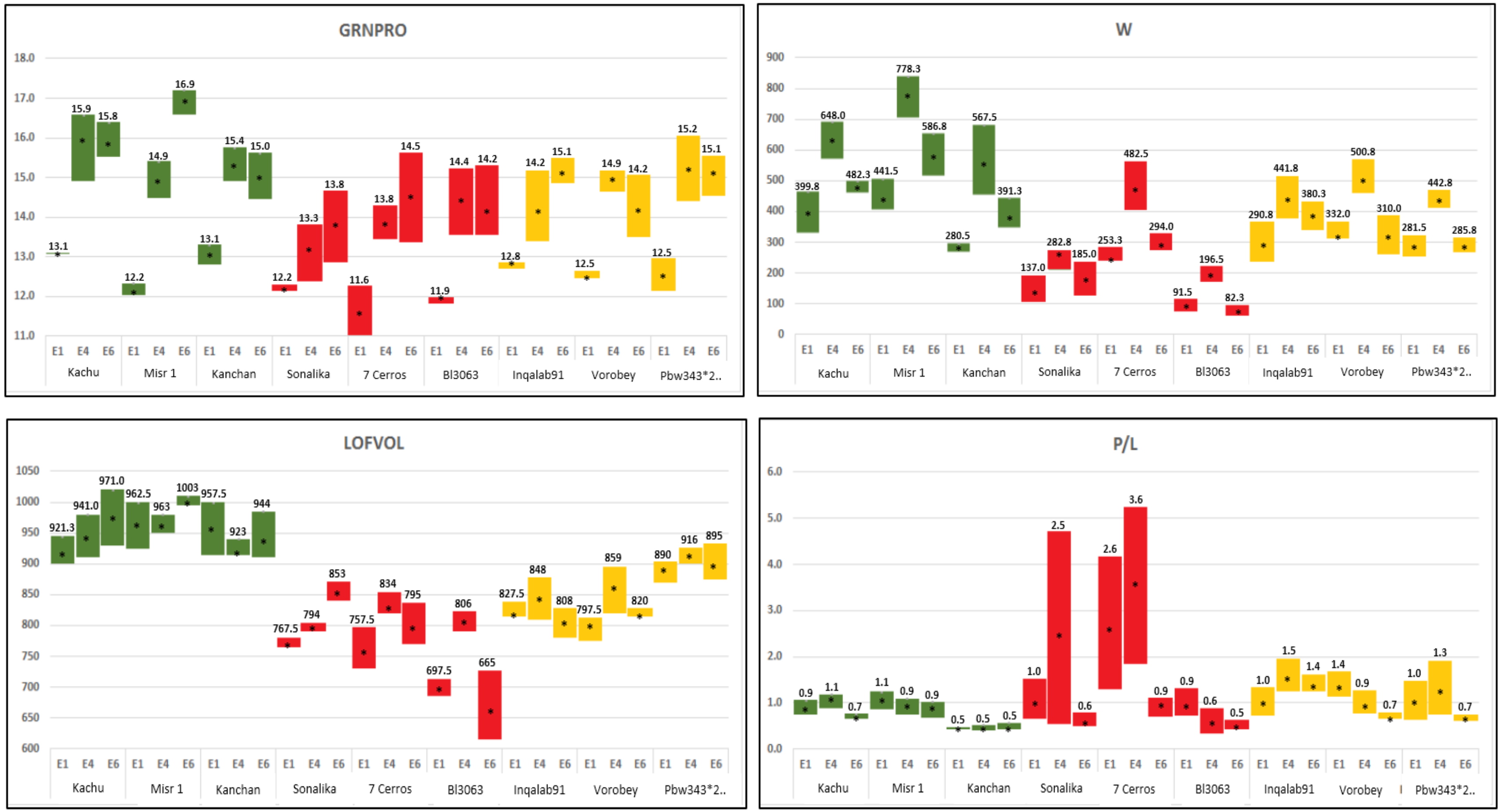
Genotype name/cross	**Alveograph W (J*10^{-4})**						**Alveograph P/L**						**Bread loaf volumen (mL)**					
	Optimum		Drought		Heat		Optimum		Drought		Heat		Optimum		Drought		Heat	
	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (Dif)	Mean	Rank	Mean	Rank (Dif)	Mean	Rank (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/Saual	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//Wbll1*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)

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

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



4. Discussion

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

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

Large ranges of values were found for most of the traits, something that does not happen in all breeding programs. The ANOVA showed strong and main environmental effects on traits related to grain filling and morphology (grain density and size) and directly affected by them (protein content and experimental flour yield). This agrees with previous studies on spring bread wheat (Mikhaylenko et al., 2000; Rozbicki et al., 2015; Studnicki et al., 2016), winter bread wheat (Bilgin et al. 2016) and durum wheat (Guzmán et al. 2016b; Rharrabti et al., 2003). The effects of severe drought, and particularly severe heat stress, on grain morphology traits (test weight and thousand kernel weight) were negative, leading to a much higher protein content level in these environments compared to the non-stressed, fully irrigated environments, which was probably due to a concentration effect (Guttieri et al., 2000; Saint-Pierre et al., 2008). The mild drought and heat stress environments also showed higher levels of protein content, although in these cases, the effects on grain density and size were moderate, which agrees with Guttieri et al. (2001) in the case of mild drought stress. These results from the severely stressed environments were expected, as drought and heat stress during grain filling are known to be responsible for shortening the grain growth period and improper grain filling, affecting the overall grain yield of the crop (Guttieri et al., 2001; Ramya et al., 2015; Rane et al., 2007). Reduced activity of the soluble starch synthase enzyme at high temperatures in the range 30-40 °C leads to a lower conversion of sucrose to starch (Jenner, 1994), which is another possible reason that explains the shriveling of grains under severe heat stress environment. Besides affecting protein content, the loss of grain plumpness under stress is the main reason for the lower flour milling yields (Guttieri et al., 2001; Spiertz et al., 2006). The different genotype by environment interactions (GxE, GxY, and GxExY, as shown in Table 1) also had a significant effect on flour yield (18% of the variation explained by those interactions), along with other grain characteristics commented above such as grain size (9%) and particularly test weight (18%). This also agrees with the stability showed by these genotypes in the exercise done in Electronic Supplementary Table 2, in which close to the half of the genotypes of the study had a significant change in their rank with respect to the other genotypes. These results do not support the strategy of selecting for these traits only under the optimum environment. Fortunately, CIMMYT runs elite and advanced yield trials under six different environments (including drought and heat), and the grains produced in these trials are evaluated by the breeders for grain size and good grain morphology (round shape, not deep crease), which probably helps to select for test weight and indirectly for flour yield under those stressed environment. Grain hardness is another parameter affecting milling quality . Compared to the full irrigation optimum environments, harder and softer grains were found in the drought and heat stress environments, respectively. Based on the literature, grains are probably harder in drought stress due to higher protein content (Peterson et al., 1992) than to smaller grain size (Gazza et al., 2008). But this does not provide a completely clear explanation as to why softer grains under heat stress were found, where protein content was higher and the grain was smaller compared to drought stress. In transgenic wheats overexpressing the HMW glutenin gene 1Dx5 Rakzsegi et al. (2005) found harder grains with a lower ratio of glutenins/gliadins than in the control. This could lead to a change in the network structure of the glutenin proteins that alters the efficiency of deposition affecting the overall seed development and finally grain hardness. In our case, the heat stress probably led to a lower ratio of glutenins/gliadins (Blumenthal et al., 1998; Li et al., 2013), opposing the mutated line described above to softer grains.

The genotypic effect was considerable and the main effect for all the traits related to gluten quality (gluten strength and extensibility, and bread loaf volume). The strong genetic control of these traits has been already reported by several authors (Souza et al. 1993; Yong et al., 2004). These results were positive in relation to the efficiency of the breeding program, as strong genetic control will make the selection process more efficient and will obtain genetic gains faster for the targeted traits in several environments. Besides, the environment effect was also significant for these traits. Both abiotic stresses led to higher gluten strength, probably due to the higher protein content levels. Particularly, in the severe drought stress environment, most of the samples had very strong gluten and, in general, somewhat more tenacious (higher alveograph P/L) gluten than in the optimum environment. This is explained by the higher protein content and probably by a higher proportion of polymeric glutenin too, as it has been shown before for bread (Panozzo et al., 2001) and durum wheat (Flagella et al., 2010). For severe heat stress, although protein content was higher than in severe drought stress environment, the gluten strength was not as high as in severe drought stress, and gluten extensibility (lower alveograph P/L) was higher than in any other environment. This is related to the findings of Blumenthal et al. (1995) and Corbellini et al. (1997), who reported a decrease in glutenin/gliadin ratio and in the percentage of very large glutenin polymers during grain-filling under heat stress conditions. Therefore, these differential changes in the amount of polymeric protein are likely the main factors that explain why gluten strength did not increase as much as under severe drought stress, and instead gluten extensibility was increased. Our results for extensibility fully agree with previous findings (Wrigley et al., 1994; Blumenthal et al., 1995; Li et al., 2013), although we did not detect the absolute weakening dough effect in severe heat stress usually reported in Australian studies, probably due to the high grain protein level reached in our severe heat stressed trials. The weakening dough effect was found in the mild heat stressed trial. Finally bread-making quality was, in general, favored by the abiotic stresses, probably due to the higher gluten strength in most of the genotypes (Mikhaylenko et al., 2000), and a more balanced or extensible gluten in others (Peterson et al. 1998).

To assess the efficiency of our breeding approach (selection done under optimum conditions), the importance of genotype by environment interactions (including interaction with year and triple interaction of genotype, environment and year) cannot be ignored, particularly for alveograph P/L, for which the variation explained by those interactions reach the 38% of the total variation. Gluten extensibility represented by alveograph P/L is an important trait within the CIMMYT breeding program for selection, as it is required for all bread wheat products, and it is used for internal end-use type classification (Guzmán et al., 2016a). Having a high level of genotype by environment interactions indicates that the response to the environment of the genotypes is not homogeneous and, therefore, carrying the selection in only one location would not lead to genetic gains for a specific trait across different environments. The analysis of the ranking of the genotypes for this trait showed a considerable part of the genotypes having a change in their ranking position in severe and drought stress environments with respect to the optimum environment. This is probably not a big concern for the severe heat environment because although the ranking of genotypes was quite different compared to the optimum environment, a large majority of the genotypes gained gluten extensibility under those conditions, which is in most cases, a desirable effect. This means that genotypes selected for good gluten extensibility under optimum conditions will likely have good or even better extensibility under heat stress environment too, as it was shown in Fig. 2 (all genotypes except Inqalab91). On the other hand, this will probably not always be the case for drought stress conditions, where there is a slight trend to increase gluten tenacity, and some genotypes that show balanced gluten in optimum environment changed to tenacious gluten under drought stress. The genotypes Inqalab91, PBW343*2/KUKUNA//PARUS /3/PBW343*2/KUKUNA or to a lesser extent Kachu (Fig. 2) are good examples of this. This is not a desirable effect and, although there is a small percentage of genotypes that show the change, an evaluation process under the two environments could be beneficial for the selection of genotypes with good extensibility for semi-arid environments. It is also necessary to understand the high and low molecular weight glutenins that are present in stably performing genotypes, which can also predict the stability. Bread-making quality was also more affected than other traits by genotype by environment interactions (18.7 % of the total variation). In this case, drought environment enhanced loaf volume in all genotypes except one, and most genotypes showed high values. Therefore, selecting for bread-making under drought stress will not add much value to the breeding process. However, in the severe heat stress environment, while most of the genotypes had a considerable increase in loaf volume, seven of the total 54 genotypes showed lower loaf volumes compared to the optimum environment (Bl3063 or Inqalab 91 in Fig.2), and around 31% of them (17 genotypes) had a significant change in their rankings. This may indicate that for a more accurate selection for this specific product (pan bread, mechanized bread industry), genotypes targeted for mega-environment 5 (heat stressed irrigated areas) could also be analyzed for bread-making quality under those conditions. Due to the lack of capacity to perform many more quality analysis in the same period of time (currently around 2,400 breeding lines are analyzed in four months at CIMMYT), using rapid tests to predict quality traits (Guzmán et al., 2016c) or new breeding technologies such as genomic selection (Battenfield et al., 2016) could provide good possibilities for the prediction of quality traits for several environments. It is also important to mention that CIMMYT provides the wheat germplasm developed in its breeding program to national partners worldwide, who will test the materials under their specific environmental conditions. The materials that show promising agronomic performance will be tested for grain quality too, and, therefore, the selection process continues out of Mexico, before a wheat genotype is released as a variety.

5. Conclusions

The research shows the importance of the genotype and the environment to explain the diversity for quality traits. It is evident that the breeding program at CIMMYT has developed spring bread wheat cultivars with diverse levels of gluten strength combined with good extensibility, and in most cases, their desirable gluten characteristics and end-use quality when they are grown under abiotic stress conditions (drought and heat) are maintained. The current selection strategy for quality traits carried out under optimum conditions seems to be suitable for obtaining acceptable quality traits across several environments for most of the traits. Under optimum conditions, a good range of variability is found for most important quality traits, which allows selecting good genotypes and discarding the bad ones. Under drought and heat stressed environments, most of the genotypes improve their quality traits, which makes selection process more difficult. Selection for gluten extensibility under drought stress and for bread-making quality under heat stress could be recommended when resources are available to ensure an optimum performance of the genotypes across diverse environments.

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

Nayelli Hernández-Espinosa[1], Suchismita Mondal[1], Enrique Autrique[1], Héctor Gonzalez-Santoyo[1], José Crossa[1], Julio Huerta-Espino[2], Ravi Prakash Singh[1], Carlos Guzmán*[1]

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

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

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

For Field Crops Research

Abstract

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

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

1. Introduction

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

The spring bread wheat (*T. aestivum* L.) breeding program of the International Maize and Wheat Improvement Center (CIMMYT) breeds high yielding, disease resistant and stress tolerant wheat germplasm and annually distributes it worldwide to national partners mainly in four target areas (mega-environments): 1) Irrigated areas (Northwestern India, Pakistan, Iran, Egypt, China, Mexico, etc.); 2) High rainfall areas (West of Asia, Eastern Africa, highlands of Mexico, etc.); 3) Semi-arid areas (North Africa, West Asia, South America, etc.); and 4) Warmer areas (Nepal, Bangladesh, Eastern Gangetic Plains of India, Southern Pakistan, Sudan, etc.) (Rajaram and van Ginkel, 1993). One key objective of the CIMMYT breeding program is to improve end-use quality in conjunction with other relevant traits to satisfy all stakeholders of the wheat value chain: farmers (bold and plump grain), millers (high test weight and high flour yield), food manufacturers (processing quality) and consumers (end-use and nutritional quality) (Guzmán et al., 2016a).

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

Several studies have focused on the effects of the environment and abiotic stresses on the expression of wheat quality, some of which are already well identified and understood. For example, heat stress has been associated with a dough weakening effect (Blumenthal et al., 1993; Corbellini et al., 1997), whereas with drought stress, there is an increase in the protein content and polymeric protein, which produces the opposite effect (Guttieri et al., 2001, 2000). However, many of these studies are characterized by having a limited number of genotypes (Corbellini et al., 1997; Guttieri et al. 2000; Li et al., 2013; Rozbicki et al., 2015), and hence, the lack of diversity in the responses of the cultivars could have led to the above conclusions. Williams et al. (2008) reviewed the studies about environment and GxE effects on bread wheat quality and recommended further research on this topic since our understanding of the environmental influence and the presence of GxE on trait expression is incomplete.

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

2. Materials and methods

2.1Field Experiment

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

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

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

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

2.2Grain parameters

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

2.3Milling

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

2.4Flour parameters, rheological and baking test

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

2.5Statistical analysis

A fixed effect linear model combined across years, environments and their interactions was performed for all 12 quality parameters using the PROC ANOVA procedure of the SAS statistical software (2010). Additionally, averages, values and least significant difference (LSD) of each quality trait for each environment were calculated averaging across years and genotypes (Fig. 1). Furthermore, mean values and least significant difference (LSD) for some quality traits and specific genotypes in three environments (E1, E4 and E6) averaged across years were calculated.

3. Results

3.1Effects of genotype, environment, year and their interactions

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

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 ($p<0.001$)

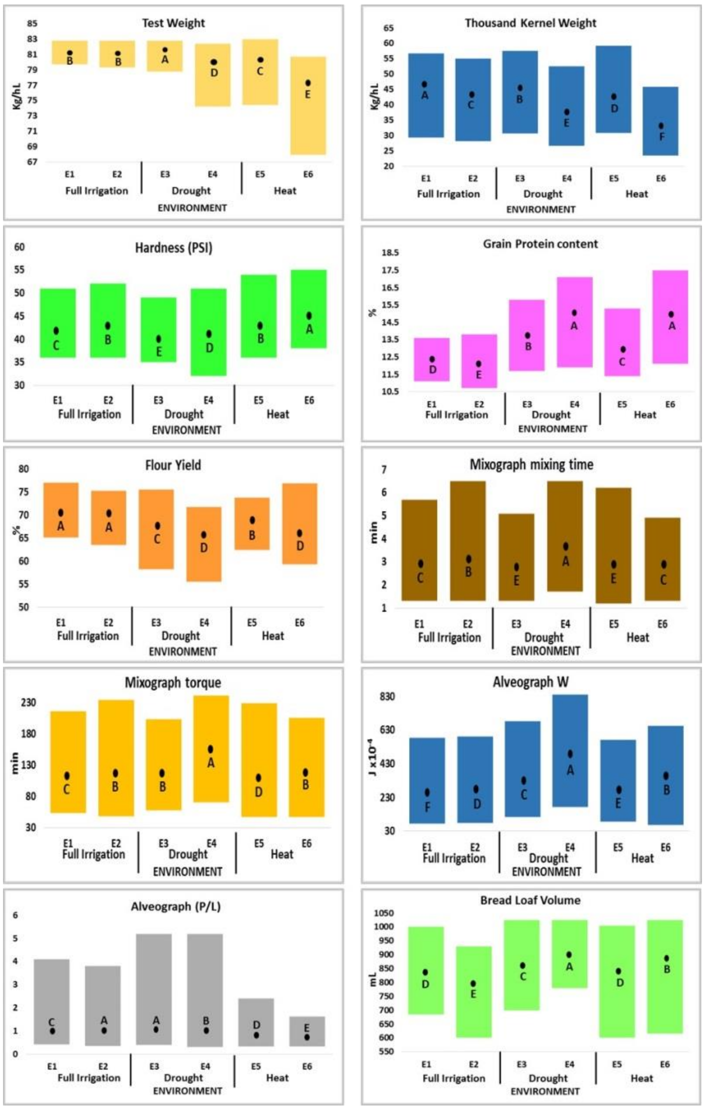
3.2Effect of environments on quality traits

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

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

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

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



3.3Differences in genotypic response to the environment

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

Table 2 shows the mean values over two years for the 54 genotypes observed for the three quality parameters mentioned above in the three environments. Based on these mean values, a rank relative to the rest of the genotypes was also assigned to each genotype (from 1 to 54). The rank of each genotype is also given for the severe drought and heat stressed environments and in brackets, the number of positions the genotype moves up or down in relation to the ranking that each genotype had in the optimum environment. For example, 'Sonalika' had the mean value for alveograph W of 137 J*10-4 being placed in the 50th position in the optimum environment. This cultivar was on the 52nd position in the severe drought environment (two positions lower in ranking with respect to E1), and on the 49th position in the severe heat stress environment (one position higher in ranking with respect to E1). This analysis of ranking approach was used to distinguish genotypes that were affected differently by the environmental stress, since overall, all genotypes had an increase in gluten strength and extensibility and loaf volume in stressed environments (Fig. 1). For the ranking of alveograph P/L, lower values (extensible gluten) were considered better than higher values (tenacious gluten).

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

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

The same exercise was done with a portion of the rest of the recorded traits: test weight, thousand kernel weight, grain protein content and flour yield (Electronic Supplementary Table 3). Overall, these traits showed less stability than the above-mentioned traits related with gluten characteristics and end-use quality. For test weight, 25 and 24 genotypes had a change larger than ten positions in drought and heat stressed environments, respectively; for thousand kernel weight, 16 and 22 genotypes; for grain protein content, 26 and 25 genotypes; and for flour yield, 21 and 18 genotypes had a change in the rank larger than ten positions in drought and heat stressed environments. The scatter plots showing the correlation between the positions in the ranking of the genotypes (Electronic Supplementary Table 2 – sheet 2) also showed that for these traits, more genotypes changed their position in the ranking. In this case, for example, if we select the best 27 genotypes for test weight under the optimum environment, of those genotypes, eight would not be among the best 27 in the severe drought stress environment and ten of them would not be among the best 27 in the severe heat stress environment. A very similar situation was found for thousand kernel weight and for grain protein content, but not for flour yield, where the correlations were higher.

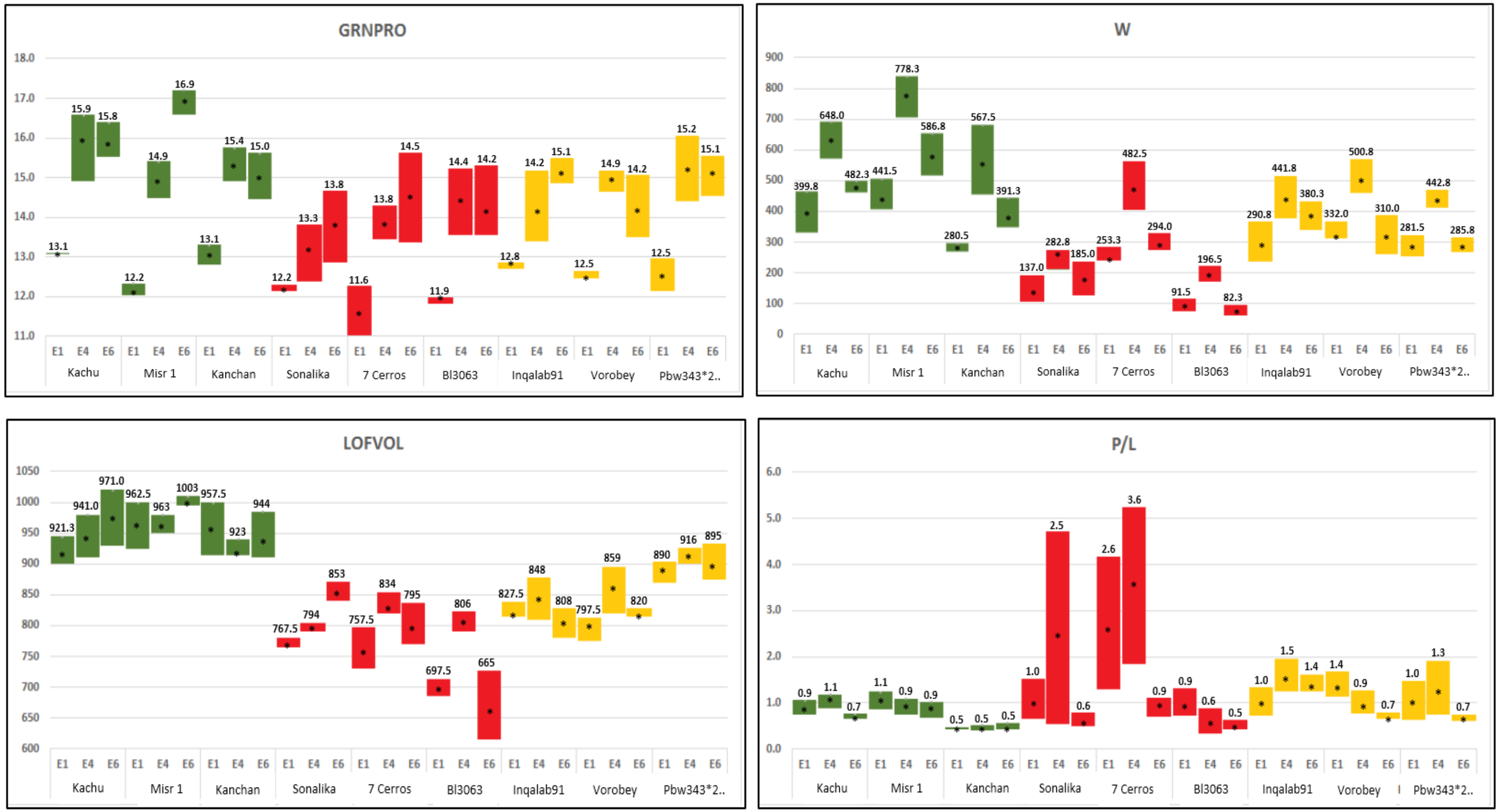
Table 2. Variability for three quality traits of 54 bread wheat genotypes across three environments (full drip irrigation or optimum, severe drought and severe heat). Average values were calculated averaging field replicates and two cropping years. The position in the ranking of each genotype is indicated. For severe drought and heat stress environment, the positions won or lost in the ranking for each genotype with respect to 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/Saual	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//Wbll1*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)

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

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



4. Discussion

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

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

Large ranges of values were found for most of the traits, something that does not happen in all breeding programs. The ANOVA showed strong and main environmental effects on traits related to grain filling and morphology (grain density and size) and directly affected by them (protein content and experimental flour yield). This agrees with previous studies on spring bread wheat (Mikhaylenko et al., 2000; Rozbicki et al., 2015; Studnicki et al., 2016), winter bread wheat (Bilgin et al. 2016) and durum wheat (Guzmán et al. 2016b; Rharrabti et al., 2003). The effects of severe drought, and particularly severe heat stress, on grain morphology traits (test weight and thousand kernel weight) were negative, leading to a much higher protein content level in these environments compared to the non-stressed, fully irrigated environments, which was probably due to a concentration effect (Guttieri et al., 2000; Saint-Pierre et al., 2008). The mild drought and heat stress environments also showed higher levels of protein content, although in these cases, the effects on grain density and size were moderate, which agrees with Guttieri et al. (2001) in the case of mild drought stress. These results from the severely stressed environments were expected, as drought and heat stress during grain filling are known to be responsible for shortening the grain growth period and improper grain filling, affecting the overall grain yield of the crop (Guttieri et al., 2001; Ramya et al., 2015; Rane et al., 2007). Reduced activity of the soluble starch synthase enzyme at high temperatures in the range 30-40 °C leads to a lower conversion of sucrose to starch (Jenner, 1994), which is another possible reason that explains the shriveling of grains under severe heat stress environment. Besides affecting protein content, the loss of grain plumpness under stress is the main reason for the lower flour milling yields (Guttieri et al., 2001; Spiertz et al., 2006). The different genotype by environment interactions (GxE, GxY, and GxExY, as shown in Table 1) also had a significant effect on flour yield (18% of the variation explained by those interactions), along with other grain characteristics commented above such as grain size (9%) and particularly test weight (18%). This also agrees with the stability showed by these genotypes in the exercise done in Electronic Supplementary Table 2, in which close to the half of the genotypes of the study had a significant change in their rank with respect to the other genotypes. These results do not support the strategy of selecting for these traits only under the optimum environment. Fortunately, CIMMYT runs elite and advanced yield trials under six different environments (including drought and heat), and the grains produced in these trials are evaluated by the breeders for grain size and good grain morphology (round shape, not deep crease), which probably helps to select for test weight and indirectly for flour yield under those stressed environment. Grain hardness is another parameter affecting milling quality. Compared to the full irrigation optimum environments, harder and softer grains were found in the drought and heat stress environments, respectively. Based on the literature, grains are probably harder in drought stress due to higher protein content (Peterson et al., 1992) than to smaller grain size (Gazza et al., 2008). But this does not provide a completely clear explanation as to why softer grains under heat stress were found, where protein content was higher and the grain was smaller compared to drought stress. In transgenic wheats overexpressing the HMW glutenin gene 1Dx5 Rakzsegi et al. (2005) found harder grains with a lower ratio of glutenins/gliadins than in the control. This could lead to a change in the network structure of the glutenin proteins that alters the efficiency of deposition affecting the overall seed development and finally grain hardness. In our case, the heat stress probably led to a lower ratio of glutenins/gliadins (Blumenthal et al., 1998; Li et al., 2013), opposing the mutated line described above to softer grains.

The genotypic effect was considerable and the main effect for all the traits related to gluten quality (gluten strength and extensibility, and bread loaf volume). The strong genetic control of these traits has been already reported by several authors (Souza et al. 1993; Yong et al., 2004). These results were positive in relation to the efficiency of the breeding program, as strong genetic control will make the selection process more efficient and will obtain genetic gains faster for the targeted traits in several environments. Besides, the environment effect was also significant for these traits. Both abiotic stresses led to higher gluten strength, probably due to the higher protein content levels. Particularly, in the severe drought stress environment, most of the samples had very strong gluten and, in general, somewhat more tenacious (higher alveograph P/L) gluten than in the optimum environment. This is explained by the higher protein content and probably by a higher proportion of polymeric glutenin too, as it has been shown before for bread (Panozzo et al., 2001) and durum wheat (Flagella et al., 2010). For severe heat stress, although protein content was higher than in severe drought stress environment, the gluten strength was not as high as in severe drought stress, and gluten extensibility (lower alveograph P/L) was higher than in any other environment. This is related to the findings of Blumenthal et al. (1995) and Corbellini et al. (1997), who reported a decrease in glutenin/gliadin ratio and in the percentage of very large glutenin polymers during grain-filling under heat stress conditions. Therefore, these differential changes in the amount of polymeric protein are likely the main factors that explain why gluten strength did not increase as much as under severe drought stress, and instead gluten extensibility was increased. Our results for extensibility fully agree with previous findings (Wrigley et al., 1994; Blumenthal et al., 1995; Li et al., 2013), although we did not detect the absolute weakening dough effect in severe heat stress usually reported in Australian studies, probably due to the high grain protein level reached in our severe heat stressed trials. The weakening dough effect was found in the mild heat stressed trial. Finally bread-making quality was, in general, favored by the abiotic stresses, probably due to the higher gluten strength in most of the genotypes (Mikhaylenko et al., 2000), and a more balanced or extensible gluten in others (Peterson et al. 1998).

To assess the efficiency of our breeding approach (selection done under optimum conditions), the importance of genotype by environment interactions (including interaction with year and triple interaction of genotype, environment and year) cannot be ignored, particularly for alveograph P/L, for which the variation explained by those interactions reach the 38% of the total variation. Gluten extensibility represented by alveograph P/L is an important trait within the CIMMYT breeding program for selection, as it is required for all bread wheat products, and it is used for internal end-use type classification (Guzmán et al., 2016a). Having a high level of genotype by environment interactions indicates that the response to the environment of the genotypes is not homogeneous and, therefore, carrying the selection in only one location would not lead to genetic gains for a specific trait across different environments. The analysis of the ranking of the genotypes for this trait showed a considerable part of the genotypes having a change in their ranking position in severe and drought stress environments with respect to the optimum environment. This is probably not a big concern for the severe heat environment because although the ranking of genotypes was quite different compared to the optimum environment, a large majority of the genotypes gained gluten extensibility under those conditions, which is in most cases, a desirable effect. This means that genotypes selected for good gluten extensibility under optimum conditions will likely have good or even better extensibility under heat stress environment too, as it was shown in Fig. 2 (all genotypes except Inqalab91). On the other hand, this will probably not always be the case for drought stress conditions, where there is a slight trend to increase gluten tenacity, and some genotypes that show balanced gluten in optimum environment changed to tenacious gluten under drought stress. The genotypes Inqalab91, PBW343*2/KUKUNA//PARUS /3/PBW343*2/KUKUNA or to a lesser extent Kachu (Fig. 2) are good examples of this. This is not a desirable effect and, although there is a small percentage of genotypes that show the change, an evaluation process under the two environments could be beneficial for the selection of genotypes with good extensibility for semi-arid environments. It is also necessary to understand the high and low molecular weight glutenins that are present in stably performing genotypes, which can also predict the stability. Bread-making quality was also more affected than other traits by genotype by environment interactions (18.7 % of the total variation). In this case, drought environment enhanced loaf volume in all genotypes except one, and most genotypes showed high values. Therefore, selecting for bread-making under drought stress will not add much value to the breeding process. However, in the severe heat stress environment, while most of the genotypes had a considerable increase in loaf volume, seven of the total 54 genotypes showed lower loaf volumes compared to the optimum environment (Bl3063 or Inqalab 91 in Fig.2), and around 31% of them (17 genotypes) had a significant change in their rankings. This may indicate that for a more accurate selection for this specific product (pan bread, mechanized bread industry), genotypes targeted for mega-environment 5 (heat stressed irrigated areas) could also be analyzed for bread-making quality under those conditions. Due to the lack of capacity to perform many more quality analysis in the same period of time (currently around 2,400 breeding lines are analyzed in four months at CIMMYT), using rapid tests to predict quality traits (Guzmán et al., 2016c) or new breeding technologies such as genomic selection (Battenfield et al., 2016) could provide good possibilities for the prediction of quality traits for several environments. It is also important to mention that CIMMYT provides the wheat germplasm developed in its breeding program to national partners worldwide, who will test the materials under their specific environmental conditions. The materials that show promising agronomic performance will be tested for grain quality too, and, therefore, the selection process continues out of Mexico, before a wheat genotype is released as a variety.

5. Conclusions

The research shows the importance of the genotype and the environment to explain the diversity for quality traits. It is evident that the breeding program at CIMMYT has developed spring bread wheat cultivars with diverse levels of gluten strength combined with good extensibility, and in most cases, their desirable gluten characteristics and end-use quality when they are grown under abiotic stress conditions (drought and heat) are maintained. The current selection strategy for quality traits carried out under optimum conditions seems to be suitable for obtaining acceptable quality traits across several environments for most of the traits. Under optimum conditions, a good range of variability is found for most important quality traits, which allows selecting good genotypes and discarding the bad ones. Under drought and heat stressed environments, most of the genotypes improve their quality traits, which makes selection process more difficult. Selection for gluten extensibility under drought stress and for bread-making quality under heat stress could be recommended when resources are available to ensure an optimum performance of the genotypes across diverse environments.

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