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Original article

Grain and flour quality of wheat genotypes grown under heat stress

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ABSTRACT

Heat stress during the grain-filling period is the main abiotic stress factor limiting grain yield and quality in wheat (*Triticum aestivum* L.). In this study, 64 wheat genotypes were exposed to heat stress during reproduction caused by delayed sowing in two growing seasons. Grain yield, 1000 grain weight (GW), grain hardness (GH), and grain-quality related traits were investigated. Heat stress caused a significant decrease in GW through reducing starch content (SC) and a non-compensating rise in protein content (PC), and thereby resulted in lower yield. In addition, significant increases in flour water absorption (WA), Zeleny sedimentation volume (ZT), ash content (AC), lipid content (LC), loaf volume (LV), wet gluten content (WG), dry gluten content (DG), gluten index (GI), and amylopectin content (APC) were found following heat stress. In contrast, decreases in grain moisture content (MC) and amylose content (AMC) induced by heat stress were observed. The heat-tolerant genotypes were superior in grain yield, GW, SC, AMC, and MC. While the sensitive genotypes contained higher PC, LV, GI and AMP. A group of wheat genotypes characterized with a higher yield, AMC, GW, and SC as well as lower PC, WA, GH, ZT, and LV; and was found to be the most heat tolerant by principal component analysis. Lighter weight and smaller grains produce a smaller starchy endosperm with lower quality (less amylose) and higher grain protein content in heat stress compared to normal conditions. Heat stress caused by delayed sowing improves some of the baking-quality related traits.

1. Introduction

Understanding how ongoing increases in global surface temperature will threaten plant production and food security is a question of profound importance. Wheat (*Triticum aestivum* L.) grain is one of the most important providers of calories and protein for human diets worldwide (Arzani and Ashraf, 2017). However, being a cool-season crop, it is sensitive to heat stress. The ability of wheat to produce unique baked products, such as bread, depends on grain quality. This attribute is assessed by physical and compositional properties such as grain hardness (GH), protein content (PC), starch content (SC), amylose content (AMC), ash content (AC), lipid content (LC), gluten index (GI), gluten content (Hernández-Espinosa et al. 2018).

The optimum growth of wheat occurs at a temperature of 18–22 °C (Hennessy et al., 2008). High temperatures (maximum daily temperatures > 30 °C), associated with climate change, adversely affect growth, development, and ultimately yield and quality of wheat (Fleitas et al., 2020). In particular, heat stress accelerates grain filling process, which leads to decreased 1000 grain weight (GW) and grain yield (Bergkamp et al., 2018; Dwivedi et al. 2017; Mahdavi et al., 2021), as well as inhibition starch synthesis in the endosperm amyloplasts and change in protein content and composition in wheat (Spiertz et al., 2006). The synthesis and accumulation of starch in the grains is tightly linked to the yield as starch contributes to almost three quarter of the grains (70%)

Abbreviations: AC, ash content; AMC, amylose content; ANOVA, Analysis of variance; APC, amylopectin content; DG, dry gluten content; GH, grain hardness; GI, gluten index; GW, 1000 grain weight; LC, lipid content; LV, loaf volume; MC, moisture content; SC, starch content; STI, stress tolerance index; PC, protein content; PC1, first principal component; PC2, second principal component; WA, water absorption; WG, wet gluten content; Yield, grain yield; ZT, Zeleny sedimentation volume.

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(Arzani and Ashraf, 2017). Wheat starch contains two polymers of glucose, namely amylose (~25%) and amylopectin (~75%), differing markedly in structure and properties (Kumari et al., 2020). Liu et al. (2011) used two wheat cultivars to assess the responses of the starch concentration and attributes to heat stress during grain filling period in greenhouse conditions. They found that heat stress negatively influenced starch biosynthesis and altered the morphology of starch granules.

The protein content of the grain and its quality are affected in a wheat plant that grows under heat stress. Tahir et al. (2006) evaluated wheat genotypes under normal and late-sowing conditions for end-use traits. They found that protein content, SDS sedimentation, and mixograph peak time were increased by high temperature during grain filling. The baking quality of wheat flour depends on the protein content and composition, but still, it can be significantly associated with lipids that bind to proteins in dough or gluten networks (Konopka et al., 2006). When plants are exposed to heat stress, membrane lipids are the most vulnerable cellular targets. Grain hardness (GH) or endosperm texture commonly is used in the trade for the classification of technological properties and end-use quality of wheat (Morris, 2002). The Zeleny sedimentation volume (ZT) shows the tendency of storage materials of the endosperm, particularly proteins, to swell and flocculate in a lactic acid solution. The ZT value is commonly used as an indirect determination method of the baking quality in wheat. Flour water absorption (WA) is another essential property of bread-making doughs and is directly related to the PC and damaged starch present in the flour (Li et al., 2020). WA is affected by high temperature (Singh et al., 2021) and associated with PC and GH (Pasha et al., 2010). The reproductive phase, such as grain-filling, is the most sensitive to heat stress in wheat (Wang et al., 2016). Therefore, bread-making quality of grain can be affected by heat stress (Wang et al., 2016).

Heat stress is the most evident abiotic stress under a scenario of global warming. Hence, the thermo-tolerant genotypes with the ability to maintain their quality are a vital component of the agricultural farming system. Nevertheless, grain quality traits and most especially protein content in wheat are quantitatively inherited; that, they are influenced by genotype, environment, and genotype-by-environment interaction (Williams et al., 2008). The current study differs from previous ones in that here we examine diverse germplasm in two-year field trial under natural warm environment (maximum air temperature > 32 °C) during the late flowering and grain filling that correspond to early spring season (see Table 1). Indeed, it is expected that heat-tolerant genotypes could also have the ability to maintain their bread-making performance, this has not been studied for many traits, and responses may differ among cultivars. In line with our research focus, CIMMYT has developed the CIMCOG (CIMMYT Mexico Core Germplasm) to facilitate breeding for grain yield and quality worldwide, especially in warmer areas of the world (Fleitas et al., 2020).

As perceived by Tahir et al. (2006), less than a handful studies focused on the effects of high temperature on wheat grain quality, but very little work has been done specifically in exposing field-grown plants to high temperatures (>35 °C) during almost the whole grain-filling stage. Nonetheless, the citations as mentioned earlier provide necessary depth to the present knowledge, but still lacks research that simultaneously addresses the following fundamental issues: (a) a spatial or temporal repetition of the experiments, which not only yields better reproducible results but also make feasible the ability to partition phenotype into the genotypic, environmental, and genotype-by-environment interaction; (b) an insight into the spatial and temporal dynamics of biological process within the grain as a whole, where interactions between all the involving parts and the bi- and multi-lateral relationships of major components can be depicted; and (c) a large number of genotypes with broad genetic diversity, which genotypic specific information can accurately be deduced. Therefore, the purposes of this study were to evaluate the grain-quality related traits of 64 field-grown genotypes of wheat in two growing seasons (2015–2017) under normal and terminal heat stress conditions and to assess the relationships among the grain quality indicators, grain hardness, weight, and yield.

2. Materials and methods

2.1. Plant materials and growth conditions

Timely sown (hereafter called "normal") and late sown (hereafter called "heat stress") field experiments were carried out under full irrigation at Mehran Agricultural Research Center (33° 7́N, 46° 6́E), located in Mehran, Ilam Province, Iran, in two years (2015–2016 and 2016–2017). The temperature and relative humidity data are provided in Table 1. Sixty advanced wheat (*Triticum aestivum* L.) lines (CIMCOG 1–60) and four native wheat cultivars grown in the region; namely, Kouhdasht (61; originated from ICARDA), Zagros (62; originated from CIMMYT), Karim (63; originated from ICARDA), and Dehdasht (64; originated from Italy) were used in this study. A square lattice design (8 × 8) arranged in a regular grid

Table 1
Maximum (Max), minimum (Min), and mean values of monthly air temperature (°C), relative humidity (%) and number of chronically high temperature days, during the two growing seasons at Mehran field site.

Month	Year	Temperature (°C)			Relative humidity (%)	Days above 32 °C *	Days above 35 °C *
		Max.	Min.	Mean			
November	2015–2016	23.5	12.1	17.8	68	–	–
	2016–2017	26.7	11.4	19.1	47	–	–
December	2015–2016	17.1	7	12.1	72	–	–
	2016–2017	18.1	6.6	12.35	56	–	–
January	2015–2016	16.3	5.8	11.1	70	–	–
	2016–2017	17.4	5.1	11.3	63	–	–
February	2015–2016	22.1	8.6	15.3	59	–	–
	2016–2017	18.5	4.9	11.7	50	–	–
March	2015–2016	25.6	11.9	18.7	52	–	–
	2016–2017	25	11.9	18.5	54	–	–
April	2015–2016	32.7	16.1	24.4	41	18	9
	2016–2017	32.3	16.9	24.6	41	17	9
May	2015–2016	38.7	23	30.8	23	9	7
	2016–2017	40	23.3	31.7	19	10	8

* Number of days showed chronic high temperatures from the early spring season to the end of physiological maturity.

and replicated twice was used in each of the normal and heat stress conditions. Each plot contained four-rows, 4 m long and 25 cm apart. In the normal experiment, sowing date was 25 November 2015 and 23 November 2016 at the optimal planting date for the area. In the heat stress experiment, the planting date was delayed to 27 December in 2015 and to 29 December in 2016. In all experiments, fields were irrigated according to schedule (about 80% field capacity) to avoid negative confounding effect of drought. Nitrogen (N) was applied as ammonium nitrate (33.5% N w/w) at a rate of 240 kg ha^{-1}, splitted in three amendments, in both the normal and heat stress experiments.

2.2. Grain weight, hardness, and yield

Grain yield (hereafter abbreviated yield) of the two middle rows of each plot was determined. The 1000-grain weight (GW) was determined using an average weight of two grain samples randomly taken from each plot. In total, 512 field plots (256 each year) were harvested. Plants were harvested on April 27, 2016 and April 29, 2017 in normal conditions and on May 9, 2016 and May 10, 2017 in heat stress conditions. Two grain samples from each plot were taken for milling. Grain hardness (GH) was determined with a NIR analyzer as described below. Grain hardness was estimated using Near-infrared reflectance (NIR) spectroscopy [NIR Analyzer Inframatic 8620 (Perten Instruments, Sweden)] the AACC method 39–70A (AACC, 2008). The stress tolerance index (STI) was calculated according to Fernandez (1992), and the data were presented in Table S1.

2.3. NIR analysis of the wholemeal flour

Grain protein content (PC, %), grain moisture content (MC, %), flour water absorption (WA, %), bread loaf volume (LV, ml), and Zeleny sedimentation volume (ZT, cm^3), were determined by the NIR Analyzer Inframatic 8620. NIR calibrated based on official AACC methods 46–13, 44–16, 56–60 for PC, MC, and ZT traits, respectively (AACC, 2008). PC and ZT were reported at a 14% moisture content basis. The WA and LV (in rapid mix test method) were calibrated using a farinograph (Brabender, model No. 827504, GmbH) using the AACC method 54–21 (AACC, 2008) and the method of Pomeranz et al. (1984), respectively.

Tolerant (9) and sensitive (8) genotypes (17 out of 64) were selected on the basis of the STI data (Mahdavi et al., 2021) and used to measure the following traits.

2.4. Starch content

Starch content (SC, %) of the grains was determined by the anthrone reagent method after extraction in ethanol and perchloric acid solutions (Hedge and Hofreiter, 1962). The 0.5 g sample of whole wheat flour was homogenized in hot 80% ethanol, centrifuged at 4000 rounds per minutes (rpm), and the supernatant discarded. This operation was repeated twice. After drying over a water bath, the residue was re-suspended in 6.5 ml of 52% perchloric acid and 5.0 ml water at 0 °C for 20 min, centrifuged at 11000 rpm and the supernatant was collected. The extraction was repeated using fresh perchloric acid, and the supernatant increased to 100 ml. Then, 0.2 ml of the supernatant was pipetted into a test tube and increased the volume to 1 ml with distilled water. Standard glucose solutions were prepared by adding 3 ml anthrone reagent to each of 0 (serves as blank), 0.2, 0.4, 0.6, 0.8, and 1 ml of the glucose working standard. The samples were heated in a boiling water bath for 20 min, cooled rapidly, and the absorbance was read at 630 nm in a spectrophotometer.

2.5. Amylose and amylopectin contents

Amylose content (AMC, %) was determined using the iodine binding colorimetric method described by Williams et al. (1970). A sample of 20 mg of wheat flour was homogenized in 10 ml KOH (0.5 N), vortexed for 5 min, and volume was increased to 100 ml with distilled water. Then, 0.5 ml of iodine reagent and 5 ml hydrochloric acid (0.1 N) were added to the 10 ml of aliquot, and the volume was made up to 50 ml. After allowing the color to develop for one hour (h), the absorbance was read at 625 nm. The AMC was calculated from a standard curve prepared using amylose and amylopectin blends.

Amylopectin content (AMP, %) was calculated by subtracting the AMC from 100 (the SC).

2.6. Lipid content

The 5 g wholemeal flour was extracted continuously in the Soxhlet apparatus using 150 ml of petroleum ether (boiling in 105 °C) according to AACC Method 30–25 with minor modifications of extraction time and temperature. The volume of 150 ml solvent was used to soak the samples thoroughly. Extraction was carried out for 4 to 6 h with 7–8 reflux cycles per h. The excess of solvent was removed by evaporation at room temperature, and the residue was dried to constant weight at 100 °C. Weights of the extracted lipid were determined after cooling in a desiccator. The lipid content (LC, %) was then expressed as a percent lipid per g of sample.

2.7. Ash content

Total ash content (AC, %) was determined as whole inorganic matter by the AACC method (08–03.01). The 3 g of the wholemeal flour was used. Total ash content was determined incineration of the sample at 600 °C for 4 h. After cooling in a desiccator, it was re-weighed. The initial weight of the sample and the weight after incinerating were used to calculate the percentage of ash.

2.8. Gluten content and quality

Gluten protein fraction was extracted from the wholemeal flour samples using the gluten washing method (AACC Method 38–10.01). Wet gluten (WG) was dried at 130 °C in an oven for 6 h, and after cooling, the dry gluten (DG) content was weighed. The WG and DG contents were expressed as the percent of sample.

Gluten index (GI) was determined using Gluten Index Centrifuge 2015 (Perten Instruments, Sweden) by the AACC method 38–12 (AACC, 2008). The wet gluten forced through the sieve and the total wet gluten (passed through and left behind on the sieve) were weighed. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the GI.

2.9. Statistical analysis

Data were analyzed by combined ANOVA with the GLM procedure of SAS software (ver. 9.4; SAS Institute Inc., Cary, NC., USA). Genotype and environment were considered as fixed effects but year and replication served as random effects. The efficiency of the lattice design was compared with the conventional randomized complete block design (RCBD) for each trait in each experiment. When the efficiency of the lattice design for a particular trait was less than or equal to 105%, the trait was analyzed by the RCBD.

Multiple comparisons of the means were done using Tukey-Kramer HSD test ($p < 0.05$). Genetic variance was estimated from the expected mean squares (EMS) obtained from the ANOVA tables for the phenotypic data of each trait. Broad-sense heritability (h_b^2) was calculated for the traits under normal and heat stress conditions using the following formula:

$$h^2{}_b = \sigma^2{}_g / \sigma^2{}_p$$

where σ_g^2 and σ_p^2 are genotypic and phenotypic variances, respectively.

Pearson correlation coefficients were calculated to estimate the pairwise relationships of the variables. Principal component analysis (PCA) and 3D plot drawing were performed using Statgraphics (XVII- Statpoint Technology, Warrington, VA, USA) and Stat Graphics (ver. 14.0) software, respectively.

3. Results

The data of 64 genotypes were used to study the following variables: yield, GW, PC, MC, ZT, WA, GH, and LV. On the other hand, the data of SC, AMC, AMP, LC, AC, WG, DG, and GI were acquired by using17 genotypes. The data shown in Table 2 indicate the significant effects of late-growing-season heat stress on GW, yield, PC, MC, ZT, GH, and LV. The WA was an exception among all the quality indicators measured by the NIR (mostly listed in prior sentence). Significant differences among the 64 genotypes were noted for all the traits. In addition, genotype × environment interactions were significant for all the above traits. There was significant genotype × year and genotype × year × environment interactions for yield and LV. Climatic conditions during the growing seasons significantly influenced GW and MC. However, no significant effect of year × environment interaction was detected on any traits. The means of the traits for the individual wheat genotypes grown in normal and heat stress environments are presented in Table S1. The heritability estimates of the investigated traits using 64 wheat genotypes ranged from 49.44 (for PC) to 67.07% (for LV) under normal conditions and ranged from 54.13 (for GH) to 66.40% (for GW) under heat stress conditions (Table 3). The heritability estimates of the traits measured on selected number of genotypes (17 out of 64) ranged from 42.21 (for LC) to 63.81% (for GI) in normal conditions, while it ranged from 39.05 (for AC) to 72.23% (for GI) in heat stress conditions (Table 4).

3.1. Grain weight, hardness, and yield

Overall means and ranges of these traits for the 64 genotypes are presented in Table 3, while Table S1 provides the means of the traits for each of the 64 genotypes. The genotypes exhibited a profound decrease in yield due to terminal heat stress, with an overall mean loss of 55%. The yield in normal conditions had a range of 2.68 to 5.48 t ha^{-1}, which declined to 1.12–2.70 t ha^{-1} in delayed planting. The genotypes no. 3, 5, 6, 11, 21, and 28 showed the highest yield under terminal heat stress and the lowest yield loss due to heat stress. Based on STI, yield and yield loss; genotype no. 2, 3, 5, 6, 11,16, 17, and 28 were considered as the most heat-tolerant genotypes. On the other hand, genotype no. 9, 13, 19, 34, 38, 40, 44, 49, and 58 were the most heat-sensitive among the 64 wheat genotypes.

GW of the 64 genotypes was declined from 38.13 to 26.95 g in response to heat stress. Grain texture hardness (GH) displays the fracture resistance of the grains. Wheat genotypes differed for GH significantly, but GH was not affected by high temperature. On the other hand, the interaction of genotype × environment was significant, indicating that the rank order of genotypes for the endosperm texture differed with the environment (normal and late sowings). Genotype no. 40 (57.43%) stood first in terms of the value of GH in normal conditions, whereas genotype no. 12 (60.58%) ranked first in delayed sowing conditions for the endosperm texture (see Table S1).

3.2. Protein content

Terminal heat stress caused by delayed sowing influenced the grain PC significantly (Table 2). Table 3 presents the overall means of PC, other quality-related traits, and yield. In addition, Table S1 shows the means of quality-related traits, yield, and STI in the 64 individual wheat genotypes evaluated under normal and heat stress conditions. Heat stress was associated with an increase in the quality attributes measured by NIR such as PC, ZT, WA, and LV (11.1%, 17.28%, 3.47%, and 16.44%, respectively) except MC, which showed a decline of 8.67%. Under heat stress conditions, PC ranged from 12.25 to 14.98% for genotype no. 6 (tolerant genotype) and no. 53, respectively. On the other hand, a narrow range (11.08 to 12.60%) of PC was observed for 64 genotypes grown in normal conditions, where, the highest increase in PC was observed in the sensitive genotypes no. 9 and 40.

3.3. Gluten content and index

Gluten content (both WG and DG) and GI were significantly influenced by delay sowing (Table 4). Terminal heat stress during grain filling increased WG, DG, and GI of 17 wheat genotypes (Table 5). WG of wholemeal wheat flour had a range of 25.2–33.57% and 28.8–36.7% under normal and heat stress conditions, respectively. While DG was found in a range of 8.4 to 10.3% and 9.7 to 12.1%. GI, an indicator of gluten quality, was increased from 62.1 to 78.75% in normal to 64.8 to 84% in heat stress conditions.

Table 2
Results of combined analysis of variance for the traits evaluated in 64 wheat genotypes grown under two environments (normal and terminal heat stress) in two growing seasons.

Source of variation	df	Mean square							
		Grain yield	GW	PC	MC	ZT	WA	GH	LV
Year (Y)	1	0.60	156.1*	1.29	2.54*	0.03	2.46	6.80	0.03
Environment (E)	1	749.9*	11610**	275*	193**	8288**	699*	758.5	1599366**
Y × E	1	0.03	20.03	0.45	0.001	0.13	0.64	9.52	2.82
Block (Y × E)	4	0.08	9.46	0.26	0.184	16.07	7.50	12.93	241.2
Genotype (G)	63	1.03***	43.99**	0.87**	0.341**	143.6**	47.27**	60.88**	16094**
G × Y	63	1.15***	1.78	0.05	0.016	2.97	0.46	5.53	586.9*
G × E	63	0.09***	19.78**	0.77**	0.383**	81.64**	11.13**	18.32**	3940.3**
G × E × Y	63	0.14**	0.95	0.04	0.033	3.27	0.36	4.67	642.1*
Residual	252	0.04	2.39	0.15	0.117	8.34	1.96	6.67	411.3
R^2		0.98	0.96	0.91	0.89	0.92	0.90	0.80	0.97
CV (%)		6.51	4.63	3.06	2.52	5.70	2.05	4.78	2.76

* Significant at $p < 0.05$, ** Significant at $p < 0.01$.
Abbreviations represent as follows: 1000 grain weight (GW), protein content (PC), moisture content (MC), Zeleny sedimentation volume (ZT), water absorption (WA), grain hardness (GH), loaf volume (LV).

Table 3
Maximum (Max.), minimum (Min.), l means, and broad sense heritability (h_b^2) of the traits of sixty-four wheat genotypes grown under two environmental (normal and heat stress) conditions.

Trait	Grain yield (t/ha)		GW (g)		PC (%)		MC (%)		ZT (ml)		WA (%)		GH (%)		LV (ml)	
Condition	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Max.	5.49	2.7	45.46	33.00	12.60	14.98	15.13	13.43	58	65.75	72.63	75.83	57.43	60.58	760	887
Min.	2.68	1.12	30.00	20.53	11.08	12.25	13.18	12.38	34	40.5	59.1	62.33	42.9	46.35	556	648
Mean ± SE	4.304 ± 0.40^a	1.88 ± 0.28^b	38.13 ± 02.10^a	26.95 ± 01.90^b	11.83 ± 0.30^b	13.30 ± 0.40^a	14.19 ± 0.30^a	12.96 ± 0.21^b	46.57 ± 03.22^b	54.62 ± 03.92^a	66.97 ± 01.82^b	69.40 ± 01.84^a	$52.77 \pm 02.14^{a*}$	55.35 ± 01.95^a	679 ± 29.63^b	791 ± 36.45^a
h_b^2 (%)	58.75	64.48	54.84	66.40	49.44	56.00	55.22	51.22	65.44	66.02	66.48	65.34	66.00	54.13	67.07	65.03

* In each trait, means with the same letters for each trait are not significantly different at $p < 0.05$ by Tukey's HSD test ($HSD_{5\%}$).
Abbreviations represent as follows: 1000 grain weight (GW), protein content (PC), moisture content (MC), Zeleny sedimentation volume (ZT), water absorption (WA), grain hardness (GH), loaf volume (LV).

The mean of ZT of 64 genotypes exposed to high temperature ranged from 40.5 to 65.8 cm^3 (Table 3, Table S1). ZT of flour from normal conditions ranged between 34.0 and 58.0 cm^3.

3.4. Starch content

SC was significantly influenced by heat stress and genotype (Table 4). SC of all 17 genotypes tested was decreased in heat stress conditions compared to normal conditions (Table 5). Genotype no. 34 produced the least SC (52.7%) when exposed to heat stress. The highest SC loss was found to be related to genotype no. 34.

3.5. Amylose and amylopectin contents

In this study, heat stress was associated with a decrease in amylose content and amylose/amylopectin ratio (Table 5). The highest reduction in AMC occurred in genotype no. 2, 28, 34, and 40 (>18.4%). In general, the mean AMC of the tolerant group was higher than the sensitive group of wheat genotypes. Genotype no. 6 had the highest AMC under normal conditions (29.8%) and showed the least AMC loss due to heat stress (5.8%). Genotypes no. 9 and 19 produced the lowest AMC under heat stress conditions. On the other hand, the AMP of all genotypes increased with an average increase of 4.94% when exposed to heat stress. The highest rise in AMP was related to genotype no. 28 (8.91%). In contrast, genotype no. 19 and 9 had the lowest increase percentage (1.87 and 2.17% increase, respectively), thereby maintained their AMC and AMP grain composition under stress conditions.

3.6. Lipid content

Heat stress due to late sowing slightly but significantly increased LC of wholemeal wheat flour of both tolerant and sensitive genotype groups. A significant difference was observed for LC among the wheat genotypes in both environmental conditions (Tables 4 and 5). Sensitive genotype no. 13 showed the highest increase (30.8%) in LC when exposed to heat stress, while the least change in LC was related to genotype no. 3and 58. Sensitive genotype no. 13 had the highest LC (3.85%) under heat stress conditions, while the sensitive genotype no. 34 had the lowest LC in normal (1.98%) and heat stress (2.34%) conditions. However, there was no significant association between the LC and LV in this study (Table 6).

3.7. Ash content

AC ranged from 0.70 to 1.36% (as seen in genotype no. 44 and 2, respectively) under normal conditions, and 0.87 and 1.77% (related to genotype no. 11 and 2, respectively) under heat stress conditions (Table 5).

3.8. Other Baking-quality related attributes

WA of the wheat genotypes was slightly but significantly increased due to heat stress (Table 3). Flour from genotype 61 showed the lowest WA in both conditions (Table S1). The highest WA capacity was found in genotype no. 20 and 35 and the lowest in no. 6 and 61.

Genotypic means of MC showed a significant decrease in response to heat stress. The overall mean changed from 14.19 in normal to 12.96% in stress conditions (Table 3 and Table S1).

LV of bread is a volume-to-weight ratio estimator. Desirable LV (over 660 ml, Pomeranz et al., 1984) depends on the gluten quantity and quality and is achieved when satisfactory conditions for yeast growth and gas generation are provided by dough. A signifi-

Table 4
Results of analysis of variance and broad sense heritability (h_b^2) for the tested traits of 17 genotypes grown under two field conditions (normal and terminal heat stress).

			Mean square						
Source of variation	df	SC	AMC	AMP	LC	AC	WG	DG	GI
Environment (E)	1	368.8**	435.2**	435.2**	3.66**	7.24**	430.8**	87.46**	1238**
Genotype (G)	16	53.08**	32.37**	32.37**	0.76**	0.272**	43.08**	3.14**	127.5**
G × E	16	2.91**	4.44**	4.44**	0.12**	0.177**	2.65**	0.80**	27.20**
Residual	102	0.192	0.28	0.28	0.011	0.006	1.02	0.14	2.41
R^2		0.98	0.97	0.97	0.93	0.96	0.92	0.91	0.94
CV (%)		0.64	2.04	0.71	3.99	6.38	3.21	3.71	2.07
h_b^2 (%)	Normal	54.95	47.26	47.26	42.21	38.58	43.63	52.95	63.81
	Heat stress	61.44	43.10	43.10	59.08	39.05	50.01	58.00	72.23

*Significant at $p < 0.05$, ** Significant at $p < 0.01$.
Abbreviations represent as follows: starch content (SC), amylose content (AMC), amylopectin content (AMP), lipid content (LC), ash content (AC), wet gluten (WG), dry gluten (DG), gluten index (GI).

Table 5
Means of grain quality traits of 17 wheat genotypes (tolerant and sensitive) in normal and heat stress conditions.

Trait	Condition	Tolerant genotypes								Sensitive genotypes									HSD
		2	3	5	6	11	16	17	28	9	13	19	34	38	40	44	49	58	
Yield (t ha^{-1})	Normal	4.43	4.55	4.29	4.86	4.57	4.53	4.49	4.78	2.68	3.88	3.38	3.55	3.99	3.76	3.82	4.01	3.49	0.693
	Stress	2.46	2.70	2.55	2.53	2.52	2.30	2.43	2.52	1.38	1.53	1.70	1.62	1.63	1.12	1.66	1.15	1.45	0.694
GW (g)	Normal	35.23	38.98	36.64	39.68	39.38	40.84	37.86	43.54	32.14	40.28	37.63	41.83	32.71	35.12	34.13	41.94	38.73	2.437
	Stress	27.09	30.23	26.63	30.31	30.75	31.06	29.69	33.00	20.53	28.09	29.00	28.5	26.25	26.50	24.63	29.17	24.11	5.200
PC (%)	Normal	12.18	11.68	12.28	11.55	11.68	11.38	11.65	12.00	11.73	12.23	12.08	12.03	12.33	11.93	11.48	12.18	12.20	0.64
	Stress	12.88	13.00	13.10	12.25	12.60	12.58	13.03	13.23	14.10	13.35	13.70	13.43	13.00	14.20	13.33	13.40	14.25	1.049
MC (%)	Normal	13.75	14.00	13.98	13.80	13.65	14.63	14.55	14.75	13.18	14.00	13.83	13.9	14.18	14.55	14.30	13.75	13.78	0.838
	Stress	13.05	13.05	13.18	12.95	13.43	13.03	13.28	13.00	12.78	13.08	12.58	12.83	13.30	12.73	12.65	12.95	12.55	0.741
ZT	Normal	50.50	44.50	52.70	49.25	45.50	45.50	45.75	48.25	50.00	47.25	52.50	50.00	50.50	46.75	40.75	49.75	49.25	6.601
	Stress	55.50	50.00	49.75	50.25	48.50	52.00	49.75	49.75	58.00	50.50	58.75	58.25	52.25	56.75	50.25	51.75	55.25	5.223
WA (%)	Normal	69.20	67.43	67.200	59.90	67.00	66.60	67.78	68.50	63.23	67.55	65.88	66.90	65.93	72.63	66.58	68.00	66.43	2.169
	Stress	70.25	69.83	68.10	62.85	67.70	69.80	68.53	69.88	70.13	69.20	69.75	71.08	68.20	73.05	68.10	68.70	71.25	3.27
GH	Normal	53.78	53.13	51.68	52.00	50.00	53.00	52.00	52.50	52.70	54.00	53.58	54.20	50.40	57.43	52.30	55.00	52.10	2.667
	Stress	56.33	56.33	52.75	48.95	52.70	56.35	53.38	55.48	55.93	55.93	54.85	57.15	55.53	58.70	55.23	57.2	56.75	2.773
LV (ml)	Normal	692.8	693.0	719.8	573.0	630.3	666.0	661.0	686.0	651.5	732.8	710.5	708.0	724.3	727.3	682	760.5	692.8	56.38
	Stress	729.8	766.8	789.8	666.5	756.5	735.8	789.3	825	781.0	825.3	782.3	813	738	878.8	835.3	833.5	844.5	50.58
SC (%)	Normal	67.25	69.78	67.39	72.35	71.95	71.96	72.49	71.25	66.28	71.28	72.63	70.78	63.48	67.8	67.9	70.98	69.53	1.106
	Stress	57.83	60.71	56.30	60.77	62.96	63.68	61.0.28	62.93	54.35	60.05	60.37	52.73	54.59	55.93	55.34	58.56	55.27	1.143
AMC (%)	Normal	27.29	27.02	29.56	29.81	28.79	29.04	29.95	31.07	22.44	26.23	23.30	28.29	26.54	26.34	27.17	27.29	29.49	1.22
	Stress	21.99	23.98	24.96	28.09	25.28	26.99	24.98	24.93	20.76	24.20	21.86	24.10	21.65	21.45	23.69	24.95	24.93	1.484
APC (%)	Normal	72.71	72.98	70.44	70.19	71.21	70.96	70.05	68.94	77.56	73.78	76.70	71.71	73.46	73.66	72.84	72.71	70.52	1.22
	Stress	78.01	76.03	75.04	71.91	74.73	73.01	75.02	75.08	79.24	75.8	78.14	75.90	78.35	78.55	76.31	75.05	75.08	1.484
LC (%)	Normal	2.40	2.86	2.52	2.30	2.74	2.32	2.32	2.80	2.10	2.94	2.47	1.98	2.68	2.24	2.86	2.35	2.83	0.278
	Stress	2.93	2.95	2.93	2.88	3.05	2.74	2.45	2.92	2.62	3.85	2.74	2.34	3.14	2.35	3.21	2.64	2.92	0.273
AC (%)	Normal	1.36	1.06	0.81	1.18	0.86	0.82	0.83	1.22	0.86	0.75	1.01	0.73	1.18	0.82	0.70	1.09	0.77	0.063
	Stress	1.77	1.40	1.08	1.29	0.87	1.39	0.92	1.43	1.58	1.28	1.61	1.38	1.28	1.72	1.66	1.67	1.54	0.265
WG (%)	Normal	29.81	30.58	32.44	30.00	31.31	28.24	28.92	29.21	27.92	30.73	33.57	27.60	30.95	33.06	25.20	26.40	27.30	1.931
	Stress	32.13	32.19	36.07	35.04	34.81	32.79	32.13	35.40	30.24	34.66	36.70	32.63	33.39	36.46	28.81	29.41	30.87	3.12
DG (%)	Normal	9.15	9.39	9.96	10.27	9.61	8.67	8.88	8.97	8.57	9.43	10.31	8.47	9.50	10.15	8.44	9.21	8.38	0.505
	Stress	10.9	11.05	12.05	12.07	10.57	10.96	9.74	11.83	10.22	11.58	10.90	11.02	10.70	11.20	9.84	10.30	9.70	1.261
GI	Normal	70.28	75.75	73.76	62.11	78.75	72.63	69.53	74.13	68.21	75.50	69.75	73.00	72.00	73.25	71.00	74.25	71.38	4.191
	Stress	74.93	83.10	78.50	64.75	80.00	79.39	76.73	76.00	73.25	76.30	78.00	82.60	78.00	83.50	84.00	82.91	79.50	3.779

*In each row, means were compared using the provided HSD at $p < 0.05$.
Abbreviations represent as follows: 1000 grain weight (GW), protein content (PC), moisture content (MC), Zeleny sedimentation volume (ZT), water absorption (WA), grain hardness (GH), loaf volume (LV), starch content (SC), amylose content (AMC), amylopectin content (AMP), lipid content (LC), ash content (AC), wet gluten (WG), dry gluten (DG), gluten index (GI).
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cant increase in the LV of 64 wheat genotypes was observed (Table 3 and Table S1). The highest LV alteration was found in genotype no. 59 (34.2%) under heat stress conditions. Under normal conditions, the bread volume was in the range of 556 and 760 ml (corresponding to genotypes no. 49 and 61, respectively), whereas its range changed from 648 to 887 ml (corresponding to genotypes no. 45 and 40, respectively) under heat stress conditions. The mean LV of sensitive genotypes was higher than tolerant genotypes. The maximum of LV related to sensitive genotypes 40, 44, 49, and 58 under high-temperature conditions. Heat tolerant genotype no. 6 showed the lowest LV in both environmental conditions.

3.9. Traits association and principal component analysis (PCA)

Negative correlations were found between yield and PC under normal and heat stress conditions (r = –0.77 and r = –0.76, respectively (Table 6). PC correlated significantly with LV (r = 0.79), WA (r = 0.72), GH (r = 0.61), and ZT (r = 0.63), which agree with the previous finding (Flagella et al., 2010).A strong correlation between SC and GW (r = 0.80) under heat stress conditions was observed. However, the correlation coefficient between GW and AMC was relatively high (r = 0.60) under heat stress conditions. AC showed negative correlation with yield (r = -0.54) and MC (r = -0.76) and positive correlation with GH (r = 0.72).

Table 6
Coefficient of correlation of grain yield, GW and quality traits of 17 wheat genotypes under normal (above diameter) and heat stress (below diameter) conditions.

Trait	Grain yield	GW	PC	MC	ZT	WA	GH	LV	SC	AMC	APC	LC	AC	WG	DG	GI
Yield	1	0.45	−0.77**	0.62**	−0.43	−0.54*	−0.41	−0.45	0.40	0.75**	−0.75**	0.18	−0.44	0.14	0.26	0.11
GW	0.58*	1	−0.16	0.09	−0.06	−0.15	−0.01	0.08	0.65**	0.56**	−0.56**	−0.02	−0.21	−0.14	−0.06	0.29
PC	−0.76**	−0.63**	1	−0.73**	0.44	0.72**	0.67**	0.79**	−0.19	−0.43	0.43	−0.32	0.52*	−0.09	−0.22	0.06
MC	0.57*	0.43	−0.73**	1	−0.55*	−0.41	−0.40	−0.44	0.11	0.37	−0.37	0.26	−0.76**	0.25	0.18	0.35
ZT	−0.58*	−0.49*	0.63**	−0.65**	1	0.583*	0.41	0.25	−0.22	−0.27	0.27	−0.41	0.49*	0.25	0.14	−0.11
WA	−0.44	−0.26	0.71**	−0.37	0.58*	1	0.94**	0.65**	−0.28	−0.35	0.35	−0.34	0.47	0.03	−0.32	0.37
GH	−0.63*	−0.25	0.61**	−0.48*	0.56*	0.81**	1	0.67**	−0.12	−0.25	0.25	−0.29	0.48	−0.15	−0.45	0.44
LV	−0.61**	−0.25	0.79**	−0.44	0.21	0.68**	0.57*	1	−0.16	−0.09	0.09	−0.14	0.31	−0.17	−0.27	0.45
SC	0.64**	0.80**	−0.19	0.11	−0.29	−0.23	−0.39	−0.16	1	0.20	−0.20	0.05	−0.25	0.13	0.20	0.06
AMC	0.49*	0.60*	−0.51*	0.25	−0.62**	−0.58*	−0.47	−0.32	0.52*	1	−1.0**	−0.06	−0.47	−0.18	−0.13	0.09
APC	−0.49*	−0.60*	0.51*	−0.25	0.62**	0.58*	0.47	0.32	−0.52*	−1.0**	1	0.06	0.47	0.18	0.13	−0.09
LC	0.12	0.03	−0.32	0.26	−0.48	−0.33	−0.27	−0.14	0.05	0.07	−0.07	1	−0.14	0.09	0.12	0.25
AC	−0.54*	−0.37	0.52*	−0.76**	0.61*	0.44	0.72**	0.31	−0.25	−0.47	0.47	−0.14	1	−0.24	−0.14	−0.22
WG	0.28	0.43	−0.15	0.20	−0.02	−0.06	−0.30	−0.13	0.26	0.04	−0.04	0.08	−0.31	1	0.85**	0.13
DG	0.38	0.44	−0.33	0.21	−0.18	−0.26	−0.27	−0.28	0.13	0.26	−0.26	0.27	−0.13	0.74**	1	−0.09
GI	−0.34	−0.05	0.35	−0.14	0.07	0.62**	0.56*	0.67**	−0.20	−0.26	0.26	−0.15	0.18	−0.25	−0.38	1

* and ** significant at $p < 0.05$ and $p < 0.01$, respectively.
Abbreviations represent as follows: 1000 grain weight (GW), protein content (PC), moisture content (MC), Zeleny sedimentation volume (ZT), water absorption (WA), grain hardness (GH), loaf volume (LV), starch content (SC), amylose content (AMC), amylopectin content (AMP), lipid content (LC), ash content (AC), wet gluten (WG), dry gluten (DG), gluten index (GI).

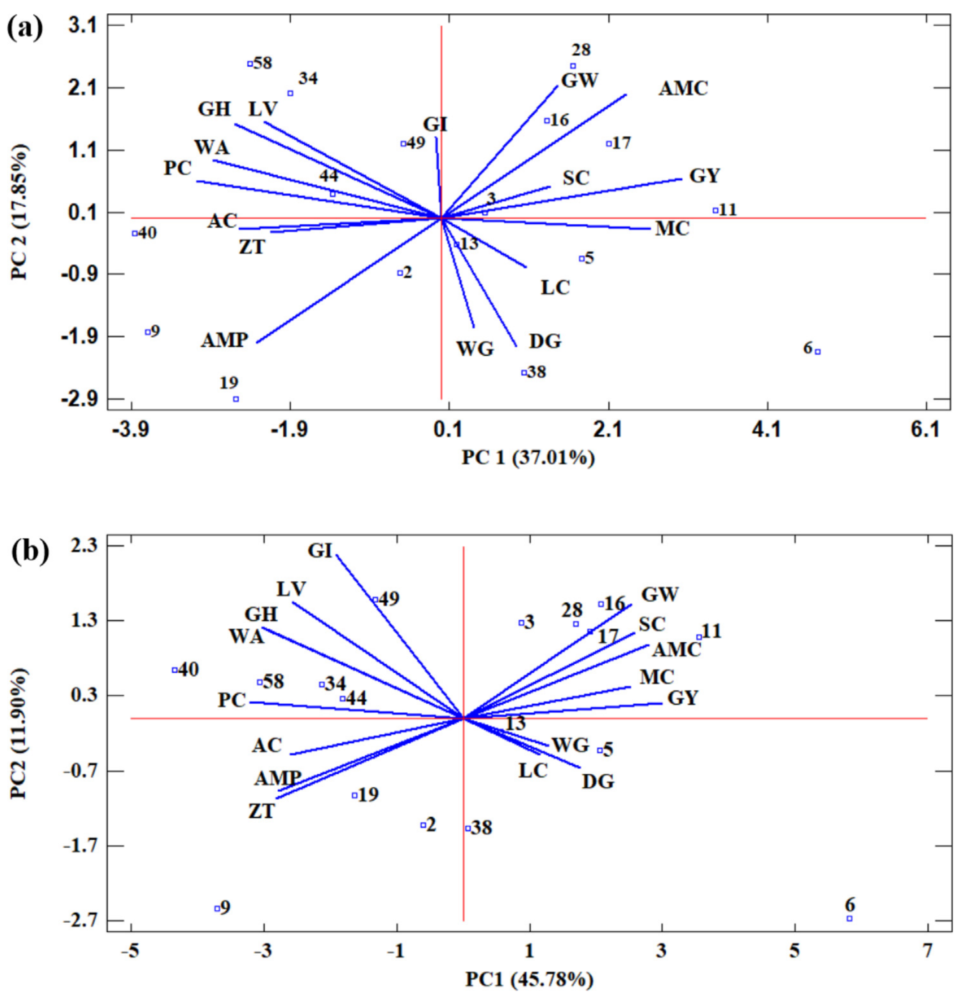

Fig. 1. The biplot display of grain yield (GY), GW and all quality traits under normal (a) and heat stress (b). Abbreviations represent as follows: GY (grain yield), 1000 grain weight (GW), protein content (PC), moisture content (MC), Zeleny sedimentation volume (ZT), water absorption (WA), grain hardness (GH), loaf volume (LV), starch content (SC), amylose content (AMC), amylopectin content (AMP), lipid content (LC), ash content (AC), wet gluten (WG), dry gluten (DG), gluten index (GI).

PCA was performed to explore relationships among genotypes and the flour quality traits of 17 out of 64 genotypes under normal and heat stress conditions (Fig. 1). Under normal conditions, the PC1 and PC2 explained 54.86% of total variation (Fig. 1a). The first PC (PC1) accounted for 37.01% of the total variation and established high positive correlations with yield, MC, and AMC and negative correlation with PC, WA, and ZT. PC2 explained 17.85% of the total variance and correlated positively with GW and AMC, GI, and LV. The biplot of PC1 and PC2 shows a group of genotypes (no. 5, 6, 11, 16, 17, 28, and 44) possessing high yield and AMC.

Under heat stress conditions, 57.68% of the total variation was explained by the first two principal components (PC1 and PC2; Fig. 1b). PC1 showed 45.78% of total variation and exhibited a positive association with yield, AMC, MC, GW, and SC; but showed a significant negative relationship with PC, GH, WA, ZT, AMP, and AC. PC2 accounted for 11.90% of the total variance and exhibited positive correlation with GI, LV, and WG. The biplot of PC1 and PC2 reveals a group of genotypes (no. 3, 6, 11, 16, 17, and 28) characterized by high yield, GW, and AMC, and low PC, GH, ZT, and LV. It was noted that genotype no. 6 was distinctly separated from the other genotypes due to having the highest AMC and the lowest PC, LV, WA, GH, and GI, which are coherent with our prior study. In other words, this heat-tolerant genotype (no. 6) stored higher AMC in the grains than other genotypes, but it was clearly weaker for PC, LV, WA, GH, and GI under heat stress conditions.

4. Discussion

The drastic effect of heat stress on yield found in the wheat genotypes in the studied region (Mehran, Ilam, Iran) is consistent with the data of high Tmax 35–38 °C in 2015–2016 or 35–40 °C in 2016–2017 during grain filling period. Yield loss due to high temperature during reproduction and grain filling period found here agrees with the previous reports on wheat (Asseng et al., 2017; Ni et al., 2017). It has been evident that chronic high temperatures (>30 °C) cause a significant reduction of yield in wheat (Dwivedi et al., 2017).

Reduction of GW in our findings are consistent with those of others who have shown decreased weight of grains in wheat plants exposed to heat stress (Dwivedi et al., 2017; Telfer et al., 2018). GW is one the key targets for deteriorating grain yield by terminal heat stress (Bahrami et al., 2021). Heat stress accelerated anthesis and physiological maturity of wheat genotypes, especially in terms of shortening the grain filling duration by 27.6% under normal to heat stress conditions (Mahdavi et al., 2021). The GW and its alterations in response to heat stress were lower in the tolerant than sensitive genotypes in our study, pointing to underlying mechanisms that drive plant protection against heat stress such as heat-shock proteins. These results demonstrate that starch was one of the potential targets of heat stress in altering the grain composition which is in accordance with previous studies (Balla et al., 2011; Singh et al., 2021). Delayed sowing means that late crop development such as flowering and grain filling stages occurs under warmer conditions (Fleitas et al., 2020). Starch in the grain endosperm is the major carbon store in plants (Dupont, 2008). Therefore, biosynthesis and accumulation of starch during the dynamic processes of grain filing is also a determinant factor in grain production and quality in wheat. GW and yield are dependent on the photosynthetic performance of the leaves, particularly flag leaf (source tissues), to supply photoassimilates for the synthesis of storage material such as starch throughout the grain-filling process (Dwivedi et al., 2017). Here, we argue that heat stress imposes a robust limit to the critical duration of the grain development, which occurs within a time span ranging from the pollination to the maturity stages. In addition to photosynthesis inhibition, heat stress may inhibit enzyme activity, i.e., sucrose synthase (SS) enzyme (Labuschagne et al., 2016), soluble starch synthase (SSS) enzyme (Hernández-Espinosa et al., 2018), and other starch biosynthesis-related enzymes (Lu et al., 2019).

The functional properties of starch depend on factors like the ratio of amylose to amylopectin (Li et al., 2013). Amylose is a key to define starch quality. In the current study, the quality of starch, as revealed by amylose content, was declined by heat stress. Likewise, Viswanathan and Khanna-Chopra (2001) also reported a decrease in the amylose content under heat stress. Lu et al. (2019) found that heat stress caused the down-regulation of the expression of 22 out of the 23 starch-related genes, including the genes encoding enzymes of the starch biosynthesis pathway (SSS, GBSS, SS, and SBE). The activity of granule-bound starch synthase (GBSS) is reduced at the grain filling period under high temperature stress in wheat (Zhao et al., 2008). The decrease in AMC of the grains was significantly related to the reduction in grain weight, indicating that amylopectin deposition is the main determining factor for the loss of grain weight under heat stress.

Increased PC due to heat stress has also been noted by other researchers (Bonfil et al., 2015; Singh et al., 2021), revealing that the heat-induced grain weight loss is more significant than the loss of protein accumulation (Zhao et al., 2008). This finding is coherent with the observation that heat-sensitive genotypes possessed a much lower yield than other genotypes in heat stress conditions and correspondingly had much higher PC in their grains. An increase in PC due to high temperatures during the grain filling period can be partly explained by altered source-to-sink C partitioning; and thereby interactions with metabolism and partitioning of N in the sources and sinks (Bonfil et al., 2015). A higher decrease in C than N partitioning to grains may also result in a higher proportion of protein than starch and sucrose in the grains. PC accumulation begins approximately on the sixth day of flowering and continues until the end of the grain-filling period (Panozzo et al., 2001). A more general explanation is granted based on specific weight change of the grain such that an increase in PC caused by heat stress may be associated with yield loss due to the reduction in starch production (Fowler, 2003).

The increase in WG, DG, and GI of wheat genotypes is in agreement with Bonfil et al. (2015) in common wheat and Sissons et al. (2018) in durum wheat. An increase in the synthesis of gliadin-like heat shock elements (HSE), which reduces the ratio of glutenin to gliadin, and ultimately lessens the GI, may be one possible explanation (Li et al., 2013). Genetic background could be the reason why heat-tolerant genotype no. 6 exhibited the lowest GI in both environmental conditions (Bonfil et al. 2015). This finding, however, cannot be rationally explained given that this genotype is a late ripening with long duration of grain filling. In the current study, it was shown that wheat grains undergo stronger gluten in their endosperm, as seen for ZT after plant treatment with heat stress during the filling period. We found that the value of ZT have a direct relationship with value of GI, similar to what was observed in some other studies in wheat (Bonfil et al., 2015; Li et al., 2013). It is also evident that there is inconsistency concerning the effect of heat stress on the ZT and GI. There are studies supporting positive effects of heat stress as does our (Bonfil et al., 2015; Li et al., 2013), while report of negative effect is also present (Balla et al., 2011). Several potential factors in the studies may explain these discrepancies. These include differences in the genetic materials, intensity and duration of heat stress, growth environment, sampling and analysis (Bonfil et al., 2015). A significant increase in the LV of wheat is in line with precious finding (Hernández-Espinosa et al., 2018; Li et al., 2013).

In this study, the increase in LC of wholemeal wheat flour is consistent with that of Wang et al. (2016), who found a positive association between heat stress and LC in wheat. The flour lipids play an important role at different stages of breadmaking (Arzani and Ashraf, 2017), and hence breadmaking quality is significantly impacted by the lipids. Lipids improve the LV of the bread by adsorbing to the surface of gas bubbles during the early dough development stages, stabilizing them, allowing dough gas retention (Min et al., 2020). Unlike starch and protein, which are chiefly present in the endosperm, a major portion of lipids (about 70%) is located in the dorsal side of the grain, including pericarp, aleurone, and embryo (Liu, 2011).

Likewise, an increase in AC due to high temperature has already been reported (Singh et al., 2021). This increase may be attributed to the alteration of the bran to endosperm ratio (Katyal et al., 2019). Wheat bran is a rich source of minerals (Arzani and Ashraf, 2017). Whether the increase in AC in wheat grains quality will translate into better nutritional outcomes remains further elucidated. Higher bran to endosperm ratio and GH may have contributed to more AC under stress conditions (Katyal at al., 2019). The overall results of this study show a slight effect of heat stress on the GH. These are consistent with a recent study that performed a single-season field experiment of timely and delayed sowings and found a profound effect of heat stress due to delayed sowing on the hard GH (Singh et al., 2021). However, we used medium to hard GH genotypes which may be a reason for the slight differences observed between our results and those of Singh et al. (2011). GH is mainly a consequence of the strength of adhesion between protein and starch in the endosperm (Singh et al., 2021).

An increase in WA of the wheat genotypes due to heat stress caused by delayed sowing is in agreement with the study of Seleiman et al. (2011), but it contrasts with the study of Singh et al. (2021). Grains of heat-tolerant genotypes were less subject to MC loss compared with heat-sensitive genotypes. The moisture content of the grains is a crucial quality marker of security of grain storage (i.e., viability, germination, and physiological health of the grain). Accordingly, preservation of the MC in hot conditions could be accounted as a reference point in protecting grain from stress damage (Ziegler et al., 2021).

The negative correlations found between yield and PC were consistent with the previous findings in wheat genotypes (Tahir et al., 2006). The inverse relationship between yield and PC might be justified by the significant reduction in GW and SC. The complex network for biosynthesis of storage protein, and its accumulation and deposition and degradation, along with their interactions with abiotic factors are needed for full explanation. PC correlated significantly with LV, WA, GH, and ZT represented that the increase in PC, LV, ZT, and, GI of sensitive genotypes was higher than those in tolerant genotypes. The results suggest genotypic-dependent heat stress effects on grain-quality related attributes in wheat. GI was correlated significantly with LV, WA, and GH under heat stress conditions. These results are coherent with those by Varga et al. (2003). Kaur et al. (2013) showed that GI was correlated only with WG, DG but not with PC and other parameters. Another study showed that GI did not show any correlation with other parameters (Bonfil et al., 2015). In response to heat stress due to late sowing, in addition to the increase in PC, a reduction in SC occurred, which could be explained by the decline in GW (Balla et al., 2011). Starch accumulation is mostly limited by the sink, while protein accumulation is source-limited. Hence, CO_2 fixation is sufficient to maintain satisfactory grain-fill (Dupont, 2008). In a previous study, we observed that tolerant genotypes were superior in CO_2 fixation than sensitive genotypes (Mahdavi et al., 2021), which can justify high GW under heat stress conditions. The strong correlation between SC and GW under heat stress conditions exhibited that tolerant genotypes produce grains with a greater GW and SC than sensitive genotypes. On the other hand, starch quality (the ratio of AMC to AMP) of tolerant genotypes was also better than sensitive genotypes.

5. Conclusion

High temperatures during the grain filling resulted in drastic grain yield loss in all 64 wheat genotypes. Heat tolerant CIMCOG line no. 3, 5, and 28 are recommended for cultivation in the area (Mehran, Iran) and other warm areas with similar agro-ecological conditions. Reducing grain yield due to heat stress associated with the reduction in GW and SC, thus expecting a lower milling yield. The synthesis of protein in grains was less affected by heat stress than that of the carbohydrate showing that the photosynthetic apparatus is thermoinhibited. Thermal stability of the photosynthetic apparatus would be hence a reason behind why yield, starch and amylose contents of the tolerant genotypes are much less affected by high temperatures. Severe heat decreased GW and yield, whereas improved flour quality traits PC, ZT, LV, WG, DG, and GI in the wheat genotypes. The observation of this study is consistent with a general trade-off between grain starch and protein. Whether this improvement in grain quality attributes will translate into better human health outcomes requires further investigation.

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CRediT authorship contribution statement

Soraya Mahdavi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ahmad Arzani:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **S.A.M. Mirmohammady Maibody:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Mahdi Kadivar:** Conceptualization, Formal analysis, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103417.

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