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Research Article

Impacts of heat shock on productivity and quality of *Triticum aestivum* L. at different growth stages

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Abstract

The transitional phase of climate change is becoming a threat worldwide. Fluctuations in temperature are frequently observed in the life cycles of field crops. The current study was intended to evaluate heat stress (HS) at sensitive stages on four wheat cultivars' growth, grain yield, and quality traits during two consecutive seasons. The experiment included four spring wheat cultivars that were exposed to HS durations (i.e., 0, 48, 96 and 144 h) at booting and anthesis stages. Pots were kept in an open-air, and labeled pots were moved to the glasshouse at the respective growth stage. After HS treatments, pots were moved from the glasshouse to the original position in the open air. The results showed that the cultivars differed in yield and grain quality traits, but their collective mean effect was significantly expressed with HS and HS duration (HSd) on the measured traits. Spike length was significantly affected by HS at the anthesis in comparison to those obtained from the control. However, the increase in HSd did not cause a significant effect on spike length compared to other treatments. Spike weight was significantly decreased in plants exposed to HS at booting and anthesis as well as by increasing HSd. In addition, grain weight and numbers were decreased in plants exposed to HS at booting and anthesis stages with a similar pattern by increasing HSd. Moreover, a reduction in amylose content was observed in grains of plants exposed to HS at booting and anthesis stages or when plants as exposed to a longer period of HSd. Nonetheless, amylopectin, wet gluten (WG) and grain N (GN) showed an increase in plants exposed to HS at booting and anthesis stages and/or extending HSd. This study concludes that plants exposed to different HS treatments during the life cycle had lower values for spike length, grains weight, and grains number per spike including amylose content in comparison to those obtained from the control. The decrease was significantly noted in the yield and grain quality of wheat when plants were exposed to HS at the booting stage in comparison to those exposed to HS at anthesis.

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Keywords: climate change; heat shock duration; grain quality; wheat yield

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide and is mainly consumed as a staple food (Seleiman and Kheir, 2018; Qiang *et al*., 2020; Seleiman *et al*., 2021a). Wheat consumption is higher in developing countries due to low-price food for human consumption (Anteneh, and Asrat, 2020; Khan *et al*., 2022). It is a rich source of energy and carbohydrate (Seleiman *et al*., 2019; Alkharabsheh *et al*., 2021; Battaglia *et al*., 2022). It provides components essential for health i.e. protein, vitamins, dietary fibers, and phytochemicals (Akmal and Goher, 2021; Seleiman *et al*., 2021b; Taha *et al*., 2021). In the recent past, climate changes are observed which have affected many crops including wheat with different ways of occurrence in a crop life cycle (Battaglia *et al*., 2021; Ding *et al*., 2021; Khan and Akmal, 2021; Kheir *et al*., 2021). The scarcity of water at the time of sowing has shifted wheat sowing from late October to the end of November and even in December in areas where the crop is sown as a rainfed crop (Anjum, and Arif, 2022). Some unexpected rainfall in March adversely affected pollination and hence a decrease in the average grain set per spike was noted (Khan and Akmal, 2021). Some areas receive unwanted rains close to the crop maturity and hence caused lodging problems for mechanical harvesting (Goher and Akmal, 2021). However, it is generally observed that temperature rise is the main cause of changing rainfall patterns and distribution (Khan *et al*., 2022). Temperature rise promotes growth but may not necessarily be effective for any stage, especially the sensitive stages i.e. booting or anthesis stage (Goher and Akmal, 2022). A sensitive stage of growth exposed to heat shock (HS) can adversely affect yield and/or grain quality (Awan *et al*., 2017). Nonetheless, HS duration is the most important when it coincides with the crop growth stages (Khan *et al*., 2022). Limited HS when coinciding with the very sensitive stage of plant growth may adversely affect yield and/or grain quality (Asseng *et al*., 2015). Adverse effects have been observed on grain yield and its quality when plants are exposed to heat shocks at the grain development stage (Goher and Akmal, 2021). It might be expected that high temperature ensures food security in some parts of the world (Prakash *et al*., 2017).

Both grain yield and grain quality of cereals could be better correlated with the climate of the area in which the crop grows (Tadesse *et al*., 2016). The expansion of industrialization has increased CO2 emissions and has also increased its concentration in the air with some other greenhouse gases (Ozlu *et al*., 2022). This raised the global net temperature (IPCC, 2013). Today, an increase in temperature (1.5 °C) has been reported and is also expected to increase by 4 °C in 2050 (Prakash *et al*., 2017). Nevertheless, some unexpected changes with temperature rise have been noticed in the crop life cycle in Pakistan (Hanif and Ali, 2014). The unexpected extreme events have sown some adverse effects on plants experiencing heat shock at the sensitive stage of growth which could be critical to sustaining future food security in most parts of the world experiencing future climate changes (Liu *et al*., 2016).

Wheat responses under the HS have been observed differently at the different growth stages of the plants (Nezhadahmadi *et al*., 2013). The ideal temperature for wheat growth is observed around 25 °C (Ullah *et al*., 2019). However, with an increase in temperature during growth and development, the plant enters the vegetative to the reproductive stage of development to complete its life cycle (Asseng *et al*., 2015). Wheat crop expresses anthesis at 24 °C (Ullah *et al*., 2019) and enters the crop reproductive development at 25 °C (Prasad and Djanaguiraman, 2014). However, these temperature fluctuations at the anthesis could be a threat to wheat grains development and to sustain good quality (Gupta *et al*., 2013). Loss in grain yield is estimated at 6% with a 1 °C rise in temperature at anthesis from the optimum (Akter and Islam, 2017). A mild HS at anthesis has shown adverse effects on grain yield (Wang *et al*. 2016) by limiting the leaf photosynthetic activity with slow leaf rubisco activities (Asseng *et al*., 2015). Rapid leaf fall is also noticed with early senescence at high

temperatures (Akter and Islam, 2017). Abrupt daily change in temperature from the usual pattern has shown some adverse effects on the flag leaf area which decreased grain number on spikes (Tovignan *et al*., 2016). A decrease in the normal photosynthetic process has also been noted with a sudden increase in temperature from the normal (Asseng *et al*., 2015) which has resulted in limited grains per spike (Sharma *et al*., 2017) lighter grains weight (Ullah *et al*., 2019) and loss in grain yield. Loss in grain yield is due to limited resource utilization timings for the grain fill period with an increase in temperature from the optimum during the crop growth (Hossain *et al*., 2013). The plant experienced temperatures over 24 °C and has shown a significant decrease in grain yield (Prasad and Djanaguiraman, 2014). The grain protein content is also adversely affected by plants facing high temperatures at the reproductive stage of growth (Goher and Akmal, 2021). Leaf N interrelates with grain protein (Iqbal *et al*., 2017). The high temperature at the anthesis stage of crops has shown shrunk grain with lighter weight which caused lower grain yield (Narayanan, 2018). It is strange to know that high temperature at the grain-filling stage of the crop has shown higher grain N (Nuttall *et al*., 2018) but a reduction in gluten (MacRitchie, 2016). It might be due to denaturing of the grain protein. Dough strength has declined within grains grown at a higher temperature during the anthesis stage of the crop which deteriorates the grain backing quality (Narayanan, 2018). The backing quality of wheat grains relays on glutenin (MacRitchie, 2016) which changes the amyloplast concentration (Sangu, 2018) Wheat is mostly consumed for backing and grain quality is, therefore, an equal concern with the quantity produced for food security.

This research was aimed to investigate the negative effects of high-temperature as heat shocks (HS) on grain yield and quality traits of some high-yielding spring wheat varieties at sensitive growth stages (i.e. booting and anthesis stages) of the crop life cycle to quantify such impact on yield and quality of wheat with the recent climate change.

Materials and Methods

Experimental location

The experiment was conducted in plastic pots in crop growth season 2017-18 and repeated in 2018-19 at the Institute of Biotechnology and Genetic Engineering (IBGE), the University of Agriculture Peshawar, Pakistan (34°1'13.50N and 71°28'53.02E, 350m above sea level). The climate of the experiment location was subtropical and received 500-700 mm natural rainfall with daily mean temperature ranging from 24±6.24 °C to 40.7±6.29 °C during the crop growth season (Nov.-May). The soil of the experimental site was silt loam with a pH of +7.6 and the organic matter content was less than 1%. The soil texture was sand (18.13%) silt (71.23%) and clay (10.64%) which was classified as Ustocrept based on the USDA classification (Anonymous, 2007). Temperatures (Max. and Min.) with precipitation during growth season were obtained from Pakistan Met. Department (PMD), Peshawar, and are shown in Figure (1).

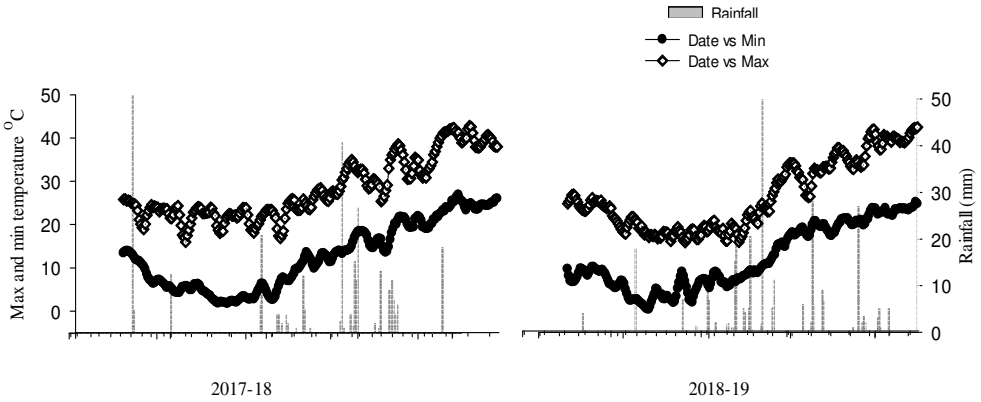

Figure 1. The temperature (°C) and rainfall (mm) during the wheat crop season (2017-18 and 2018-19). Source: Pak. Met. Department, Peshawar

Design and treatments

The experiment was a completely randomized design (CRD) with 4 repeats in plastic pots (18 cm x 20 cm). Soil and farm yard manure (FYM), received from the University Research Farm, was thoroughly mixed and filled in pots with a known quantity. Sowing was done on Nov. 25, 2017, and Nov. 09, 2018; initially with 10 seeds and thinned out to 5 after emergence. Four commercially high-yielding wheat varieties ('Pirsabak-2005', 'Pakhtunkhwa-2015', 'Pakistan-2013', and 'DN-84') were used for this study. Plants within pots were allowed to grow in open field conditions. For the treatment, HS one set of plants was shifted inside the glasshouse at the booting stage to stay there for 48 h, 96 h, and 144 h. On completion of the defined HS duration, the plants in pots were re-shifted back to their original position in the open field. Similarly, the other set of plants was shifted to a glasshouse at the anthesis and allowed to stay for HS for 48 h, 96 h, and 144 h. On completion of the defined HS duration pots were shifted back to the open air and allowed to complete the crop life cycle. The temperature inside and outside the glasshouse was periodically noted for every 3h on each day for the HS durations. One control treatment (No HS) was also included for which the plants in pots remained in the open air throughout the crop life cycle. Treatment HS was imposed at the booting stage (i.e. 2nd week of March) and at the anthesis stage (i.e. 1st week of April) for 48 h, 96 h, and 144 h, respectively. The temperature was recorded (n=5/d) during the HS durations with an auto-recorded thermometer (Humidity/Clock HTC-01) already installed inside the glasshouse and outside the field. The mean difference in temperature both inside the glass house and outside in field conditions were 1.5, 1.5, and 2.0 °C for 48, 96, and 144 h, respectively at the booting stage for year 1 of the experiment and 1.2, 1.3, and 2.0 °C in year 2. Similarly, mean temperature differences inside from the outside glass-house were 1.8, 4.3, and 4.6 °C for 48, 96, and 144 h, respectively at the anthesis stage for years 1 and 2.3, 2.6, and 2.7 °C for year 2. Plants were irrigated on an alternate day with a measured quantity of water during crop growth. Plants were harvested on May 03, 2018, and May 05, 2019.

Measurement

Spike length (cm) was observed on five representative tillers in a pot by manually measuring the length from the base to the tip excluding the awn. On complete drying, spike weight was recorded. Spike weight (g), grain weight (g), and grain number were noted on the same representative spikes in each pot. Grain yield was recorded on 10 spikes in a pot. The calorimetric iodine method was used (Juliano, 1971) to estimate grain amylose and amylopectin content. Briefly, grains were ground and passed through a 100 mm sieve in a grinding mill (Cyclone Mill Twister, 50/60 Hz). A ground sample (100 mg) was collected in a tube and added with 1.0 ml (95 %) ethanol and 9 mL NaOH (I M). To gelatinize, starch was kept for 20 min in a water bath. The boiled mixture was transferred to a flask and distilled water was added to make the volume 100 mL, stationed for 23 h at room temperature. For error correction, a blank (without a sample) was prepared. After thorough stirring,

5 mL solution was pipetted in another flask, added with 1 mL of glacial acetic acid (I M) and 2 mL iodine solution, adjusted 900 mL volume by adding distilled water. The sample was thoroughly mixed and kept for 20 min to develop a dark blue color. The spectrophotometer was calibrated by running a blank at 620 nm spectrum, and samples were run thereafter. The value of absorbance was then converted to amylose content using a standard calibration curve developed for pure potato amylose (Figure 2).

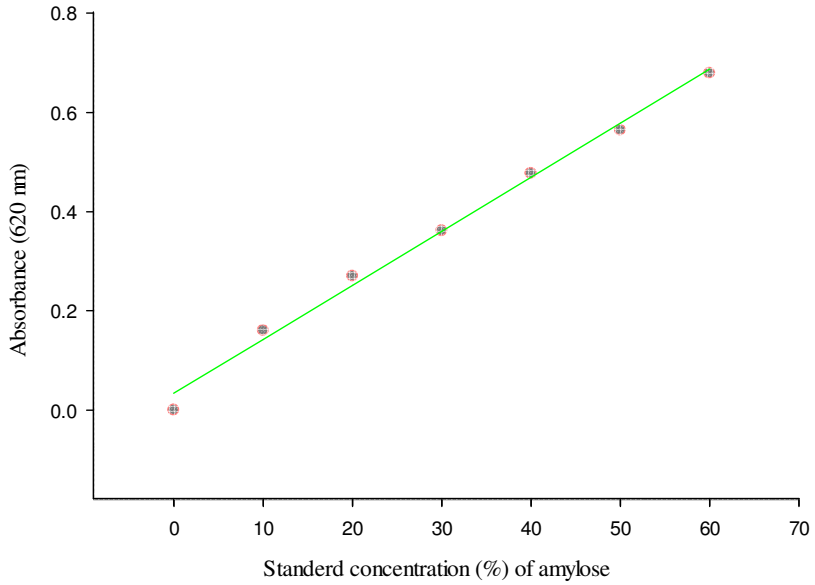

Figure 2. The standard curve developed for amylose in wheat grains solid dot shows the actual reading

Quality analysis

Amylose and amylopectin contents were also determined as per the standard procedure already used (Goher and Akmal, 2021). Briefly, a 12.5 mg sample was taken in tubes, added with 5 mL (45%) perchloric acid, and dissolved completely. Make the final volume 50 mL by adding distilled water. A 6.25 mL amylose stock was taken from 50 mL solutions and added with 18.75 mL ultra-pure water to make 6.25 mg mL^{-1} amylose solution, repeated the same procedure for the amylopectin solution. Using the stock solution, the standard solution of amylose and amylopectin was prepared in percentages of 0, 10, 20, 30, 40, 50, and 60 to a final volume of 5 mL for the standard curve. Transfer 40 µl of each standard mixture to a microtiter plate, add a 50 µl of iodine solution (2 g KI + 1 g I2 in 900 mL of ultra-pure water), and mixed each sample including a blank (perchloric acid) by pipetting. Read absorbance at 620 nm at spectrophotometer and calculated the amylose ratio for each concentration. To determine gluten (%), a hand wash method (AACC, 2004) was used. From each sample, 20 g of ground powder was taken and added to 14 mL water to form a wet dough. After about an hour, the dough was kept untouched, and all soluble matter and starch were removed by washing it in the running water in a tub. The resultant gluten ball was kept in a tarred, flat-bottomed dish and weighed as wet gluten. For estimation of dry gluten, the gluten ball was dried in the oven at 100 °C. Wet gluten (%) was determined as a ratio of wet gluten weight in sample weight and expressed in percentage. Grain N-content (%) was also determined by using Kjelflex-K360 (Buchi, Switzerland) following the protocol of Kjeldahl (Jones, 1991). A 0.2 g sample with 1.3 g digestion mixture (20 g $CuSO_4$+100 g K_2SO_4+0.2 g Se) was digested with 3 mL concentrated H_2SO_4 in the digestion tube, kept in digestion assembly till the clearness of the sample and changing color. The digest was filtered and distilled water was added to make the volume 100 mL. A 100 mL solution was added to the sample tube of Kjelfelx-K360 and distilled with 40% NaOH (prepared by adding

400 g of NaOH in 1 L distilled water), collected in a flask having 4% H_3BO_3 (40 g of H_3BO_3 in 1 L distilled water), and titrated against 0.1N HCL (100 mL of 1N standard impulse was added to 1L distilled water) with 877 titrino plus. Reading for the total N of the sample was adjusted with blank. For crude protein, the value of N content was multiplied with a factor 6.25.

Statistical analysis

Data collected during the study were statistically analyzed with a modified Excel Computer Software Program (ECSP) using the Fishers Analysis of Variance (ANOVA) technique and the mean where found significant ($p \leq 0.05$) was further evaluated using the least significant difference (LSD) test at 5 % probability (Steel *et al*., 1997).

Results

Primary yield traits

Spike length (cm), the primary yield trait, showed variations ($p \leq 0.05$) among different cultivars (C) and their plants when experienced heat shock (HS) of limited durations (HSd). Spike length was the maximum in 'Pakistan-2013', followed by 'Paktunkhwa-2015' with no change ($p \leq 0.05$) from 'Pirsabak-2005'. The minimum spike length was observed in 'DN-84' (Table 1). On average across the cultivars, spike length markedly increases ($p \leq 0.05$) under HS given at the anthesis to little increase with HS at the booting stage when compared with control i.e. no HS plants. While averaged over cultivars and HS, spike length increased ($p \leq 0.05$) by increasing the HSd from 48 h to 96 h and thereafter to the maximum of 144 h. Treatment effects of the single years and two years averages were the same. Interaction (C x HS) was significant for spike length (Figure 3). Where the three cultivars increased spike length when the plant experienced HS at anthesis over the booting stage but the DN-84 which showed almost the same spike length of plant experienced to HS at booting or anthesis stage.

Spike weight did differ ($p \leq 0.05$) within cultivars but was also influenced by imposed treatments HS and HSd (Table 1). The maximum spike weight was observed in Pakistan- in 2013, followed by Pakhtunkhwa-2015 which did not vary ($p \leq 0.05$) from 'Pirsabak-2005'. The lowest spike weight was noticed in DN-84. While averaged across cultivars, spike weight decreased ($p \leq 0.05$) with HS given at the booting stage to marked at the anthesis when compared with the control treatment. However, a consistent decrease ($p \leq 0.05$) was observed in the spike weight when the plant experienced with HSd from 48 h to 144 h. Interesting was the treatment interaction (HS x HSd and C x HS) effects on spike weight (Fig. 3). Spike weight showed a reduction ($p \leq 0.05$) when the plant experienced HS at the anthesis than a botting stage for all cultivars but with variable changes. However, a decrease in spike weight was observed with increasing HSd for both stages of the HS given either at booting or the anthesis.

Grain weight (g spike^{-1}), an equally important primary yield trait, revealed differences ($p \leq 0.05$) in cultivars and plants experienced with HS as well as its duration (Table 2). The maximum grain weight was noted for Pakistan-2013, followed by Pakhtunkhwa-2015 with no change ($p \leq 0.05$) in grain weight from 'Pirsabak-2005'. The minimum grain weight of a spike was noted in 'DN-84'. While averaged across cultivars, the spike grain weight markedly decreased ($p \leq 0.05$) in plants experienced with HS at booting as well as at the anthesis as compared to the control treatment. Grain weight significantly ($p \leq 0.05$) and decreased with extending the HSd from 48 h to 144 h. Interactions of treatment (HS x HSd and C x HS) significantly varied ($p \leq 0.05$) for the grain weight of a spike (Figure 4). Grain weight markedly reduced ($p \leq 0.05$) in plants experienced HS at the anthesis than the booting stage. Whereas, increase the HSd from 48 h to 144 h, grain weight per spike markedly decreased at booting as well as at the anthesis stage.

Table 1. Spike length (cm) and weight (g) of selected wheat cultivars experienced limited heat shock (HS) at booting and anthesis in 2017-18 and 2018-19

Cultivars (C)	Length (cm)			Weight (g)		
	2017-18	2018-19	Mean	2017-18	2018-19	Mean
Pirsabak-2005	6.76	6.92	6.84 bc	1.15	1.57	1.36 b
Pakhtunkhwa-2015	7.04	6.91	6.98 b	1.32	1.38	1.35 b
Pakistan-2013	8.12	7.60	7.86 a	1.44	1.70	1.57 a
DN-84	6.54	6.81	6.67 c	0.84	1.32	1.08 c
LSD (0.05) for C	0.32	0.28	0.20	0.03	0.04	0.02
Heat shock (HS)						
Booting	6.93	6.89	6.91 b	1.34	1.67	1.50 a
Anthesis	7.30	7.23	7.27 a	1.03	1.32	1.18 b
LSD (0.05 for HS	0.23	0.20	0.15	0.02	0.03	0.02
HS durations (HS_d)						
48 h	6.78	6.76	6.77 c	1.32	1.60	1.46 a
96 h	7.11	7.02	7.06 b	1.18	1.50	1.34 b
144 h	7.47	7.40	7.43 a	1.06	1.38	1.22 c
LSD (0.05) for HS_d	0.34	0.30	0.18	0.03	0.04	0.02
Years mean	7.12	7.06	NS	1.19	1.49	**
Contrast						
Control	6.28	6.28	6.28	1.63	1.88	1.75
Rest	7.12	7.06	7.09	1.19	1.49	1.34
Significance	**	**	**	**	**	**
Level of significance ($p \leq 0.05$) for treatment interaction						
Y x C	-	-	**	-	-	**
HS x HS_d	NS	NS	NS	NS	*	*
Y x HS	-	-	NS	-	-	*
Y x HS_d	-	-	NS	-	-	NS
Y x HS x HS_d	-	-	NS	-	-	NS
C x HS	**	**	**	**	**	**
C x HS_d	NS	NS	NS	NS	NS	NS
C x HS x HS_d	NS	NS	NS	*	NS	**
Y x C x HS	-	-	*	-	-	*
Y x C x HS_d	-	-	NS	-	-	NS
Y x C x HS x HS_d	-	-	NS	-	-	NS

Means followed by a common letter within a category are not significant using the least significant difference (LSD). * and ** represent significance level ($p \leq 0.05$) and ($p \leq 0.01$); NS = Non-significant

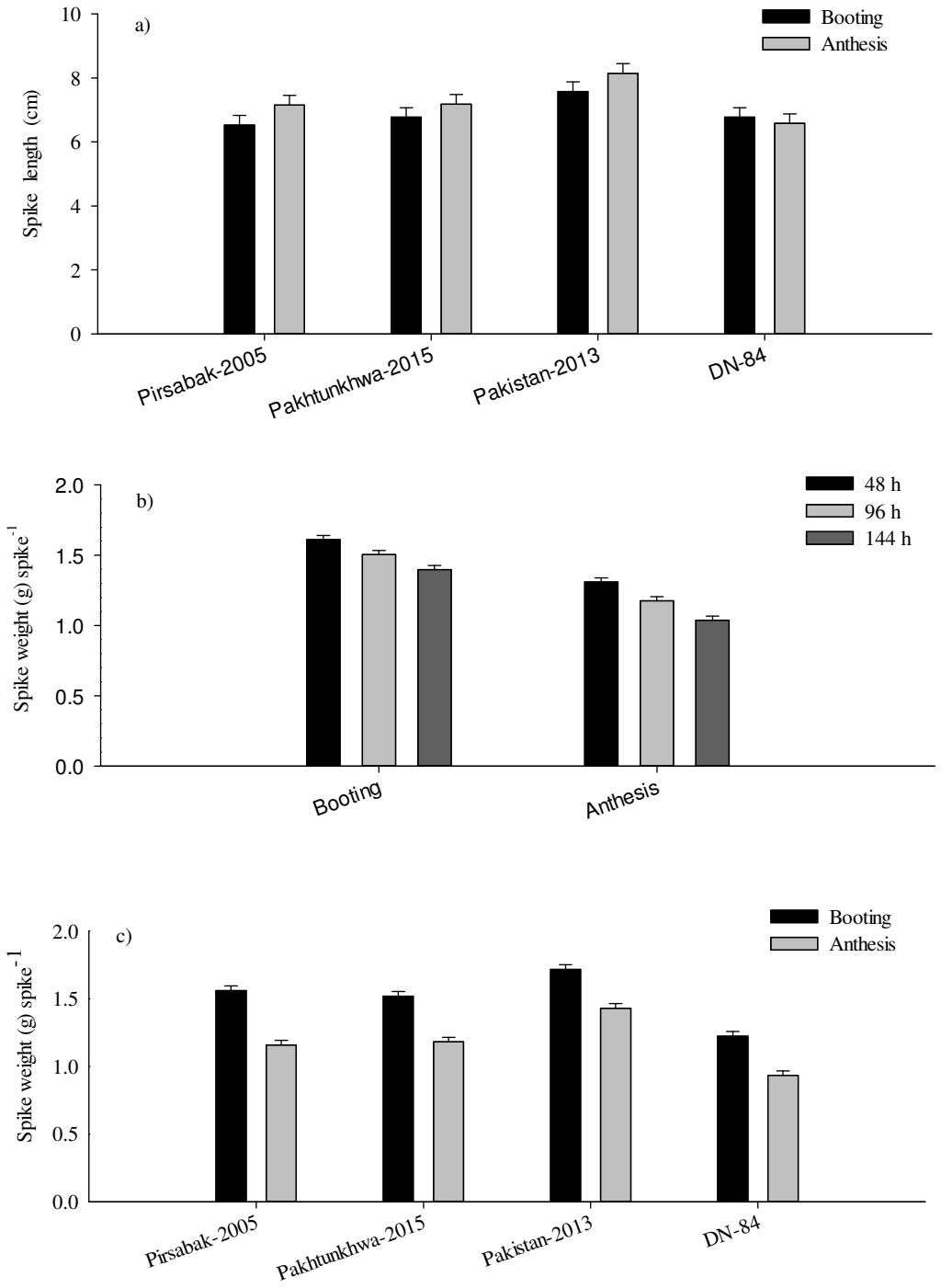

Figure 3. Interaction of (a) cultivars × heat shocks for spike length (cm), (b) heat shocks x heat shock durations and (c) cultivars × heat shocks for spike weight (g) spike^{-1} in two years' average data. LSD values of means are shown on bars

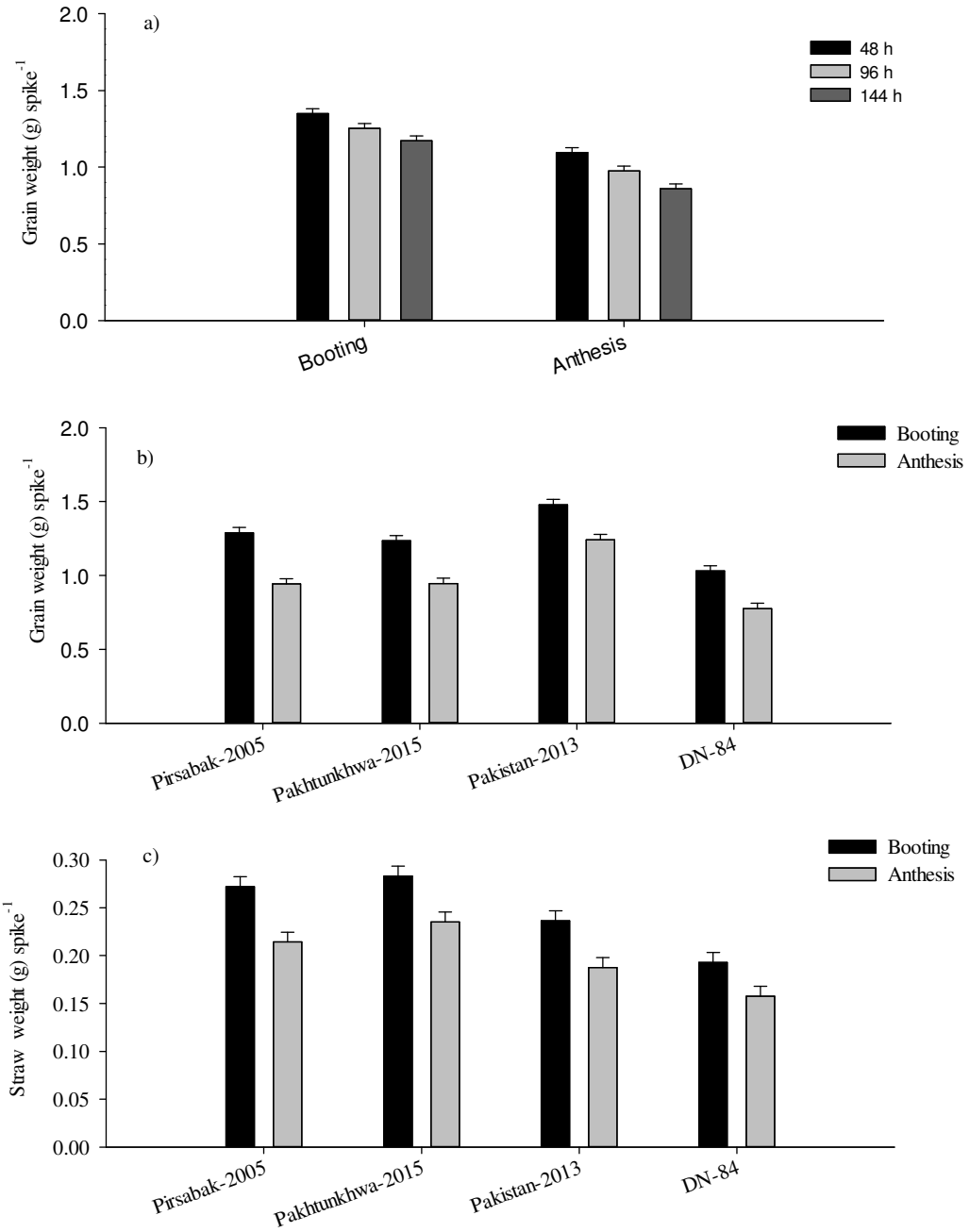

Figure 4. Interaction of (a) heat shocks × heat shock durations (b) cultivars × heat shocks for grain weight (g) spike^{-1} and (c) cultivars × heat shocks for the straw weight (g) spike^{-1} in 2017-18 and 2018-19. LSD values are shown in bars.

Table 2. Grain weight (g spike^{-1}) and grain number (spike^{-1}) of wheat cultivars exposed to heat shock (HS) at booting and anthesis during 2017-18 and 2018-19

Cultivars (C)	Grain weight (g) spike^{-1}			Grain number spike^{-1}		
	2017-18	2018-19	Mean	2017-18	2018-19	Mean
'Pirsabak-2005'	0.91	1.32	1.12 b	18.99	20.95	19.97 d
'Pakhtunkhwa-2015'	1.06	1.12	1.09 b	21.54	21.99	21.77 c
'Pakistan-2013'	1.25	1.48	1.36 a	29.18	29.92	29.55 a
'DN-84'	0.67	1.14	0.90 c	23.93	25.96	24.96 b
LSD (0.05) for C	0.03	0.04	0.02	1.17	1.00	0.716
Heat shock (HS)						
Booting	1.10	1.41	1.26 a	24.98	26.72	25.85 a
Anthesis	0.84	1.11	0.98 b	21.84	22.69	22.27 b
LSD (0.05 for HS	0.02	0.03	0.02	0.826	0.705	0.54
HS durations (HS_d)						
48 h	1.09	1.35	1.22 a	25.24	26.79	26.01 a
96 h	0.96	1.26	1.12 b	23.40	24.91	24.15 b
144 h	0.86	1.17	1.02 c	21.59	22.42	22.01 c
LSD (0.05) for HS_d	0.04	0.04	0.02	1.24	1.06	0.66
Year means	0.97	1.26	**	23.41	24.70	**
Contrast						
Control	1.36	1.59	1.47	30.68	32.05	31.36
Rest	0.97	1.26	1.12	23.41	24.70	24.06
Significance	**	**	**	**	**	**
Level of significance (p≤0.05) for treatment interaction						
Y x C	-	-	**	-	-	**
HS x HS_d	NS	*	*	NS	*	*
Y x HS	-	-	NS	-	-	NS
Y x HS_d	-	-	NS	-	-	NS
Y x HS x HS_d			NS	-	-	NS
C x HS	*	**	**	**	**	**
C x HS_d	NS	NS	NS	NS	*	NS
C x HS x HS_d	NS	NS	*	NS	NS	NS
Y x C x HS	-	-	NS	-	-	**
Y x C x HS_d	-	-	NS	-	-	*
Y x C x HS x HS_d	-	-	NS	-	-	NS

Means followed by a common letter within a category are not significant using the least significant difference (LSD). * and ** represent significance level (p≤0.05) and (p≤0.01); NS = Non-significant

Grain number (spike^{-1}) was observed different for the different cultivars (Table 2). Likewise, changes in grain number were seen with treatment HS and HSd. The maximum grain number was reported for 'Pakistan-2013' in, followed by 'DN-84', and 'Pakhtunkhwa 2015' with the minimum grain number for Pirsabak-2005. While averaged over cultivars, grain number markedly reduced (p≤0.05) with HS at booting as well as at the anthesis compared to no HS i.e. the control treatment. Grain number decreased (p≤0.05) markedly with extending HSd from 48 h to 144 h. Interaction (HS x HSd & C x HS) showed variations (p≤0.05) in grain number (Figure 5). Grain number decreased (p≤0.05) in plants experienced with HS at the anthesis stage as

compared to the booting stage in all cultivars but did no change in 'Pirsabak-2005' with HS at any stage of the growth. An increase HSd showed a decrease in grain number with marked at booting than mild at the anthesis stage of the plant growth.

Quality traits

The grain quality of this study addressed amylose, amylopectin, gluten, and grain N- content (%) which plays a role in backing. Treatment showed significant ($p \leq 0.05$) changes in amylose for cultivars, HS, and HSd (Table 3). 'Pakistan-2013' showed the highest amylose in grains, followed by 'DN-84' which did not vary ($p \leq 0.05$) from 'Pirsabak-2005'. Similarly, amylose in grains of 'Pirsabak-2005' was the same ($p \leq 0.05$) with 'Paktunkhwa-2015'. Amylose, however, decreased ($p \leq 0.05$) in wheat grain when the plant experienced HS at booting and the anthesis stages as compared to the control treatment. Amylose in grains decreased by extending the HSd from 48 h to 144 h. Interactions (HS x HSd & C x HS) showed a significant change in grain amylose (Figure 5). Amylose in grains decreased with HS at the anthesis stage than the booting stage in all cultivars and likewise a decrease was observed in amylose with increasing HSd from 48 h to 144 h at both stages but more at the anthesis than the booting stage.

Amylopectin in grains also differs ($p \leq 0.05$) for the wheat cultivars, HS and HSd (Table 4). Amylopectin was high in 'Pakhtunkhwa 2015' but did not differ ($p \leq 0.05$) from 'Pirsabak 2005', followed by DN-84 which also was the same ($p \leq 0.05$) as Pirsabak-2005. The lowest amylopectin was noted in grains of 'Pakistan-2013'. Amylopectin in grains increased ($p \leq 0.05$) mild to marked of plants experienced with HS at booting and the anthesis stages than control treatment where no HS was given. Amylopectin in grains significantly increased with increasing HSd from 48 h to 144 h. Interaction (HS x HSd and C x HS) were significant for amylopectin (Figure 6). Amylopectin in grains was more in HS given at the anthesis stage than the booting stage of the crop in almost all cultivars but with varying rates. Likewise, amylopectin in grains increased with extending HSd from 48 h to 144 h but was mild to marked at booting and anthesis stages.

Wet gluten (WG) is an important trait of wheat that expressed various ($p \leq 0.05$) among cultivars and plants experienced with HS and HSd (Table 4). Among cultivars, 'Pakistan-2013' revealed the highest WG content, followed by 'Pukhtunkhwa-2015', and the lowest WG in 'Pirsabak-2005'. WG of the 'Pirsabk-2005' and 'DN-84' was the same ($p \leq 0.05$). While averaged across the cultivars, WG showed an increase within grains exposed to HS at booting and the anthesis stages as compared to the control treatment. By increasing the HSd from 48 h to 144 h, WG showed a significant increment. Interaction of treatment (C x HS and C x HSd) showed a change ($p \leq 0.05$) in the grain WG (Figure 6). WG of wheat grains was high in plants experienced with HS at the anthesis stage than booting but with different ratios in the different cultivars and likewise the increase in WG was observed with extending HSd with varying rates for the different cultivars.

Grain N (GN), evidence of protein indicator, also exhibited variations ($p \leq 0.05$) among the different cultivars and they are experienced with HS and HSd (Table 4). GN was observed the highest in 'Pakistan-2013', followed by 'Pukhtunkhwa-2015', and the lowest in 'Pirsabak-2005', which did not show a difference ($p \leq 0.05$) in GN of the DN-84. Averaged across cultivars, GN increased with HS at booting and finally at the anthesis as compared to the control treatment. Likewise, an increase was noticed in GN with increasing HSd on plants from 48 h to 144 h. Interaction (C x HS) expressed variations for GN (Figure 7). GN was reported higher in plant experienced with HS at the anthesis as compared to the booting stage in all cultivars but with different ratios.

Table 3. Grain amylose (%) and amylopectin (%) of wheat cultivars exposed to heat shock (HS) at booting and anthesis during 2017-18 and 2018-19

Cultivars (C)	Amylose content (%)			Amylopectin content (%)		
	2017-18	2018-19	Mean	2017-18	2018-19	Mean
'Pirsabak-2005'	20.08	20.89	20.49 bc	79.92	79.11	79.51 ab
'Pakhtunkhwa-2015'	19.48	21.11	20.29 c	80.52	78.89	79.71 a
'Pakistan-2013'	21.28	22.34	21.81 a	78.72	77.66	78.19 c
'DN-84'	19.05	22.14	20.59 b	80.95	77.86	79.41 b
LSD (0.05) for C	0.30	0.49	0.28	0.30	0.49	0.28
Heat shock (HS)						
Booting	21.23	22.96	22.10 a	78.77	77.04	77.90 b
Anthesis	18.71	20.27	19.49 b	81.29	79.73	80.51 a
LSD (0.05 for HS	0.23	0.38	0.22	0.23	0.38	0.22
HS durations (HS_d)						
48 h	20.85	22.17	21.51 a	79.15	77.83	78.49 c
96 h	20.04	21.58	20.81 b	79.96	78.42	79.19 b
144 h	19.03	21.10	20.06 c	80.97	78.90	79.94 a
LSD (0.05) for HS_d	0.28	0.46	0.27	0.28	0.46	0.27
Year means	19.97	21.62	**	80.03	78.38	**
Contrast						
Control	22.70	23.40	23.05	77.30	76.60	76.95
Rest	19.97	21.62	20.80	80.03	78.38	79.20
Significance	**	**	**	**	**	**
Level of significance ($p \leq 0.05$) for treatment interaction						
Y x C	-	-	**	-	-	**
HS x HS_d	**	NS	*	**	NS	*
Y x HS	-	-	NS	-	-	NS
Y x HS_d	-	-	*	-	-	*
Y x HS x HS_d	-	-	NS	-	-	NS
C x HS	**	**	**	**	**	**
C x HS_d	NS	NS	NS	NS	NS	NS
C x HS x HS_d	NS	NS	NS	NS	NS	NS
Y x C x HS	-	-	**	-	-	**
Y x C x HS_d	-	-	NS	-	-	NS
Y x C x HS x HS_d	-	-	NS	-	-	NS

Means followed by a common letter within a category are not significant using the least significant difference (LSD). * and ** represent significance level ($p \leq 0.05$) and ($p \leq 0.01$); NS = Non-significant

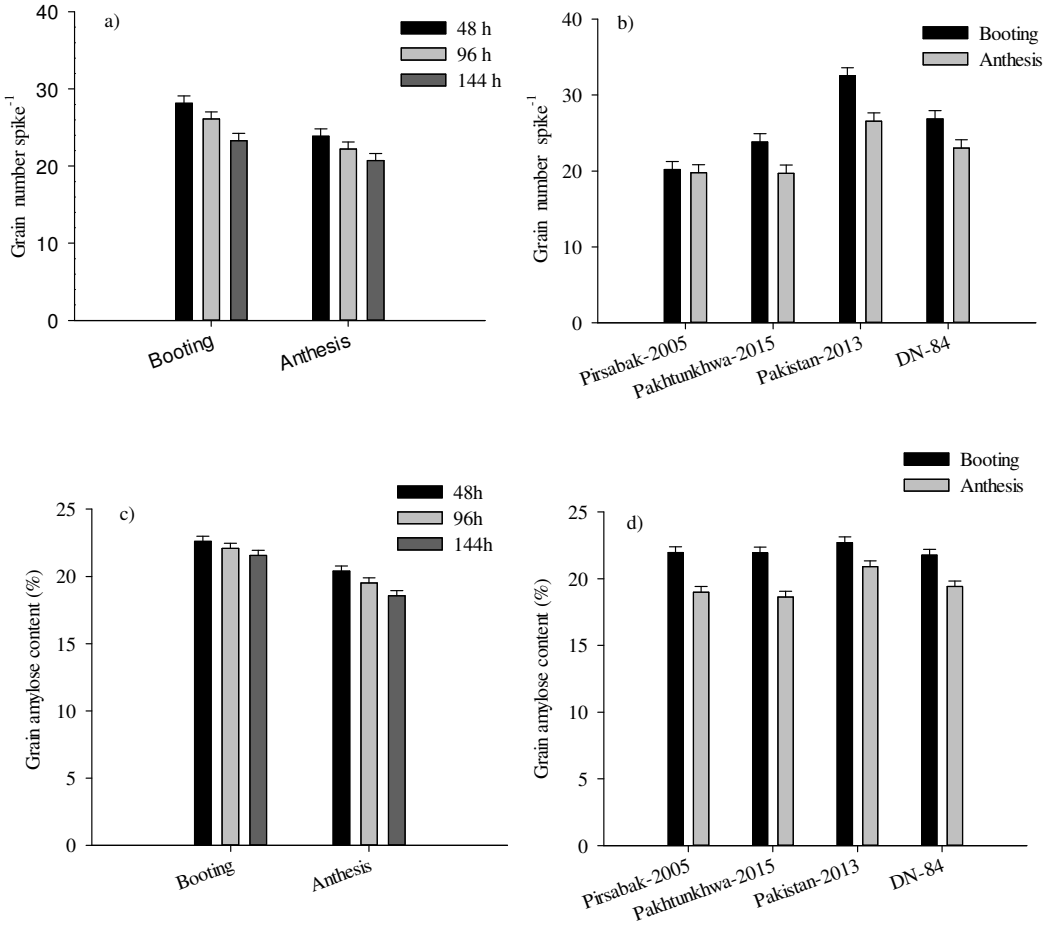

Figure 5. Interaction of (a) heat shocks × heat shock durations (b) cultivars × heat shocks for grain number spike^{-1} (c) cultivars × heat shocks and (d) heat shocks × heat shock durations for grain amylose content (%) in 2017-18 and 2018-19. LSD values are shown in bars

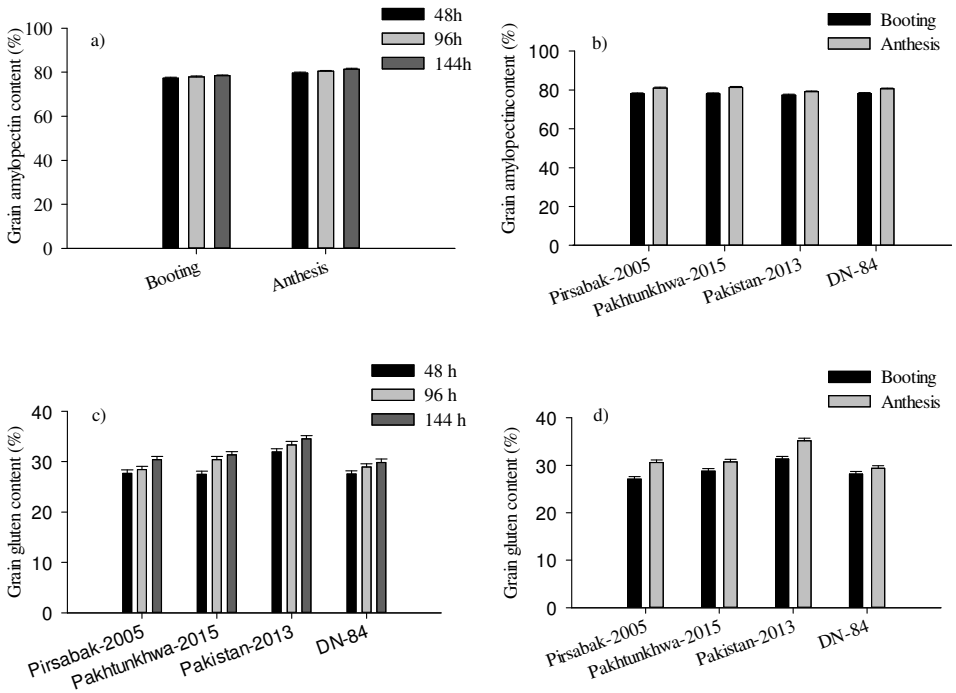

Figure 6. Interaction of (a) heat shocks × heat shock durations (b) cultivars × heat shocks for grain amylopectin content (%), (c) cultivars × heat shock durations and (d) cultivars x heat shocks for grain gluten content in 2017-18 and 2018-19. LSD values are shown in bars

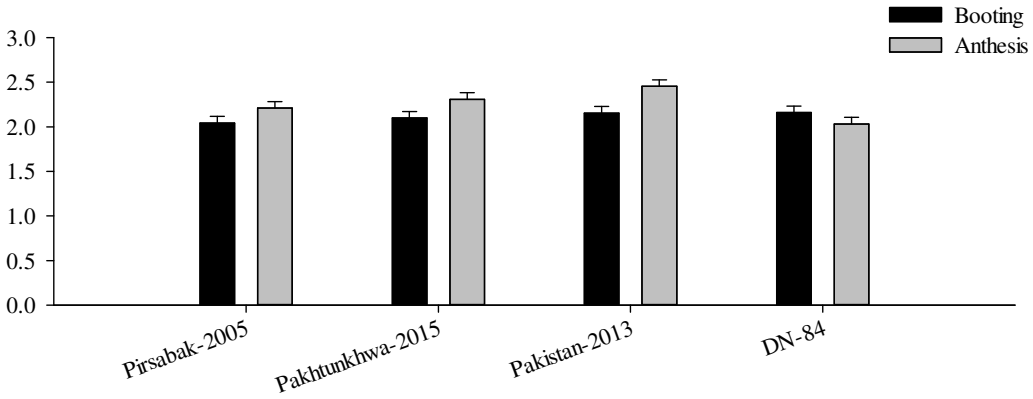

Figure 7. Interaction of cultivars × heat shocks for grain nitrogen content (%) in 2017-18 and 2018-19 LSD values are shown as bars

Table 4. Grain wet gluten (%) and N-content (%) of wheat cultivars exposed to heat shock (HS) at booting and anthesis during 2017-18 and 2018-19

Cultivars (C)	Wet gluten (%)			N-content (%)		
	2017-18	2018-19	Mean	2017-18	2018-19	Mean
'Pirsabak-2005'	29.46	28.24	28.85 c	2.25	2.01	2.13 c
'Pakhtunkhwa-2015'	30.74	28.77	29.76 b	2.29	2.11	2.20 b
'Pakistan-2013'	34.18	32.41	33.29 a	2.37	2.24	2.30 a
'DN-84'	29.14	28.44	28.79 c	2.15	2.04	2.10 c
LSD (0.05) for C	0.45	0.55	0.35	0.04	0.09	0.05
Heat shock (HS)						
Booting	29.22	28.50	28.86 b	2.23	2.00	2.11 b
Anthesis	32.54	30.43	31.48 a	2.30	2.21	2.25 a
LSD (0.05 for HS	0.34	0.42	0.27	0.03	0.07	0.04
HS durations (HS_d)						
48 h	30.03	27.32	28.67 c	2.14	1.97	2.05 c
96 h	30.86	29.72	30.29 b	2.28	2.08	2.18 b
144 h	31.75	31.36	31.55 a	2.38	2.25	2.31 a
LSD (0.05) for HS_d	0.42	0.52	0.33	0.04	0.08	0.04
Year means	30.88	29.46	**	2.26	2.10	**
Contrast						
Control	27.86	26.96	27.41	1.86	1.88	1.87
Rest	30.88	29.46	30.17	2.26	2.10	2.18
Significance	**	**	**	**	**	**
Level of significance ($p \leq 0.05$) for treatment interaction						
Y x C	-	-	**	-	-	*
HS x HS_d	NS	NS	NS	*	NS	NS
Y x HS	-	-	**	-	-	**
Y x HS_d	-	-	**	-	-	NS
Y x HS x HS_d	-	-	NS	-	-	NS
C x HS	**	**	**	**	**	**
C x HS_d	NS	**	**	***	NS	NS
C x HS x HS_d	NS	NS	NS	NS	NS	NS
Y x C x HS	-	-	**	-	-	**
Y x C x HS_d	-	-	**	-	-	*
Y x C x HS x HS_d	-	-	NS	-	-	NS

Means followed by a common letter within a category are not significant using the least significant difference (LSD). * and ** represent significance level ($p \leq 0.05$) and ($p \leq 0.01$); NS = Non-significant

Discussion

Primarily yield is assumed from grain weight and number on a column. Wheat spike is the primary yield-determining trait and its length and weight express the final product (Goher and Akmal, 2021). Spike length and weight are, therefore, the most significant traits affecting yield. A change in spike length may affect the yield but not necessarily because an increase in the spike length may be due to loose spikelets on rachis (Wolde

et al., 2021). It may also be due to changes in spikelets on a spike and hence we have observed variations in spike length of different cultivars. While averaged on cultivars, the spike length showed an increase with HS and extending HSd. Temperature rise boosted growth with rapid cell enlargement and hence has shown longer spikes with HS treatment at booting and the longest at the anthesis (Fan *et al*., 2022). An increase in HSd has, therefore, contributed to an equal increase in the spike length (Schmidt *et al*., 2020). Spike weight, on the other hand, is a product of grain and straw which, therefore, showed a different trend. Cultivars did differ in spike weight which is obviously due to their grain number and sizes (Riaz *et al*., 2021) because different cultivars with more grains have lighter weight and/or otherwise (Tura *et al*., 2020). 'Pakistan-2013' expressed the highest grain weight, followed by 'Pirsabak-2005', and the lowest grain weight was noticed for 'DN-84'. Grain fill duration and rate of assimilate accumulation together with enzyme activities are the main sources to contribute to the grain weight (Ullah *et al*., 2022). They were adversely affected when plants experienced HS in sensitive stages i.e. the grain fill durations (Sehgal *et al*., 2018). Changes in grain weight are associated with its susceptibility or resistance to HS and its maximum duration (Sharma *et al*., 2019). Literature also confirms an increase in grain number with lighter weight (Schittenhelm *et al*., 2020). The lighter weight with HS is also observed due to a decrease in soluble starch synthase at grain growth and development. A developing grain is adversely affected by HS damages the endosperm cells and low starch accumulation which resulted in losses in grain weight (Narayanan, 2018). Higher temperature close to anthesis has decreased the pollinations due to infertility of the florets or limited timings for effective fertilization (Yang *et al*., 2022) 30 °C temperature at the time of anthesis has shown partially to complete sterility in wheat (Kumar *et al*., 2020). As compared to the booting stage, the anthesis is a more sensitive stage of crop growth and hence has shown a marked significant decrease in grain weight (Schittenhelm *et al*., 2020). Both grain number and weight are interrelated to each other. HS is mild with limited duration and plants develop a mechanism to escape its effect.

Both grain number and weight are interrelated to each other. HS is mild with limited duration and plants develop a mechanism to escape its effect (Wang and Liu, 2021). Nevertheless, with an increase in the duration of HS plant fails to fight and hence causes damage to developing grains. HS at the time of florets fertilization may limit the grain set and hence affects the number with infertility and/or reduce the grain size. In this study, HS at the anthesis of the crop has markedly reduced grain weight (4- 7%) with an artificial rise of 1.5-2.0 °C temperature from regular air temperature which also agrees with literature where air temperature above 25 °C has shown lighter grains (Ullah *et al*., 2019). The marked reduction was noted in grain weight when the plant experienced HS at the anthesis stage due to the slow mobilization of assimilated from sink to sources (Bergkamp *et al*., 2018). Literature has also confirmed smaller grains with few numbers in a wheat spike under HS which are mainly due to early ripening and/or infertile pollen (Li and Lei, 2022). Grain weight decreased from control to HS and extended the HSd. This decrease in grain weight is due to the shrinking of grain fill duration with increasing temperature of 1-2 °C under the HS (Hussain *et al*., 2021). Losses in grain weight are obvious to limited grain fill duration with raised temperature (Narayanan, 2018). A little rise in temperature from optimum may disrupt the physiological process of developing grains which adversely affects size (Jagadish, 2020). Grain development is critical to the environment with HS and extending its duration damages physiological processes adversely affecting both grain number and weight by limiting assimilates to accumulate, consequently smaller grains size (Poudel and Poudel, 2020). HS at or after anthesis adversely affects pollen fertility and/or slows down assimilates accumulation which results in a loss in grain weights (Sehgal *et al*., 2018). The high temperature at or after anthesis adversely affects grain development due to limiting photo-assimilate partitioning (Xu *et al*., 2021) and hence resulting in smaller grains (Guo *et al*., 2018). Variations in the grain number of spikes are due to changes in the ratio of empty spikelets due to loss of pollen viability (Aiqing *et al*., 2018). HS at the anthesis markedly reduced grain number due to pollen infertility index (Poudel and Poudel, 2020). Whereas HS before anthesis usually shows little or no effects on grain (Bergkamp *et al*., 2018). Pollen is very much sensitive to temperature and slight HS may adversely affect its fertility and

hence the grain number on a spike (Fabian *et al*., 2019). This also resulted in lower grain numbers on spikes (Hutsch *et al*., 2019). HS has also shown rapid grain growth with lighter sizes on wheat spikes (Narayanan, 2018). Cultivars differ in timings of pollen fertility (Sharma *et al*., 2019) which results in differences in fertilized florets (Fabian *et al*., 2019), and synthesis or thereafter is the most sensitive stage of grain development causing more losses in yield (Djanaguiraman *et al*., 2020). The intensity of heat at the time of anthesis plays a major role and is found more crucial at the anthesis than booting (Akmal and Goher, 2021). Maximum empty spikelets observed in plants experienced a mild HS at anthesis (Guo *et al*., 2018) which is mostly due to limited assimilates translocation with poor photosynthesis of the flag leaf (Wang and Liu, 2021).

Grain quality traits

Amylose and amylopectin (%) in wheat grains exhibited significant variations with HS and HSd treatments. Both amylose and amylopectin are interrelated, the one increase adversely affects the others (Akmal and Goher, 2021). Differences in both amylose and amylopectin of the cultivars are due to the grain size and the grain outer layer (Bala *et al*., 2018). Changes in the ratio i.e. increase of amylose have markedly influenced starch characteristics which affect the grain quality (Poudel and Poudel, 2020). The ratio of amylose to amylopectin is mostly associated with the grain size and the chemical composition for example the total starch in the grain (Zi *et al*., 2018). Between the stages of booting and anthesis stages, a reduction in amylose with HS to an increase in amylopectin is mainly due to the enzymatic activities of the growing grain. Plants in HS disturbed the grain amylose to amylopectin ratio by decreasing amylose (Schittenhelm *et al*., 2020). HS has shown adverse effects on photo-assimilate production and its translocation to sink (Blake *et al*., 2018). Damage was noted on tissue dehydration with limited assimilate production in HS (Balla *et al*., 2019). Amylose, therefore, showed a decrease by extending HS duration. It also shows the sensitivity of amylose to amylopectin by disturbing their ratio. Plants at 30 °C temperature have shown poor starch accumulation (Lal *et al*., 2021). A decrease is noted in grain amylose when plants are exposed to HS at the anthesis stage of the crop (Sehgal *et al*., 2018). Starch composition is disturbed with HS due to a change in the timing of anthesis (Lu *et al*., 2019). An increase in amylopectin with HS means that the ratio of the grain outer layer to the inner starch narrowed down (Giunta *et al*., 2020). We have seen that plants at a temperature of 35 °C for 5 d have shown changes in endosperm structure, which adversely affects grains quality with higher amylopectin contents (Sehgal *et al*., 2018) which is due to limited starch synthesis in storage cells in HS that disturbed amylose to amylopectin ratio (Sehgal *et al*., 2018). The anthesis stage and thereafter grain growth are sensitive to grain assimilates accumulation (Nuttall *et al*., 2018). Moreover, grain formation starts after fertilization and HS thereafter disturbed the starch accumulation rate (Lu *et al*., 2019). Under HS negative effects were observed on starch accumulation in developing grains, by shrinking grain fill duration, which reduced size with a limited endosperm (Akter and Islam, 2017). Grain growth slows down at HS (+30 °C temperature) due to slow enzyme activity i.e. ADP-Glucose Pyro-phosphorylase (AGPase) and soluble starch synthase, which reduced grain starch and disturbs the ratio of amylose to amylopectin (Sattar *et al*., 2020). Amylopectin increases with HS in grain due to faster growth (Sattar *et al*., 2020) which resulted in a decrease in size and weight (Balla *et al*., 2019). Accumulation of both starch and protein depends on endosperm cell size and rate (Farooq *et al*., 2017), therefore, HS and HSd have shown changes in amylose to amylopectin (Lu *et al*., 2019).

Wet gluten and N (%) were affected ($p \leq 0.05$) with HS and HSd. Protein in grain ranges from 13-15% of which 80% is gluten i.e. storage protein (glutenin and gliadin). Dough strength (i.e. loaf volume) and flour extensibility also depend on the ratio of storage protein (Qazi *et al*., 2021). Changes may occur in the composition of storage protein by differences in the amount of N accumulation within grains (Sehgal *et al*., 2018). Wet gluten was higher in grains affected with HS at the anthesis than booting and/or control treatment. Maximum gluten under HS may be due to more N accumulation in developing grains (Hernandez-Espinosa *et al*., 2018) because grain growth (35 °C) has deteriorated quality (the dough strength) by denaturing of protein

(Narayanan, 2018) which decreased gliadin and increase gluten (Kino *et al*., 2020). N in grain and grain protein is an important trait and is closely associated (Nuttall *et al*., 2018). Both grain weight and size cause changes in protein (Tura *et al*., 2020). Differences in grain size and weight are obvious to cultivars (Iqbal *et al*., 2017), but the higher temperature has accelerated growth with higher N due to the dilution factor (Bala *et al*., 2018). Temperature is key to growth and plays important role in assimilating synthesis (Wang and Liu, 2021). High temperature with HS has, therefore, shown high gluten (Hernandez-Espinosa *et al*., 2018). Usually, with HS, grains' size shrinks, and biochemical composition is disturbed (Poudel and Poudel, 2020). High gluten has been seen in high temperatures (Wang and Liu, 2021) Extension in HS duration has increased both gluten and N-content (Sattar *et al*., 2020). Grain development at high temperatures completes in a short time to decrease grain weight and size by increasing N by dilution.

Conclusions

This study focused on temporal heat shock (HS) at booting and anthesis stages of spring wheat cultivars' life cycle for a limited duration (48, 96, and 144 h) and documented changes in grain yield and quality. HS adversely affected grain weight, size, and amylose content with a mild to marked degree at the booting and anthesis stage when plants were exposed to HS. Increasing the duration of HS from 48 h to 144 h significantly and negatively affected the weight and number of grains per spike as well as grain amylose content as compared with the control treatment. In contrast, straw fraction of spike, amylopectin, wet gluten, and grain-N expression was increased when plants were exposed to HS at booting to mild at anthesis in comparison to those obtained from control.

Authors' Contributions

Conceptualization: RG, HMA, MFS and MA; Data curation: RG, HMA, MFS and AAD; Formal analysis: RG and MA; Investigation: RG, HMA, MFS and MA; Methodology: RG, GRK and MA; Resources: RG and MA; Software: RG, HMA, MFS, DOW and GRK; Supervision: MA; Writing - original draft: RG; Writing - review and editing: RG, HMA, MFS, AAD, HG, DOW, GRK and MA. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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