







Differential effects of high-temperature stress on the morphophysiological traits of different Wheat (*Triticum aestivum* L.) genotypes

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Abstract

High-temperature stress (HTS) is one of the significant constraints in crop production under climate change. The temperature rises from 32 °C during the wheat reproductive and grain-filling stage, leading to yield penalties. In the present investigation, ten different wheat genotypes were evaluated at three different times of the same cropping year to assess the effect of HTS on developmental patterns and yield. The physiological characterization assessed chlorophyll content (CC) and cell membrane stability (CMS). However, the Canopy temperature depression (CTD) was measured at the three development stages, pre-anthesis, anthesis and post-anthesis, under HTS conditions to identify promising genotypes for developing new breeding lines. Simultaneously the morphological characterization viz. days to 50 % heading (HD), days to 50 % flowering (FD), plant height (PH), flag leaf length (FLL), flag leaf width (FLW), spike length (SL), spike numbers (SN), spike weight (SW), grain number per spike (GN/S), grain weight (GW), test grain weight (TGW) and grain yield per plot (GY/P) were analyzed to correlate the yield under HTS. Statistical analysis was done using a factorial ANOVA in all the genotypes and K910-30 showed better responses with CC, CMS and TGW values under HTS conditions. Simultaneously, the assessment of morphological traits revealed that the K910-30 genotype performed better than other genotypes. The results of the present investigation will be helpful in future studies dealing with improved high-temperature stress tolerance in wheat. They might be beneficial for identifying wheat genotypes that can withstand high-temperature stress.

Keywords: abiotic stress; crop improvement; dryland cropping; genetic variability

Introduction

Wheat (Triticum aestivum L.) delivers about 19 % of the calories and 21 % of the protein to the world's population (1). Thus, it is one of the most essential cereals, ranked after rice and maize. The rice and wheat cropping is the primary cropping system grown in rotation on 13.5 Mha in the Gangetic plains of India (2). This system produces about 50 % of the total food grain and feeds 40 % of India's population (3). However, due to the proximity of the equator and the late sowing of wheat in India, wheat crops face high-temperature stress (HTS) during the grain-filling stage, leading to a reduction in yield. In the last few decades, the world has witnessed climate changes, which are rapidly changing crops and agronomic practices (4). As per the projection, South Asia will be deficient in 22 Mt. of food-grain production by 2030 (5). The global air temperature is predicted to increase by 0.2 °C every ten years and it will increase the temperature by 1.8 to 4.0 °C, which is higher than the current level by 2100 (6). A temperature rise of 3 to 4 °C might cause a 15 to 35 % loss of crop yield in Africa and Asia and 25 to 35 % in the Middle East (7, 8). As a sessile organism, plants cannot move to favourable environments, substantially affecting their growth and developmental processes.

The rice-wheat cropping system has started showing a reduction in the marginal yields due to different abiotic stressors and high-temperature stress is one among them. The HTS leads to rapid wheat maturation during the anthesis and grain filling stage, reducing grain size, weight and yield (9, 10). Wheat production is estimated to reduce by 6 % with the rise of each degree in temperature (11). However, only a few studies have been conducted in India to assess the long-term impact of heat stress on wheat's physiological traits and yield potential. A detailed overview of morpho-physiological responses of wheat under HTS may help formulate

appropriate strategies for the development of heat-tolerant cultivars of wheat. An understanding of morpho-physiological traits related to yield and stress tolerance is required to enhance wheat yields. Physiological characteristics are also crucial in selecting breeding lines to increase yield (12).

Screening of contemporary scientific findings revealed that among the physiological traits affected by HTS, it severally affects cell membrane stability (CMS), chlorophyll content (CC) and canopy temperature depression (CTD), seriously hampers wheat production and, ultimately, yield (13-15). High temperatures adversely affect plant metabolism at each phenological stage, reducing yield. A positive correlation between high temperature-induced thylakoid membrane damage and chlorophyll content was reported in 12 winter wheat genotypes (16). As per the report, a stable cell membrane that remains functional during stress seems to control adaptation to high temperatures and is related to heat and drought tolerance (17). High-temperature stress and drought frequently result in photoinhibition via a reduction in CC. Moreover, the cell membrane is one of the first targets for different stressors, including HTS, so the stability of the cell membrane is a crucial factor in studying the effect of HTS. High temperature-induced membrane fluidity is a key factor for oxidative damage (18). Increased membrane fluidity is closely related to electrolyte leakage and damage in thylakoids and grana, reducing photosynthesis (18, 19). Hence, plant CMS can indicate stress tolerance and the injury rate to the cell's plasma membrane (20). Therefore, the measurement of membrane thermal stability was used to screen and evaluate different genotypes for thermal tolerance, which would help breed stress tolerance wheat genotypes to double farmers' incomes.

Because of the above-mentioned physiological traits, the present investigation was designed (i) to investigate the effect of HTS on CC, CMS, CTD and days to maturity of different genotypes of wheat (*Triticum aestivum* L.); and by using this data, the responses of these genotypes were evaluated in terms of their resistance to temperature stress (ii) to examine the association between CMS, CC, CTD and yield (iii) to identify the best-performing genotypes for further genetic studies and breeding for the development of high-Temperature stress tolerant lines of wheat.

Material and methods

Experimental layout

This work was done during the winter of 2013-14 at the experimental field of the Department of Genetics and Plant Breeding, SHUATS, Prayagraj, India, located at 25.57° N latitude, 81.51° E longitude and 98 m above sea level following the details given in Table 1. The soil in this district is sandy loam and alkaline. The seedlings of three different sowings were exposed to a gradual increase in temperature ranging from 28.6 °C to 31 °C, 26.4 °C to 42.6 °C and 18.48 °C to 47.5 °C from the time of sowing to harvest (Meteorological data; Table 2). Polyethylene sheets were used to keep rain out. A required fertilizer (NPK) dose of 120:60:60 NPK- kg/ha was used based on soil analysis for each sowing. Each genotype was sown at a seed rate of 300 seeds per square meter in the early sowing (D1)

and at 350 and 380 seeds per square meter for the later sowings D2 and D3, respectively. The seedlings were sown in 30 plots of 7.5 m 2 (Table 1) under environmental conditions of 25.17 $^{\circ}$ C/10.78 $^{\circ}$ C average day/night temperature, an average 10±14 hr photoperiod and 91.62 %/48.96 % average max/min relative humidity. The experiments were carefully handled to avoid unwanted stressors.

Genotypes and environmental condition

Seeds of 10 wheat genotypes (K-9162, NW-1014, HUW-658, NW-4035, AAI-16, AAI-13, AAI-12, AAI-11, K-910-30, K 911) were provided by the Department of Genetics and Plant Breeding, SHUATS, India. These genotypes were sown at three different times. The first sowing (D¹) was done on Nov 30, 2013, where the average temperature and relative humidity were recorded as ~21 °C and 67 %, respectively (Table 1-2). The second sowing (D²) was done after 23 days of the first sowing on 24-12-2013. However, the third sowing (D³) was done 17 days after the second sowing on 10-01-2014. The average temperature and relative humidity during the second and third sowing were recorded as 18.5 °C, 67.6 % and 16.7 °C, 77.0 %, respectively (Table 1-2). All the experiments were conducted in three replications using a factorial ANOVA for statistical analysis.

Measurement of phenological traits

Phenological traits such as days of 50 % heading (HD), days of 50 % flowering (FD), plant's height (PH), number of tillers/m² (TS), flag leaf dimension (length and width), spike length (SL), days to maturity (MD) and spike dry weight (SDW) were recorded. The HD and FD were recorded from the time of sowing to the time of 50 % head emergence and 50 % emergence of the primary ear from the flag leaf, respectively. The PH was measured in cm from the soil level to the base of the spike. The TS was measured at 70 days after sowing. The flag leaf dimension (length and width) was measured in cm at 50 % anthesis. Flag leaf length was measured from the collar junction of the blade to the leaf sheath of the sword and the width was measured from one margin to the other margin of the same leaf. The average SL was recorded in cm at the time of anthesis by measuring the average spike length of five

Table 1. The layout of the experiment and chronological records

| , , , , , , , , , , , , , , , , , , , , | | | | | | | | | | |
|-------------------------------------------------------|-------------------------------|--|--|--|--|--|--|--|--|--|
| Season | Winter-spring | | | | | | | | | |
| Number of genotypes | 10 | | | | | | | | | |
| Design | RBD (Randomized block design) | | | | | | | | | |
| Replications | 3 | | | | | | | | | |
| Row-to-row distance | 25.0 cm | | | | | | | | | |
| Plant-to-plant distance | 5.0 cm | | | | | | | | | |
| Number of plots | 30 | | | | | | | | | |
| Plot size | 7.50 m ² | | | | | | | | | |
| Total gross area | 351.0 m ² | | | | | | | | | |
| Net area | 225.0 m ² | | | | | | | | | |
| Main irrigation channel | 1.0 m | | | | | | | | | |
| Fertilizer dose (NPK) | 120:60:60 NPK- kg/ha. | | | | | | | | | |
| (D¹) Date of sowing 1stTimely sowing | 30/11/2013 | | | | | | | | | |
| (D²) Date of sowing 2 nd Delayed sowing | 24/12/2013 | | | | | | | | | |
| (D³) Date of sowing 3 rd Delayed sowing | 10/1/2014 | | | | | | | | | |
| Date of harvesting 1st | 06-04-2013 | | | | | | | | | |
| Date of harvesting 2 nd | 20-04-2014 | | | | | | | | | |
| Date of harvesting 3 rd | 20-04-2014 | | | | | | | | | |
| | | | | | | | | | | |

Table 2. Meteorological data recorded during experimental period winter-spring 2022-23

| Month and work | Tempe | rature °C | Relative hu | Rainfall (mm) | | |
|----------------|-------|-----------|-------------|---------------|-------|--|
| Month and week | Max. | Min. | Max. | Min. | | |
| November 2013 | 27.34 | 14.9 | 91.13 | 42.30 | 3.0 | |
| December 2013 | 27.11 | 9.98 | 91.08 | 44.11 | 7.20 | |
| January 2014 | 21.19 | 12.27 | 92.28 | 61.71 | 12.20 | |
| February 2014 | 25.82 | 13.49 | 85.99 | 49.81 | 8.30 | |
| March 2014 | 30.76 | 16.96 | 85.39 | 49.74 | 9.15 | |
| April 2014 | 40.14 | 22.09 | 93.47 | 53.42 | 8.32 | |

Rain, average maximum (Max) and minimum (Min) temperature and relative humidity

plants on the main calm from the base of the spike to the top of the last spikelet, excluding awns. The days of physiological maturity were recorded from the date of sowing to the date of maturity. The SW was recorded by harvesting five spikes of 75 cm from inner rows and the main stem and tiller spikes were separated from the aerial part and placed into an oven at 65 °C to estimate the spike dry weight (SDW).

Measurement of physiological traits

The effects of HTS on the physiological traits were studied by measurement of total chlorophyll content (CC), cell membrane stability (CMS) and canopy temperature depression (CTD). CTD is directly proportional to photosynthesis and affects carbohydrate synthesis. Total chlorophyll was extracted using a dimethyl sulphoxide (DMSO) extraction procedure. 50 mg of chopped leaf tissue was incubated with 10 mL of DMSO at 65 °C for 3 hr (21). Chlorophyll concentration was measured at preanthesis, anthesis and post-anthesis by a UV-1280 UV-visible spectrophotometer (Shimadzu Scientific Instruments, USA) at 645 and 663 nm, following the procedure (22). The Chl a/b ratio was calculated as described and the chlorophyll content (CC) was calculated by using the following Equations 1-3 (23):

$$\begin{array}{c} \text{Chlorophyll a} \\ \text{(mg/g fresh weight)} \end{array} = (12.7 \times A_{663} - 2.63 \times A_{645}) & \frac{V}{1000 \text{xw}} \\ \text{Eqn. 1} \\ \text{Chlorophyll b} \\ \text{(mg/g fresh weight)} \end{array} = (22.9 \times A_{645} - 4.48 \times A_{663}) & \frac{V}{1000 \text{xw}} \\ \text{Eqn. 2} \\ \text{Total Chlorophyll} \\ \text{(mg/g fresh weight)} \end{array} = (22.2 \times A_{645} - 8.02 \times A_{663}) & \frac{V}{1000 \text{xw}} \\ \text{Eqn. 3} \\ \end{array}$$

(A= absorbance at the given wavelength)

The CMS test under heat stress conditions was performed by standard procedure (24, 25). Three 10 cm long flag leaf samples were taken from the plants in each genotype. Two groups of 12 leaf discs were prepared from the selected leaf samples. These samples were rinsed with double-distilled water. Leaf discs of the first group were submerged in 10 mL of deionized water in test tubes as a control for precisely 24 hr at 25 °C in darkness, whereas leaf discs of the second group were placed in a test tube containing 10 mL double-distilled water. Then they were subjected to heat stress for 50 min in a water bath at 52 °C. After the treatment, 10 mL of deionized water was added to all tubes, which were then incubated overnight at 10 °C to allow diffusion of the electrolytes from the plant material to the water. Then, the conductance at 25 °C was recorded with a conductivity meter (DXF 200, TA instruments, New Castle, USA). Subsequently, the samples were autoclaved at 15 PSI

pressure for 20 min to cause complete electrolyte leakage and their conductance was re-recorded. The thermos stability expressed as relative injury was calculated by using the Equation 4 formula (26).

T is treatment, C is control and 1 and 2 refer to the first and second conductance readings before and after autoclaving. The CTD was measured at the pre-anthesis and post-anthesis stages using an IR2-S infrared turf thermometer (Turf-Tec International, USA). All outcomes were determined by subtracting the canopy temperature from the air temperature.

CMS or relative injury (%) =
$$1-[{1-(T1/T2)}/ {1-(C1/C2)}\times 100$$
 (Eqn. 4)

Analysis of yield parameters

The crops were harvested after reaching complete physiological maturity (13 % grain moisture content) measured through a moisture meter. The timely sowed (D^1) plants were harvested one month earlier, while the delayed ones were harvested fifteen days later (D^2) and thirty days later (D^3). The crops sown and harvested later (D^2 and D^3) were exposed to high-temperature stress (HTS). The daily high temperatures were > 40 °C at the time of harvest. The spikes were threshed by hand and other yield parameters were assessed. The grain number per spike (GN/spike) was estimated twice using an automatic seed counter (SC/0312/135, OSAW industrial product). Total grain yield per plot (GY/plot) was calculated from all three rows in grams.

Statistical analysis

The statistical analysis between the late sowing and the early sowing of the wheat genotypes was done using a factorial ANOVA on Windows's GraphPad Prism (version 5.00) platform. In each case, the *p-value* was statistically considered to be: *p <0.05(significant), ** *p <0.01(moderate significance) and *** *p <0.001 (highly significant). A Pearson correlation analysis determined the correlation between treatments (27).

Results and discussion

HTS influenced total chlorophyll content (CC), cell membrane stability (CMS) and canopy temperature depression (CTD)

Global warming has led to high temperatures, posing a serious plant threat. Photosynthesis is highly sensitive to high -temperature stress, which often inhibits before the inhibition of all other cellular functions (28, 29). As chlorophyll content is positively correlated with plants' photosynthetic potential, a reduction of total chlorophyll content leads to decreased photosynthesis capacity observed under different abiotic stress conditions (30, 31). In the present investigation, ten

different wheat genotypes were sown three times during the same cropping season for insight into the effect of hightemperature stress (HTS) on their phenological and physiological characteristics. The total chlorophyll content (CC), cell membrane stability (CMS) and canopy temperature depression (CTD) were considered as physiological parameters to assess the effect of HTS on wheat. In this study, we found that the highest CC was measured for the K910-30 genotype with an average value of 5.24 mg/g at the D1 sowing date, 4.93 mg/g at the D2 sowing date and 4.79 mg/g at the D3 sowing date. However, the lowest CC was recorded for the K-9162 genotype, where the average CC was measured as 3.0 mg/g at the D1 sowing date, 3.37 mg/g and 3.44 mg/g at the D2 and D3 sowing date, respectively (Table 3). A gradual decrease in CC was observed in all the genotypes with a gradual increase in the sowing date, where the average value of CC for all genotypes was measured as 4.24 ± 0.14 mg/g in the D¹ sowing date. It was reduced to 3.75 ± 0.33 mg/g and 3.48 ± 0.24 mg/g for the D2 and D3 sowing dates, respectively. Decreased chlorophyll content under heat stress is usually attributed to destroying chlorophyll synthetic enzymes and thylakoid membranes. In the present investigation, we also found that HTS had a significant effect on CC (p<0.05) (Table 3). The gradual reduction in CC was observed in this study and it might be due to the gradual increase in temperature with three sowing times. The decrease in CC might be attributed to photoinhibition, photo destruction of pigments and disruption of the thylakoid membrane (16, 32).

Simultaneously, cell membrane stability was also measured in all the genotypes under HTS. The biological membranes are highly ordered structures of lipid and protein complexes. High-temperature stress can directly change the properties of membranes, including their fluidity, through the modulation in the lipid composition and their respective interaction with proteins and other biomolecules. The maximum CMS was recorded for the K-910-30 genotype with a value of 52.3 % in D¹ sowing time, 51.0 % in D² sowing time and 48.7 % in D³ sowing time. However, the minimum cell membrane stability was measured in the AAI-12 genotype as 33 % in D1 sowing time and 30.0 % in both D2 and D3 sowing time (Table 3). However, a significant decrease was observed in the CMS in all the genotypes from the late to very late sown (D1 to D2) condition compared to the timely sown condition (Table 3). The results of the present study agree with a previous report that found a negative correlation between high-temperature stress and CMS in different plants, such as Basil and Cotton (33, 34).

Canopy temperature depression (CTD) is a promising trait for identifying plants' abiotic stress tolerance potential and is correlated with crop transpiration potential (35). The CTD is also influenced by several factors, including the capacity of the plants to extract water and the differences in transpiration under different phenological stages. In the present study, a significant difference in the mean CTD was recorded between all the sowing dates (D1, D2 and D3) at the pre-anthesis, anthesis and post-anthesis stage. The mean CTD at the pre-anthesis stage showed the lowest increase of 5.5-5.6 °C for the AAI-13 genotype, followed by 5.4-5.6 °C for the AII-11 genotype under early showing conditions (Table 4). However, the increment rate in mean CTD was significantly higher for the K-9162 genotype (3.2-5.6 °C) under the D1 lateshowing condition (Table 4). Simultaneously, CTD was also found to be significantly associated with grain yield (0.71-79) and test grain weight (TGW) (0.01-0.50) (Table 5).

The grain yield was correlated with TGW in the (D1, D2 and D³) sown condition. The minimum value of CTD was found for genotype K-910-30 (4.3 °C). At the anthesis stage, the differences in mean CTD were relatively close for the NW-1014, HUW-658, NW-4035, AAI-11, K-910-30 and K-911 genotypes across all sowings, ranging from 4.3-4.7 °C. This shows that at the anthesis stage, there is a positive correlation between ambient temperature and canopy temperature. The correlation between the mean CTD of genotype for each sowing was positive (r = 0.47). However, a negative correlation was noticed between ambient temperature and canopy temperature in the K-9162, AAI-16, AAI-13 and AAI-12 genotypes, which exhibited a maximum mean CTD at the early sowings of 5.2 °C (D1) and 4.5 °C for the later sowings (D1 and D²). The correlation between CTD and the mean days to anthesis for the genotypes was averaged across sowings and it was 0.82. A high CTD led to a higher yield in the various wheat genotypes; however, the CTD is less clear under water-limited environments. We hypothesized that a higher CTD would be associated with leaf conductance and relative greenness. At the post-anthesis stage, all genotypes had a significant drop in the mean CTD. The mean CTD in D1 (early showing) was 3.72 °C ±0.45. The minimum value of the mean CTD at each

Table 3. Comparisons of physiological traits among 10 different genotypes of wheat under HTS

| Constuns | CMS (%) | | | | CC (mg/g) | | | PY (kg) | | GN/Spike | | | |
|-------------|----------|----------------|----------------|------|-----------|------|------|----------------|------|----------|----------------|-------|--|
| Genotype | D¹ | D ² | D ³ | D¹ | D^2 | D³ | D¹ | \mathbf{D}^2 | D³ | D¹ | D ² | D_3 | |
| K-9162 | 40.7 | 38 | 36.7 | 3 | 3.37 | 3.4 | 1.8 | 0.8 | 1.5 | 61.7 | 58.9 | 56.3 | |
| NW-1014 | 45 | 44.7 | 43.3 | 4.17 | 4.3 | 4.5 | 1.8 | 1.1 | 1.5 | 63.1 | 54.4 | 51.9 | |
| HUW-658 | 37 | 34.7 | 33 | 4.07 | 3.7 | 3.9 | 1.4 | 1.2 | 1.3 | 51.1 | 46.4 | 42.8 | |
| NW-4035 | 41 | 40.7 | 41 | 4.5 | 4.3 | 4.3 | 1.3 | 1.4 | 1.3 | 58.9 | 53.2 | 49.4 | |
| AAI-16 | 37.7 | 36 | 36 | 3.73 | 3.4 | 3.2 | 1.6 | 1.13 | 1.7 | 51.3 | 46.5 | 43.5 | |
| AAI-13 | 36.3 | 33.7 | 36 | 3.4 | 3.4 | 3.2 | 1.46 | 1.2 | 1.43 | 58.7 | 53.4 | 50.8 | |
| AAI-12 | 33 | 30 | 30 | 3.2 | 3.24 | 3 | 1.2 | 0.63 | 1.2 | 64.2 | 58.6 | 55.4 | |
| AAI-11 | 35 | 31.7 | 33 | 3.2 | 3.18 | 3.2 | 1.6 | 1.5 | 1.7 | 51.3 | 50 | 44.7 | |
| K-910-30 | 52.3 | 51 | 48.7 | 5.24 | 4.93 | 4.79 | 2.03 | 1.7 | 1.7 | 63.5 | 58.4 | 56.2 | |
| K-911 | 47.3 | 44 | 44.7 | 4.53 | 4.6 | 4.6 | 2.94 | 2.77 | 2.37 | 53.8 | 48.9 | 45.3 | |
| F-test | S | S | S | S | S | S | S | S | S | S | S | S | |
| S. Ed(±) | 0.88 | 1.56 | 43.19 | 0.14 | 0.33 | 0.24 | 0.41 | 0.4 | 0.33 | 2.52 | 2.86 | 3.12 | |
| C.D. at 5 % | 1.86 | 3.29 | 90.74 | 0.29 | 0.7 | 0.5 | 0.86 | 0.84 | 0.68 | 5.3 | 6.01 | 6.56 | |

High temperature stress (HTS), Timely sowing (D1), Delayed sowing, (D2 and D3), Cell membrane stability (CMS), Chlorophyll content (CC), Plot yield after harvesting (PY), Number of grains per spike (GN/Spike), (F-test - Fisher test, S. Ed(±) standard error of differences, C.D. at 5 %- Critical difference at 5 %)

development stage was exhibited by the K-910-30 genotype for all sowings and the maximum value was shown by the AAI -13 genotype at all stage the pre-anthesis, anthesis and post-anthesis stages (Table 4). As CTD is a critical trait that indicates the canopy cooling capacity of the plants, it can explain the yield of different wheat genotypes under HTS. This implies that genotypes that achieve transpiration cooling even under HTS conditions might efficiently extract soil moisture through efficient root system architecture. Predicting yield in different wheat genotypes can be better when CTD is measured during the reproductive stage than at the early crop growth stages. Results of the present experiment indicated that in addition to staying green features, CTD could also be used as a key trait of leaves to screen and select the genotype with higher adaptability to HTS.

Grain yield

The grain yield of all the genotypes (except NW - 4035) under earlier sowing conditions was higher than that of their counterparts under late sowing. However, the grain yield was significantly varied between the late sowing conditions (D1 > D2 > D3) (Supplementary data 3). The observed changes in the grain yield might be due to the hotter conditions, as the mean temperature from the date of sowing to the date of harvesting was approximately 18 °C for the early sowing and 47 °C for the later sowing (D² and D³). The days until maturity in the early sowing were approximately 111, whereas the days until maturity were reduced to 106 and 94 days in the late sowings for D2 and D3, respectively. This acceleration in maturity significantly reduced the grain number and size; the same results were also observed for the 50.0 % heading stage (D1, 83> D2, 77> D3, 61 days) and the flowering period (D1, 86> D2, 81>D3, 64 days). The correlation between the traits was investigated further. Genotype and high-temperature stress (HTS) were both significantly correlated with grain yield (p<0.01) of the genotypes. K-910-30 genotype showed the highest mean grain yield (2 kg) and AAI-12 showed the lowest (1.2 kg) per experimental plot (Table 3). The comparison of the mean grain yield of all genotypes under different sowings periods showed that K-9162 produced 1 kg in the D2 late sowing condition, which was the highest decrease in grain yield as compared to the timely D¹ sowing at 1.8 kg (Table 3). Except

for the genotype NW- 4035, the earlier sowing of all other genotypes yielded more than the later sowings ($D^1 > D^2$ and D^3); the reduction in the yield might be attributed to the hightemperature stress, as the mean temperature between sowing and harvesting was 18 °C for D1 and 47 °C for D2 and D3 respectively for all the genotype. Simultaneously, all the related parameters, such as grain number and 1000 grain weight, were also significantly affected by HTS (Supplementary Table 4). The TGW ranged from 31 to 40.56 g, depending on genotype and planting time (p<0.05). The minimum grain weight was recorded in genotypes HUW-658 (31.85 g), AAI-11 (31.5 g) and K-911(31.2 g), However higher grain weight (31.2 g) was recorded in (40 g) in K-910-30. Moreover, the highest number of tillers was observed in K-910-30 and the lowest was in HUW-658. The present study found that the number of tillers was recorded by 10 to 11 % in D2 and D3 conditions. GN was contributed by the main stem as well as by the tillers. The GN in timely sowing, D1, was 57 grains per spike. However, it was reduced to 51 grains per spike in D2 and D3 sowings. Research indicates that exposure to high temperatures during the maturation stage leads to a reduction in GN. In the present study, the highest GNs were recorded in the three genotypes, AAI-12 ($D^1 = 64.2$, $D^2 = 58.6$ and $D^3 = 55.4$), K-910-30 $(D^1 = 63.5 D^2 = 58.6 \text{ and } D^3 = 55.4) \text{ and NW-1014} (D^1 = 58.9 D^2 =$ 53.2 and D^3 = 49.9) in all sowing condition. However, in all sowing conditions, HUW-658 produced the lowest GNs (D1 51.1, D2 46.4, D3 42.8).

The correlation between the traits

The results of the correlation analyses between the traits are illustrated (Table 5). The correlations between grain yield (GY) and canopy temperature depressions (CTD) were significant (r=0.71) for late sowing conditions for all the genotypes (Table 5). Moreover, there was a positive correlation between CC and HD (r=0.92), MD (r=0.929) and anthesis (r=0.90). CC showed a significant positive correlation with spike length, weight and grain numbers (r0.99). The results also showed a clear relationship between (TGW) and CC, HD, MD, FD, PH, SL and weight at both the early and late sowing stages. The cell membrane stability (CMS) was closely correlated with the plant height, flag leaf and spike, as well as length and weight (r>0.90). GY was negatively correlated with HD FD and TGW.

Table 4. Effect of HTS on pre anthesis canopy temperature depression CTD (°C) anthesis CTD and post anthesis CTD. Timely sowing D1 and late sowing (D2, D3) treatment under salinity stress

| Compleme | CTD (°C) | at Pre anthe | sis stage | CTD (°C | C) at anthes | is stage | CTD (°C) at Post anthesis stage | | | |
|-------------|----------|--------------|-----------|---------|--------------|----------|---------------------------------|-------|------|--|
| Genotype | D^1 | D^3 | D¹ | D^2 | D^3 | D^1 | D^2 | D^3 | | |
| K-9162 | 3.2 | 5.6 | 5.2 | 5.2 | 4.6 | 4.4 | 4.2 | 2.4 | 2.1 | |
| NW-1014 | 4.7 | 5.6 | 5.3 | 4.4 | 4.5 | 4.6 | 3.4 | 2.3 | 2.6 | |
| HUW-658 | 4.5 | 5.6 | 5.5 | 4.5 | 4.5 | 4.6 | 3.5 | 2.4 | 2.4 | |
| NW-4035 | 3.9 | 5.4 | 5.5 | 4.7 | 4.5 | 4.4 | 3.5 | 2.5 | 2.6 | |
| AAI-16 | 4.3 | 5.5 | 5.3 | 5.2 | 4.6 | 4.4 | 3.6 | 2.4 | 2.1 | |
| AAI-13 | 5.5 | 5.6 | 5.6 | 5.2 | 4.7 | 4.6 | 4.6 | 2.7 | 3.4 | |
| AAI-12 | 5.3 | 5.5 | 5 | 5.2 | 4.6 | 4.5 | 3.5 | 2.2 | 2.5 | |
| AAI-11 | 5.4 | 5.6 | 5.5 | 4.5 | 4.4 | 4.4 | 3.5 | 2.6 | 2.7 | |
| K-910-30 | 3.2 | 4.4 | 4.9 | 4.3 | 4.3 | 4.3 | 3.1 | 2.1 | 2 | |
| K-911 | 4.8 | 5.5 | 5.4 | 4.7 | 4.4 | 4.6 | 4.3 | 2.6 | 2.4 | |
| F-test | S | S | S | S | S | S | NS | S | S | |
| S. Ed(±) | 0.16 | 0.19 | 7.44 | 0.19 | 0.14 | 0.24 | 0.27 | 0.19 | 0.37 | |
| C.D. at 5 % | 0.34 | 0.4 | 15.62 | 0.4 | 0.3 | 0.5 | 0.56 | 0.4 | 0.78 | |

Each data value represents the mean ± S.E. of three different sowing. Bars with different letters indicate the level of statistical significance at P < 0.05

The genotypic correlations showed a positive correlation between the grain yield with HD (r=0.50) and GY (36, 37). It was suggested that the morphological traits could be an essential criterion for developing a high-yielding durum wheat variety.

Association between physiological and morphological traits with yield.

To illustrate the differences in values of traits between timely sowing (D1) and late sowings (D2 and D3) in terms of performance and physiological characteristics, the mean values of three replicas, as determined by the mean performance of the 10 bread wheat genotypes, are presented in (Table 5). The associations between yield and the measured physiological and morphological traits were statistically significant for all 10 genotypes, as determined by correlation analyses (Table 5). This table demonstrates a substantial decrease in yield and phenology under a hightemperature stress (HTS) condition. Heat stress consistently reduced the crop cycle length (days between emergence and physiological maturity). The yield was 15 % lower in the delayed sowings than in the timely one. The physiological data for chlorophyll content (CC) and cell membrane stability (CMS) indicated that reduction in CC and CMS was associated with reduced performance in terms of yield.

Conclusion

This assessment of the effect of high-temperature stress on various physiological characteristics of different wheat genotypes allows us to conclude that high-temperature stress (HTS) influences their physiological parameters. Most of these parameters decreased linearly with an increase in temperature. Chlorophyll content (CC) and cell membrane stability (CMS) were lower under HTS. The data also revealed that CMS and canopy temperature depressions (CTD) under HTS had positive correlations with TGW, which signifies that higher values of CMS and CC at the seedling stage might be considered a suitable indicator of genotypes performing better under HTS. K-910-30 was the most tolerant genotype toward HTS among all the genotypes investigated. However,

Table 5. Correlation coefficients between traits of wheat under HTS

HUW-658 was the least tolerant. Therefore, we concluded that physiological traits could be used to indirectly predict the best selection of crop genotypes. These traits will help to increase the yield and fulfil the mounting demand for wheat, ultimately providing food security in the coming decades.

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Authors' contributions

AS experimented and drafted the primary manuscript. PKS and SSP participated in the study design. SS, PM and RGB performed the statistical analysis. JREC drafted the manuscript. JRR, PWR and JB finalized the draft. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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| | cc | CMS | CTD | HD | MD | FD | PH | FLL | FLW | TS | SL | SW | GN/S | TGW | GY/PLOT |
|---------|------|------|------|-------|-------|--------|------|------|------|------|------|------|------|-------|---------|
| СС | 1 | | | | | | | | | | | | | | |
| CMS | 0.95 | 1 | | | | | | | | | | | | | |
| CTD | 0.91 | 0.99 | 1 | | | | | | | | | | | | |
| HD | 0.92 | 0.76 | 0.68 | 1 | | | | | | | | | | | |
| MD | 0.92 | 0.78 | 0.70 | 0.99 | 1 | | | | | | | | | | |
| FD | 0.90 | 0.73 | 0.64 | 0.99 | 0.99 | 1 | | | | | | | | | |
| PH | 0.99 | 0.91 | 0.86 | 0.95 | 0.96 | 0.94 | 1 | | | | | | | | |
| FLL | 0.91 | 0.99 | 1 | 0.68 | 0.70 | 0.64 | 0.86 | 1 | | | | | | | |
| FLW | 0.96 | 0.99 | 0.99 | 0.77 | 0.79 | 0.74 | 0.92 | 0.99 | 1 | | | | | | |
| Ts | 0.92 | 0.99 | 0.99 | 0.71 | 0.72 | 0.67 | 0.88 | 0.99 | 0.99 | 1 | | | | | |
| SL | 0.99 | 0.91 | 0.85 | 0.96 | 0.96 | 0.94 | 0.99 | 0.85 | 0.91 | 0.87 | 1 | | | | |
| SW | 0.99 | 0.91 | 0.86 | 0.95 | 0.96 | 0.94 | 0.99 | 0.86 | 0.92 | 0.87 | 0.99 | 1 | | | |
| GN/S | 0.99 | 0.94 | 0.90 | 0.93 | 0.93 | 0.91 | 0.99 | 0.90 | 0.95 | 0.91 | 0.99 | 0.99 | 1 | | |
| TGW | 0.77 | 0.55 | 0.45 | 0.95 | 0.95 | 0.97 | 0.84 | 0.45 | 0.57 | 0.48 | 0.84 | 0.84 | 0.79 | 1 | |
| GY/PLOT | 0.37 | 0.63 | 0.71 | -0.01 | 0.009 | -0.062 | 0.27 | 0.71 | 0.61 | 0.69 | 0.25 | 0.26 | 0.35 | -0.29 | 1 |

^{*}P< 0.05. CC: Chlorophyll content; CMS: Cell membrane stability; CTD: Canopy temperature depression; HD: Days of heading; MD: Days to maturity; FD: Days to flowering, PH: Plant height; FLL: Flag leaf length; FLW: Flag leaf width; TS: Tiller per meter square; SL: Spike length; SW: Spike weight; GN/S: Grain number per spike; TGW: Test grain weight; GY/ PLOT: Grain yield per plot

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