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Research Article

Ameliorating heat stressed conditions in wheat by altering its physiological and phenotypic traits associated with varying nitrogen levels

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Abstract

Currently, more than half of the global nations cultivating wheat crops are facing severe consequences of climate change and its associated heat stress in terms of quantitative and qualitative yield losses. Plants exposed to heat stress need a balanced and adequate amount of mineral nutrients to counter its ill effects. Therefore, the present study was designed to investigate the potential effects of heat stress applied during the vegetative growth period (Zadoks growth scale: ZGS 5-60) on physiological and phenotypic traits of wheat (*Triticum aestivum*) crop subjected to variable rates of nitrogen (N). In this experiment, wheat plants of cv. 'Punjab-2011' were exposed to two levels of temperature i.e. heat stress (HS) and control or non-heat stress (NHS), and three N rates (N_{50} = 50 kg ha^{-1}, N_{100}= 100 kg ha^{-1} and N_{150} = 150 kg ha^{-1}). The experiment was executed under controlled conditions in a completely randomized design (CRD) with six replications. One set of eighteen pots containing wheat seedlings was placed in a compartment of the greenhouse under heat-stressed conditions, while another set was placed in another compartment under non-heated conditions. The greenhouse compartments were equipped with a heating and cooling system to maintain desired ecological

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conditions. Pots in heated chamber were kept for 60 days from emergence (ZGS = 5-60), and then shifted to non-heated chamber till harvesting. The temperature in heat stress treatment was almost 2 ± 0.47 °C higher than in non-heated treatment. The results indicated that HS significantly reduced the photosynthetic rate by 42.52%, leaf photosynthetic efficiency by 56.82%, chlorophyll scores by 20.11%, relative water contents (RWC) by 12.81%, tillers by 48.21%, grain weight by 21.47% and grain yield by 68.20% relative to NHS conditions. These reductions were more prominent in plants subjected to a limited N dose rate (50 kg N ha^{-1}). Furthermore, the results also revealed higher transpiration rate, stomatal conductance, and membrane ruptures under HS with N_{50} treatment. However, N_{150} treatment compensated for the detrimental effects of HS on wheat plants by improving the photosynthetic rate and efficiencies, higher RWC, more stability of membrane and pigments, more tillers, and higher grain weight, and grain yield of wheat. Additionally, grain yield was negatively correlated with transpiration rate, stomatal conductance, internal CO_2 concentration, and membrane leakage. In conclusion, a high dose rate of N under high temperatures during vegetative growth could alleviate the magnitude of penalties to grain yield and enhance the potential of wheat crops to withstand heat-induced detrimental effects.

Keywords: heat stress; N rates; phenotypic traits; physiological traits; wheat

Introduction

During the last century, the mean global surface temperature has increased by 0.6 °C and a further increase of 1.4 to 5.8 °C is also anticipated by the end of current century. Pakistan is among the top ten countries which are vulnerable to extreme climatic events and global climate change (Kreft *et al.,* 2017). In Pakistan, an upsurge of approximately 0.57 °C in mean temperature had also been recorded from 1961-2014. Moreover, it is also projected that future temperatures will rise by 2.8/2.2 °C day/night temperatures at the end of 2069 especially in rice-wheat zones of Pakistan (Ahmad *et al.,* 2015; Nazir *et al.,* 2021). This rapid increase in temperature would be the biggest environmental challenge for future sustainable production of major cereal crops such as wheat, rice, barley and oat (Fu and Huang, 2003).

The rising temperature beyond threshold limit normally cause irreversible damage to crop plants particularly in terms of stunted growth and development which ultimately lead to significant yield reductions (Laghari *et al.,* 2010; Khosa *et al.,* 2022). It had been anticipated that 2-3 °C increment in mean global temperature would reduce the grain yield of wheat by 14-17% during mid-century period (2040-69) (Ahmed *et al.,* 2015). Heat stress is responsible for rapid cell destruction in wheat plants along with other significant damages such as reduced seed germination, stunted plant growth, sterility of pollen and abortion of plant ovaries (Mukhtar *et al.,* 2020; Ding *et al.,* 2021). Moreover, the growth-sensitive stages of wheat plant such as anthesis, grain filling, and reproduction are also prone to adverse effects of heat stress and rising temperature (Ahuja *et al.,* 2010; Mittler and Blumwald, 2010). Heat stress imposed at germination, seedling and grain filling stage is more detrimental for wheat crop as compared to rest of the growth stages (Munns *et al.,* 2006). It significantly affects the stability of vital cellular component such as proteins, membranes, lipids and nucleic acid (Asseng *et al.,* 2003; Zaman *et al.,* 2022) and also interrupts normal functioning of various physiological and biochemical processes (Hamam and Khaled, 2009; Ruelland and Zachowski, 2010; Fleitas *et al.,* 2020). Furthermore, heat stress could drastically mutilate plant photosynthetic activity, stomatal conductance, transpiration rate, and grain filling rate in wheat crop (Ruelland and Zachowski, 2010). The prime reason associated with disruption of these vital plant processes could be outburst of singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals which are major active oxygen species (AOS) producers under a wide range of abiotic stressed conditions (Yasmeen *et al.,* 2014). These AOS impose a significant detrimental impact on the membranous structures of crop plant (Shaukat *et al.,* 2021). Moreover, Asseng *et al.* (2003) evaluated the response of 30 wheat genotypes at varying temperatures ranging from 15 °C to 32 °C with the help of crop simulation models, and reported that wheat grain yield could reduce up to 28% and 55% respectively against an increase of 2 °C and 4 °C rise in temperature. The greater decline in wheat yield due to unequivocal rising of temperature is mainly attributed to the production of active oxygen species (AOS) (Farooq *et al.,* 2009).

Plants need adequate and proper supply of mineral nutrients for driving essential physiological processes and maintaining their structural stability (Hassan *et al.,* 2020). Nutrients are a vital components of different plant systems and play important role in coordination of several developmental and physiological processes in plants such as respiration, photosynthesis, root and shoot growth, seed germination and flower development (Khan *et al.,* 2023). Moreover, exogenous applications of certain minerals are also involved in improving heat tolerance in crop plant by regulating stomatal conductance and upregulation of metabolic and physiological processes (Khan *et al.,* 2023).

Among different nutrients, nitrogen (N) is an essential structural component of numerous organic compounds such as proteins, Rubisco, chlorophyll, nucleic acids as well as some phytohormones (Pessarakli, 2001). Moreover, N fertilization is also a vital agronomic strategy to enhance the productivity of crop plants (Ata-Ul-Karim *et al.,* 2016). An appropriate level of N is compulsory not only to regulate plant growth and development but also to cope wider range of environmental stresses (Zhu *et al.,* 2014; Boschma *et al.,* 2015). It also has the potential to ameliorate the negative impacts of different abiotic stresses by maintaining higher leaf water relation and membrane stability (Saneoka *et al.,* 2004; Zhang *et al.,* 2007). Previous studies have also indicated the improved heat tolerance of crop plants by exogenous supplementation of NH_4NO_3 due to their higher scavenging potential of antioxidants (Fu and Huang, 2003) and inhibition of lipid peroxidation (Zhang *et al.,* 2007).

Wheat (*Triticum aestivum* L.) is one of the most important cereal and staple food crop providing a significant proportion of nutrition to ever-burgeoning world population (Anjum *et al.,* 2005; Seleiman *et al.,* 2019; Taha *et al.,* 2021; Jones *et al.,* 2023). In Pakistan, it is also the most widely cultivated and consumed cereal crop. The optimum sowing time of wheat in Pakistan is month of November (Afzal *et al.,* 2011), but due to current global climatic variations, a shift in its sowing time had also been noticed (Ahmad *et al.,* 2015). Moreover, five years historic weather data showed significant fluctuations in temperature even in the month of November and December. Extreme weather events, though ephemeral, have potential to cause shifts in the structure of plant communities and affects their growth and yield (Wang *et al.,* 2008). In certain cases, the rapid and sudden climatic events happening during critical growth stages of a plant might inflict more damage to crops than seasonal ecological changes (Karl *et al.,* 1997). Keeping in view the global climatic variations, it can be anticipated that in near future wheat crop might face the consequences of heat stress even in month of December.

Previously no study has explored the effect of heat stress imposed at vegetative stage of wheat plants on their gas-exchange parameters, foliar chlorophyll (*chl*) and water contents, membrane stability index (MSI) and grain yield. It was hypothesized that N addition could ameliorate the negative effects of heat stress by improving photosynthetic rate and efficiency, leaf *chl* and water contents, membrane stability, grain yield and related attributes of wheat plants. Hence, the current study was organized with aims to examine the detrimental effects of vegetatively imposed heat stress (ZGS 5-60) on gas-exchange measurements, tolerance traits, yield and related traits of wheat under variable doses of N.

Materials and Methods

Study site

The current experiment was conducted at Climate Change Studies and Glasshouse Laboratory, operated by Department of Agronomy, University of Agriculture, Faisalabad (31.4400° N, 73.0748° E). The soil used in this controlled experiment was collected at 0-15 cm depth from agronomic research area. The soil type was silt loam (coarse-silty, mixed, hyperthermic Typic Calciargids) in the layallpur soil series. The collected soil was well-drained, and the fraction of clay, silt and sand in the top 10 cm of the soil were 10, 56, and 34%, respectively. Other parameters in the soil profile were: bulk density, 1.24 g cm^{-3}; organic carbon content, 0.53 g kg^{-1}; total nitrogen (N) content, 4.0 g kg^{-1} and pH, 8.3.

Experimental setup

Thirty-six concrete pots (height: 24 cm, top diameter: 25 cm, bottom diameter: 17 cm) were used in the experiment which were filled with 6 kg of sieved soils (2 mm mesh). Further, two sets of eighteen pots were made: one set placed in a chamber/partitioning of greenhouse indicated as heated chamber and second set in another chamber indicated as controlled or non-heated chamber. The pots in both sets were arranged in completely randomized design (CRD) with factorial arrangements. Pots in heated chamber were kept for 60 days from emergence (Zadoks growth scale – ZGS = 5-60), then shifted to non-heated chamber till harvesting. Three varying rates of N i.e., N_{50} = 50, N_{100} = 100 and N_{150} = 150 kg N ha^{-1} were randomly applied to both sets of pots. The greenhouse designated as heated was equipped with auto-heating system. Temperature data were recorded four times per day (9:00 am, 12:00 noon, 3:00 pm and 5:00 pm) with digital thermometer (Beurer Thermo Hygrometer, HM-16) in the heated chamber pots at four different points. The temperature in heat stress treatment was almost 2 ± 0.47 °C higher than non-heated treatment (Figure 1).

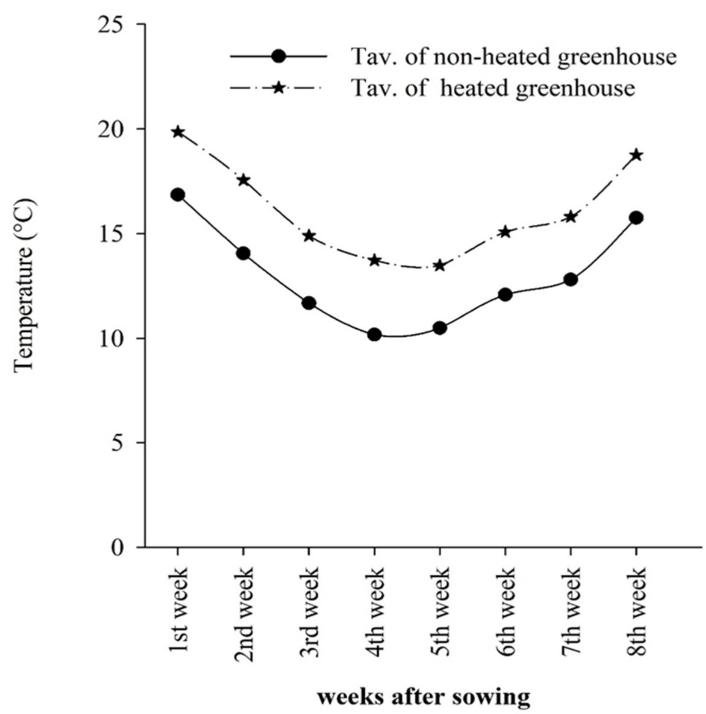

Figure 1. Average temperature variations from emergence to 60 days under heated and non-heated greenhouse conditions

Crop husbandry

Before filling the collected sieved soils in each pot, all fertilizers including nitrogen (50, 100 and 150 kg ha^{-1}), potassium (60 kg ha^{-1}) and phosphorus (80 kg ha^{-1}) were thoroughly mixed with potting soil. The seeds of wheat cv. 'Punjab-2011' were purchased from Punjab Seed Corporation with seed purity and germination percentages over 95%. All the pots were sprinkled irrigated with appropriate quantity of water to attain optimum moisture for sowing. The dry seeds of wheat cv. 'Punjab-2011' were sown on 20th November, 2018. After seedling emergence, a total of four healthy vigorous plants were maintained in each pot. Weeding was done manually at maximum tillering stage (Zadoks growth scale – ZGS = 24–27) (Talukder *et al.,* 2014). Irrigations were scheduled as; first irrigation at crown root initiation (25 days after sowing; DAS), second irrigation at jointing (45 DAS), third irrigation at booting stage (80 DAS), fourth irrigation at heading (110 DAS) and final irrigation at milking stage (130 DAS) of the crop. At maturity (155 DAS), plants were harvested and threshed manually.

Plant measurements

Allometric traits

At maturity, productive tillers per plant were counted and averaged for each pot. All plants in each pot were harvested and weighed to measure the biological biomass of each treatment. For grains per spike, five representative spikes were threshed, and grains were counted and averaged. Afterwards, all remaining plants were threshed manually, and grain yield (gm) per pot was measured. A sample of thousand grains was taken, and 1000-grain weight was measured.

Physiological traits

Physiological performance, plant pigments, and stress tolerance indicators i.e., membrane integrity and relative water contents (RWC) were assessed using photosynthetic gas-exchange, foliar chlorophyll content, foliar membrane leakage and water saturation. Gas exchange and chlorophyll measurements were made at 30, 45, 60, 75 and 90 DAS on the most recently expanded and matured leaf of representative plants in each pot. Gas exchange traits including leaf net photosynthetic rate (Pn), transpiration rate (T), leaf photosynthetic efficiency (Pn/T), Leaf CO_2 concentration (Ci) and stomatal conductance (gs) were recorded using an LCi T photosynthesis system (ADC BioScientific Ltd. UK), between 10:00-14:00 local time at light 1000-1500 μmol m^2s^{-1} PAR, 410 ppm CO_2, leaf temperatures of 20-30 °C and humidity in both chambers ranged between 45-60%. Moreover, chlorophyll scores were measured with chlorophyll tester (CI-202). During each observation day, four sequential measurements of gas-exchange traits were made at 30-second intervals at the same plant's leaf, while chlorophyll scores were recorded from random locations on each measured leaf. Later, mean of four measurements were taken.

Furthermore, membrane integrity and RWC were recorded by destructive sampling of flag leaves of two plants at 100 DAS. Membrane permeability was determined in term of electrolyte leakage using a method proposed by Blum and Ebercon (1981). In this method, six segments of leaves with equal size were immersed in distilled water for 12 h. After this, the measurements of electrical conductivity (EC_1) of the solution were made using EC meter. The samples were shifted to autoclave with water for 01 h at 50 °C. Next, the samples were cooled down at room temperature for 2 h, and conductivity of killed tissues (EC_2) was measured. Membrane permeability was calculated as the ratio between EC_1 and EC_2. For RWC, fresh leaves were weighed to get fresh weight (W_f). Later, these leaves were soaked in water for 4 h to record saturated weight (W_s). Leaves were then dried in an oven at 70 °C for 24 h to record dry weight (W_d). RWC was calculated as:

$$\mathrm{RWC} = \frac{(W_f)\ (W_d)}{(W_s)\ (W_d)} \times 100$$

Statistical analysis

The experiment was carried out in a completely randomized design (CRD), and every treatment's combination was replicated six times. A two-way analysis of variance (for HS and N fertilizer) following least significant difference (LSD) test was applied to compare the mean values of each measured trait with the help of Statistix 8.1 software. The statistical significance among treatments were based on a probability level of 5% ($P \leq 0.05$). Further, pearson correlation co-efficient was also estimated to assess the strength of relationship among recorded traits of wheat. Visualization of data was aided by SigmaPlot (Systat Software, San Jose, CA).

Results

Gas exchange measurements

The result of the experiment revealed that net photosynthetic rate (Pn), transpiration rate (T), stomatal conductance (Gs), internal CO_2 concentration (Ci) and leaf photosynthetic efficiency were significantly ($P \leq 0.05$) influenced by heat stress (HS) and N fertilization (Figure 2 a-e). Heat stress imposed a general decrease in Pn, and this decrease was more pronounced in plants subjected to 50 kg N ha^{-1} (N_{50} treatment). However, a statistically similar Pn values were recorded from plants receiving 100 kg N ha^{-1} (N_{100}) and 150 kg N ha^{-1} (N_{150}). In general, HS reduced Pn by 42.52% in plants grown under all N treatments relative to non-heated plants under same N treatments (Figure 2a). Interestingly, T, Gs and Ci were more pronounced in plants receiving N treatments placed in HS condition, but this increase in T, Gs and Ci was less pronounced under N^{150} treatment (Figure 2b-d). HS imposed a decreasing trend in leaf photosynthetic efficiency (E) of plants grown in N_{150} to N_{50} treatments compared with plants of non-heat stress and N treatments. Generally, HS reduced E by 56.82% relative to non-heated plants under N fertilization (Figure 2e).

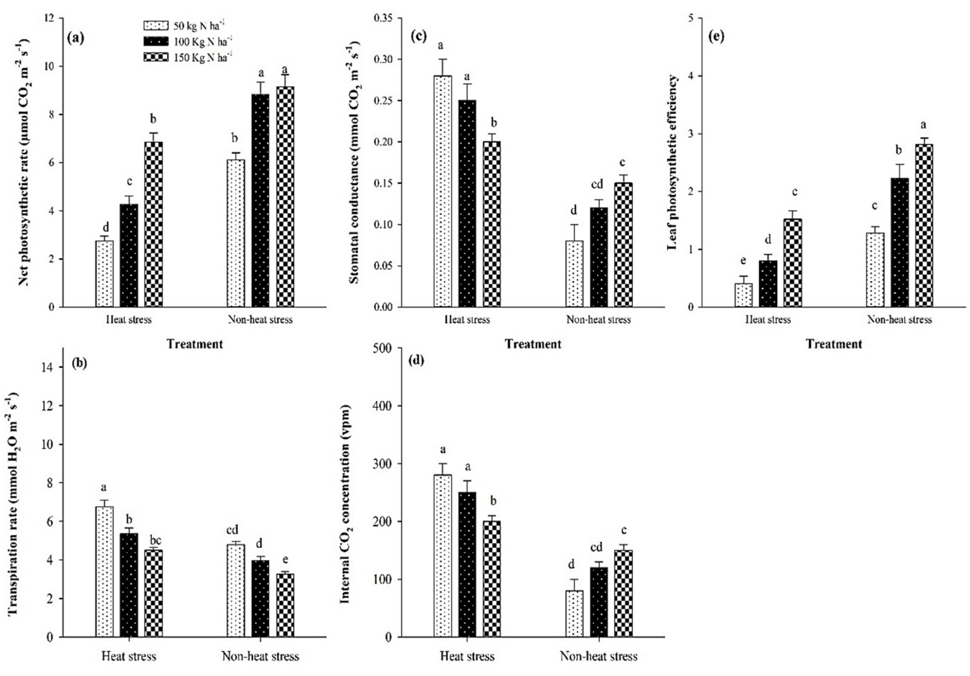

Figure 2. Effect of heat stress on (a) net photosynthetic rate (μmol CO2 m^{-2} s^{-1}), (b) Transpiration rate (mmol H_2O m^{-2} s^{-1}), (c) stomatal conductance (mmol CO_2 m^{-2} s^{-1}), (d) internal CO_2 concentration (vpm) and (e) leaf photosynthetic efficiency of wheat under different N treatments

Bars bearing different lower-case letters are significantly different at $P \leq 0.05$. Vertical bars indicate SD values.

Stress tolerance traits (chlorophyll score, membrane leakage and relative water content)

The chlorophyll score, membrane leakage and relative water content (RWC) also differed significantly ($P \leq 0.05$) in heated and non-heated plants at various N treatments (Figure 3a-c). Maximum value of chlorophyll score (0.94) was measured from N_{150} under non-heat stress condition, and it was statistically similar with plants grown at N_{150} and N_{100} treatments under heated and non-heated conditions respectively (Figure 3a). Generally, HS reduced chlorophyll scores by 20.11% relative to the controlled condition. Furthermore, similar trends were also found with RWC, and in this case HS reduced leaf RWC by 12.81% compared to the non-heated plants (Figure 3b). More leakage of membrane was observed from N_{50} treatment under HS condition, however, high N rates resulted comparatively lower leakage under both HS and NHS conditions (Figure 3c).

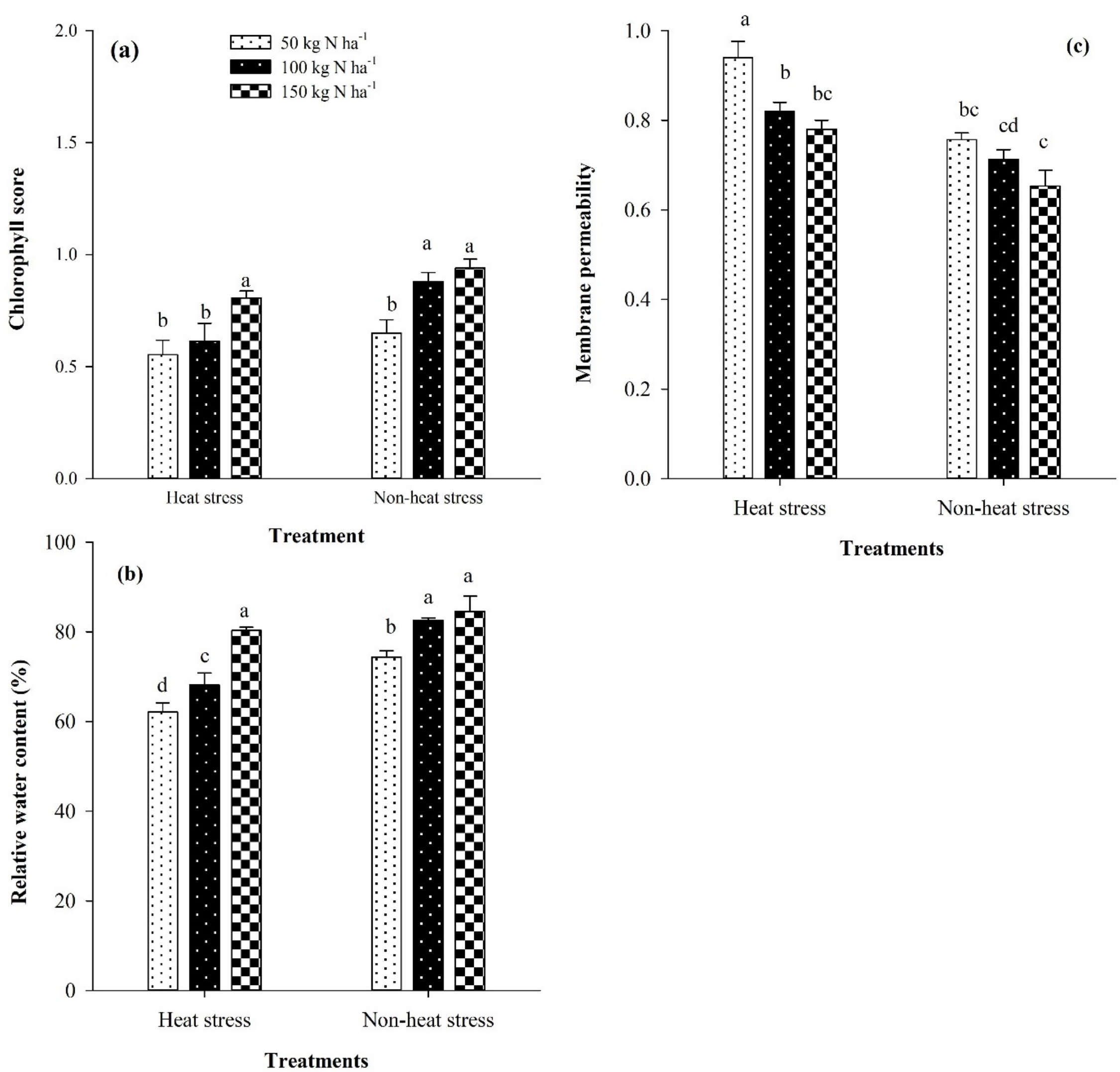

Figure 3. Effect of heat stress on (a) chlorophyll score, (b) relative water content (%) and (c) membrane leakage of wheat under different N treatments

Bars bearing different lower-case letters are significantly different at $P \leq 0.05$. Vertical bars indicate SD values.

Allometric traits

Heat stress and N fertilization significantly ($P \leq 0.05$) affected plant height and productive tillers of wheat crop (Figure 4a-b). Plants with reduced height were recorded from all N treatments under heat stress condition. In general, HS reduced plant height by 13.38% relative to non-heat stressed conditions (Figure 4a). Additionally, HS resulted in pronounced reduction in tillers under all N rates in comparison with that from plants grown under non-heat stress at various N treatments. Similarly, HS reduced productive tillers by 48.21% under all N treatments relative to the non-heated plants grown under same N doses (Figure 4b).

Similarly, grains per spike and 1000-grain weights also demonstrated significant ($P \leq 0.05$) difference under heat and all N treatments (Figure 5a-b). Heat stress resulted in pronounced decline in number of grains at N_{50} and N_{100} treatments, however, plants with N_{150} treatment produced similar number of grains as found in N_{100} treatment under normal condition. Heat stress in general, imposed a reduction in number of grains by 17.84% relative to the plants grown under normal condition (Figure 5a). Similarly, HS also reduced the grain weight by 21.47% relative to normal condition, and this reduction in grain weight was more pronounced in plants subjected to N_{50} and N_{100} treatments (Figure 5b).

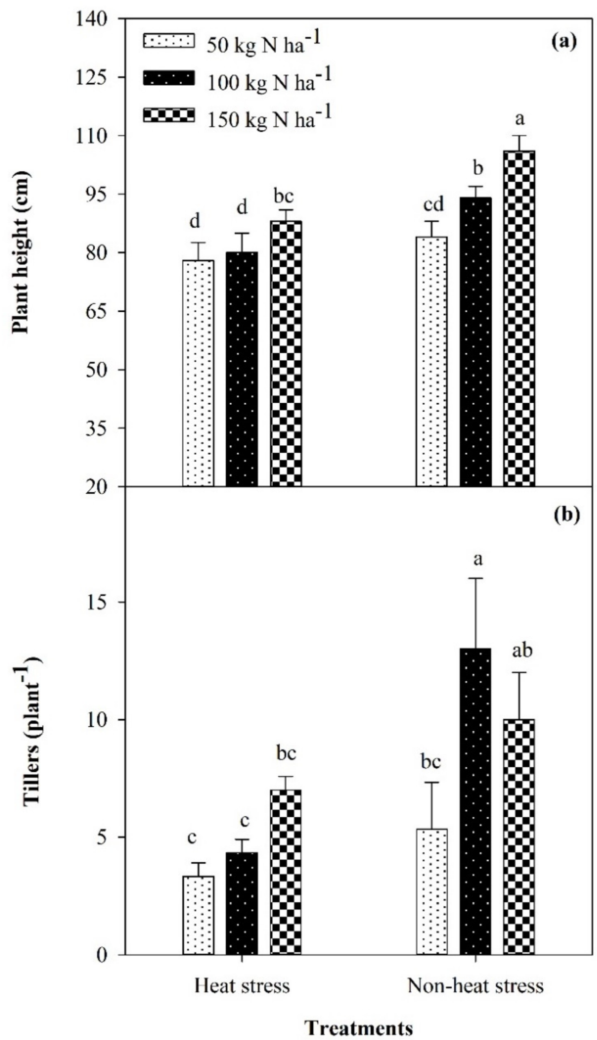

Figure 4. Effect of heat stress on (a)plant height and (b) productive tiller palnt-1 of wheat under different N treatments

Bars bearing different lower-case letters are significantly different at $P \leq 0.05$. Vertical bars indicate SD values.

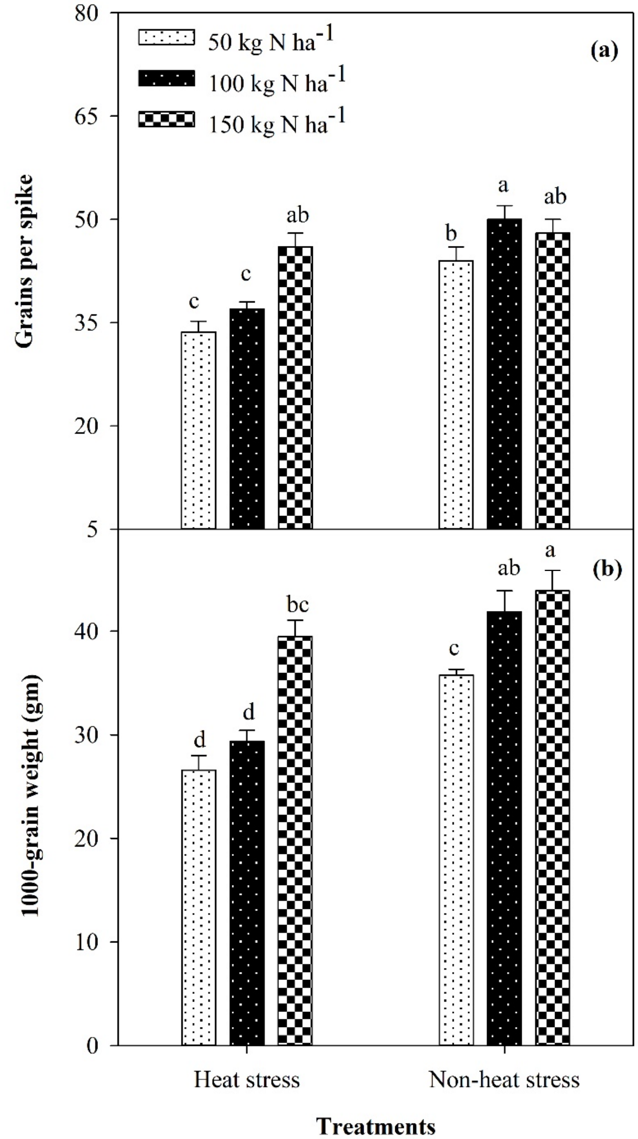

Figure 5. Effect of heat stress on (a) grains per spike and (b) 1000-grain weight of wheat under different N treatments

Bars bearing different lower-case letters are significantly different at $P \leq 0.05$. Vertical bars indicate SD values.

Moreover, HS and N fertilization had a significant ($P \leq 0.05$) effect on grain yield and harvest index (HI) of wheat crop (Figure 6a-b). Maximum grain yield was noted from plant subjected to N_{150} treatment followed by N_{100} treatment under normal condition. However, HS reduced grain yield by 68.20% under all N treatments relative to normal condition, and this decline in grain yield was more pronounced in N_{50} and N_{100} treatments (Figure 6a). Further, HS also reduced the HI by 23.42% relative to normal conditions (Figure 6b).

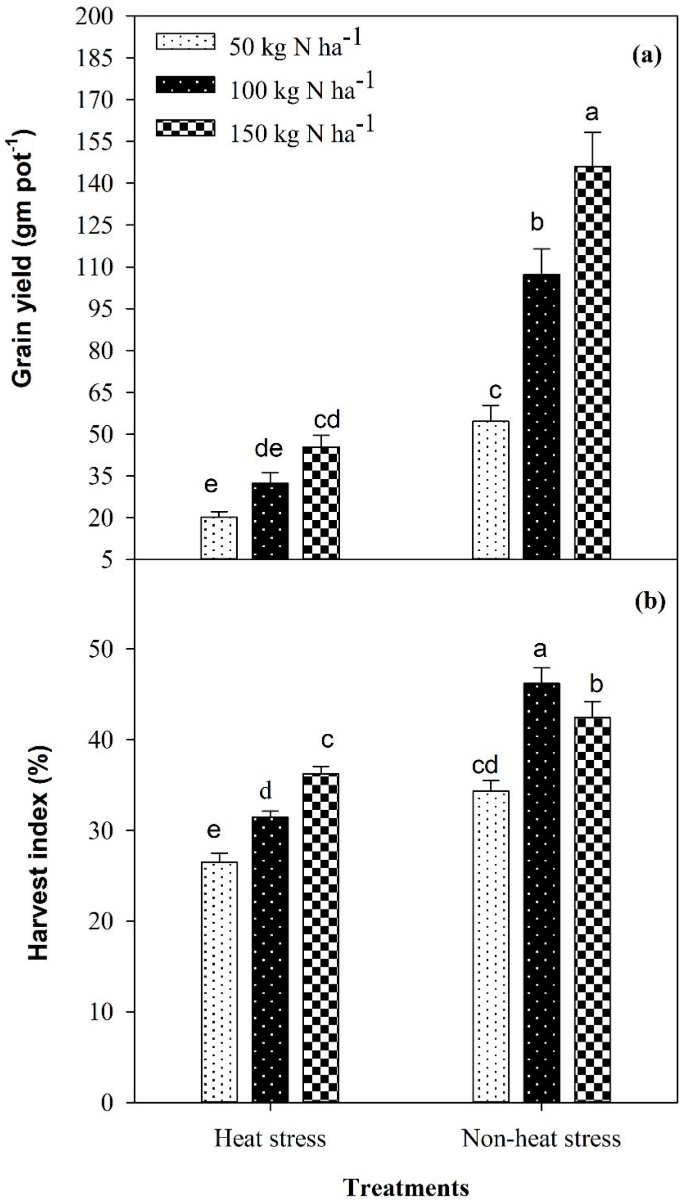

Figure 6. Effect of heat stress on (a) grain yield and (b) harvest index of wheat under various N treatments
Bars bearing different lower-case letters are significantly different at $P \leq 0.05$. Vertical bars indicate SD values.

Pearson's correlation (r)

Grain yield of wheat was significantly ($P \leq 0.05$) but negatively correlated with transpiration rate, stomatal conductance, internal CO_2 concentration and membrane leakage. However, grain yield was linearly and positively correlated with grains per spike, 1000-grain weight, net leaf photosynthetic rate, foliar water and chlorophyll contents as the values r ranged from 0.79 to 0.89 (Table 1). Similar negative relationships of grains per spike, 1000-grain weight and net photosynthetic rate were found with transpiration rate, stomatal conductance, internal CO_2 concentration and membrane leakage. In this case, the value of r ranged from -0.70 to -0.94. However, grains per spike, 1000-grain weight and net photosynthetic rate were linearly and positively correlated with relative water content and chlorophyll concentration. Furthermore, stomatal conductance and transpiration rate were linearly and positively correlated with membrane permeability (Table 1).

Table 1. Pearson correlation coefficient (r) among characters recorded during this study

Character	Character									
	GY	**GPS**	**TGW**	**Pn**	**T**	**Gs**	**Ci**	**MP**	**RWC**	**Chl**
GY	1.00									
GPS	0.79**	1.00								
TGW	0.82**	0.91**	1.00							
Pn	0.89**	0.95**	0.96**	1.00						
T	-0.88**	-0.91**	-0.88**	-0.94**	1.00					
gs	-0.58*	-0.77**	-0.69**	-0.70**	0.75**	1.00				
Ci	-0.58*	-0.77**	-0.69**	-0.70**	0.75**	1.00**	1.00			
MP	-0.88**	-0.90**	-0.86**	-0.92**	0.99**	0.75**	0.75**	1.00		
RWC	0.83**	0.96**	0.94**	0.96**	-0.94**	-0.67**	-0.67**	-0.92**	1.00	
Chl	0.87**	0.90**	0.87**	0.92**	-0.88**	-0.50*	-0.50*	-0.86**	0.95**	1.00

GY, grain yield; GPS, grain per spike; TGW, thousand-grain weight; Pn, net photosynthetic rate; T, transpiration rate; gs, stomatal conductance; Ci, internal CO_2 concentration; MP, membrane permeability; RWC, relative water content; Chl, chlorophyll contents

*Statistically significant at $P \leq 0.05$, **Statistically significant at $P \leq 0.01$

Discussion

Climate change is emerging as a significant threat to the productivity of major field crops, particularly altering the sowing periods of major cereal crops. Keeping in view the ongoing changes in global climate, it is imperative to accelerate efforts to mitigate the harmful impacts of high temperatures on crop growth (Kumar *et al.,* 2013; Asseng *et al.,* 2015). The rising global average temperature poses a serious threat to wheat production (Lobell *et al.,* 2008; Ortiz *et al.,* 2008). Pakistan, due to its geographical position, is particularly susceptible to the effects of climate change as compared to other Asian countries (Ahmad *et al.,* 2015; Poudel and Poudel, 2020; Rehman *et al.,* 2021; Ullah *et al.,* 2022). It is predicted that in future there would be a change in sowing time of wheat crop due to climate shifts, thus, planting wheat in early November might lead to potential early exposure to heat stress. Generally, wheat develop various physiological mechanisms to address harvest-end heat stress. These mechanisms encompass early maturity (Joshi *et al.,* 2007; Mondal *et al.,* 2013), lowered canopy temperature and substantial biomass accumulation (Pinto and Reynolds, 2015), along with production of higher stem water-soluble carbohydrates, which facilitates the conversion of assimilates into yield (Blum *et al.,* 1994). Elevated temperatures induce various changes in the physiological, biological, and biochemical processes in wheat (Asseng *et al.,* 2015). In wheat, HS leads to inferior seed germination, shortened grain-filling duration, reduced grain numbers, inactivation of the Rubisco enzyme, lowered photosynthetic capacity, reduced rate of assimilate translocation, premature leaf senescence, decreased chlorophyll content, and ultimately results in reduced yield (Hossain *et al.,* 2013; Kumar *et al.,* 2016; Pandey *et al.,* 2019). Therefore, current study focused on assessing the impact of heat stress induction during vegetative stage of wheat crop (ZGS 5-60) by maintaining a temperature that is 2 ± 0. 47 °C higher than untreated control (Figure 1).

Photosynthesis stands out as the paramount physiological process in plants, and its efficiency is significantly impacted by higher temperatures. It is reported that within wheat, the sensitivity to heat stress is most pronounced in the stroma and thylakoid lamellae (Mathur *et al.,* 2014). The translocation of photosynthetic products to various plant parts is essential for growth and development. However, under HS, the rate of assimilate translocation from source to sink is hindered due to a decrease in membrane stability, disturbing the photosynthetic rate due to accelerated degradative processes (Ruelland and Zachowski, 2010; Farooq *et al.,* 2011; Zlatev and Lidon, 2012). In our study, HS reduced the net accumulation of photosynthates by 42.52% compared to the non-heated conditions at various N treatments (Figure 2a). When net photosynthesis is inhibited, the most of photosynthetic reserves are consumed in continuous respiration (Abid *et al.,* 2016). This study also reported the higher levels of transpiration rate and stomatal conductance in plants under heated conditions (Figure 2b-c), possibly linked with more rapture of cell membrane under HS (Figure 3c). Despite the reduction of nitrogen levels under HS (Ordóñez *et al.,* 2015), study reported that the application of N posed beneficial effects on plants subjected to high temperatures (Wang *et al.,* 2008). In this study, improvements in net photosynthate accumulation and leaf photosynthetic efficiency by supplying higher rate of N were observed (Figure 2a-c). Previous studies showed that the limited N availability is linked with the degradation of *chl* contents (Gonzalez-Real and Baille, 2000; Makino, 2011; Abid *et al.,* 2016), which is demonstrated in present work with lower *chl* scores under heated conditions, particularly when subjected to lower concentration of Nitrogen (Figure 3a). Elevated temperatures (above 34°C) expedite leaf senescence by reducing chlorophyll biosynthesis. In contrast, chlorophyll fluorescence is closely linked to yield and serves as a measure of photosynthetic efficiency. Therefore, chlorophyll fluorescence can be valuable indicators for selecting heat-tolerant genotypes (Pandey *et al.,* 2019).

High temperatures also impact the water dynamics and content in plants, leading to cell dehydration HS due to a decrease in osmotic potential (Ahmad *et al.,* 2010). In present investigation, lower concentrations of N resulted in reduced water contents in plants' leaves and accelerated the degradation of plasma-membrane (Figure 3b-c). Therefore, high internal CO_2 concentration due to high stomatal conductance could not be beneficial for increasing photosynthates production in plants under heated environment. Nevertheless, a prominent increase in *chl* scores, RWC and membrane stability was measured by increasing amount of N nutrient (Figure 3a-c). Nitrogen being a vital constituent of Chl, proteins and Rubisco (Pessarakli, 2001), has a potential to affect whole plant metabolism under HS environments (Ordonez *et al.,* 2015). Therefore, in our study, supplying of higher amount of N over recommended dose has enhanced the thermostability of membrane, more *Chl* scores, photosynthetic rate and RWC. Grassi and Magnani (2005) also reported that adequate supply of N under abiotic stress environments could enhance the final output of crops by stabilizing photosynthetic machineries.

In wheat, high temperature stress adversely impacts both seed germination and plant establishment (Hossain *et al.,* 2013). Higher temperature up to 45 °C negatively affects embryonic cells, causing suboptimal germination and emergence, resulting in a weakened crop stand (Essemine *et al.,* 2010). The influence of high temperatures influences the survival of productive tillers, contributing to a decline in overall yield and experiences a substantial decrease of 53.57 and 15.38 per cent in both grain yield and tiller number, respectively (Din *et al.,* 2010). Additionally, heat stress induces a reduction in root growth, further impacting overall crop production (Huang *et al.,* 2012). In this study, heat stress has resulted in substandard crop vigor, short-heighted plants, reduced tillers, less grains and grain weight as well as lower grain yield of wheat. Previously, Laghari *et al.* (2010) also reported that heat stress during early vegetative growth of wheat (ZGS 5-60) severely influenced the tillering capacity of wheat plants. Under HS, this study also recorded reduced plant height and tillers in wheat by 10.7 and 48.240 per cent, respectively, as compared to control group. Further, it was noted that this reduction in plant height and tillers was comparatively more in plants treated with lower rates of N (Figure 4a-b). However, increasing the availability of N under heat stress conditions has improved the productive tillers by 18-28%. Thus, increase in N supply could alleviate heat-induced damages as were observed under N50 treatment. The earlier study of Wu *et al.* (2011) indicated that stress imposed at vegetative growth periods affected tillering potential and hampered final crop productivity under low nitrogen supply treatments. In the present study, the plants with high N supply exhibited their potential to produce a greater number of grains and grains weight (Figure 5a-b) by improving photosynthetic activities under stressful environment. Further, a significant reduction in grain yield by 68.21% was observed under heat stress compared to control conditions across all N treatments (Figure 6a). Increased N levels led to a reduction in the extent yield loss. It was observed that, decline in crop yield under heat stress during vegetative stage (ZGS 5-60), was attributed to the simultaneous impacts on grain number and grain weight either directly or indirectly. Results of this study showing significant reduction in wheat yield under HS were in line with previous study of Ordonez *et al.* (2015). Moreover, the reduction in grain yield might be associated with reduced photosynthetic rate (Mittler and Blumwald, 2010), less *chl* scores (Ahuja *et al.,* 2010), reduced tiller numbers, limited grains per spike and decreased 1000-grains weight (Wang *et al.,* 2008). In HS treatments, majority of the tillers underwent mortality during the later stages of wheat, resulting in a decrease in the net number of tillers per plant with ears at maturity.

In wheat, during grain filling, extreme temperatures can cause a loss in grain yield of up to 23 per cent (Mason *et al.,* 2010). HS adversely impact both quality and quantity of wheat grains, causing decrease in grain number, and ultimately leads to a reduction in the harvest index (Lukac *et al.,* 2012; Lizana and Calderini, 2013). Wheat productivity undergoes significant reduction due to the detrimental effects of high temperatures during the growth process, even for a shorter period of time (Janjua *et al.,* 2010; Sharma *et al.,* 2016). In this study, the data from the Harvest index (Figure 6b) indicate that, under HS treatment and limited supply of nitrogen (N_{50}), the reserves were primarily utilized for the maintenance of growth and development. Consequently, harsh environmental conditions during vegetative stage led to reduced morphological traits, physiological activities, grain yields and related traits. In contrast, The N_{150} treatment under heated treatment demonstrated significantly higher grain yield compared to N_{50} and N_{100} treatments. The improved physiological and yield traits of wheat under heat stress with higher N supply suggest that the detrimental effects of heat stress imposed at vegetative growth can be ameliorated through Nitrogen increments, presenting a cost-effective strategy for farming community.

Conclusions

The present study revealed that the negative effects of heat stress during vegetative growth phase of wheat crop was counteracted by increasing the counteracted by increasing field applications of nitrogenous fertilizers. This study suggested that sufficient N supply under HS enhances rate and efficiency of photosynthesis, chlorophyll scores, membrane stability and water content, while simultaneously minimizing the down-regulation of photosynthetic processes and yield losses. Observations indicated that increased internal CO_2 concentration and stomatal conductance under heat stress did not confer benefits to the final productivity of crop. Results showed that, higher stomatal conductance under heat stress has resulted in an increase in internal CO_2 and transpiration rate. Further, grain yield of wheat was negatively correlated with transpiration rate, stomatal conductance, internal CO_2 concentration and membrane rupture. Hence, in addition to morphological, physiological and agronomic studies, molecular investigations are imperative to comprehend the underlying processes driven by Nitrogen that enhance wheat's potential to alleviate the detrimental effects of heat stress.

Authors' Contributions

Conceptualization; M.S., S.Q.M., Z.M. and A.A.; Data curation; Z.M., A.H., A.Z.G., M.S.H., S.F.L., K.R. and S.Q.M.; Formal analysis; A.A., A.H. and Z.M.; Funding acquisition; A.A., A.Z.G., M.S.H. and S.F.L.; Investigation; S.Q.M. and M.S.; Methodology; M.S., K.R.; and Z.M.; Project administration; M.S. and K.R.; Resources; S.Q.M.; Software; A.A. and A.H.; Supervision; M.S.; Validation; S.Q.M., A.Z.G., M.S.H. and S.F.L.; Visualization; A.A. and M.S.; Writing - original draft; M.S., A.A., A.Z.G., M.S.H., S.F.L. and A.H.; Writing - review and editing: K.R., A.Z.G., M.S.H., S.F.L. and Z.M. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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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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