

Wheat cultivars exposed to high temperature at onset of anthesis for yield and yield traits analysis

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Abstract. Temperature fluxes at some critical growth stages adversely affect the crop yield. Heat stress (HS) of limited duration shows mild to marked effects on crop yield. The study focused on evaluating HS of limited durations on wheat crop effective from anthesis. Four wheat cultivars (Pirsabak-2005, Pakhtunkhwa-2015, Pakistan-2013, and DN-84) and three advanced lines (P-2, P-12, and P-18) were subjected to HS for 48, 72, and 96 h evaluating changes in the yield and yield contributing traits. The experiment was conducted in a randomized complete block design during 2017–18 and 2018–19 at Agronomy Research Farm, the University of Agriculture Peshawar Pakistan. At the onset of anthesis, plants were exposed to HS in the plastic tunnel for limited durations. The temperature inside and outside tents was recorded periodically. The mean across the years showed a significant effect on yield traits by HS imposition at anthesis stage. The mean data across the two years showed a significant effect of HS on yield and yield contributing traits. In comparison with control, spike weight (g) reduced by 29, 40, and 49% under limited HS of 48, 72, and 96 h, respectively. Grains per spike were decreased by 45, 61, and 69% and grain weight by 29, 36, and 45% from control to imposed HS of 48, 72, and 96 h, respectively. Overall grain yield decreased by 44, 61, and 70% upon exposed to 48, 72, and 96 h of HS, respectively. The differences among the cultivars for yield and yield contributing traits were different under various HS conditions. The study concluded that HS effective from anthesis has an adverse effect on grain weight and number and hence the grain yield. Among the cultivars, Pakistan-2013 showed better resistance to HS of limited duration when exposed at the anthesis stage of the crop growth.

Key words: heat stress, heat-stress duration, grain weight, grain number, wheat yield.

INTRODUCTION

Wheat is susceptible to heat stress at critical growth stages (Gupta et al., 2013) resulting in yield losses. Plant growth is adversely affected by heat stress through the sudden increase in temperature, particularly at the start of the reproductive stages of plant growth (Asseng et al., 2015). Yield loss has been reported up to 6% with 1 °C rise in temperature from optimum at critical growth stages (Akter & Islam, 2017). An increase in temperature has shown adverse effects on plant meristem, which resulted in a decrease in leaf photosynthetic activity (Haque et al., 2014) but an increase in leaf fall was also

observed (Kosova et al., 2011). Change in daily temperature has shown adverse effects on the flag leaf of the wheat crop, which resulted in yield loss (Zhao et al., 2007). Senescence of the leaf has also increased due to high temperature with structural changes in the chloroplast, breakdown of vacuoles, damaging cell membrane, and intrusion of cellular stability (Khanna-Chopra, 2012). Some common adverse effects were also observed under heat stress like reduction in the photosynthetic process, changes in stomatal behavior, reduction in flag leaf area, premature leaf fall, and loss of pollen viability, etc. (Asseng et al., 2011).

Research has confirmed rapid spike growth at a temperature over 20 °C (Semenov, 2009) with complete pollen sterility in wheat at above 30 °C (Kumar et al., 2020). The reason for pollen sterility was due to the disintegration of the pollen cell and microspore (Anjum et al., 2008). The structure of cell endosperm and aleurone layer has shown changes by differences in the day and night temperatures (Dias et al., 2008). Heat stress for more than 3 days effective from anthesis has resulted in abnormal anther and functionally futile florets (Hedhly et al., 2009; Yu et al., 2014). Heat stress (+ 1–2 °C) has caused a reduction in grain size and numbers due to limited grain fill duration in wheat (Lukac et al., 2012). The high temperature at the post-anthesis stage of wheat has also resulted in early maturity, limited grain fill period, loss in grain number, and weight (Nasehzadeh & Ellis, 2017; Mazurenko et al., 2020). Heat stress also decreased biomass (Akter & Islam, 2017) due to the limited source accumulation rate (Prasad & Djanaguiraman, 2014). Heat stress at anthesis has shown about a 23% reduction in the above-ground biomass of wheat crops (Banerjee & Krishnan, 2015). Considering the available weather data in Pakistan (1960 to 2010) for future projection through model approach for future climate change (2030 and 2040) a temperature rise is expected from 1 to 2 °C that may affect the wheat crop (Hanif & Ali, 2014).

This study aimed to expose plants to heat stresses by artificially raising the growth temperature for limited durations by making temporary polyethylene plastic tents over the plants effective from the onset of the anthesis stage of the crop. We want to estimate the change that has taken place in the primary yield traits that adversely affect the grain yield of the promising wheat cultivars. It has been observed that in changing climate the abnormal HS is frequently observed in the field during the crop entered from vegetative to reproductive stages of growth.

MATERIAL S AND METHODS

Site description

A 2-year field experiment was conducted during winter 2017–18 and 2018–19 at Agronomy Research Farm, the University of Agriculture Peshawar-Pakistan. The location of the experimental site was at 34° 1'13.50 "N" and 71° 28'53.02 "E", 350 m above sea level. Climate is subtropical, received 500–700 mm annual precipitation, with daily mean temperature varying from 24 ± 6.24 °C to 40.7 ± 6.29 °C during the wheat crop growth (November to May). The soil of the experimental site was silt loam having pH > 7.6, organic matter < 1%. Sand (18.13%) silt (71.23%) and clay (10.64%), and classified as *Ustocrept* based on USDA classification (Anonymous, 2007). Data regarding seasonal temperature (Max. and Min.) and rainfall of the crop growth period were obtained from the local Metrological Department (Fig. 1).

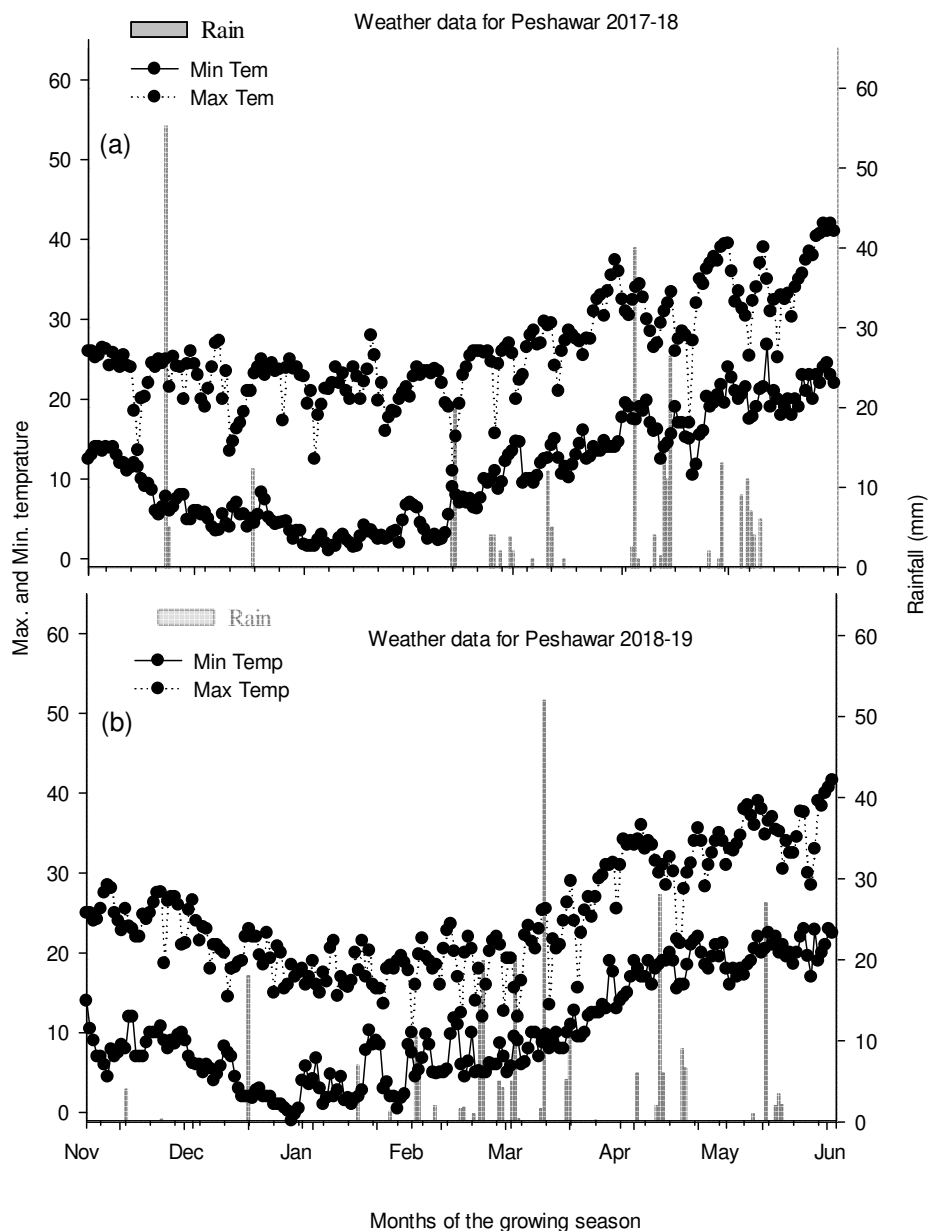


Figure 1. Temperature and rainfall of the crop growth seasons are presented in the separate windows during the experimental years (a) 2017–18 and (b) 2018–19 with bars for rainfall (mm) and lines for temperature minimum (T Min °C) and maximum (T Max °C).

Experimental design and treatments

The experiment was conducted in a randomized complete block design (RCBD) using three replications. Seven wheat cultivars/lines were exposed to three heat stress (HS) durations by raising the growth temperature in plastic tents for limited durations (48, 72, and 96 h) effective from the anthesis stage of the crop growth. Seeds were sown

on Nov. 30, 2017, and Nov. 10, 2018, at Agronomy Research Farm, the University of Agriculture, Peshawar with the recommended rate (100 ha^{-1}) for all cultivars. Sowing was done with the help of a hand drill in 6 rows, 5 m long and 30 cm apart. The field was prepared as recommended for wheat sowing. Fertilizers including urea (46% N), single superphosphate (20% P_2O_5), and murate of potash (60% K) were added during seedbed preparation to provide fixed amounts of 120 kg ha^{-1} N, 80 kg ha^{-1} P, and 50 kg ha^{-1} K, respectively. Nitrogen was applied in two equal splits at sowing and 50 days after sowing (DAS). Four high yielding wheat cultivars (Pirsabak-2005, Pakhtunkhwa-2015, Pakistan-2013, and DN-84) including three advanced lines (P-2, P-12, and P-18) were exposed to heat stress (HS) through artificially raising the temperature inside the tents for limited durations (48, 72, and 96 h) effective at the onset of anthesis stage. One control treatment with normal growth (no HS) was also included.

Heat stress (HS)

Temporal heat stress (HS) was established by raising the inside plastic tents temperature for the plant growth effective from the crop anthesis stage. Four high-yielding wheat cultivars such as Pirsabak-2005, Pakhtunkhwa-2015, Pakistan-2013, and the DN-84 (as a check) along with three selected lines (P-2, P-12, and P-18) were evaluated. All 4 cultivars including the P-12 line were uniform in anthesis except lines (P-2 and P-18), which were relatively late to start anthesis and maturity. The pedigree of the check is the Mexico line from CIMMYT (Siddiq et al., 2006). Plants in 2 rows of the limited length in an experimental unit were covered under a plastic tent made over steel rods and 2-ply transparent polyethene sheets for higher HS imposition. An auto-measuring temperature clock (*Humidity/Clock HTC- 01*) was also installed within and outside tents for temperature. On completion of HS for the defined duration, plastic sheets were removed and the crop was allowed to grow as a normal crop for the rest of the growth period. Control plots were not treated with HS for the entire crop growth duration in the field.

The temperatures inside and outside the tents were periodically recorded four times a day during the HS durations. All readings were averaged for a single mean. Mean differences of all the readings within tents from outside temperature were $+3.08$, $+4.04$, and $+4.41$ °C in the year 2017–18 and $+2.42$, $+3.18$, and $+3.53$ °C in the year 2018–19 for all cultivars (Pirsabak-2005, Pakhtunkhwa-2015, Pakistan-2013, DN 84, and P-12) and $+7.16$, $+7.23$ and $+7.37$ °C for the year 2017–18 and $+5.47$, $+5.91$ and $+6.25$ °C for the year 2018–19 for the relatively late anthesis cultivars (P-2 & P18) for the duration of 48, 72, and 96 h HS, respectively. Differences in temperatures inside from the outside within the plastic tent vary for the different hours of the HS durations and are shown in Fig. 2. The absolute mean temperatures for 48, 72, and 96 h during the HS within the tents were 36.0 ± 10.2 , 35.4 ± 8.8 and 35.4 ± 8.1 (2017–18), 37.7 ± 7.38 , 38.3 ± 7.04 , and 37.2 ± 7.93 (2018–19) for all cultivars, 37.5 ± 7.21 , 37.8 ± 7.48 , and 38.5 ± 7.74 (2017–18), and 35.9 ± 6.31 , 35.8 ± 5.90 and 37.0 ± 5.54 (2018–19) for the late anthesis cultivars during HS of 48, 72, and 96 h, respectively.

All other cultural operations such as irrigation, fungicide, weeding, and nutrients, etc. were applied uniformly as recommended for the wheat crop in the area. Harvesting was done manually at the crop maturity on May 4, 2018, for normal cultivars and May 10, 2018, for late-maturing advanced lines in 2017–18, and May 5, 2019, for normal and May 13, 2019, for relatively advanced lines in 2018–19.

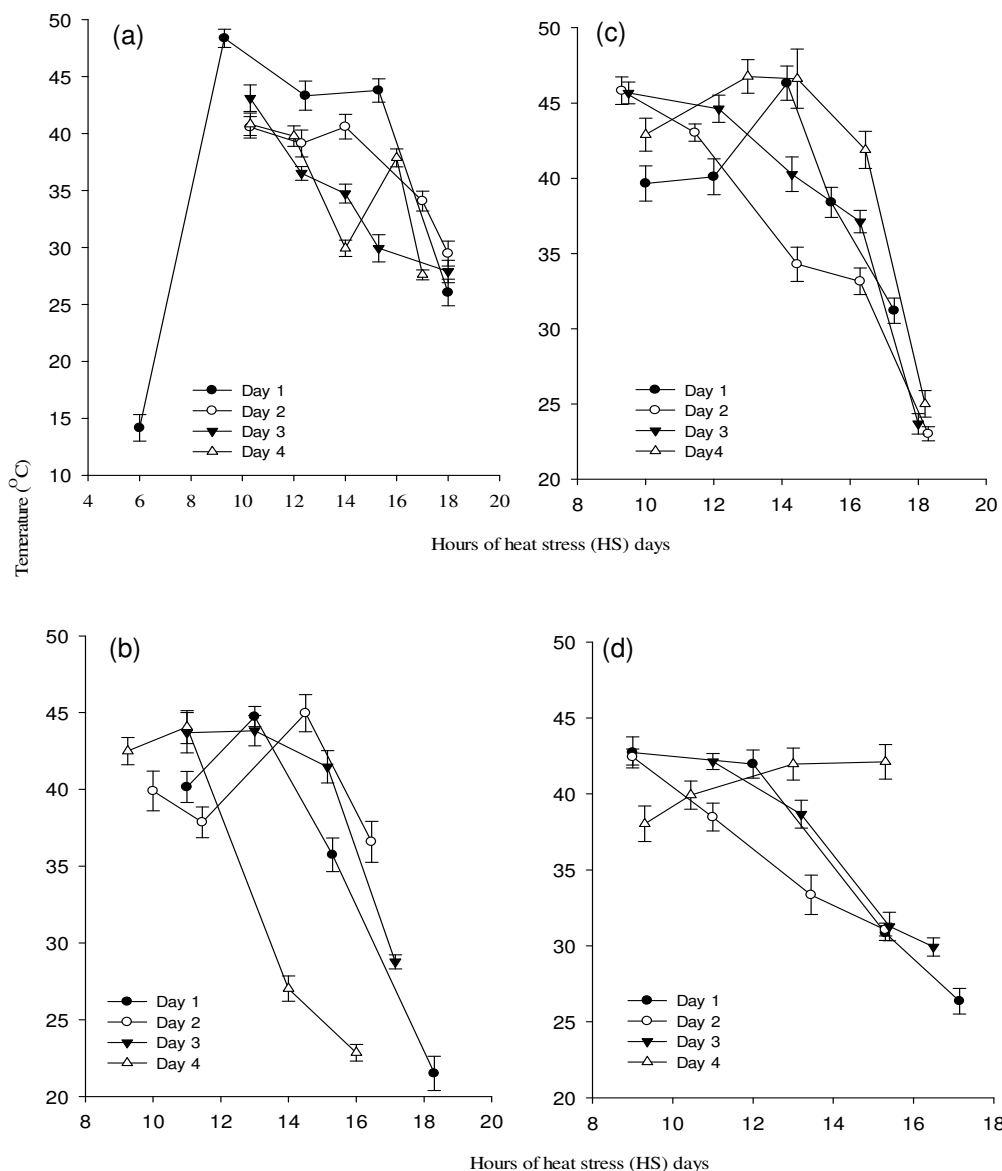


Figure 2. Periodic temperature change observed during heat stress (HS) from inside and outside the plastic tents over plants in the field. Figures (a and b) for cultivars (Pirsabak-2005, Pakhtunkhuwa-2015, Pakistan-2013, DN-84 and P-12) and windows (c and d) for late anthesis cultivars (P-2 & P-18) during 2017–18 2018–19, respectively.

Sampling and measurements

Days to anthesis were counted from sowing to 80% tillers in an experimental unit that reached the stage of anthesis. Maturity was counted from sowing to the stage when peduncle turned yellow and grain in spikes was hard. Ten spikes from an experimental unit were randomly selected and preserved. Data of spike length (cm), spike weight (g), grain weight (g), and grain number were recorded in the lab after properly drying the

samples. To record the above-ground biomass and yield, two rows of one-meter length in an experimental unit were harvested, bundled, and sun-dried for 10 days. Biomass was weighed and threshed using a mini-lab thresher. Both biomass and grain yield were subsequently adjusted with samples taken and oven-dry in a forced circulating hot air oven for about 46 h at 70 °C. Grains were separately weighed for each plot and expressed in kg ha⁻¹. Harvest index (HI) was determined as the ratio of grain to biomass. Data were statistically analyzed using the Fisher's analysis of variance technique and the mean found significant ($p < 0.05$) were separated using the least significant difference (*LSD*) test (Steel et al., 1997).

RESULTS AND DISCUSSION

Days to anthesis and maturity

Days to anthesis were significantly affected by different cultivars (C; Table 1).

Table 1. Days to anthesis and -maturity of wheat cultivars as influenced by the heat stress durations effective from anthesis stage of the crop growth

Cultivars (C)	Days to anthesis			Days to maturity		
	2017–18	2018–19	Mean	2017–18	2018–19	Mean
Pirsabak-2005	115.3	118.3	116.8 d	146.0	153.3	149.7 c
Pakhtunkhwa-2015	119.7	124.7	122.2 c	150.0	159.7	154.8 b
Pakistan-2013	112.3	117.3	114.8 f	143.7	152.3	148.0 e
DN-84	111.3	115.7	113.5 g	142.7	150.7	146.7 f
P-2 (Line)	122.0	132.7	127.3 a	157.7	170.7	164.2 a
P-12 (Line)	112.7	119.3	116.0 e	143.7	154.3	149.0 d
P-18 (Line)	121.7	130.0	125.8 b	159.3	170.0	164.7 a
<i>LSD</i> (0.05) for C	0.4	0.7	0.4	0.6	0.9	0.5
Heat stress (HS)						
Control	116.4	122.6	119.5	149.0	158.7	153.9
48 h	116.4	122.6	119.5	149.0	158.7	153.9
72 h	116.4	122.6	119.5	149.0	158.7	153.9
96 h	116.4	122.6	119.5	149.0	158.7	153.9
<i>LSD</i> (0.05) for HS	NS	NS	NS	NS	NS	NS
Year mean	116.4 b	122.6 a	**	149.0	158.7	**
Level of significance ($p < 0.05$) for treatment interaction						
HS x Y	-	-	ns	-	-	ns
C x HS	-	-	ns	-	-	ns
C x Y	-	-	**	-	-	**
C x HS x Y	-	-	ns	-	-	ns

Means followed by different letters within a category of treatments are statistically dissimilar using the least significant difference (*LSD*) test ($p < 0.05$);

* and ** represents the significance at ($p < 0.05$) and ($p < 0.01$) probability, NS = Non-significance.

The average across the years showed the maximum days to anthesis for P-2, followed by P-18, Pakhtunkhwa-2015, Pirsabak-2005, P-12, and Pakistan-2013. Minimum days to anthesis were observed for DN-84. Cultivars differed in anthesis due to the growth cycle (Cossani & Reynolds, 2015). In contrast, days to anthesis did not differ from control to heat stress (HS) imposed for about 48, 72, or 96 h. Days to anthesis were greater in the year 2018–19. It might be due to that crop in year 2017–18 was planted

late and cumulative temperatures of growth were greater in year 2017–18 than 2018–19. It needs to be clarified here that temporary plastic tents made over the crop fully covering it, have subsequently increased the humidity within tents, which contrary to HS promoted rust infection on the plants. To avoid this high humidity within tents, in 2018–19 plastic length of the tents was restricted about 25cm above the ground surface over the plants. Interaction (Y x C) revealed a marked change in days to anthesis in all cultivars with greater magnitude in 2018–19 (Fig. 3, a).

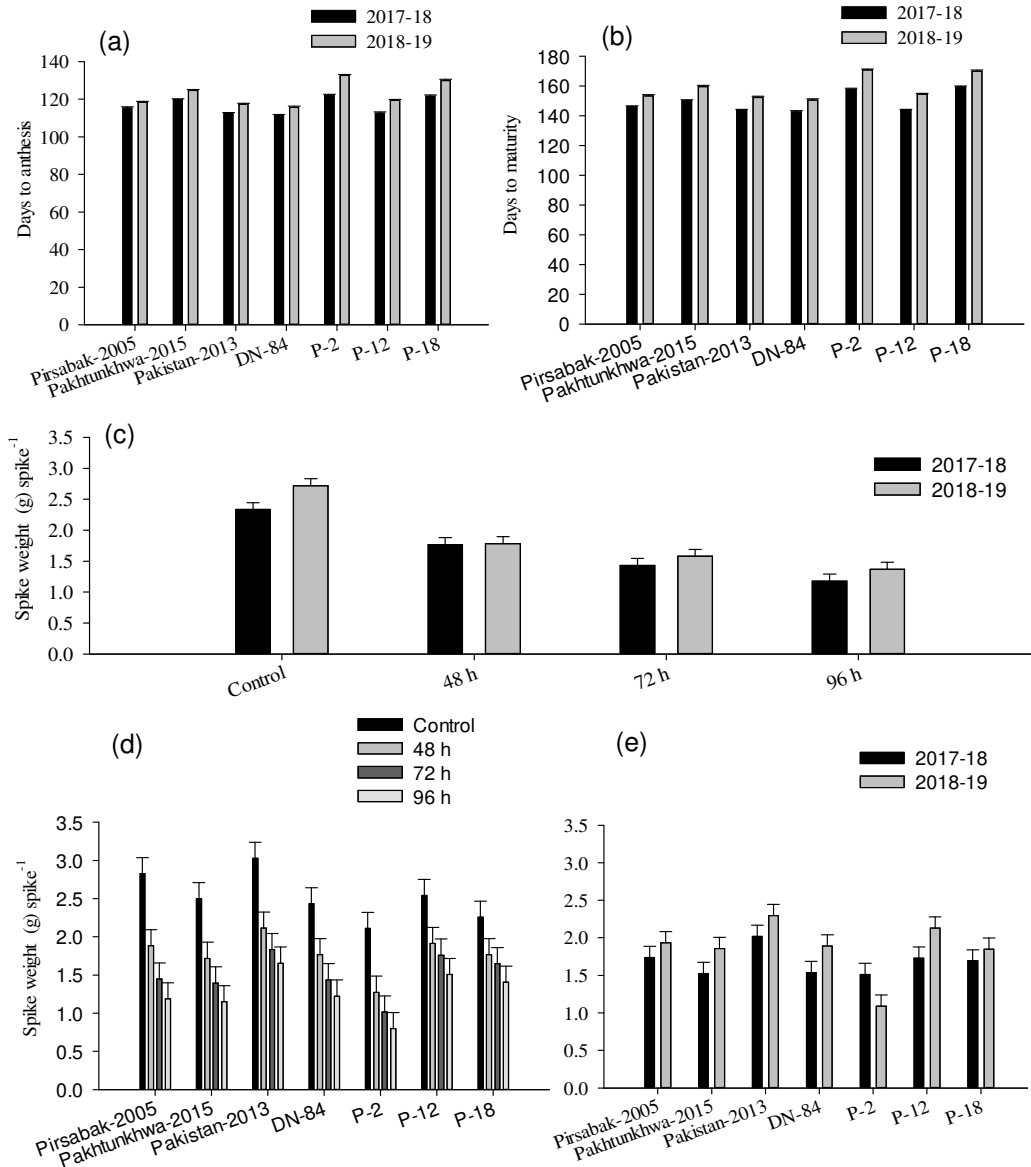


Figure 3. Interactions of treatments (a) cultivars x years for days to anthesis (b) cultivars x years for days to maturity (c) heat stress x years (d) cultivars x heat stress (e) cultivars x years for weight (g) spike⁻¹ of the cultivars.

Days to maturity showed significant changes for cultivars (Table 1). On two years' averages, the maximum days to maturity were recorded for P-2, which was statistically similar to P-18, followed by Pakhtunkhwa-2015, Pirsabak-2005, P-12, and Pakistan-2013. Minimum days to maturity were noted for cultivar DN-84. Changes in days to maturity of cultivars are based on their genetic differences as reported earlier (Sikder, 2009; Bhattarai et al., 2017). They observed that different cultivars of the same species differed in days to maturity within a similar climate. Moreover, changes in seed size make them different to imbibe and hence to emerged. This created differences of about 1–4 days irrespective of the same planting date. Days to maturity did not differ from control to HS of any duration. Days to maturity were higher in 2018–19, which might be due to changes in mean daily temperature of duration between seasons and also due to timely planting of crop unlike 2017–18, where sowing was delayed due to heavy rains. Treatment interaction (Y x C) showed significant changes in days to maturity among the cultivars with markedly more days were observed in 2018–19 (Fig. 3, b).

Spike weight

A significant difference was found in spike weight (g spike^{-1}) for cultivars and HS (Table 2). On two years averages, the spike weight was markedly higher in Pakistan-2013, followed by P-12 with similar ($p < 0.05$) in Pirsabak-2005. The lowest spike weight was noted in P-2. Philipp et al. (2018) observed differences in spike weight of the different cultivars, which was due to changes in grain number, grain sizes, and grain weight. Spike weight was reduced from control to the imposing HS for 48, 72, and 96 h. A similar finding of the reduction in spike weight under HS was observed by Semenov et al. (2014). They explained that spike weight associates with HS, as a decline in fertile spikelet was noted, which resulted in a decrease in the grain number and their weight. According to them, it was common that grains growth in HS situation resulted in shrivelled and smaller grains with lighter weight. Grain number per spikelet has reduced which decreased grains per spike (Philipp et al., 2018). Spike weight was observed higher in 2018–19, which was due to differences in the climatic conditions of the seasons. Similar changes in yield contributing traits due to variations in the environment have been reported earlier (Sikder, 2009; Nasehzadeh & Ellis (2017).

Interaction (HS x Y) exhibited a reduction in the spike weight in 2017–18, which was observed in comparison with control for any of the HS treatments (Fig. 3, c). This may be due to frequently abrupt changes in the absolute temperature due to unexpected rainfalls at the stage of the crop anthesis, and thereafter, in the grain development stage. The crop was sown late and availed less time to complete its growth cycle hence spike growth followed by grain number and weight were affected upon exposed to HS. Our results are in line with the published literature on the wheat crop (Semenov et al., 2014). They explained that overall the seasonal changes have an adverse effect on grain growth and especially in the early development stages. Interaction (C x HS) was also found significant in spike weight (Fig. 3, d). Spike weight showed a reduction in a linear fashion with extending HS (i.e. 48, 72, and 96 h) duration on crop effective from anthesis when compared with control. Nevertheless, Pakistan-2013, P-12, and P-18 were found relatively better to reduce spike weight in HS. Wheat grain fill duration may be attributed to sink-limiting for sources under the HS. These results are supported by the earlier study (Abdelrahman et al., 2020), explaining that the source-limited condition during the grain development is important to bring a change in spike weight by adversely affecting grain

number and size. Mohammadi (2012) already observed differences among genotypes to maintain higher seed weight, despite passing through HS for a limited duration. However, it should not couple with the crop-sensitive stages of plant growth. The HS effect can be classified as a high level of heat tolerance when there is no significant effect by HS on yield traits. This might be possible that the crop is not at the critical stage of growth and/or the HS is not so severe that adversely affects the yield traits.

Interaction of treatment (C x Y) showed a relatively higher spike weight in all cultivars during 2018–19 except P-2 (Fig. 3, e). The HS was found higher in 2017–18, which might have caused differences in the spike weight of wheat due to surpassing from a critical stage of the crop growth such as early developing grains. A similar severe adverse effect of the HS in fertilization and/or initial grain growth has caused losses to a complete failure of grains in developing spike (Mohammadi, 2012).

Grain weight

Grain weight (g spike⁻¹) differed for cultivars and HS (Table 2). On two years averages, Pakistan-2013 showed the highest grain weight, followed by P-12, DN-84, and the lowest grain weight was observed in P-2. Rates of weight losses in the developing grain differed in different cultivars. Cultivar possessing stability in grain weight under HS did not affect but those found sensitive to HS has greatly lost grain weight (Hossain et al., 2013). Grain weight showed marked reduction when compared with the control for every next degree of the imposed HS such as 48, 72, and 96 h, respectively. Heat stress has adversely affected grain growth and development. At the onset of anthesis and/or early grain developing, increasing HS duration has shown almost a linear reduction in the wheat grain size and number. It might be due to adversely affecting the assimilate translocation for grain set, which showed a reduction in grain growth rate and duration (Akter & Islam, 2017). Our results were supported by the findings of Dias & Lidon (2009) who explained that HS, in general, has speed-up the grain-fill rates by curtailing the grain-fill duration, which resulted in lower grain weight. A higher grain weight was noted in 2017–18. The prevailing cumulative temperature has played a significant role in grain growth; therefore, we found differences between the years. Moreover, the time of anthesis for the cultivars differed within seasons and the timing of imposing HS within seasons and cultivars have shown variations in the grain weights.

Interaction (HS x Y) showed higher grain weight in 2018–19 for control treatment and lower for the crop exposed to HS for about 48, 72, and 96 h (Fig. 4, a). Increase HS duration has reduced the translocation of assimilates which outcomes in a gradual reduction in grain weight with extending HS durations on the crop. The HS index was noted higher during 2018–19 as the temperature on imposition of the HS to crop was +35 °C for the maximum of the reading recorded in HS. This might have led to a drop in grain weight and the development of a higher number of smaller to shrivelled grains and/or higher empty florets due to pollen abortion. Dias et al. (2008) reported similar results that wheat crops subjected to HS of 31/20 °C in day/night temperatures have shown marked changes in the structure of endosperm cells and aleurone layers, which resulted in the shriveled grains. Treatment interaction (C x HS) also showed significant changes in grain weight for the years (Fig. 4, b). As expected, extended HS duration has resulted a significant loss in grain weight of all cultivars. However, Pakistan-2013 showed relatively a lower reduction in grain weight by extending the HS durations from control to every next level of the HS. This pattern was more or less mimicked for the

other cultivar like P-12. The formation and translocation of assimilates reduced in the prolonged HS and/or extending its duration, hence all cultivars have shown an adverse effect on grain weight leading to smaller grain and low yield under HS (Abdelrahman et al., 2020). Thus, extending HS duration at any critical stage of the plant growth has resulted in significant loss to complete failure of grain growth.

Table 2. Spike weight and grain weight (g) spike⁻¹ of wheat cultivars as affected by heat stress duration effective from anthesis stage of the crop growth

Cultivars (C)	Spike weight (g) spike ⁻¹			Grain weight (g) spike ⁻¹		
	2017–18	2018–19	Mean	2017–18	2018–19	Mean
Pirsabak-2005	1.74	1.94	1.84 bc	0.54	0.52	0.53 d
Pakhtunkhwa-2015	1.53	1.86	1.69 d	0.57	0.55	0.56 d
Pakistan-2013	2.02	2.30	2.16 a	1.02	0.99	1.01 a
DN-84	1.54	1.90	1.72 d	0.75	0.60	0.68 c
P-2 (Line)	1.51	1.09	1.30 e	0.30	0.30	0.30 f
P-12 (Line)	1.72	2.13	1.93 b	0.89	0.77	0.83 b
P-18 (Line)	1.69	1.85	1.77 cd	0.46	0.39	0.42 e
<i>LSD</i> (0.05) for C	0.15	0.15	0.11	0.06	0.05	0.04
Heat Stress (HS)						
Control	2.34	2.72	2.53 a	1.02	1.16	1.09 a
48 h	1.77	1.79	1.78 b	0.68	0.53	0.60 b
72 h	1.43	1.58	1.51 c	0.50	0.38	0.44 c
96 h	1.18	1.37	1.28 d	0.39	0.28	0.33 d
<i>LSD</i> (0.05) for HS	0.12	0.11	0.08	0.04	0.03	0.03
Year mean	1.68	1.86	**	0.65	0.59	*
Level of significance ($p < 0.05$) for treatment interaction						
HS x Y	-	-	**	-	-	**
C x HS	NS	**	**	**	**	**
C x Y	-	-	**	-	-	**
C x HS x Y	-	-	NS	-	-	**

Means followed by different letters within a category of treatments are statistically dissimilar using the least significant difference (*LSD*) test ($p < 0.05$);

* and ** represents the significance at ($p < 0.05$) and ($p < 0.01$) probability, NS = Non-significance.

Interaction of treatments (C x Y) showed the same trend for cultivars, with higher grain weight in 2017–18 but a relatively marked variations for DN-84 and P-12 between seasons (Fig. 4, c). The relative absolute temperature in 2018–19 at the anthesis stage of the wheat crop was quite higher than 2017–18, and hence HS inside plastic tents were severe, which has negatively affected the grain growth due to disruption of the photosynthetic machinery in the top leaves particularly the flag leaf that resulted in partially filled florets with fewer grains and hence reduced the grain size and grain weight in most cultivars. Three-way interaction (C x HS x Y) also showed a marked reduction in grain weight of the cultivars by extending HS durations in both years with higher reductions observed for 2018–19 (Fig. 4, d). Pakistan-2013 and P-12 were found relatively stable to resist the imposed HS, however, P-2 was found more susceptible. It has been explained earlier that HS has adversely affected the grain formation in florets and hence the growth (Semenov et al., 2014). Imposed HS in 2018–19 was relatively stronger in intensity and had shown marked variations in wheat grain weight.

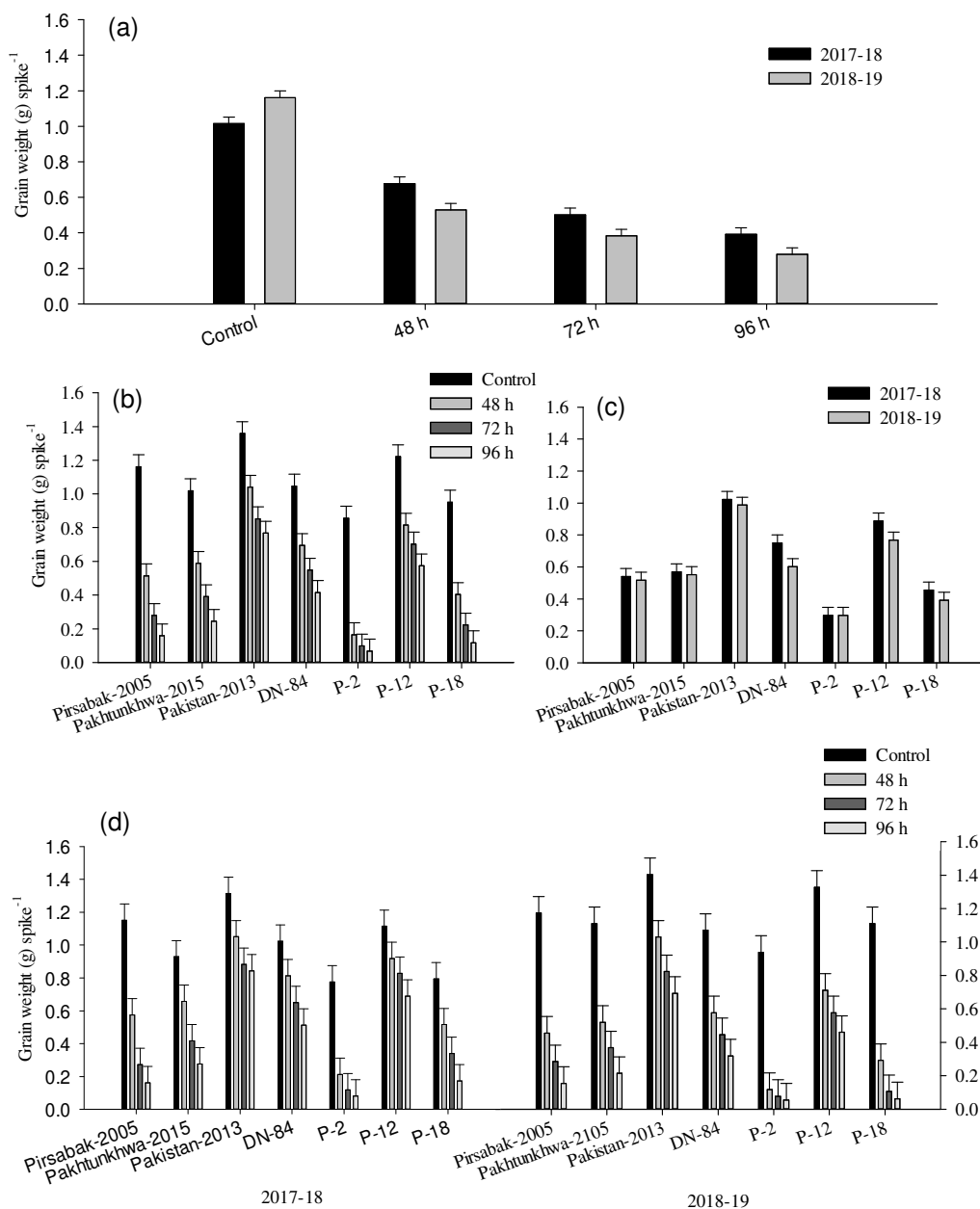


Figure 4. Interactions of treatment (a) heat stress x year, (b) cultivars x heat stress, (c) cultivars x year, (d) cultivars x heat stress x year for grain weight (g spike⁻¹) of cultivars.

Grain number

Grain number (spike⁻¹) exhibited marked variations in wheat cultivars and HS (Table 3). The higher numbers were observed in Pakistan-2013, followed by P-12 and DN-84 with non-significant changes. The lowest grain number was noted in P-2. It has been confirmed that grain number reduced with HS but varied among wheat cultivars. Plants treated with HS have shown a reduction in the grain number (Lukac et al., 2012;

Mazurenko et al., 2020), whereas, cultivars did differ to stand with HS. A temperature of +20 °C, at the time of anthesis, has accelerated spike growth which has reduced the grain number and hence resulted in a limited spikelet size (Semenov, 2009). Grain number has shown a reduction in all wheat cultivars with increment in HS from control to every next stage such as 48, 72, and 96 h, which are in line with the findings of Anjum et al. (2008). They reported that high temperature at anthesis is harmful to pollen survival. It caused pollen sterility and hence limited with grain number. The difference in grain number of the cultivars is obvious in the rate of sterility in florets when the temperature exceeds +30 °C. The rates of sterility may differ within cultivars due to differences in the timing of their anthesis (Kumar et al., 2020). Both structure and function of anthers are poorly affected under the mild HS and severely when HS undergoes a prolonged duration of +3-days at the critical growth stage of the crop like anthesis or grain development (Hedhly et al., 2009). Grains number did not show any statistical change between the years of this study.

Table 3. Grain number spike-1 and grain yield (kg ha⁻¹) of wheat cultivars as affected by heat stress duration at anthesis stage of the crop growth

Cultivars (C)	Grain number spike ⁻¹			Grain yield (kg ha ⁻¹)		
	2017–18	2018–19	Mean	2017–18	2018–19	Mean
Pirsabak-2005	19.37	21.67	20.52 de	1,489	1,481	1,484 d
Pakhtunkhwa-2015	21.00	21.92	21.46 d	1,522	1,468	1,495 d
Pakistan-2013	32.45	33.42	32.93 a	2,907	2,848	2,878 a
DN-84	34.47	26.75	30.61 b	2,039	1,797	1,918 c
P-2 (Line)	16.27	23.92	20.09 e	813	796	805 f
P-12 (Line)	33.45	29.75	31.60 b	2,439	2,308	2,373 b
P-18 (Line)	25.17	25.82	25.49 c	1,268	1,037	1,153 e
<i>LSD</i> (0.05) for C	2.17	1.49	1.30	170.1	113.6	101.1
Heat stress (HS)						
Control	35.38	35.56	35.47 a	2,885	3,303	3,094 a
48 h	26.16	27.71	26.94 b	1,870	1,562	1,716 b
72 h	22.5	22.71	22.60 c	1,351	1,059	1,205 c
96 h	20.06	18.71	19.39 d	1,024	0.781	0.902 d
<i>LSD</i> (0.05) for HS	1.64	1.13	0.99	128.60	86.9	76.5
Year mean	26.0	26.2	NS	1,782	1,676	*
Level of significance ($p < 0.05$) for treatment interaction						
HS x Y	-	-	*	-	-	**
C x HS	**	**	**	**	**	**
C x Y	-	-	**	-	-	ns
C x HS x Y	-	-	**	-	-	ns

Means followed by different letters within a category of treatments are statistically dissimilar using the least significant difference (*LSD*) test ($p < 0.05$);

* and ** represents the significance at ($p < 0.05$) and ($p < 0.01$) probability; NS = Non-significance.

Interaction (HS x Y) showed marked reductions in grain number during 2017–18 at 48 h HS but no changes thereafter for extending HS to 72 h. Control treatments did not show any changes in grain number between the years (Fig. 5, a). A significant difference between the years was noticed in prevailing temperature at anthesis and hence has caused variations for HS. Similarly, cultivars and various HS durations showed a clear difference

for grain number in both years. The interaction (C x HS) also exhibited a marked reduction in the grain number of cultivars exposed to HS but at different rates (Fig. 5, b).

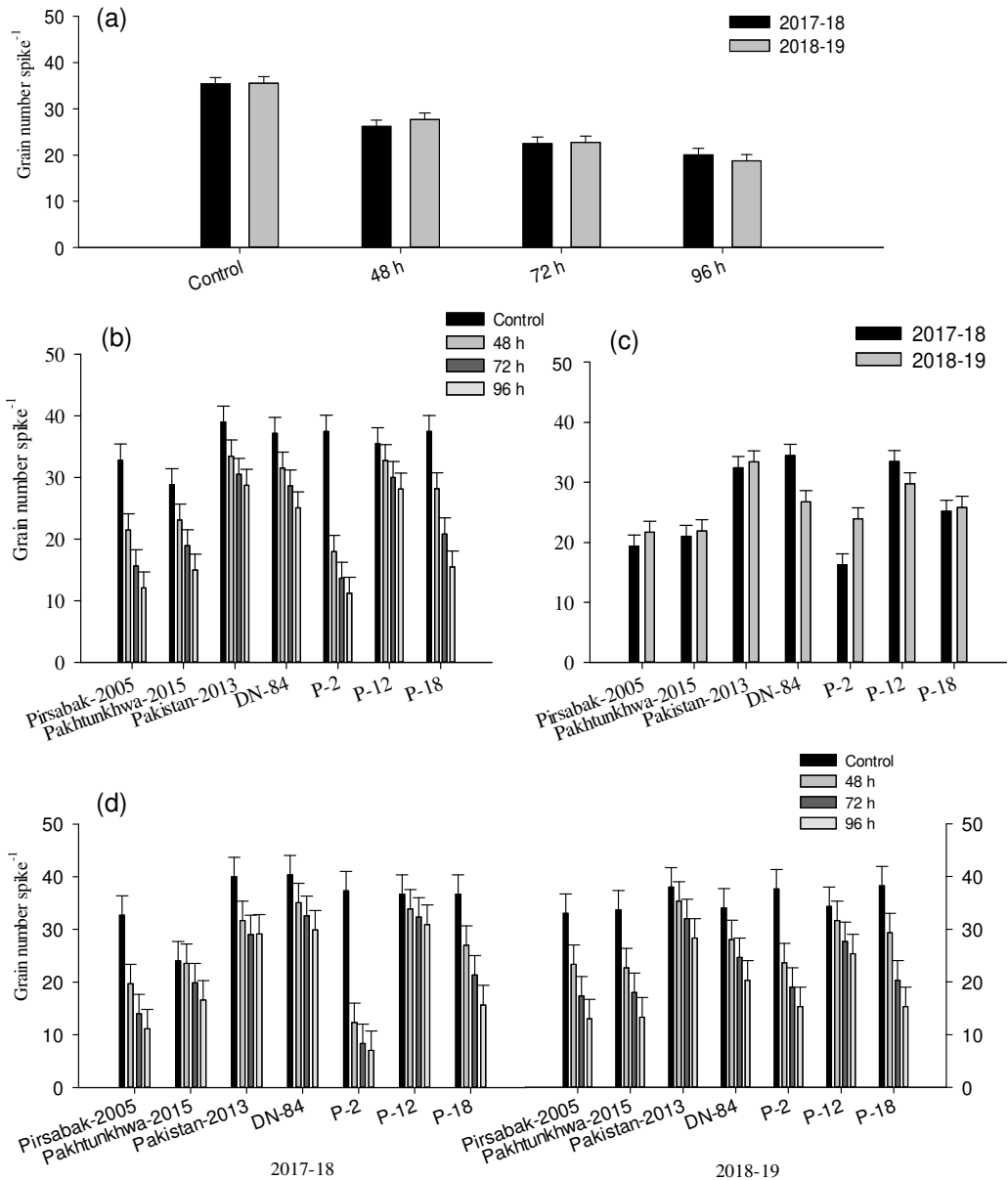


Figure 5. Interaction of treatments (a) heat stress x year, (b) cultivars x heat stress, (c) cultivars x years, (d) cultivars x heat stress x year for grain number spike⁻¹ of cultivars.

Grain number per spike decreased with the HS but, was almost at a stable rate for the cultivar Pakistan-2013, P-12, and DN-84 as compared to the rest of the cultivars. It showed that cultivars were stable to resist HS at anthesis. The pollen viability is an important factor not to be affected with HS within cultivars (Semenov et al., 2014). Otherwise, a

decrease in grain number in wheat spike may accelerate to a complete failure of the grains (Kumar et al., 2020). Relatively faster variations in grain number within spikes exposed to HS from control have shown the rate of susceptibility of the cultivar facing HS at the crop critical growth stages. The interaction (C x Y) has revealed higher grain numbers within the spikes of all cultivars except DN-84 and P-12 in 2018–19 (Fig. 5, c). Cultivars did vary in grain number within spikes by prevailing temperature and its critical stage of the growth when plants undergo anthesis or grain development (Moshatati et al., 2017). The interaction (C x HS x Y) showed a gradual reduction in grain number of the cultivars but with different rates between seasons (Fig. 5, d). An unusual variation in the grain number was noticed in wheat cultivar Pakhtunkhwa-2015 and P-2, which reflects their susceptibility to HS exposed at the anthesis stage of the crop growth. Similar variations in grain number within wheat spikes under temporarily elevated HS have been reported by Mohammadi (2012) which were due to loss in pollen fertility (Bhattarai et al., 2017).

Grain yield

As observed from the yield traits, grain yield (kg ha^{-1}) exhibited changes within cultivars and HS (Table 3). Grain yield was reported the highest for Pakistan-2013, followed by P-12, DN-84, and Pakhtunkhwa-2015. The lowest grain yield was recorded in P-2. The HS starting from the anthesis stage of the crop growth which reflected marked reductions in the yield. It was due to the limited duration of the resource utilization for grain fill periods and their accumulation as grain within spikes (Dias & Lidon, 2009; Yin et al., 2009). A higher yield of wheat cultivars might be due to a relative delay in the senescence phase of the cultivar with a relatively higher grain filling rate under the HS. It has been observed that assimilates partitioning is limited in the adverse growth conditions for grains development (Farooq et al., 2011). Grain yield showed a reduction when compared with control to any HS imposed on plants for 48, 72, and 96 h. Marked reduction in yield observed with exposing plants to a temperature above $24\text{ }^{\circ}\text{C}$ at the crop anthesis stage (Prasad & Djanaguiraman, 2014), which were due to a significant effect on yield contributing traits of the plants. The anthesis stage is one of the most critical factors of the grain yield. From anthesis to grain development stages, the climate photoperiod expands, and the daily temperature increases relatively at a very faster rate for the next few days in comparison with the previous growth duration such as stem elongation. This showed an adverse effect on grain number and weight which resulted in a marked reduction of grain yield (Nasehzadeh & Ellis, 2017; Bergkamp et al., 2018). Grain yield was observed higher in 2017–18 as mean temperatures of two years differed and HS was probably found less fatal in 2017–18 which resulted in a higher yield. The absolute temperature in 2017–18 was comparatively low at the anthesis stage, which might have decreased the severity of HS effects and therefore have better yield.

Interaction (HS x Year) reflected a better yield of the control treatment in 2018–19. Nonetheless, a decrease in yield was observed with imposing HS on wheat, which raised the surrounding temperature in a plastic tent and it might become fatal for the pollen sterility as well as assimilates accumulation in grains by extending the HS duration (Fig. 6, a). Grain yield in 2018–19 was reported higher despite the higher absolute temperature. The reason could be that crops were infected with rust due to HS and the rainy season in the month of April-May which resulting higher variations

in yield under controlled conditions in 2017–18. Treatments interaction (C x HS) exhibited a significant change in grain yield across average of the two years (Fig. 6, c).

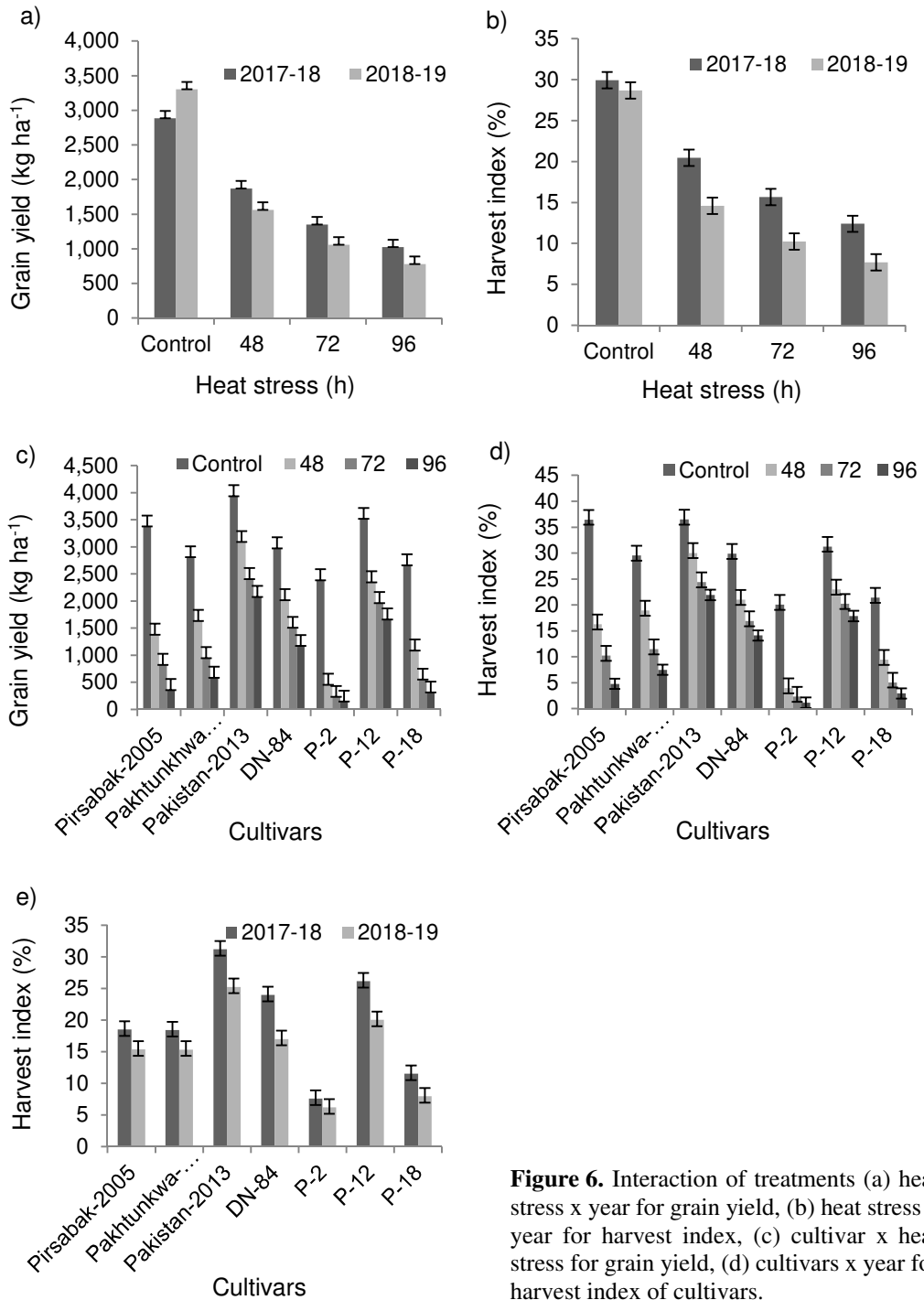


Figure 6. Interaction of treatments (a) heat stress x year for grain yield, (b) heat stress x year for harvest index, (c) cultivar x heat stress for grain yield, (d) cultivars x year for harvest index of cultivars.

Grain yield markedly reduced for wheat cultivars with extending the HS duration with a relatively higher reduction in lines P-2 and P-18 than the rest of the cultivars. Each cultivar responded differently to changes in HS which depending on its potential and coinciding with the growth. Similar findings have been already observed by Nasehzadeh & Ellis (2017). According to them, the susceptible cultivars showed greater losses in yield when exposed to HS by adversely affecting their pollen fertility and decreasing their rate of assimilates accumulation in grains. Temperature changes have been noted within years at the crop anthesis stages, which have shown differences in the yield of the cultivars. The cumulative effect of the HS corresponds to critical stages of growth of the cultivar which has created mild to marked variations in the cultivars subjected to HS between years. The higher ambient temperature is directly related to HS. It is also obvious that as the HS duration prolonged the adverse effect on yield would be greater.

Biomass yield

Above-ground biomass (kg ha^{-1}) also reflected a significant variation for wheat cultivars and HS treatment (Table 4). The highest biomass was recorded in the advanced line P-18, which was statistically similar with the line P-2, followed by P-12, and Pakistan-2013. Our findings are in line with the published reports (Banerjee & Krishnan, 2015; Rakutko et al., 2020). They observed differences in biomass among the cultivars. It is estimated that a reduction of 23.5% biomass is expected when plants are subjected to HS at the start of anthesis, which limits the growth of flag leaf within HS conditions (Bergkamp et al., 2018). The plant biomass decreased with increasing HS as compared to the control for every next level of the HS such as 48, 72, and 96 h but varying greatly among cultivars. Imposing HS (+40 °C) may have also lead to scorching on leaves and sunburn, reducing branches, twigs, and stems elongation, starting early senescence and greater leaf abscission, which reflected significant losses in plant biomass (Moshatati et al., 2017). Biomass was recorded higher in 2018–19, which might be due to the reason that the crop had enjoyed the optimum growth period and hence has gathered the maximum assimilates to produce higher biomass. However, uneven changes at maturity stages of the cultivars were noted between years with differences for the same cultivar in 2018–19. Rust infestation has also damaged the plants within tents in 2017–18 due to higher humidity created by the fully covered tents. However, all other possible treatment combinations for the interactions were found non-significant in yielding the above-ground biomass production.

Harvest index

Harvest index (%), which is the ratio of grain yield to above-ground biomass, was significant for the cultivars and HS treatment (Table 4). Better harvest index was derived for the cultivar Pakistan-2013, followed by P-12, and DN-84. Increased HS at the start of the crop anthesis stage has shown marked reductions in grain filling durations within the cultivars, and has reflected a lower grain as well as biomass yield followed by a lower harvest index. Prasad & Djanaguiraman (2014) also observed that higher temperature (+24 °C) around the plant anthesis stages may lead to floret sterility, which resulted in a lower grain yield than total biomass and has shown a reduction in harvest indices. Harvest index consistently decreased with extending HS duration from the control treatment to any HS duration. Harvest index is directly related to grain production, which was reduced under the HS due to declining both grain weight and the grain number.

Similar results were also published by Prasad et al. (2011). They reported that the imposition of short duration HS for a limited period has adversely affected grains as compared to the total above-ground biomass, and has shown a reduction in harvest index. A higher harvest index was noted in 2017–18 as the absolute temperature was different between years and was relatively stable at the time of the crop anthesis and early grain filling in 2017–18. Plants have responded with an effect on the transportation of assimilates from source to sink, which might have resulted in more filled grains and a higher harvest index. This is clearly explained by Reynolds et al. (2005) and also by Farooq et al. (2011). Interaction (HS x Y) also reflected a better harvest index in 2017–18 for control as compared to the rest of the treatments (Fig. 6, b). The intensity of temperature during grain development is the main factor affecting grain weight.

Table 4. Biomass yield (kg ha⁻¹) and harvest index (%) of wheat cultivars as affected by heat stress effective from the anthesis stage of the crop growth

Cultivars (C)	Biomass yield (kg ha ⁻¹)			Harvest index (%)		
	2017–18	2018–19	Mean	2017–18	2018–19	Mean
Pirsabak-2005	7,746	9,199	8,472 d	18.51	15.36	16.93 d
Pakhtunkhwa-2015	8,077	9,177	8,627 d	18.41	15.33	16.87 d
Pakistan-2013	9,256	11,176	102,156 b	31.2	25.24	28.22 a
DN-84	8,407	10,460	9,434 c	23.97	17.00	20.49 c
P-2	10,606	12,174	11,390 a	7.57	6.18	6.88 f
P-12	9,246	11,322	10,284 b	26.14	20.04	23.09 b
P-18	10,787	12,548	11,668 a	11.5	7.94	9.72 e
<i>LSD</i> (0.05) for C	631.1	340.5	354.5	2.10	1.02	0.93
Heat stress (HS)						
Control	9,823	11,683	10,753 a	29.93	28.67	29.30 a
48 h	9,448	10,977	10,212 b	20.45	14.6	17.53 b
72 h	8,765	10,582	9,673 c	15.68	10.23	12.95 c
96 h	8,606	10,218	9,412 c	12.4	7.69	10.04 d
<i>LSD</i> (0.05) for HS	477.1	257.4	268.0	1.59	0.77	0.71
Year mean	9,161	10,865	***	19.61 a	15.30 b	**
Level of significance ($p < 0.05$) for treatment interaction						
HS x Y	-	-	ns	-	-	**
C x HS	ns	ns	ns	**	**	**
C x Y	-	-	ns	-	-	**
C x HS x Y	-	-	ns	-	-	ns

Means followed by different letters within a category of treatments are statistically dissimilar using the least significant difference (*LSD*) test ($p < 0.05$).

* and ** represents the significance at ($p < 0.05$) and ($p < 0.01$) probability, NS = Non-significance.

The temperature in 2018–19 was higher than 2017–18 for all HS durations and hence has shown markedly adverse effects on the yield traits, which resulted in a decrease in harvest index. A lower grain yield was reported in 2018–19, followed by a lower harvest index compared to 2017–18. Treatment interaction (C x HS) exhibited a significant reduction in harvest index of cultivars subjected to the HS at a critical stage of the plant growth, but with varying rates due to resistances of the cultivars for pollen fertility and/or timing of crop anthesis during the day temperature (Fig. 6, d). Harvest index markedly reduced with the imposition of HS in P-2 and P-18 relative to the rest of the cultivars in this study as their grain yield was also lower. Similar differences within cultivars in the

harvest index on exposing the crop to HS at the time of its growth and development were also noted by Gupta et al. (2015). Gibson & Paulsen (1999) also reported marked changes in the crop harvest index on exposure to HS (+20 °C) from anthesis to maturity. The crop harvest index was decreased at a faster rate on facing HS during the growth and development, subject to the climate and crop growth stage, which resulted in a faster declining the grain yield than the biomass (Gupta et al., 2015). Grain yield decreased with changes in the number and weight of the developing grains within spikes. This is due to a decrease in the rate of starch synthesis at the grain filling duration (Hogy et al., 2013), hence lost food reserves stored in grains. Interaction (C x Y) reflected a higher harvest index in all cultivars during 2017–18 (Fig. 6, e). The cultivar with better grain yield has shown healthy traits with relatively marked HS in 2018–19 showing a higher harvest index. Heat stress is the main factor affecting grain growth and development and it was observed relatively higher in 2018–19, which adversely affected the growth, yield, and yield traits and finally the harvest index.

CONCLUSIONS

It is concluded that the period between anthesis to grain filling is the most critical stage of the wheat crop. Plants when undergoes heat stress for a shorter duration during this critical stage of the plant growth, resulted in marked losses in the grain weight (45, 61, and 69%) and grain number (29, 36, and 45%) of spike with extending HS duration from control to 48 h, to 72 h, and thereafter from 72 to 96 h, respectively. This showed a considerable reduction in the grain yield and subsequently increased production cost. Extending the duration of HS during the critical stages of plant growth has resulted in a marked reduction in wheat productivity. However, cultivars differed in yield losses under the imposed HS which shows that potential exists within cultivars for future breeding to make available the best resistant cultivar that could be planted in areas facing adverse weather effects in terms of heat stress on yield losses.

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