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RESEARCH ARTICLE

# Physiological and biochemical effect of elevated night temperature stress on bread wheat (*Triticum aestivum* L.)

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ABSTRACT

A laboratory experimentation was done in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University to evaluate elevated night temperature effect in wheat (*Triticum aestivum* L.) to see the response in some antioxidant and biochemical parameters in two wheat cultivars viz. HUW-234 (comparatively tolerant to heat stress) and sonalika (comparatively susceptible to heat stress). A significance difference was recorded in the parameters pertaining to antioxidant system includes catalase, SOD and APX activity were found to increase substantially when the plants kept under stress conditions (25$^0$C/24$^0$C day and night temperature) than the plants kept under controlled condition (25$^0$C/15$^0$C day and night temperature). The percent increase in the antioxidant enzyme activity was found to be more in the tolerant genotype (HUW-234) than in the susceptible genotype (Sonalika). There was 23.52 % increase was reported in the catalase activity by tolerant genotype when kept under stress conditions than those kept under control. Significant reductions were reported in most of the photosynthetic and membrane parameters *viz*., chlorophyll 'a', chlorophyll 'b' and membrane thermostability index. Similar percent reduction was reported with respect to biochemical parameters (total protein content, starch content and soluble sugar content) in the susceptible variety kept under high night temperature regime. Based on the pooled data of two-year experimentation it can be concluded that variety HUW-234 was found to be more tolerant than variety sonalika to HNT stress.

**Keywords:** High night temperature; Wheat; Catalase activity; Anti-oxidant system

## INTRODUCTION

A most important stable and popular crop grown worldwide among the farmers is Wheat (*Triticum aestivum* L.) and it is a livelihood for millions of farmers and an important dietary requirement in almost every household. Among the major cereals, globally, the area and production of wheat is highest than the other cereal crops and contributes more calories and protein requirement of human diet. Among the abiotic stresses subjected to several crops, high temperature stress is limiting yield in wheat crop grown across different agro-climatic zones in the world (Akhter et al., 2017). The loss of 1.5 per cent of India's GDP due to climate change was recorded during the recent past (Thakkar, 2017). Rice and wheat both are subjected to yield reduction about of 6-10 per cent by 2030. (IPCC AR 5, 2014; Dujeshwer et al., 2021). An estimate says that the global mean surface air temperature will rise by 1.8$^0$C to 5.8$^0$C by the completion of century (IPCC, 2013). Further, it is also estimated that future climate would be increased in frequency of episodes including high temperature with mean surface temperature (Easterling, 1997). Now a days, high temperature frequency of 30-40$^0$C is very common that leads to yield reduction about 50 per cent or more (Wardlaw, 1970). Most of the wheat growing areas are subjected to elevated temperature stress regime during the reproductive phase of crop. Reproductive phase is more prone to high temperature and can cause serious damage specially during anthesis or just before anthesis. Asymmetric rise in global temperature is experienced while rising daily minimum temperature much on higher pace than daily maximum temperature (Pareek et al.,2020).

Less radiant heat loss causes faster rate of increase in night temperature than increase in day temperature. Future climate simulation models forsee greater rate of night

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temperature increases than the day temperature (Mall et al., 2021). There is significant impact of high night time temperature regime on physiological, bio-chemical, growth and yield parameters in wheat and the effect of HNT stress in wheat is not studied so far (Tewari, 1998). Some cases of increase in night temperature have been already documented *e.g.*, International Rice Research Institute (IRRI) reported a substantial increase of 1.13$^0$C in night temperature over a period of 25 years (1979-2003) in the Philippines. Likewise, an increased rate of HNT about 0.18$^0$C per decades over a period of 45 years (1950-1995) in Libya. The global warming causing episodes of heat stress in multiple crops causing yield reduction and crop quality deterioration. (Al Khatib and Paulson, 1990). Several studies highlighted the impact of long- and short-term impact of high temperature stresses on crop yield and quality. However, most of these studies assumes no difference in day versus night temperature influence in crops. (Niu and Xiang, 2018).

HNT results in a number of detrimental effects on crop physiology e.g., reduces crop yield by restricting photosynthetic efficiency, sugar, starch content and increased respiration rate, suppressing development of floral bud, resulting in a greater number of pollen variability, male sterility etc. hastening crop maturity (Al-Khatib and Paulson, 1984). Another effect of HNT stress causing reduction in antioxidant capacity in plants that contributes to final yield reduction. Enzymatic and non-enzymatic antioxidants are capable of minimizing toxic effect of ROS under normal physiological condition. (Ahmad and Prakash, 2018). Stress condition staggering the antioxidant level resulted in cell damage. At conclusion, a series of physiological processes are affected due to heat stress and resulted in yield reduction in plants.

In future, increase in global warming could be beneficial for wheat productivity to a certain degree as suggested by experts. But it can cause negative impact on productivity where the optimum temperature is already surpassed. For instance, by 2050, a probable climate shift can be observed in the Indo Gangetic Plains of India by 2050, that are popularly known for its favourable, low rainfall and higher wheat productivity potential mega environment contributes about 15% of world production with 51% of area might be convert into heat stressed, irrigated and short season mega environment (Keeling, 1994). Such climate shift in the region can cause significant yield reduction and will draw the dependencies on heat tolerant varieties and change in crop management practices to a large extent by the farmers. To ensure the food security, it is necessary to mitigate climate change effect in wheat crop by crop management interventions like heat tolerant cultivars suitable under conservation agriculture (Ferris, 1998).

A thorough analysis on previous temperature data trend depicts the diurnal asymmetry in maximum and minimum temperature (Praveen et al. 2022). Several investigations resulted that grain weight reduction due to HNT stress in wheat and Milling quality of grain at grain filling stage is more sensitive to temperature anomalies in several agro-ecological regions. (Prasad et al., 2005; Gracia et al., 2015).

High and low temperature events generally cause physiological and biochemical dysfunctions and metabolic disorders, resulting in a loss of yield and a degradation of grain quality (Coast at al., 2015). Previous findings report on such effects in a variety of crop plants, including rice (Shi et al., 2016), wheat (Lobell et al., 2007), tomato (Li et al., 2015), strawberry, and lettuce. In last few years, many research works have paid emphasis on the effects of the night temperature on different crops (Zhou et al., 2018; Gaiotti et al., 2018; Tombesi et al., 2019). It is observed by significant number of researchers that low and HNTs caused a greater percent reduction in the biomass with differing patterns. For instance, Jing et al. (2016) observed that the effects of a HNT regime were mainly related to percent biomass allocation and distribution to seeds, whereas the effects of a low night temperature condition were more connected with the total biomass. The results from Loka and Oosterhuis (2010) reflected that two high temperature conditions (27 °C and 30 °C) caused a greater increase in the respiration rate (by 49% and 56% respectively), compared to the control (24 °C).

In addition, climate model forecasts that there will be relatively higher increase in night time temperature than day time temperature. The data pertaining to last century global daily minimum temperature rapidly increased more than twice in comparison to increase in daily maximum temperature. Many research outcomes have proven the fact that the historic yield of rice and wheat was strongly correlated to minimum (night time) temperature as compared to daytime temperature. (Mohammed and Tarpley, 2009). In view of the above fact, the present research work was undertaken with the objective to assess the impact of HNT stress on physio-biochemical, antioxidant system and productivity of bread wheat genotypes.

## MATERIALS AND METHODS

Two-disease free wheat genotypes namely, HUW-234 and sonalika were procured from the Department of Genetics and Plant Breeding, I. Ag. sc., BHU, Varanasi. The research was conducted in two-part one comprising pot-based experiment in the polyhouse facility of Horticulturist unit the institute and another in the laboratory experiment in the Department of Plant Physiology, Institute of Agricultural Science, BHU (25.2568 °N, 82.9868 °E) Varanasi, India (Fig. 1) during rabi season of 2016 and 2017. The trial completed in the first year was validated and replicated in subsequent year as well. The seeds were sown in pots of 15 cm diameter. The pots were kept in the growth chambers (3m x 3m) at optimum temperature ($25^{0}$C/$15^{0}$C day and night) till ear-head emergence. After ear-head emergence, plants under treatment were subjected to HNT (high night temperature) stress ($25^{0}$C/$24^{0}$C). Different physiological parameters *viz*., antioxidant enzymatic activity (Catalase, APX and Super Oxide Dismutase), biochemical (Protein, Total Soluble Sugar and Starch content), photosynthetic and membrane parameters (chlorophyll 'a', chlorophyll 'b') and MSI were estimated as per the procedure below:

Estimation of catalase: was done in leaf samples at 60 DAE, 75 DAE and 90 DAE (to study temporal variation) in normal and stressed plants as per protocol of Aebi et al. (1983). The enzyme activity was expressed as per formula given below:

$$\text{Enzyme units} = \frac{\delta A_{230/min} \times 1000}{43.6\ \text{mL reaction mixture}}$$

Estimation of ascorbate peroxidase: Quantification of APX enzyme was done as per the protocol of Nakano and Asada (1981). Enzyme activity of ascorbate peroxidase was quantified in terms of amount of ascorbic acid oxidized (initial absorbance – final absorbance = quantity of ascorbic acid oxidized) per minute per mg protein.

Estimation of superoxide dismutase (SOD): SOD activity was measured as per the the protocol proposed by Dhindsa et al. (1981). Enzyme unit (EU) calculation was quantified as per the formula given below:

EU= {Absorbance without enzyme in light- (Absorbance with enzyme in light- Absorbance in Dark)}/Absorbance without enzyme in light/2

The EU was expressed on per g fresh weight basis as well as on the basis of per mg protein (specific activity) in the plant sample.

Estimation of total protein content: Quantification of Total protein content was done as per method suggested by Bradford (1976). The amount of total soluble protein was expressed in mg/g of sample.

Estimation of soluble sugar content: Estimation of soluble Sugar Content was done as per the protocol suggested by Somogyi et al. (1952). Amount of reducing sugar calculated using glucose being the standard.

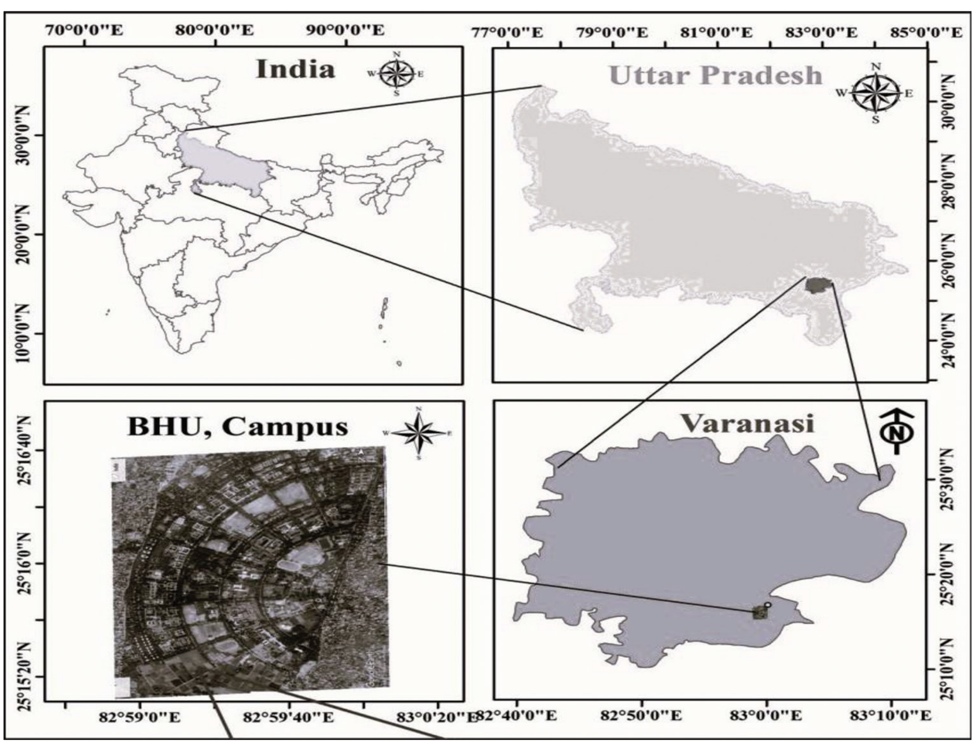


Fig 1. Location of the experiment.

**Fig 1a.** Wheat crop exposed in elevated night temperature under controlled condition during the experimentation.

Estimation of starch: Starch content in leaves was determined at 60, 75 and 90 DAE under all the treatments by following anthrone method put forwarded by Dubois et al. (1956). The total amount of the starch in the given samples was quantified by standard curve as detailed in sugar estimation and expressed in terms of glucose.

Estimation of chlorophyll: Chlorophyll 'a' and 'b' content in leaves was estimated by following the method of Arnon (1949).

The Chlorophyll content was estimated by the formulae as given below.

Chlorophyll 'a' Content =

$$\left(12.7X\ A663-2.69\ \ X\ A645\right)X\frac{V}{1000XW}$$

Chlorophyll 'b' content =

$$\left(22.9X\ A645-4.68\,X\ \ A663\right)X\frac{V}{1000XW}$$

Membrane stability index (MSI):MSI was estimated in leaf samples as per the protocol suggested by Sairam et al. (1994).MSI was calculated as follows:

$$\text{MSI} = [1 - (C1/C2)] \times 100$$

### Data analysis

Data were analysed using SPSS v26.0. The experimental design used for the experiment was Factorial completely randomized design (FCRD). Least significant difference (LSD) of 5 per cent was accepted for significance to compare the treatments means. The date presented are pooled of two-year of experimentation.

## RESULTS AND DISCUSSION

### (i) Effect of HNT on superoxide dismutase activity (EU mg$^{-1}$ min$^{-1}$):

Antioxidant system of a plant is a unique defence mechanism to counter any deleterious effect of abiotic stresses on its yield performance. A number of antioxidant enzymes and compounds are activated in response to a particular stress which reduces the threat by detoxification, destruction and reduction in the generation of tree radials and maintenance of cell redox potential to avoid any cellular damage to crop plants. Among the various antioxidant enzymes being tested, SOD was estimated both under control and stress condition to both the varieties in both the years, which revealed that the variety HUW-234 reported higher i.e., 6.87% increase in the SOD activity under stress condition over control (Fig. 2). The results reflects that the SOD activity in the variety HUW-234 was more responsive to HNT stress than the variety sonalika therefore HUW-234 is expected to perform better under HNT stress and resulted in quick upregulation of antioxidant enzyme system. The activity of enzyme SOD was reported in both the variety but % increase was much less in the variety sonalika in comparison to HUW 234, it can be inferred from above result that the antioxidant defence system of variety HUW 234 was activated quickly than sonalika under HNT stress (Nakano and Asada, 1981).

### (ii) Effect of HNT on Catalase (EU g$^{-1}$ DW min$^{-1}$)

Catalase was also reported similar with that of SOD with higher percent increase being reported in HUW-234 than in sonalika (Fig. 3). There was 22.85% increase in the Catalase activity in the variety HUW-234 under HNT stress over control. The cultivar sonalika also recorded increasing catalase activity, but percent increase was lesser than reported in the cultivar HUW-234. Similar data findings were also reported by Haider et al. (2021).

### (iii) Effect of HNT on ascorbate peroxidise (EU g$^{-1}$ DW min$^{-1}$)

Unlike SOD and catalase, the activity of APX was reported to be higher in the variety sonalika than in HUW-234. HUW-234 also reported increased APX activity in response to imposition of HNT stress but the increase was less than the variety sonalika (Fig. 4). Other biochemical parameters traits such as soluble sugar and starch content showed similar trend. HUW 234 also reported in APX activity in response to imposition of HNT but the increase was less than sonalika. This outcome was also justified by the results of Nakano and Asada (1981).

### (iv) Effect of HNT on membrane thermostability index (MSI)

It is one of the critical physiological parameters which reflects the biological status of membrane permeability,

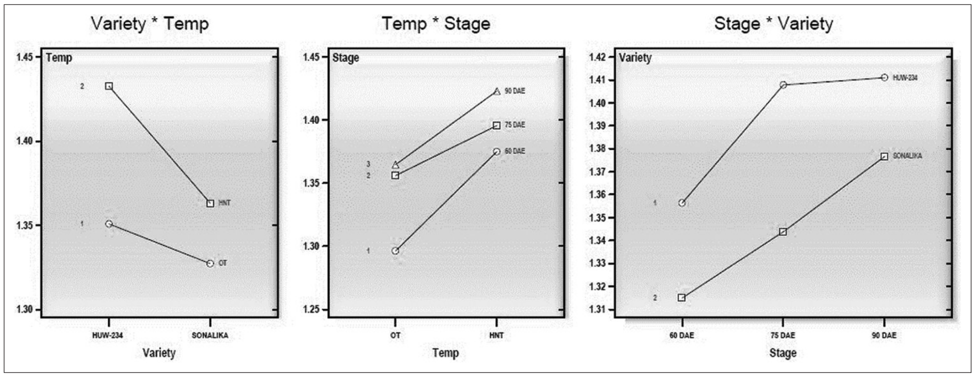


**Fig 2.** Effect of elevated night temperature stress on antioxidant enzymes- Superoxide Dismutase Activity (EU $mg^{-1}$ DW $min^{-1}$).

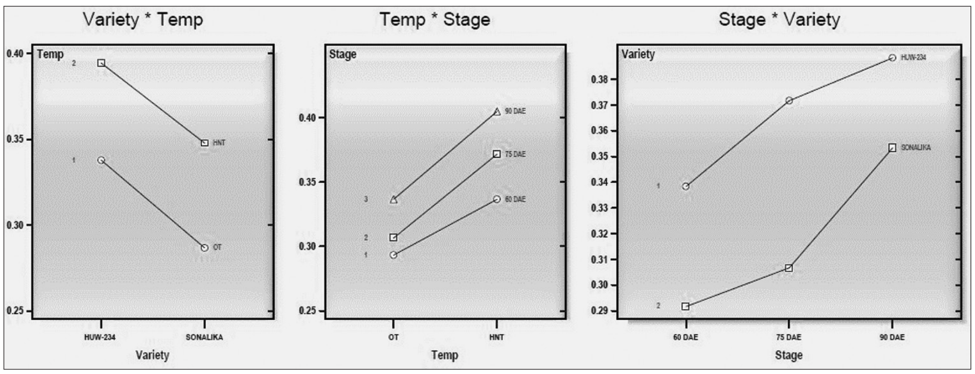


**Fig 3.** Effect of elevated night temperature stress on antioxidant enzymes- Catalase Activity (EU $g^{-1}$ DW per min).

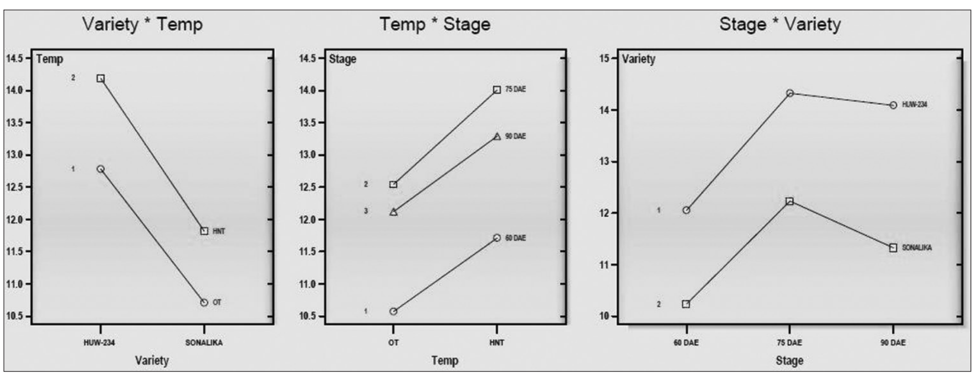


**Fig 4.** Impact of elevated night temperature stress on antioxidant enzymes- Ascorbate Peroxidase Activity (EU per g DW $min^{-1}$).

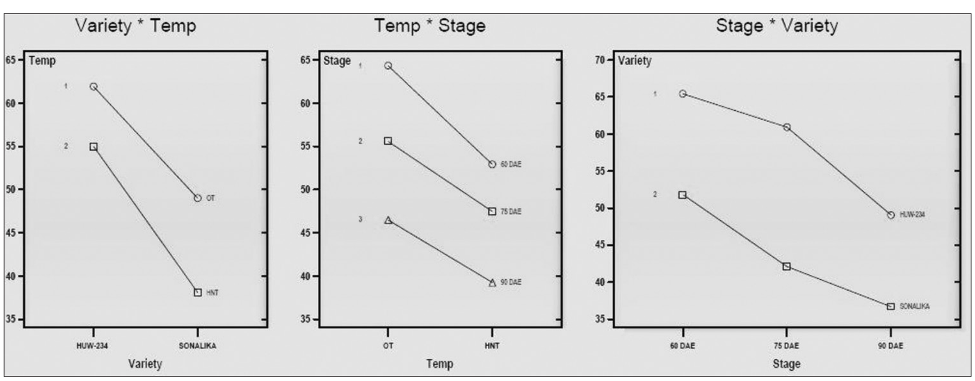


**Fig 5.** Effect of elevated night temperature stress on antioxidant enzymes- Membrane Thermostability Index.

membrane selectivity and extent of membrane leakage. The variety sonalika showed higher percent decrease in MSI over control which reflects that sonalika is more prone to membrane leakage under HNT stress than variety HUW-234 (Fig. 5). The physiological reason for sonalika showing more susceptibility to HNT with reference to lower value of MSI is that sonalika being traditionally grown variety and susceptible to membrane damage, membrane leakage under HNT stress. The finding was supported by the work of Krause and Weiss (1984).

### (v) Chlorophyll a and b content (mg $g^{-1}$ FW)

Significant reduction in chlorophyll 'a', and 'b' content was reported in the variety Sonalika which were subjected to elevated night temperature condition over control (Fig. 6). The reason behind sonalika showing lower value of chlorophyll pigment because the photosynthetic pigment of sonalika is highly prone to variation in temperature regime specially in marginal shift in night temperature. Relatable results were reported by Tiwari and Tripathi (1998) and Mamrutha et al. (2020).

### (vi) Total leaf protein content, soluble sugar and starch content (mg $g^{-1}$ DW)

Leaf protein content, total soluble sugar and total starch content biochemical parameters and shown in fig. 7. With respect to biochemical parameters traits, total protein content is an important reflection of the status of nitrogen metabolism in the crop which ultimately has a bearing on the nutritive quality of the grains. The variety sonalika reported 15.79 % decrease in the total protein content under HNT regime over control. HUW-234 also reported almost related percent reduction in protein content but variety sonalika was more susceptible to HNT stress than HUW-234 on total protein content (Fig. 7). For soluble sugar content, unlike in the protein content, variety HUW-234 reported higher percent decrease than the variety sonalika in both the experimental years. There was 17.19% reduction in the sugar content in the variety HUW-234 under HNT stress than the variety sonalika (Fig. 8). The data relating to starch content revealed that the sonalika reported more percent reduction in the starch content than HUW-234 which is in conformity with the result of total protein content and less grain filling duration being available to sonalika under stress condition (Fig. 9). Less total protein content in sonalika in comparison to HUW 234 because of nitrogen metabolism in sonalika seams to more prone to HNT stress than nitrogen metabolism operating in HUW 234. Moreover, protein synthesis is a manifestation of nitrogen metabolism and amino acid synthesis which seems to impaired in the variety sonalika, whereas, HUW 234 could coup-up with the increase in HNT regime. Similar finding was reported by Porter and Gawith (1999).

## High night temperature effect on enzymatic activity

Antioxidant system of both the wheat variety was found to be activated in response to HNT stress. There was 22.85% increase in the Catalase activity in the variety HUW- 234 under HNT stress over control. The variety sonalika also recorded increased catalase activity but percent increase was lesser than the variety HUW- 234. The variety Sonalika showed greater percent increase in the APX activity at 90 DAE over control as compared to HUW- 234. HUW- 234 also reported increase in the APX activity in response to imposition of HNT stress. The variety HUW-234 reported higher *viz*.6.87% increase in the SOD enzyme activity under stress condition than what was observed in control. The variety sonalika also reported increase in SOD activity but percent increase was comparatively lesser than that reported in the variety HUW-234. SOD activity was reported to be maximum at 60 DAE in both the wheat varieties. Under the high temperature stress, ROS and antioxidant enzyme activity aggravates. High temperature stress decreases all above enzymatic activity compared to OT. The recent results points to the fact that enzymatic activity gets reduced by ENT stress, aggravates more formation of ROS, SOD and other functional enzymes (Djanaguiraman et al., 2018).

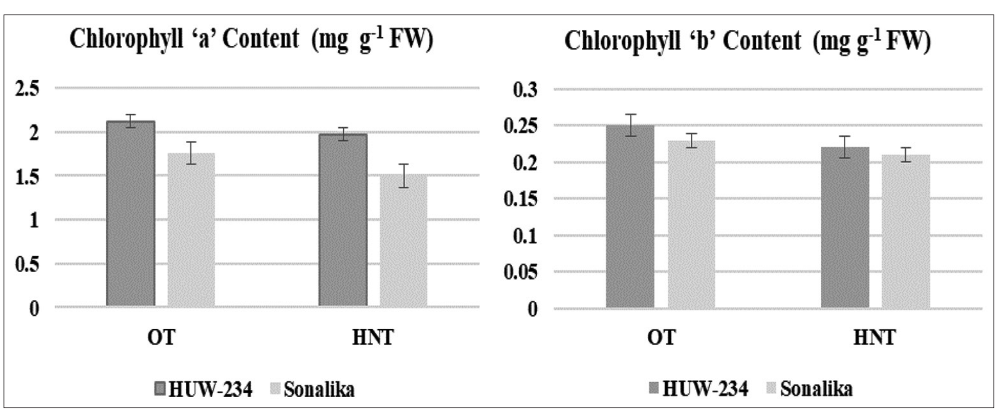


Fig 6. Effect of elevated night temperature on chlorophyll a & b content (mg $g^{-1}$ FW).

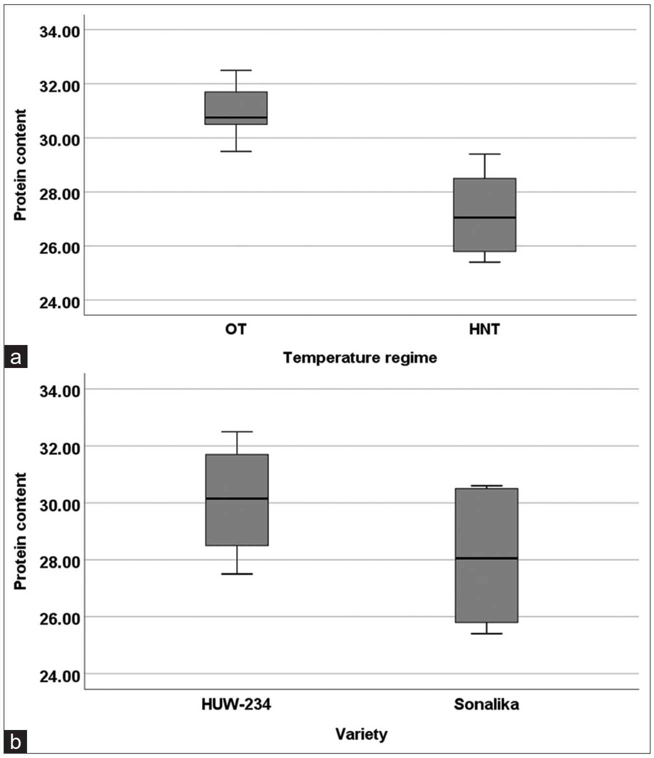


Fig 7. Effect of elevated night temperature on total leaf protein content (%). Box plot for protein content (a) Protein content by temperature (b) Protein content by variety

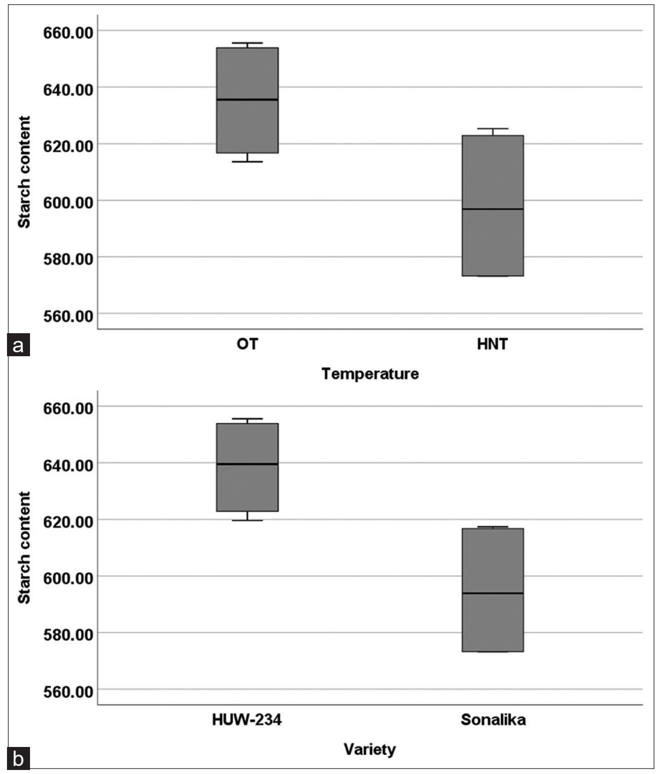


Fig 9. Effect of elevated night temperature on starch content (mg $g^{-1}$ DW). Box plot for starch content (a) starch content by temperature (b) starch content by variety

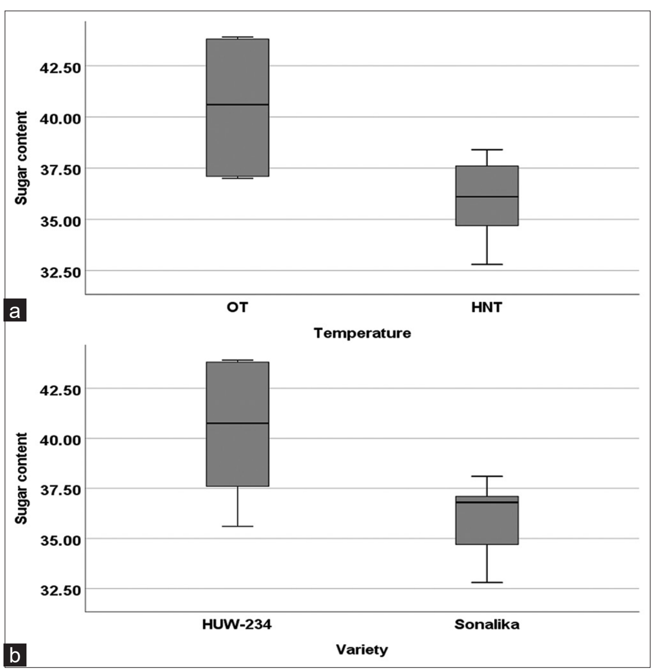


Fig 8. Effect of elevated night temperature on sugar content (%). Box plot for soluble sugar content (a) sugar content by temperature, (b) sugar content by variety

## High night temperature effect on biochemical content

The leaf protein content of both the varieties showed significant reduction with variety sonalika showing 15.79% decrease over control. Whereas, HUW- 234 reported only 9.25 % decrease in the protein content under ENT regime over control. HUW- 234 reported high percent decrease in the total soluble sugar content than the variety sonalika under stress condition in both the years. The variety sonalika reported lesser percent decrease in the sugar content than the variety HUW- 234. 6.96% decrease in the starch content was reported in the variety sonalika under HNT regime over control. HUW- 234 also reported similar reduction in the starch content in the both the experimental years under HNT condition. Under elevated temperature and drought stress condition enzymatic changes involved in the metabolic conversion of sucrose to starch reduced starch accumulation and reduced time required for the process of grain filling in wheat, eventually led to decreased starch content and grain yield. (Lu et al., 2019; Hein et al. 2020).

## High night temperature effect on photosynthetic activity and membrane thermostability index

The value regarding chlorophyll a content measured at 60, 75 and 90 DAE reflected that there was about maximum reduction in chlorophyll 'a' content in variety sonalika at 75 DAE under HNT regime. Similar observations were also recorded in the second year with maximum reduction at 75 DAE in the variety sonalika under stress conditions. The chlorophyll 'b' values also reported similar changes as recorded in chlorophyll 'a' but with some variation observed at 60 DAE. Both the varieties showed significant reduction in MSI with variety sonalika showing 19.39% decrease at 75 DAE over control. Similar data were obtained in the second year as well. An increased temperature during growth is directly correlated to decrease in photosynthetic rate by both stomatal and non-stomatal causes resulted in decreased chlorophyll content. (Jumrani et al., 2017). Chlorophyll is basically located in the thylakoid membranes, where it produces complexes with the proteins of PSII and PSI and excessive damage to thylakoid membranes under HNT stress may lead to loss of chlorophyll pigment. (Prasad et al., 2008; Chen et al., 2017).

## CONCLUSION

Wheat is the most important stable cereal crops in the world and it is produced and consumed by more than 60 % of world population like any other crop weed is also subjected to various kind of abiotic stresses among which HNT stress is the most emerging abiotic stress threats threatening the yield potential of many wheat genotype across Indian plains. Most of the work relating to temperature stress in wheat crop is being carried out for day temperature regime, however, elevated night temperature has now been proved to be a critical factor restricting the yield performance of wheat crop. Hence, this work has analysed the impact of HNT stress on key physiological, biochemical and antioxidant enzyme system of wheat crop where significant alteration has been observed in total protein content, soluble sugar content and starch content of wheat genotype (Sonalika and HUW-234) which were subjected to HNT stress about $24^{0}$C as compared to control ($15^{0}$C), similarly, greater loss in chlorophyll content was observed in plants exposed to HNT stress condition. A study on membrane leakage property was also undertaken where MSI values of plant decreased to significant level. On the other hand, most of the antioxidant enzyme like. Catalase, SOD, APX where up regulated and these enzyme shows greater activity in plant under HNT stress than in those which were kept under control indicating significant role of these antioxidant enzyme system as a way to counter the deleterious effect of HNT stress.

This may be finally concluded that the HNT stress altered the physiological and biochemical parameters to a greater extent resulting in sever loss of photosynthetic efficiency and final yield in both the variety. The variety sonalika found to be more susceptible to HNT stress than the variety HUW-234 which was found to be more tolerant to HNT regimes. HNT of about $24^{0}$C was reported to be highly deleterious for realising optimum physiology, biochemistry and productivity of wheat crop.

### Abbreviations

HNT-High Night Temperature.
SOD- Superoxide dismutase
APX- Ascorbate Peroxidase
MSI- Membrane Thermostability Index
DAE- Day after emergence
ROS- Reactive oxygen species
LSD- Least significance difference


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### Author's contribution

MAF and PP conceived and designed the study. JP, AKS, HSJ and DK wrote the manuscript. PP and JP provided guidance on the whole study and DK improved the manuscript. All authors read and approved the final version of the manuscript.

## DECLARATIONS

### Conflict of interest

The authors declare they do not have any conflict of interest.

### Ethical approval

All the authors have agreed to submit it.

### Consent to participate

Before submission of the paper, all authors have given the consent to publish.

### Consent to publish

All the authors have given their consent to publish.

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