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

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Abstract

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

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

1. Introduction

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

At the International Maize and Wheat Improvement Center (CIMMYT) durum wheat breeding draws on a large, genetically wide, gene pool to develop germplasm, which is widely distributed among breeding programs of durum-producing countries. The priority of the wheat breeding program is to develop high-yielding, disease-resistant varieties that can tolerate drought, heat stresses, and produce high grain quality. The latter is essential in durum wheat varieties to be accepted by industry, local food manufacturers and consumers. The most important parameters affecting industrial quality for pasta-making from durum wheat grain are probably gluten quality (strength) and the yellow color of semolina. Although environmental and processing conditions play a significant role in these two traits, research has shown that they are under strong genetic control. The favourable dough or gluten properties of durum lines have been associated mainly with the presence of specific glutenins coded by the *Glu-B1* and *Glu-B3* loci (Ammar, Kronstad & Morris, 2000; Boggini & Pogna, 1989; Boggini, Tusa & Pogna, 1995; Brites & Carrillo, 2001; Peña, Zarco-Hernandez, Amaya-Celis & Mujeeb-Kazi, 1994). Semolina color will depend to a great extent on the genes involved in pigment accumulation (enzymes involved in pigment biosynthesis as phytoene synthase and pigment degradation as lipoxygenase or polyphenol oxidase) (see Ficco et al., 2014 for a review). Apart from pasta, durum wheat is widely used to elaborate other non-baked goods as couscous and bulgur. In the former high yellow color is appreciated while in bulgur light yellow color is preferred (Belibagli et al. 2009). Both products also demand high protein content to avoid stickiness and to have high water absorption capacity (Bayram and Öner 2006; Ounane et al. 2006).

Although durum wheat generally exhibits inferior bread-making quality compared with bread wheat (*T. aestivum* L. ssp. *aestivum* .) in terms of loaf volume and crumb structure (Boggini et al., 1995; Peña et al., 1994), approximately 24% of the global durum wheat production, and up to 70-90% in some Middle East countries, is used for bread-making (Quaglia, 1988). Speciality breads made with durum are common in the south of Italy (Boggini et al., 1995), and this popularity is spreading to other countries (Sissons, 2008). In the regions of West Asia and North Africa 50% of durum wheat is processed into single and two-layered flat breads and 35% is used for leavened breads (Liu, Shepherd & Rathjen, 1996). In those countries durum breads are very popular with consumers probably due to their sensory properties, particularly to their pleasant aroma (Rao, Pozniak, Hucl & Briggs, 2010) and special taste. They are slow to go stale (Pasqualone, Summo, Bilancia & Caponi, 2007) and consequently their longer shelf-life due to the high water absorption of durum wheat flour related to increased content in damage starch, is another desirable characteristic of durum wheat breads (Boyacioğlu & D´Appolonia, 1994). Besides, although yellow bread wheat flours are typically undesirable for bread-making, in the case of durum wheat breads a distinctive yellow colour is an important factor influencing whether it is accepted by the consumer (Brescia et al., 2007; Pasqualone, Caponio & Simeone, 2004).

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

Besides processing and end-use quality, nutritional quality is also becoming an important priority in wheat breeding. Wheat is good source of diverse beneficial compounds including fibre, phytochemicals and micronutrients. Among different micronutrients, iron and zinc are deficiency in the diet of two billion people (WHO 2012), and because of this have become the focus of micronutrient biofortification, which serves to enhance iron and zinc grain concentration. Modern wheat cultivars have been shown to be poor sources of these micronutrients for meeting daily requirements for humans (Cakmak, Pfeiffer & McClafferty, 2010). Not much research has been carried out about durum wheat and iron and zinc content. According to Ficco et al. (2009), there is some genetic variation to breed for iron and zinc in durum wheat, although more studies screening for larger genetic variability and examining the environment effect are required.

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

2. Materials and methods

2.1 Plant Materials/Agronomic trials

A trial consisting of six CIMMYT durum wheat cultivars (Mexicali C75, Yavaros C79, Altar C84, Atil C2000, Jupare C2001 and Cirno C2008) and two bread wheat ones (Kachu and Roelfs F2007), were sown in 2012-2013 and 2013-2014 crop seasons in Ciudad Obregon, Sonora, in northwestern Mexico. The trial was planted with two replicates in a randomized complete Block Design under six different environmental conditions: full drip irrigation (optimum conditions), full basin irrigation, reduced irrigation or medium drought stress, severe drought stress, medium heat stress, and severe heat stress. All the trials were planted in November except medium heat stress (planted in January) and heat stress (planted in February). All the trials had full irrigation (>500 mm) except medium drought stress (300 mm) and severe drought stress (180mm). Weed, diseases, and insects were all well controlled. In all the trials, N 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 except in severe drought stress (50 N units). At maturity whole plots were harvested and 1kg of seed from each of the wheat lines was used for analyzing the quality traits.

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

2.2 Grain and flour parameters

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

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

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

2.3 Rheological tests

Sodium dodecyl sulfate (SDS) sedimentation volume was carried out according to the modified protocol described in Peña et al. (1990) using 1g of flour or of whole-meal flour. Additionally, 35g flour samples were run 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 60g flour samples and constant water absorption (55%). Higher water absorption than in the official methodology (50%) was used to compensate for the typically greater water absorption caused by high levels of starch damage occurring during milling of the very hard durum wheat grain (Ammar et al., 2000; Dexter, Preston, Martin & Gander, 1994, Peña et al., 1994).

2.4 Bread-making characteristics

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

All the phenotypic data recorded is shown in ESM2.

2.5 Statistical Analysis

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

The basic conventional fixed effect linear model for describing the univariate mean response of genotypes in environments and years, and their interactions is $y_{ijrk} = \mu + R(EY)_{rjk} + Y_k + G_i + E_j + (GY)_{ik} + (EY)_{jk} + (GE)_{ij} + (GEY)_{ijk} + \varepsilon_{ijrk}$, where y_{ijrk} is the response of the ith genotype (i=1,2,…,I) in the rth replicated (r=1,2,…,L) of the jth environment (j=1,2,…,J) and the kth year (k=1,2,…,M), μ is the grand mean over all genotypes, environments, and years, $R(EY)_{rjk}$ is the effect of the rth replicate within the jth environment and the kth year, Y_k is the effect of the kth year, G_i is the effect of the ith durum wheat line, E_j is the effect of the jth environment, $(GY)_{ik}$ is the effect of the interaction between the ith durum wheat line with the kth year, $(EY)_{jk}$ is the effect of the interaction between the jth environment with the ith durum wheat line, $(GE)_{ij}$ is the effect of the interaction between the ith durum wheat line with the kth year, $(GEY)_{ijk}$ is the effect of the triple interaction among the ith durum wheat line with jth environment and the kth year, and ε_{ijrk} is the error assumed to be normally and independent distributed NID $(0, \sigma^2)$, where σ^2 is the error variance, assumed to be constant.

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

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

3. Results

3.1 Effects of genotype, environment, year and their interactions

The results of the analysis of the variance (Table 1) showed that almost all the factors had a significant effect on the different traits. Genotype and environment were the most important factors explaining the variation found, followed by GxE and ExY interaction. The environment effect was particularly high in traits as test weight, thousand kernel weight, Zn content and alveograph P, which explained more than 47% of the variation. In the case of Fe content, flour yellowness, SDS sedimentation volume, mixograph optimum dough mixing time and torque, alveograph L, the genotype effect was predominant and responsible for at least 25% of the variation, and in some cases as flour SDS sedimentation and bread loaf volume explained more than 55% of variation. For this latter trait the GxE effect was also particularly important. GxE effect also explained about 12% of the variation in traits Fe and Zn content, flour SDS sedimentation volume, mixograph optimum dough mixing time and torque, and alveograph P, L and P/L. The ExY effect was high for test weight (30%) and also important for thousand kernel weight, flour yellowness, flour SDS sedimentation volume and alveograph W. The triple interaction GxExY effect was significant for mixograph optimum dough development time and torque and alveograph W. The effects of the particular year and the GxY were not significant for all of the traits.

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

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

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

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

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

In traits traditionally related to grain size (grain protein, Fe and Zn content), the response of the genotypes was not consistent in all environments. For grain protein content, the environments with lower grain size (severe drought stress and severe heat stress) had significantly higher values. This did not happen in the case of Fe content, for which severe heat stress showed also the lowest value. On the contrary, for Zn content severe heat stress resulted in the highest value (56 mg/kg). In the case of severe drought stress, higher values than in the other environments were found for both micronutrients. All the genotypes showed large ranges for grain protein content. Jupare did not show a wide range for Zn or Fe content; Yavaros and Cirno were also stable across environments for Fe content. Atil was the genotype, which had higher average grain protein and Fe content, while Altar was for Zn content.

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

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

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

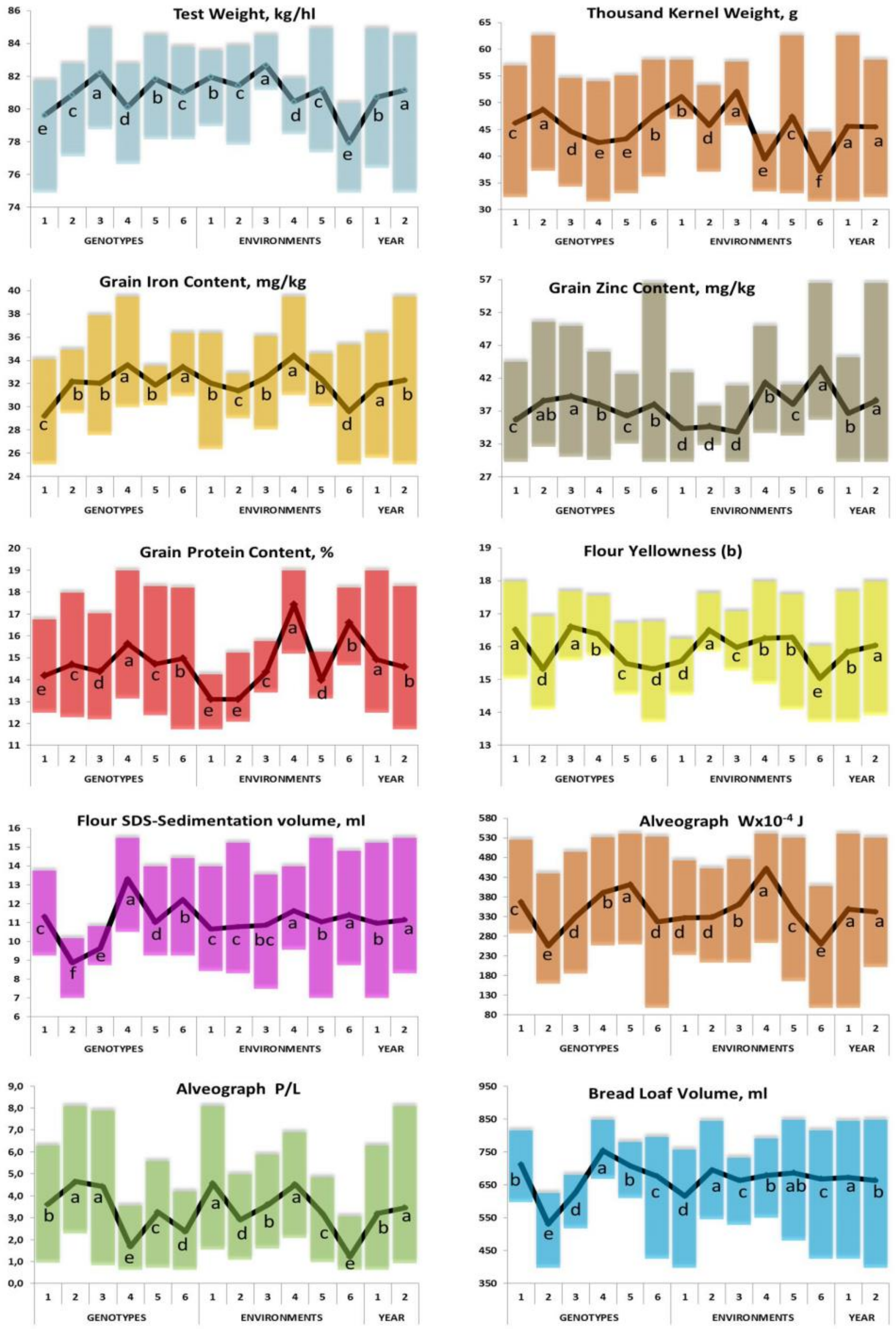

Figure 1- Maximum, average and minimum values for different quality traits obtained by each genotype in the whole trial, and in each environment and year by the six genotypes. The range of values (maximum and minimum) is represented by bars. The average values in each case are represented with a continuous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

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

3.3 Glutenins composition and bread-making analysis

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

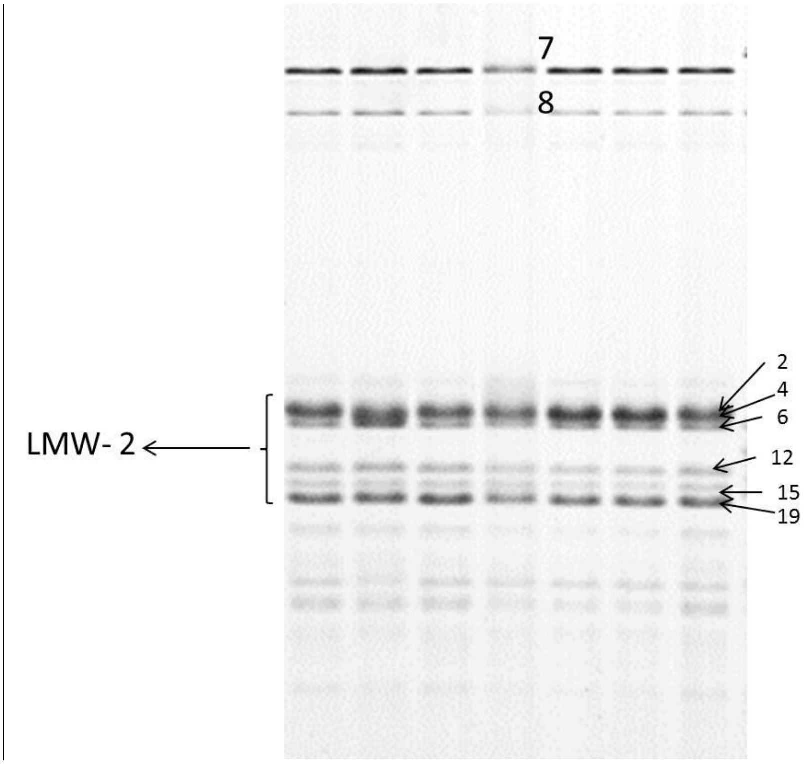

Figure 2 – SDS-PAGE electrophoresis of HMW and LMW glutenins of durum wheat samples. Lanes are as follow: 1, Mexicali C75; 2,Yavaros 79; 3, Altar 84; 4, Atil C2000; 5, Jupare C 2001; 6, Cirno C 2008.

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

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

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

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

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

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

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

3.4 Relationships between quality parameters

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

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

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

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

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

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

4. Discussion

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

All the above results strengthen the well-established idea that some quality traits, particularly those related with processing and pasta-making quality have a very strong genetic control and therefore the genotypes respond consistently across environments for these characteristics. Most of those traits (flour SDS sedimentation volume, mixograph optimum dough mixing time and torque, alveograph W) also showed consistent correlations between them in the different environments. In a previous study (Li et al., 2013) it was revealed that in general drought stress favored gluten strength and yellowness (processing and pasta-making quality), which was confirmed with our study. On the contrary, heat stress was found to reduce gluten strength by both studies. Li et al. 2013 found a significant increase in flour yellowness in heat stressed environments, probably due to a "concentration effect" of the pigment carotenoid. In our experiment, flour yellowness was reduced in severe heat stress trial, which disagree with Li et al. (2013). This discrepancy with this study carried out in the same location could be due to a different tolerance to heat stress of the genotypes used in both studies, or to other environmental changes. Temperatures in May, when grain filling for severe heat stress trial happened, were something higher in our study (maximum temperatures around 1.5 ºC above), which could affect the carotenoid pigment synthesis.

In the case of Fe and Zn content had a different response in our study. For Fe content, both genotype and environment were equally important, followed by GxE and GxExY. For Zn content environment was much more determining factor. These results are different from the study of Ficco et al. (2009) carried out in the Mediterranean region. In that case GxE was the most important factor for both micronutrients, followed by genotype, with only a slight environmental impact. This is likely due to the differences in environmental conditions used for testing in both studies, which were very highly contrasted in this study. In the study, Zn content was favored by smaller grain ("concentration effect" in severe drought stress and severe heat stress), but Fe content only in the case of severe drought stress. In the case of severe heat stress, in spite of showing the smaller grain size values, Fe content decreased respect to optimum condition trial (drip full irrigation). This may reflect differences in the passive iron transport through the soil-plant system compared to Zn. Azaz et al. (2012) found also the micronutrients "concentration effect" with water stress management. Joshi et al. (2010) reported that iron and zinc concentrations were also found to be influenced by higher temperature. It is also important to note that a significant trend in grain micronutrients concentration related to the release year of the varieties was not found (data not shown). Some studies have shown that modern breeding focused on grain yield has reduced the grain micronutrient concentrations in bread wheat (Fan, Zhao, Fairweather-Tait, Poulton, Dunham & McGrath, 2008; Garvin, Welch & Finley, 2006), which is in agreement with the results from Ficco et al. (2009). Additionally, with the limited number of genotypes in this study the variation for micronutrients could not be accurately assessed and further studies are required. At the same time, studies of other grain components related to the potential bioavailability of these micronutrients (i.e. phytic acid) are required.

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

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

In the case of bread-making, our results showed that this ability depends largely on the genotype, even in this study in which all cultivars had the same composition of glutenins (Glu-B1 7+8 and LMW-2). This finding is in agreement with Rao et al. (2010) who showed that high bread-making quality is heritable in tetraploid wheat and that as a result, selection in durum breeding programs for improved bread-making potential should be possible. Ammar et al. (2000) found also a strong genotypic effect, but their research showed the glutenin composition was heterogeneous among the lines tested. Glutenin composition has been said to be the key determinant of gluten strength in durum wheat, and also in bread-making. There is no clear consensus about the best *Glu-B1* allele for bread-making: Ammar et al. (2000) found 6+8 better than 7+8; Boggini & Pogna (1989) indicated 7+8 > 20 >6+8; Peña et al. (1994) found 7+8> 6+8 >20. In the case of LMW glutenins, it is well established that LMW-2 type confers higher gluten strength and consequently higher bread volumes. The lines used in our research had all the subunit 7+8 and LMW-2 types, so glutenin composition cannot be used in this case to explain the big differences found in dough properties and bread-making among the genotypes of this study. This indicates that there are other qualitative or quantitative grain factors that also play a role in determining dough viscoelastic properties, which should be identified in more extensive studies. Our data also strengthens the idea expressed by Ammar et al. (2000), who due to the conflicting conclusions about the best glutenins alleles for durum wheat bread-making pointed out that the use of glutenins as markers to select for improved bread-making in durum wheat might not be justified. The high relationship between bread loaf volume and SDS sedimentation volume (0.81), suggests that this test could be a much more efficient tool for rapid and low-cost identification of suitable durum wheat genotypes for bread-making. The efficiency of this methodology for this purpose has been already demonstrated with CIMMYT germplasm (Peña et al. 1994, r = 0.81) and with cultivars of diverse origin (Ammar et al. 2000, r = 0.56). We also showed the high efficiency to predict bread-making (r = 0.78) of the same test run with whole-meal flour, which would allow, as in the case of yellowness determination, the save of time and resources in the selection process.

Overall, the durum wheat lines in this study showed inferior values for loaf volume and lower crumb structure quality compared to the bread-wheat checks. The less viscoelastic properties of the gluten and excessive starch damage could be the main reasons to explain this. However, some durum genotypes in specific environment as severe heat stress had almost the same performance that bread-wheat checks used in this study (Kachu and Roelfs), which are considered optimal for the bread-making process. This fact agrees with previous studies (Ammar et al. 2000; Peña et al. 1994), which have already proven that the long-established belief that durum wheat is not suitable for pan bread may be incorrect (Boggini et al. 1995). Based on the high correlations values obtained, gluten strength (alveograph W) and extensibility (alveograph L) were identified as key factors to enhance loaf volume, as well as the balance between gluten tenacity and extensibility (alveograph P/L). In previous studies, durum wheat flours were showed to a have high alveograph P/L values, leading to bread more compact (Brescia et al. 2007). Gluten extensibility with adequate levels of gluten strength is required in durum wheat for bread-making. Atil, the variety with higher extensibility by far, and Mexicali in severe heat stress trial (that have a great extensibility increase in this environment), were the ones showing better breads. This data supports the idea that to develop durum wheat cultivars with similar bread-making quality to those of bread wheat lines, it is necessary to achieve a better balance of tenacity and extensibility in conjunction with increased strength (Ammar et al. 2000; Dexter et al. 1994; Rao et al. 2010). For this purpose, the glutenin alleles or rheological tests traditionally used to enhance gluten strength for pasta-making quality may not be more suitable for bread-making, as durum wheat lines with strong gluten also tends to exhibit tenacious and inextensible gluten (Rao et al. 2001). Besides, the importance of gluten extensibility in bread-making is what confers to durum wheat in severe heat stress an increase in the bread volumes compared to bread wheat, which showed their best performance in severe drought stress. Durum wheat samples lack extensibility in control and drought stressed environments while bread wheat cultivars had balanced gluten on those environments. Heat stress caused the increase of extensibility in both, favoring the bread-making quality of durum wheat lines but not that of bread wheat, whose more extensible gluten was not better for bread-making. For unknown reasons (no significant changes in grain protein content or other traits), extensibility was also higher in basin full irrigation trial with respect to drip full irrigation, which also led to better bread volumes in this environment. Thus, this fact should be also taken into account when growing durum wheat for bread-making. Severe drought stress, because increasing gluten strength but not extensibility, led in most of the cases to inferior bread volumes compared with the rest of the environments. Both drought and heat stresses are often present in the main areas where durum wheat is grown (Mediterranean countries and Middle East), where local bread accounts for half of the durum wheat consumption.

It is also significant that the results obtained in bread-making with durum wheat are not the consequence of a planned and designed breeding program for this purpose. Durum wheat breeding programs are focused on obtaining lines with pasta-making quality (high gluten strength), which means there is probably lot of room for improvement in durum bread-making if systematic selection is applied. Extensibility could be increased applying selection for this trait with tools not extensively used in durum wheat quality breeding as the alveograph. Introgression of *Glu-D1* or *Glu-D3* glutenins from bread wheat is other possibility (Pogna et al. 1996).The development of durum lines with good bread-making quality could increase the commercial value of this crop and open alternative markets in years of high production. Moreover, durum wheat flour is a derivate in semolina production, so its use in bread-making could also offset the cost of semolina production. This is particularly interesting for those countries where durum has a superior yield performance compared to bread wheat. In addition to this, for CIMMYT bread wheat breeding program, the development of durum lines with enhanced bread-making quality it is also convenient, because durum lines are being used to transfer a variety of traits to bread wheat through intra-specific (bread x durum) and inter-specific (synthetics) hybridization. Using parental durum lines with good bread-making quality in those crosses would contribute to ensure desirable bread-making quality in the novel lines developed.

5. Conclusions

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

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

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Abstract

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

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

1. Introduction

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

At the International Maize and Wheat Improvement Center (CIMMYT) durum wheat breeding draws on a large, genetically wide, gene pool to develop germplasm, which is widely distributed among breeding programs of durum-producing countries. The priority of the wheat breeding program is to develop high-yielding, disease-resistant varieties that can tolerate drought, heat stresses, and produce high grain quality. The latter is essential in durum wheat varieties to be accepted by industry, local food manufacturers and consumers. The most important parameters affecting industrial quality for pasta-making from durum wheat grain are probably gluten quality (strength) and the yellow color of semolina. Although environmental and processing conditions play a significant role in these two traits, research has shown that they are under strong genetic control. The favourable dough or gluten properties of durum lines have been associated mainly with the presence of specific glutenins coded by the *Glu-B1* and *Glu-B3* loci (Ammar, Kronstad & Morris, 2000; Boggini & Pogna, 1989; Boggini, Tusa & Pogna, 1995; Brites & Carrillo, 2001; Peña, Zarco-Hernandez, Amaya-Celis & Mujeeb-Kazi, 1994). Semolina color will depend to a great extent on the genes involved in pigment accumulation (enzymes involved in pigment biosynthesis as phytoene synthase and pigment degradation as lipoxygenase or polyphenol oxidase) (see Ficco et al., 2014 for a review). Apart from pasta, durum wheat is widely used to elaborate other non-baked goods as couscous and bulgur. In the former high yellow color is appreciated while in bulgur light yellow color is preferred (Belibagli et al. 2009). Both products also demand high protein content to avoid stickiness and to have high water absorption capacity (Bayram and Öner 2006; Ounane et al. 2006).

Although durum wheat generally exhibits inferior bread-making quality compared with bread wheat (*T. aestivum* L. ssp. *aestivum* .) in terms of loaf volume and crumb structure (Boggini et al., 1995; Peña et al., 1994), approximately 24% of the global durum wheat production, and up to 70-90% in some Middle East countries, is used for bread-making (Quaglia, 1988). Speciality breads made with durum are common in the south of Italy (Boggini et al., 1995), and this popularity is spreading to other countries (Sissons, 2008). In the regions of West Asia and North Africa 50% of durum wheat is processed into single and two-layered flat breads and 35% is used for leavened breads (Liu, Shepherd & Rathjen, 1996). In those countries durum breads are very popular with consumers probably due to their sensory properties, particularly to their pleasant aroma (Rao, Pozniak, Hucl & Briggs, 2010) and special taste. They are slow to go stale (Pasqualone, Summo, Bilancia & Caponi, 2007) and consequently their longer shelf-life due to the high water absorption of durum wheat flour related to increased content in damage starch, is another desirable characteristic of durum wheat breads (Boyacioğlu & D´Appolonia, 1994). Besides, although yellow bread wheat flours are typically undesirable for bread-making, in the case of durum wheat breads a distinctive yellow colour is an important factor influencing whether it is accepted by the consumer (Brescia et al., 2007; Pasqualone, Caponio & Simeone, 2004).

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

Besides processing and end-use quality, nutritional quality is also becoming an important priority in wheat breeding. Wheat is good source of diverse beneficial compounds including fibre, phytochemicals and micronutrients. Among different micronutrients, iron and zinc are deficiency in the diet of two billion people (WHO 2012), and because of this have become the focus of micronutrient biofortification, which serves to enhance iron and zinc grain concentration. Modern wheat cultivars have been shown to be poor sources of these micronutrients for meeting daily requirements for humans (Cakmak, Pfeiffer & McClafferty, 2010). Not much research has been carried out about durum wheat and iron and zinc content. According to Ficco et al. (2009), there is some genetic variation to breed for iron and zinc in durum wheat, although more studies screening for larger genetic variability and examining the environment effect are required.

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

2. Materials and methods

2.1 Plant Materials/Agronomic trials

A trial consisting of six CIMMYT durum wheat cultivars (Mexicali C75, Yavaros C79, Altar C84, Atil C2000, Jupare C2001 and Cirno C2008) and two bread wheat ones (Kachu and Roelfs F2007), were sown in 2012-2013 and 2013-2014 crop seasons in Ciudad Obregon, Sonora, in northwestern Mexico. The trial was planted with two replicates in a randomized complete Block Design under six different environmental conditions: full drip irrigation (optimum conditions), full basin irrigation, reduced irrigation or medium drought stress, severe drought stress, medium heat stress, and severe heat stress. All the trials were planted in November except medium heat stress (planted in January) and heat stress (planted in February). All the trials had full irrigation (>500 mm) except medium drought stress (300 mm) and severe drought stress (180mm). Weed, diseases, and insects were all well controlled. In all the trials, N 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 except in severe drought stress (50 N units). At maturity whole plots were harvested and 1kg of seed from each of the wheat lines was used for analyzing the quality traits.

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

2.2 Grain and flour parameters

Grain morphological characteristics were evaluated with digital image system SeedCount SC5000 (Next Instruments, Australia). Thousand kernel weight (g) and test weight (kg/hl) were obtained. Grain iron and zinc content (mg/kg) were measured using a bench-top, non-destructive, energy-dispersive X-ray fluorescence spectrometry (EDXRF) instrument (model X-Supreme 8000, Oxford Instruments Plc, Abingdon, UK), previously standardized for high throughput screening of iron (Fe) and zinc (Zn) in whole wheat grain (Paltridge et al., 2012). Grain protein (%) and moisture content were determined by near-infrared spectroscopy (NIR Systems 6500, Foss Denmark) calibrated based on official AACC methods 39-10 and 46-11A (AACC, 2010). Grain samples previously conditioned at 16% of moisture were milled into flour using Brabender Quadrumat Jr (C. W. Brabender OHG, Germany). Whole-meal flour samples were also obtained with a UDY Cyclone mill carrying a 0.5 mm screen. The protein and moisture content in flour was estimated using near-infrared spectroscopy (NIR Systems 6500, Foss Denmark) calibrated based on the AACC methods commented above. Grain protein and flour protein content values were reported at 12.5% and 14% moisture basis, respectively. Flour yellowness and whole-meal flour yellowness were obtained as the b value of a Minolta color meter (Konica Minolta, Japan).

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

2.3 Rheological tests

Sodium dodecyl sulfate (SDS) sedimentation volume was carried out according to the modified protocol described in Peña et al. (1990) using 1g of flour or of whole-meal flour. Additionally, 35g flour samples were run 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 60g flour samples and constant water absorption (55%). Higher water absorption than in the official methodology (50%) was used to compensate for the typically greater water absorption caused by high levels of starch damage occurring during milling of the very hard durum wheat grain (Ammar et al., 2000; Dexter, Preston, Martin & Gander, 1994, Peña et al., 1994).

2.4 Bread-making characteristics

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

All the phenotypic data recorded is shown in ESM2.

2.5 Statistical Analysis

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

The basic conventional fixed effect linear model for describing the univariate mean response of genotypes in environments and years, and their interactions is $y_{ijrk} = \mu + R(EY)_{rjk} + Y_k + G_i + E_j + (GY)_{ik} + (EY)_{jk} + (GE)_{ij} + (GEY)_{ijk} + \varepsilon_{ijrk}$, where y_{ijrk} is the response of the ith genotype (i=1,2,…,I) in the rth replicated (r=1,2,…,L) of the jth environment (j=1,2,…,J) and the

k^{th} year (k=1,2,…,M), μ is the grand mean over all genotypes, environments, and years, $R(EY)_{rjk}$ is the effect of the r^{th} replicate within the j^{th} environment and the k^{th} year, Y_k is the effect of the k^{th} year, G_i is the effect of the i^{th} durum wheat line, E_j is the effect of the j^{th} environment, $(GY)_{ik}$ is the effect of the interaction between the i^{th} durum wheat line with the k^{th} year, $(EY)_{jk}$ is the effect of the interaction between the j^{th} environment with the i^{th} durum wheat line, $(GE)_{ij}$ is the effect of the interaction between the i^{th} durum wheat line with the k^{th} year, $(GEY)_{ijk}$ is the effect of the triple interaction among the i^{th} durum wheat line with j^{th} environment and the k^{th} year, and $\mathcal{E}_{ijrk}$ is the error assumed to be normally and independent distributed NID $(0, \sigma^2)$, where σ^2 is the error variance, assumed to be constant.

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

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

3. Results

3.1 Effects of genotype, environment, year and their interactions

The results of the analysis of the variance (Table 1) showed that almost all the factors had a significant effect on the different traits. Genotype and environment were the most important factors explaining the variation found, followed by GxE and ExY interaction. The environment effect was particularly high in traits as test weight, thousand kernel weight, Zn content and alveograph P, which explained more than 47% of the variation. In the case of Fe content, flour yellowness, SDS sedimentation volume, mixograph optimum dough mixing time and torque, alveograph L, the genotype effect was predominant and responsible for at least 25% of the variation, and in some cases as flour SDS sedimentation and bread loaf volume explained more than 55% of variation. For this latter trait the GxE effect was also particularly important. GxE effect also explained about 12% of the variation in traits Fe and Zn content, flour SDS sedimentation volume, mixograph optimum dough mixing time and torque, and alveograph P, L and P/L. The ExY effect was high for test weight (30%) and also important for thousand kernel weight, flour yellowness, flour SDS sedimentation volume and alveograph W. The triple interaction GxExY effect was significant for mixograph optimum dough development time and torque and alveograph W. The effects of the particular year and the GxY were not significant for all of the traits.

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

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

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

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

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

In traits traditionally related to grain size (grain protein, Fe and Zn content), the response of the genotypes was not consistent in all environments. For grain protein content, the environments with lower grain size (severe drought stress and severe heat stress) had significantly higher values. This did not happen in the case of Fe content, for which severe heat stress showed also the lowest value. On the contrary, for Zn content severe heat stress resulted in the highest value (56 mg/kg). In the case of severe drought stress, higher values than in the other environments were found for both micronutrients. All the genotypes showed large ranges for grain protein content. Jupare did not show a wide range for Zn or Fe content; Yavaros and Cirno were also stable across environments for Fe content. Atil was the genotype, which had higher average grain protein and Fe content, while Altar was for Zn content.

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

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

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

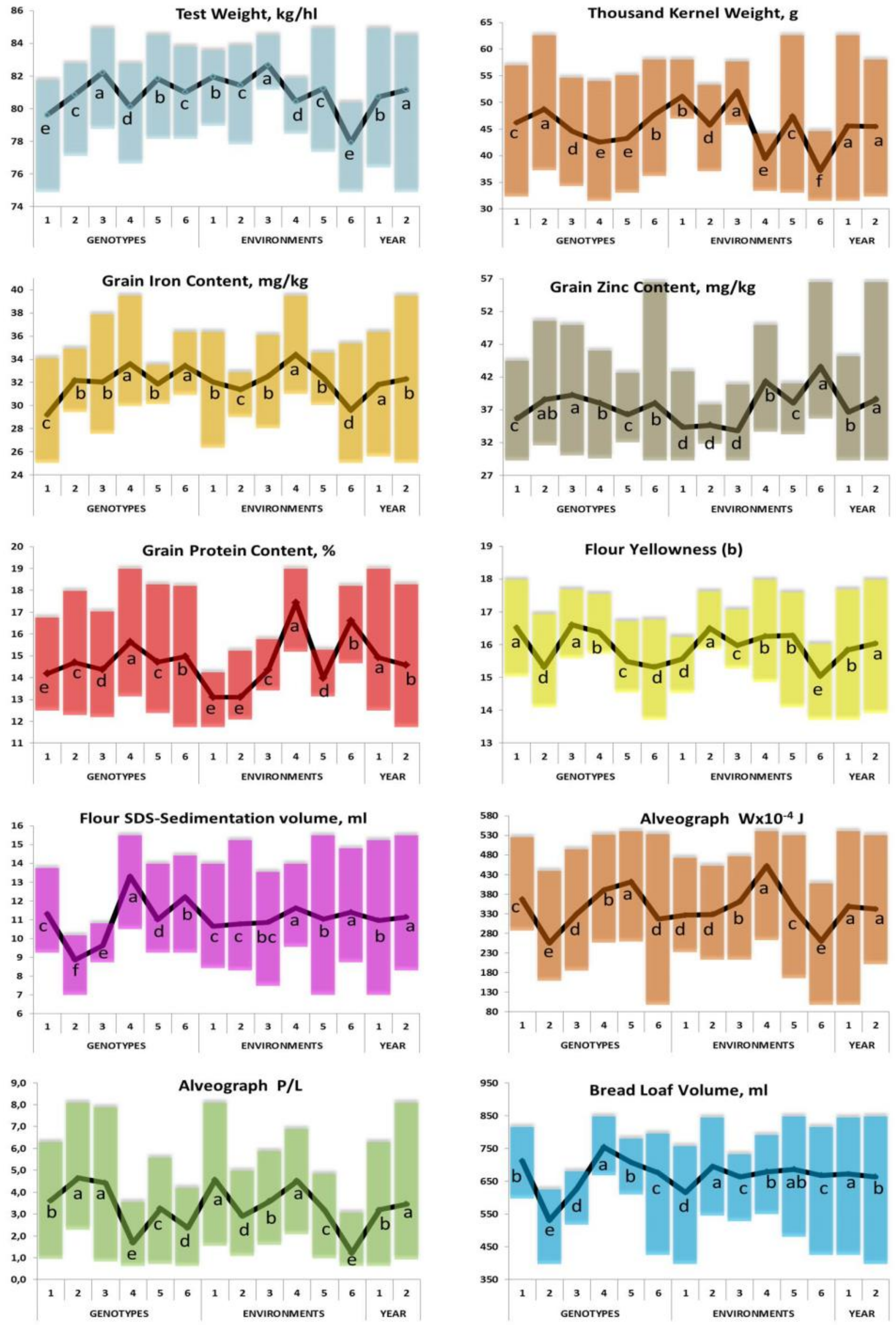

Figure 1- Maximum, average and minimum values for different quality traits obtained by each genotype in the whole trial, and in each environment and year by the six genotypes. The range of values (maximum and minimum) is represented by bars. The average values in each case are represented with a continuous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

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

3.2 Glutenins composition and bread-making analysis

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

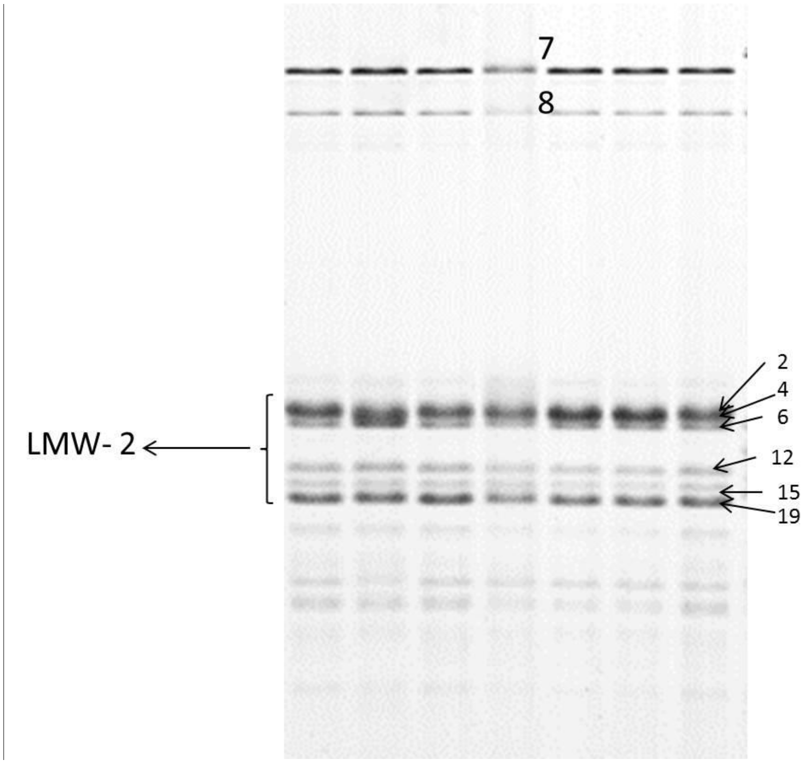

Figure 2 – SDS-PAGE electrophoresis of HMW and LMW glutenins of durum wheat samples. Lanes are as follow: 1, Mexicali C75; 2,Yavaros 79; 3, Altar 84; 4, Atil C2000; 5, Jupare C 2001; 6, Cirno C 2008.

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

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

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

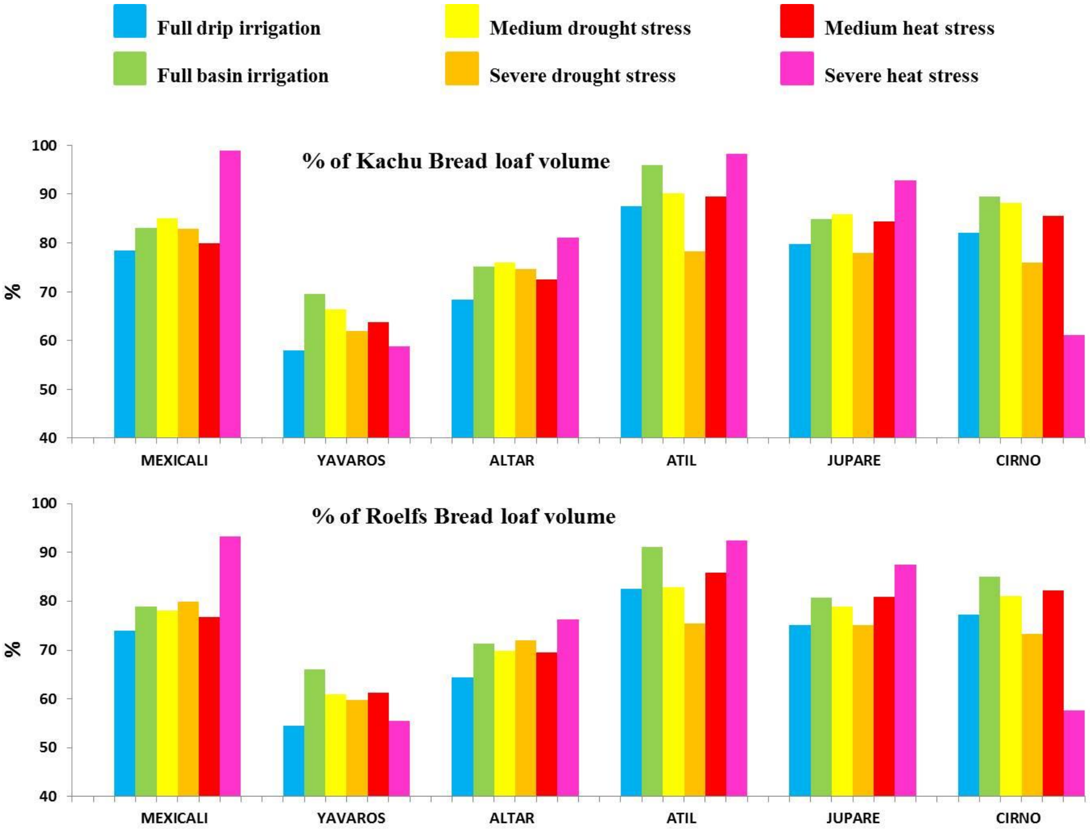

Figure 3 – Bread-making of durum wheat varieties. Colored bars show the percentage of each bread loaf volume obtained with durum wheat cultivars respective to Kachu and Roelfs bread wheat checks. Each color represents a different environment as indicated in the legend.

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

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

Severe heat stress	816	866	661	801	499	758	808	480

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

3.3 Relationships between quality parameters

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

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

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

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

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

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

4. Discussion

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

All the above results strengthen the well-established idea that some quality traits, particularly those related with processing and pasta-making quality have a very strong genetic control and therefore the genotypes respond consistently across environments for these characteristics. Most of those traits (flour SDS sedimentation volume, mixograph optimum dough mixing time and torque, alveograph W) also showed consistent correlations between them in the different environments. In a previous study (Li et al., 2013) it was revealed that in general drought stress favored gluten strength and yellowness (processing and pasta-making quality), which was confirmed with our study. On the contrary, heat stress was found to reduce gluten strength by both studies. Li et al. 2013 found a significant increase in flour yellowness in heat stressed environments, probably due to a “concentration effect” of the pigment carotenoid. In our experiment, flour yellowness was reduced in severe heat stress trial, which disagree with Li et al. (2013). This discrepancy with this study carried out in the same location could be due to a different tolerance to heat stress of the genotypes used in both studies, or to other environmental changes. Temperatures in May, when grain filling for severe heat stress trial happened, were something higher in our study (maximum temperatures around 1.5 ºC above), which could affect the carotenoid pigment synthesis.

In the case of Fe and Zn content had a different response in our study. For Fe content, both genotype and environment were equally important, followed by GxE and GxExY. For Zn content environment was much more determining factor. These results are different from the study of Ficco et al. (2009) carried out in the Mediterranean region. In that case GxE was the most important factor for both micronutrients, followed by genotype, with only a slight environmental impact. This is likely due to the differences in environmental conditions used for testing in both studies, which were very highly contrasted in this study. In the study, Zn content was favored by smaller grain (“concentration effect” in severe drought stress and severe heat stress), but Fe content only in the case of severe drought stress. In the case of severe heat stress, in spite of showing the smaller grain size values, Fe content decreased respect to optimum condition trial (drip full irrigation). This may reflect differences in the passive iron transport through the soil-plant system compared to Zn. Azaz et al. (2012) found also the micronutrients "concentration effect" with water stress management. Joshi et al. (2010) reported that iron and zinc concentrations were also found to be influenced by higher temperature. It is also important to note that a significant trend in grain micronutrients concentration related to the release year of the varieties was not found (data not shown). Some studies have shown that modern breeding focused on grain yield has reduced the grain micronutrient concentrations in bread wheat (Fan, Zhao, Fairweather-Tait, Poulton, Dunham & McGrath, 2008; Garvin, Welch & Finley, 2006), which is in agreement with the results from Ficco et al. (2009). Additionally, with the limited number of genotypes in this study the variation for micronutrients could not be accurately assessed and further studies are required. At the same time, studies of other grain components related to the potential bioavailability of these micronutrients (i.e. phytic acid) are required.

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

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

In the case of bread-making, our results showed that this ability depends largely on the genotype, even in this study in which all cultivars had the same composition of glutenins (Glu-B1 7+8 and LMW-2). This finding is in agreement with Rao et al. (2010) who showed that high bread-making quality is heritable in tetraploid wheat and that as a result, selection in durum breeding programs for improved bread-making potential should be possible. Ammar et al. (2000) found also a strong genotypic effect, but their research showed the glutenin composition was heterogeneous among the lines tested. Glutenin composition has been said to be the key determinant of gluten strength in durum wheat, and also in bread-making. There is no clear consensus about the best *Glu-B1* allele for bread-making: Ammar et al. (2000) found 6+8 better than 7+8; Boggini & Pogna (1989) indicated 7+8 > 20 >6+8; Peña et al. (1994) found 7+8> 6+8 >20. In the case of LMW glutenins, it is well established that LMW-2 type confers higher gluten strength and consequently higher bread volumes. The lines used in our research had all the subunit 7+8 and LMW-2 types, so glutenin composition cannot be used in this case to explain the big differences found in dough properties and bread-making among the genotypes of this study. This indicates that there are other qualitative or quantitative grain factors that also play a role in determining dough viscoelastic properties, which should be identified in more extensive studies. Our data also strengthens the idea expressed by Ammar et al. (2000), who due to the conflicting conclusions about the best glutenins alleles for durum wheat bread-making pointed out that the use of glutenins as markers to select for improved bread-making in durum wheat might not be justified. The high relationship between bread loaf volume and SDS sedimentation volume (0.81), suggests that this test could be a much more efficient tool for rapid and low-cost identification of suitable durum wheat genotypes for bread-making. The efficiency of this methodology for this purpose has been already demonstrated with CIMMYT germplasm (Peña et al. 1994, r = 0.81) and with cultivars of diverse origin (Ammar et al. 2000, r = 0.56). We also showed the high efficiency to predict bread-making (r = 0.78) of the same test run with whole-meal flour, which would allow, as in the case of yellowness determination, the save of time and resources in the selection process.

Overall, the durum wheat lines in this study showed inferior values for loaf volume and lower crumb structure quality compared to the bread-wheat checks. The less viscoelastic properties of the gluten and excessive starch damage could be the main reasons to explain this. However, some durum genotypes in specific environment as severe heat stress had almost the same performance that bread-wheat checks used in this study (Kachu and Roelfs), which are considered optimal for the bread-making process. This fact agrees with previous studies (Ammar et al. 2000; Peña et al. 1994), which have already proven that the long-established belief that durum wheat is not suitable for pan bread may be incorrect (Boggini et al. 1995). Based on the high correlations values obtained, gluten strength (alveograph W) and extensibility (alveograph L) were identified as key factors to enhance loaf volume, as well as the balance between gluten tenacity and extensibility (alveograph P/L). In previous studies, durum wheat flours were showed to a have high alveograph P/L values, leading to bread more compact (Brescia et al. 2007). Gluten extensibility with adequate levels of gluten strength is required in durum wheat for bread-making. Atil, the variety with higher extensibility by far, and Mexicali in severe heat stress trial (that have a great extensibility increase in this environment), were the ones showing better breads. This data supports the idea that to develop durum wheat cultivars with similar bread-making quality to those of bread wheat lines, it is necessary to achieve a better balance of tenacity and extensibility in conjunction with increased strength (Ammar et al. 2000; Dexter et al. 1994; Rao et al. 2010). For this purpose, the glutenin alleles or rheological tests traditionally used to enhance gluten strength for pasta-making quality may not be more suitable for bread-making, as durum wheat lines with strong gluten also tends to exhibit tenacious and inextensible gluten (Rao et al. 2001). Besides, the importance of gluten extensibility in bread-making is what confers to durum wheat in severe heat stress an increase in the bread volumes compared to bread wheat, which showed their best performance in severe drought stress. Durum wheat samples lack extensibility in control and drought stressed environments while bread wheat cultivars had balanced gluten on those environments. Heat stress caused the increase of extensibility in both, favoring the bread-making quality of durum wheat lines but not that of bread wheat, whose more extensible gluten was not better for bread-making. For unknown reasons (no significant changes in grain protein content or other traits), extensibility was also higher in basin full irrigation trial with respect to drip full irrigation, which also led to better bread volumes in this environment. Thus, this fact should be also taken into account when growing durum wheat for bread-making. Severe drought stress, because increasing gluten strength but not extensibility, led in most of the cases to inferior bread volumes compared with the rest of the environments. Both drought and heat stresses are often present in the main areas where durum wheat is grown (Mediterranean countries and Middle East), where local bread accounts for half of the durum wheat consumption.

It is also significant that the results obtained in bread-making with durum wheat are not the consequence of a planned and designed breeding program for this purpose. Durum wheat breeding programs are focused on obtaining lines with pasta-making quality (high gluten strength), which means there is probably lot of room for improvement in durum bread-making if systematic selection is applied. Extensibility could be increased applying selection for this trait with tools not extensively used in durum wheat quality breeding as the alveograph. Introgression of *Glu-D1* or *Glu-D3* glutenins from bread wheat is other possibility (Pogna et al. 1996).The development of durum lines with good bread-making quality could increase the commercial value of this crop and open alternative markets in years of high production. Moreover, durum wheat flour is a derivate in semolina production, so its use in bread-making could also offset the cost of semolina production. This is particularly interesting for those countries where durum has a superior yield performance compared to bread wheat. In addition to this, for CIMMYT bread wheat breeding program, the development of durum lines with enhanced bread-making quality it is also convenient, because durum lines are being used to transfer a variety of traits to bread wheat through intra-specific (bread x durum) and inter-specific (synthetics) hybridization. Using parental durum lines with good bread-making quality in those crosses would contribute to ensure desirable bread-making quality in the novel lines developed.

5. Conclusions

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

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