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The Influence of Terminal High Temperature Stress on Membrane Stability, Chlorophyll Content, Canopy Temperature and Yield of Wheat (*Triticum aestivum* L) Genotypes

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ABSTRACT

Wheat genotypes Halna, HD2967, K911, K9006, NW 1014 and HD 2733 were grown in field condition in differential date of sowing for detection genetic variability for high temperature tolerance traits in wheat during Rabi season 2021-22. The high temperature stress was given to wheat genotypes by 60 days late from normal sowing (15 November) for exposing terminal stage of crop under high temperature stress. The high temperature stress significantly influenced membrane stability index (MSI), canopy temperature depression (CTD), total chlorophyll content and ultimately grain yield irrespective of wheat varieties. The high MSI, CTD and less reduction in chlorophyll were recorded in Halna, K911and NW 1014. It is also linked with long stay green duration at grain filling stage. The maximum percent reduction in total chlorophyll content and grain yield were observed in HD2967, HD2733 and K9006 as it showed low MSI and CTD under high temperature stress. The high Catalase and Peroxidase activities were appeared in Halna, K 911 and NW 1014 over other varieties under high temperature. The high MSI and CTD had positive correlation with grain yield. Therefore, high MSI, CTD, Catalase, Peroxidase activity and long green duration can be used as physiological traits for evaluating wheat varieties for high temperature tolerance.

Keywords Heat, Terminal stress, Genotypes, Membrane stability, Yield.

INTRODUCTION

Wheat is one of the major food crops of India as well as many parts of the word. It is the important crop in food security mission in India. It consists second position in area and production after rice in the country. Wheat is grown over 31.62 mha area in the country with average productivity 34.46 q/ha (DAFW 2021-22). The northern plain zones are the major wheat growing states in India and largely

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affected by terminal high temperature stress. The Uttar Pradesh has first rank in area and production. The highest productivity of wheat was found in Punjab (50.77 q/ha), followed by Haryana (46.43 q/ha), Uttar Pradesh (32.69) and Madhya Pradesh (31.43) (Singh *et al.* 2019).

Now a day's the reproductive stage high temperature stress is becoming one of the major constraints for wheat production in Punjab, Uttar Pradesh, Madhya Pradesh and Rajsthan. The main reason of terminal heat stress is global warming and delayed sowing of wheat varieties up to late month of January. These two reasons forcibly exposed anthesis stage of wheat crop under heat stress regimes (Tiwari *et al.* 2017). Every one-degree centigrade rise in temperature above optimum temperature losses 4-5 % grain yield (Ottman *et al.* 2012). So, on an average heat stress during reproductive stage causes 30-35 % reduction or even more due to heat stress condition. Heat stress faster the phasic changes of wheat crop from vegetative stage to reproductive stage and shortened grain filling duration (Dias and Lidon 2009, Fahad *et al.* 2017). Early leaf senescence reduces the photosynthetic activity of plant and affects the photosynthates formation and its translocation to demanding sink (Liu *et al.* 2016). The early senescence at reproductive stage causes more losses in wheat yield comparatively vegetative stage heat stress (Pandey *et al*. 2015).

The heat stress produces high amount of superoxide radicals causes lipid per oxidation, chlorophyll degradation, protein oxidation, nucleic acid degradation and various enzymatic activities and consequently cell death under intensive heat stress regimes, under such condition yield associated traits of plants highly affected (Farooq *et al.* 2011). The tolerant plant produces the high activities of super oxide dismutase, catalase, peroxidase and other antioxidant enzymes in high temperature stress (Caverzan *et al.* 2016). High antioxidant enzyme activity reduces the ROS content by scavenging it by various adopting deferential mechanisms under oxidative stress and reduces the heat stress impact on early senescence (Gupta *et al.* 2013).

The anthesis stage of wheat is most sensitive stage for heat stress comparatively vegetative stage as losses occurs at this stage merely recoverable (Farooq *et al.* 2011). The pollen and ovule formation is highly affected when temperature rises beyond 30^{o}C for 3 days as compared to normal temperature (20^{o}C). Terminal heat stress also shortened the reproductive stage by force maturity. It reduces grain filling duration, number grains per spike, grain weight and finally yield of the wheat crop (Qaseem *et al*. 2020). The optimum grain filling temperature is 12-22^{o}C and increase in day/night temperature 30^{o}C/20^{o}C affects the grain growth due to poor efficiency of starch formation enzymes and consequently grain become shrivelled, thin and it quality reduces (Hampton *et al.* 2013).

MATERIALS AND METHODS

The wheat genotypes Halna, HD2967, K911, K9006, NW 1014 and HD 2733 were sown for characterization of high temperature stress tolerance traits in wheat in rabi season 2021-22 at Students Instruction Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224229, Uttar Pradesh, India. Wheat genotypes were sown under normal and 60 days very late sown condition so that late sown crop reproductive stage exposed to high temperature environment. The agronomic and plant protection requirement were managed as per need of crop. The temperature stress ranges from 37 to 40^{O}C at reproductive stage in very late sown crop.

Membrane stability index

Membrane stability Index (MSI) was recorded in fresh leaves by the method of Sadalla *et al.* 1990. 100 mg fresh leaf samples were cut in small pieces of 2 cm and emerged in 50 ml of deionised water in test tubes in three replications of each genotype and kept in water for one hour at 40^{o}C. The electrical conductivity was recorded at normal temperature by electrical conductivity meter (C1). The same sample was kept at 121^{o}C for 15 minutes. The tubes were cooled at room temperature and electrical conductivity was recorded (C2). MSI of each sample was calculated by the following formula:

$$MSI = 1 - \frac{C1}{C2} \times 100$$

Total chlorophyll content

The total chlorophyll content was recorded with the help of chlorophyll meter (SPAD-502 Plus, Konica Minolta) of each genotype as SPAD value. The SPAD-502Plus determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions. The SPAD502Plus measures the absorbances of the leaf in the red and near-infrared regions. The total chlorophyll of each genotype was recorded under stress condition against control by keeping the leaf properly as per instruction manual of the instrument.

Catalase activity and peroxidase activity

The Catalase activity was measure as per protocol of Sinha 1972. The fresh leaf of 200 mg of plant sample were weighed and homogenized with 10 ml phosphate buffer of 0.1 M (pH 7.0) and centrifuge at 10000 rpm at 4°C for 10 minutes. Supernatant was collected and store at 4°C temperature. The reaction mixture was prepared as per protocol and optical density was recorded at 570 nm wave length against control.

Peroxidase activity

The peroxidase enzyme assay was recorded according to method of (Curne and Galston 1959). The enzyme catalyse the substrate by removal of hydrogen with combine with hydrogen peroxide. The fresh leaf sample of 200 mg were weighed and homogenised with10 ml phosphate buffer of 0.1 M (pH 7.0) and centrifuged at 10000 rpm. The aliquat was used for enzyme assay as per protocol. The optical density was noted at 530 nm wave length against control.

The thousand grain weight was recorded by counting 1000 grain of each genotypes separately and weighed at around 12 % moisture as test weight. Grain yield of randomly five plants selected were recorded separately and average out to one as grain yield plant^{-1} at physiological maturity.

RESULTS AND DISCUSSION

The wheat varieties showed a significant variation in MSI (fig.1). The high MSI was noted in Halna

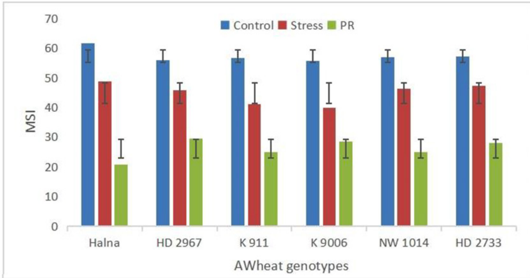


Fig.1. The effect of terminal high temperature stress on membrane stability index (%) of wheat genotypes.

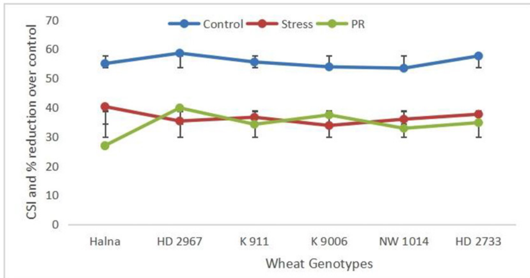


Fig.2. Effect of terminal high temperature stress on total chlorophyll content (SPAD value) of wheat genotypes.

(61.53%), K 911(56.70%) and NW1014 (55.93%) and less in HD 2967 (49.78%), HD 2733(58.7%) and K9006 (61.4%) under control condition. Under terminal stress condition, the less reduction in membrane stability was recorded in Halna (20.86%), K 911(25.04%) and NW1014 (32.77%) over other varieties. The integrity and fluctuation of biological membranes are susceptible to elevated temperature, as heat stress altered the tertiary and quaternary structures of membrane protein (Kumar *et al.* 2012). Such alteration enhances the permeability of membranes, as evident from increased loss of electrolytes. The increased solute leakage or low MSI, as an indication of decreased cell membrane thermostability while high MSI used as measure of high temperature tolerance (Wahid *et al.* 2007).

The chlorophyll content varied from variety to variety under control and stress condition (Fig.2). The high SPAD value was recorded in HD2733 (57.60), HD 2967 (58.57), K9006 (55.60) under control condition. These varieties also showed high reduction under terminal heat stress like HD 2967 (39.78%), K9006 (37.60%), and HD2733 (34.72%)

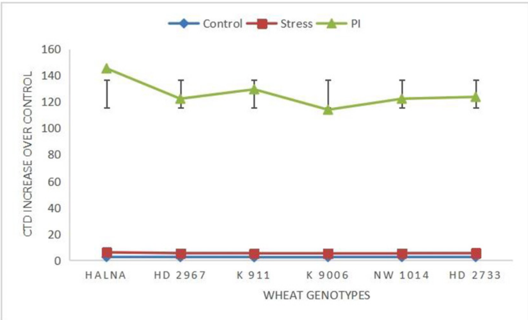
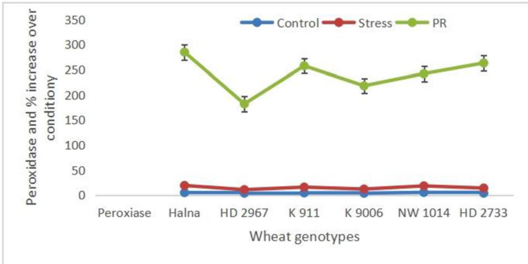


Fig.3. The effect of canopy temperature depression (oC) variability on wheat genotypes in terminal high temperature stress.



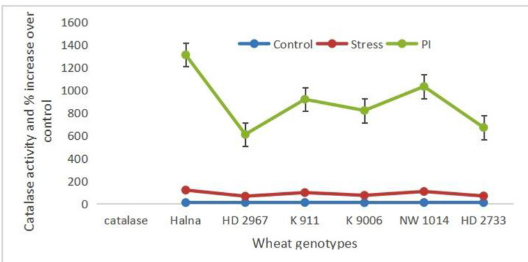
*One unit is defined as 1 μmol of H_2O_2 reduced per minute.

Fig.5. Influence of terminal high temperature stress on peroxidase activity (unit mg^{-1} protein) in wheat genotypes.

while minimum was observed in Halna (26.85%), NW1014 (32.77%) and K 911(34.15%). Halna, K 911 and NW 1014 had long green duration at grain growth stage over HD 2967, HD2733 and K9006 genotypes. Chlorophyll synthetic enzymes are heat sensitive and less functional during severe heat stress. It needs optimum temperature for proper function. When temperature increases the heat sensitive enzymes denatured and chlorophyll synthesis inhibited while senescence related process enhances (Marutani *et al.* 2012, Qaseem *et al.* 2020). The total chlorophyll content was less affected in heat tolerance varieties as it specific proteins formation, might be heat shock protein that protect the integrity of heat sensitive enzymes and remain in high temperature regimes (Sharma *et al.* 2015).

The CTD varied among wheat genotypes under high temperature stress (Fig.3.). The high CTD was noted in Halna, K 911 and NW1014 while less in PBW343 and K9006 under stress regimes. The high CTD value wheat genotypes showed minimum reduction in grain yield over control (Thapa *et al.* 2018). Leaf temperatures are depressed below air surface. To maintain high rates of photosynthesis a genotype must have an effective vascular system for transpiration of water as well as transport of nutrients and assimilates. It shows better correlation with grain number and yield (Zhang *et al.* 2018, Sohail *et al.* 2020).

The Catalase and Peroxidase activity remains nearly same under normal condition while abruptly increase many times under high temperature environment in wheat varieties (Fig. 4 and 5). Under terminal heat stress, high Catalase activity was recorded in Halna, NW 1014 and K 911 comparatively HD 2967, HD2733 and K9006 wheat varieties. The percent reduction in chlorophyll content and membrane leakage were also found less in high catalase and peroxidase enzyme activity varieties and stay green during reproductive phage. Plants exposed to heat stress often leads to the generation of destructive ROS, including singlet oxygen (1/2 O2), superoxide radical (O_2−), hydrogen peroxide (H2O2), and hydroxyl radical (OH−) responsible for generating oxidative stress (Suzuki *et al.* 2011). Reactive oxygen species disturb



*One unit is defined as 1 μmol of H_2O_2 reduced per minute.

Fig. 4. Effect of terminal high temperature stress on catalase activity (unit mg^{-1} protein) in wheat genotypes.

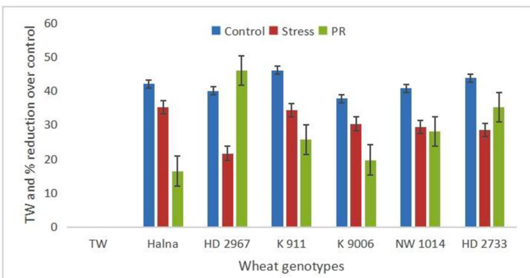


Fig.6. Effect of terminal high temperature on thousand grain weight (g) of wheat genotypes.

the membrane stability by lipid peroxidation sensitive genotypes (Sattar *et al.* 2020). Superoxide radicals accumulate excessively in high temperature stress and damage the plasma membrane. It also activates of reactive oxygen producing enzyme RBOHD which is responsible organised cell death (Mittler *et al*. 2011).

The test of wheat genotypes varied under control stress condition (Fig.6). The high thousand grain weight was noted in K911 (46.06 g) followed by HD2967 (43.76 g) and Halna (42.03). High temperature stress reduced the test weight irrespective of wheat genotypes. High reduction in thousand grain weight was noted in HD2967 (45.96 g), HD2733 (35.43g) while less in Halna (16.42 %) and K 911 (16.62%). High temperature stress during grain filling stage significantly reduces the grain weight and grain yield by 39% and 44% respectively over normal temperature. This result is similar to finding of Prasad and Djanaguiraman 2014 and Fabian *et al.* 2019).

Wheat genotypes had differential response for grain yield potential under high temperature environment. High temperature markedly decreased the grain yield plant^{-1} (Fig.7.). The maximum percent reduction under heat stress was recorded in HD 2967(32.5 %) and less in Halna (14.6 %). But high membrane thermo tolerant varieties showed less reduction in grain yield in comparison to membrane thermo sensitive varieties. Elevated temperature reduces the duration between anthesis and physiological maturity which is associated with a decrease in grain weight (Zampieri *et al.* 2017). Reduction in grain weight (~1.5 mg per day) occurs for every 1oc above 15-20oc and heat stress during the grain growth phase is more harmful than the vegetative phase due to direct effect on grain number and dry weight (Pandey *et al.* 2019, Poudel an Poudel 2020).

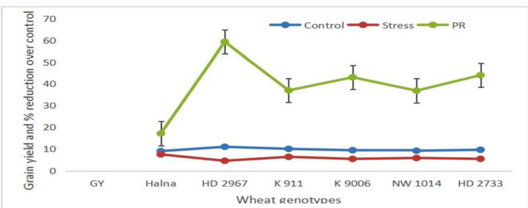


Fig.7. Influence of terminal high temperature stress on grain yield plant^{-1} (g) of wheat genotypes.

CONCLUSION

High temperature stress markedly decreases the growth, chlorophyll, starch content and yield irrespective of the wheat varieties. But the percent reduction was very high in HD 2733 and K9006 in comparison to Halna, K911 and NW 1014. The heat tolerant varieties Halna, K911 and NW 1014 showed high MSI and less reduction in growth, yield and stay long green during reproductive stage. The high MSI, antioxidant enzyme activity, stay green duration and less reduction in yield might be taken as screening criteria for selection of high temperature tolerant wheat genotypes in wheat breeding programme.

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