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Impact of elevated temperatures on water status, membrane integrity and antioxidant activity in flag leaves of wheat varieties (*Triticum aestivium*) sown at differential time intervals

T Chandra[1], J P Jaiswal[2], A Dubey[3], S C Shankhdhar[1], D Shankhdhar[1*]

[1] Department of Plant Physiology, College of Basic Sciences and Humanities, GB Pant University of Agriculture and Technology Pantnagar, US Nagar, Uttarakhand, India

[2] Department of Genetics and Plant Breeding, College of Agriculture, GB Pant University of Agriculture and Technology Pantnagar, US Nagar, Uttarakhand, India

[3] Department of Biochemistry, College Basic Sciences and Humanities, GB Pant University of Agriculture and Technology Pantnagar, US Nagar, Uttarakhand, India

Abstract

Flag leaf is considered as powerful source of assimilates in wheat (*Triticum aestivium*) which helps in translocation of photosynthates in developing grains. However, its performance is afflicted by elevated temperatures, resulting reduced assimilate supply. Delayed sowing in wheat causes sudden elevation in air temperatures during various growth stages of wheat life cycle which disturbs flag leaf performance and reduces grain yield & quality. Researches shows that delayed sowing causes reduction in grain yield by 60 kg ha-[1]. Therefore, identifying varieties with improved flag leaf performance even under elevated temperature conditions is a need of hour, to minimize the yield loss due to delay in sowing conditions. In this context performance of flag leaves in eight wheat varieties (UP2628, HD3086, UP2967, UP2784, UP2526, UP2565, UP2748 and HD3059) sown at different time intervals were analyzed. As a result of delayed sowing, an elevation of 5ºC, 1.45ºC, 4.07 ºC and 3.71ºC in average air temperature at tillering, heading, anthesis and grain filling were registered. At the time of anthesis, water status and stability of membrane in flag leaves were found hampered upto 48.46% and 63.96% due to elevated temperatures. The increment in lipid peroxidation (79.66%) activation of antioxidant defense system (150% & 340%) also started off while during grain filling, water status (48.49%) and stability of cell membrane (69.03%) found more disturbed with much increase in lipid peroxidation (69.62%) was seen. However, the antioxidant defense system (SOD and CAT) also found activated (72.73% and 108.81% respectively) under elevated temperatures in compared to normal sowing conditions. The flag leaves of wheat varieties UP2565 and UP2748 showed heat adaptive response &considered as good performer under elevated temperatures and can be suggested as a promising source for further use in selection of heat-tolerant wheat genotypes.

Keywords: wheat, sowing timing, elevated temperatures, flag leaf performance, membrane stability, antioxidant activity

Introduction

Delayed sowing in wheat is one of the major issues for reduction in grain yield especially when wheat is under any cropping system such as rice-wheat and cotton-wheat (Ali *et al*., 1993) [1]. Elevation in air-temperature during sensitive growth stages (pre-anthesis and post-anthesis) affects overall growth, yield and quality of heat sensitive crops and could reduce the yield by the rate of 60 kg ha-[1] due to shorter growing period (Tsukaguchi *et al*., 2003) [31]. Due to delayed sowing, temperature reaches above the optimum conditions; at anthesis (15.40 to 21.80 °C) and at grain filling (17.50 to 20.40 °C) and alter the cellular functions in plants, hamper the physiological processes such as cell division, elongation and differentiation in the cells (Asseng *et al*., 2003) [3]. Being the major site of photosynthetic activity leaves appears to have a direct relation with plant grain yield however top most leaf (flag)contributes the most in photosynthetic assimilates production in wheat. It also plays a pivotal role in assimilate partitioning during grain filling and responsible for 41 to 43% for deciding grain weight. Under elevated temperature, leaves experiences forced senescence as a result of it, it causes poor assimilate synthesis, reduction in translocation of photosynthates to developing grains which ultimately affects the crop productivity (Chandra, 2006) [7]. Due to delayed sowing, elevated temperature is also associated with reduced water availability to the grow up the crop. Relative water content (RWC) is the most appropriate measure for measuring plant water status with respect to physiological consequence of cellular water deficit condition when plant experience elevation in temperature (heat stress). It also expresses the effect of osmotic adjustments performed by individual crop varieties under stressful condition for conserving their cellular hydration. Dry environment with higher temperature causes high vapor pressure deficit, which responsible for higher evapotranspiration which results reduced leaf relative water content and leaf water potential in flag leaves of cereal crop like wheat (Almenselman *et al*., 2009) [2]. This enhancement in transpiration induces water deficiency in leaves causing a decrease in water potential and leading to perturbation of many physiological processes of crop (Hossain *et al*., 2013) [13]. A negative relation between transpiration rate and increasing temperature (from 15/10ºC to 40/30 ºC) was also found with decreased growth in various crop species. Higher temperature (35/25ºC) after tillering stage in wheat genotypes leads to a significant reduction in water potential which concludes that the investigated varieties were susceptible to heat stress (Sattar

et al., 2017) [28].

Plant physiological and biochemical processes depend on proper cell membrane functioning. When plant experiences elevated temperatures, the membranes of plant cells get damaged. Exposed heat causes protein denaturation & enzymes inactivation in cell membrane system resulting sudden changes in membrane permeability and integrity which causes reduction in ion flux, electrolyte leakage, reduction in relative water content, production of toxic compounds, and disturbance in plant homeostasis that reduces cell viability. Reduction in cell viability inhibits plant growth and induces leaf wilting, reduced leaf area and promote leaf abscission. Increase in cell permeability and leakage of ions out of the cell can be measured by measuring membrane stability index which is also used as a screen test for stress tolerance (Bartels and Sunkar, 2005) [4]. According to some experiments it was proved that membrane thermo stability test could be a suitable measure for selection of heat tolerant wheat genotypes as it is an indicator of heat tolerant behavior of plants. Tolerance behavior towards elevated temperatures in 5 different wheat genotypes were studied with the help of membrane thermostability test which is based on percent leakage of electrolytes in flag leaves under heat stress condition. PBW452×LoK 54 was found the most tolerant with least relative injury of 26%. Under high temperature, leakage of ions occurred due to disturbance in cell membrane which is identified by measurement of electrical conductivity measures (Reddy *et al*., 2008) [23].

In plant system, free radicles generated due to excess heat and steal electrons from lipid cell membrane causing lipid peroxidation (oxidative degradation of lipids) and causes cell damage. Such oxidative stress responsible for protein degradation, membrane rupture and enzyme inactivation led to decreased rate of leaf photosynthesis in various crop species, especially those are susceptible towards elevated temperatures such as wheat, maize, sorghum, sugarcane etc (Sairam *et al*., 2000) [28]. Chloroplast (PSII and PSI reaction centers of thylakoid membrane) being a significant site for production of various reactive oxygen species (ROS) under stress conditionleads to generation triplet stage of chlorophyll (Chl*), singlet oxygen ($^1O_2$), superoxide radicle (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Liu and Huang, *et al*., 2000) [16]. Lipid peroxidation in the cells can be measured by Malondialdehyde assay, (MDA content) and used as marker to check the oxidative lipid injury caused by heat stress. From previous reviews, elevation in temperatures significantly decreases relative water content and increases the level of lipid peroxidation (Malondieldehyde) and H_2O_2 content in leaves of crop species while the crop species which shows lower level of lipid peroxidation even under stress conditions had higher degrees of heat tolerance in them (Sairam *et al*., 2004) [25]. Crop cultivars showed elevation in lipid peroxidation under heat stress condition also showed higher hydroxyl radicles which can further react to all biomolecules like pigments, proteins and DNA which ultimately decreases plant overall performance and yield (Moller *et al.*, 2007) [20]. In wheat plant system with delay in sowing timing causes increased production of reactive oxygen species and causes cellular damage. As a result of its transcript and translation of many proteins related to reactive oxygen species (ROS)-scavenging enzymes also found increased in order to protect cells from oxidative damage. Coordinated function of antioxidant enzymes such as SOD (superoxide dis-mutase) and catalase help in processing ROS and regenerate redox metabolites (Ristic *et al*. 2008) [24]. Antioxidant defense system is also a part of the high thermal stress adaptation and resistance and highly correlated with the acquisition of thermotolerance. In metabolic studies, ROS production in chloroplast act as signal to inform the nucleus machinery to activate the expression of genes to aid in the protection of plants against environmental stresses (Pogson *et al*., 2008) [21]. Development of detoxification system breaks down the highly toxic ROS into less toxic or harmless product. An antioxidant molecule capable of slowing or preventing the oxidation of another molecule. If is not controlled, oxidation reaction produces free radicals and start chain reactions that damage the cells. (Almenselmania *et al*., 2009) [2]. Superoxide dis-mutase (SOD) catalyzes the breakdown of superoxide anion into oxygen and hydrogen peroxide. Further hydrogen peroxide in plants is taken care via catalase enzyme where hydrogen peroxide converted non-harmful product oxygen and water. According to previous studies SOD and CAT activity was found higher in tolerant wheat cultivar in comparison to susceptible cultivars. SOD and CAT activity also found to be increased in case of late sowing and very late plantings. Highest SOD and CAT activity was recorded at 15 days after anthesis in very late planting followed by anthesis stage and lowest activity was recorded at vegetative stage (Farooq *et al*., 2011) [9]. Parameters such as relative water content, membrane stability index, lipid peroxidation and antioxidant defense system in flag leaf is considered as a good marker for identifying thermotolerant behavior of various crop species. With the help of such parameters the extent of plant performance under elevated temperatures can be easily analyzed. Under such scenario understanding of physiological aspects that enable plants to adapt to heat stress and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and could use in breeding programs.

Plant material and experimental details

To analyze the effect of elevated temperatures on membrane stability and enzymes activity in flag leaves, eight wheat varieties (UP2628, HD3086, UP2967, UP2784, UP2526, UP2565 UP2748 and HD3059) were analyzed under different sowing timings i.e November, 20th and December. 22nd 2018 as normal and elevated temperatures, respectively. A field experiment was perfomed in triplicate RBD with 4 rows 3m^2 / plot area during Rabi season of 2018-19 at Dr. N.E. Borlaug Crop Research Center G.B.P.U.AT Pantnagar. By using random sampling technique flag leaves were collected at the time of anthesis and grain filling stage (15DAA).

Relative water content (RLW) was calculated from the method determined by (Slatyer and Barrs 1965) [30].

$$\text{RWC (\%)} = \frac{FW-DW}{TW-DW} \times 100$$

Where FW, DW, and TW are the fresh weight, dry weight, and turgid weight of leaf, respectively.

Membrane stability index was examined according to the procedure described by (Sairam *et al.*, 1997) [26]. Leaf discs (4 mm) from each variety were taken and placed in petri plates containing 20 ml of distilled water (in two sets). One

set incubated in water bath at 40°C for 30 minutes and another set at 100°C for 1 hour. Electrical conductivity was measured in each case. MSI was calculated by applying formula,

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

Where, C1 and C2 are the electrical conductivity at 40º C and 100ºC respectively.

Lipid peroxidation was estimated by measuring the amount of Malondialdehyde (MDA) content by the method described by Health and Packer, 1968 [12]. Leaf sample were homogenized in 2 ml of TBA-Thio-barbituric acid (0.25% TBA in 10 % TCA (Trichloro Acetic Acid)). Homogenate was heated at 95ºC for 30 minutes and cooled rapidly in ice bucket. After centrifugation (at 10,000g for 30 minutes) supernatant was collected and absorbance recorded at 532nm and 600 nm against blank containing 0.25% TBA in 10 % TCA. The MDA content was calculated by using following formula with extinction coefficient of $155mM^{-1}$ cm^{-1}.

Superoxide dismutase was assayed by using procedure given by (Beauhamp and fridovich, 1971) [5], leaves were homogenized in a medium composed of 0.1M potassium phosphate buffer (pH 7.0), and supernatant used as enzyme extract. Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) in the presence of riboflavin in light (Giannapolitis and Ries, 1977) [10]. One unit of SOD activity is defined as the amount of the enzyme that causes inhibition of 50% NBT reduction rate by monitoring the absorbance at 560 nm with the help of spectrophotometer.

Catalase activity was assayed by using procedure given by (Chance and Maehly, 1995) [6]. The leaves were homogenized in a medium composed of 100 mM sodium potassium phosphate buffer (pH 6.8) and supernatant used as enzyme extract. For measuring CAT activity, the assay solution (3 ml) contained 100 mM potassium phosphate buffer (pH 7.0), 0.1µM EDTA, 20mM H_2O_2 and 0.2 ml enzyme extract. The decrease in absorbance of the reaction solution at 240 nm was recorded after every 5 seconds for 1 minutes. Using extinction coefficient 36 M^{-1} cm^{-1} catalase activity was calculated. CAT activity is the amount of enzyme which breaks down 1mM of H_2O_2 per minute under assay condition.

Statistical analysis

Data collected for all parameters were analysed statistically by using the Two-way Analysis of Variance technique with R software (R x 64 4.0.3) and STPR 2. Least Significant Difference (LSD) test at 5% probability level was applied to compare the treatment's means. Data for correlation analysis was done in SPSS software version 26.

Results

Correlational Analysis between Parameters under both sowing conditions

At anthesis, RWC was found negatively correlated with MDA (r= - 0.41) while positive correlation was observed with MSI (r=0.32)under normal conditions indicates generous water accessibility in plant system accountable for less lipid peroxidation and fine membrane stability whileunder elevated temperatures, a more positive correlation of RWC with MSI (r=0.57) and SOD (r=0.29)was established which may be due to generation of heat stress condition however, mutual correlation was improved by increasing SOD activity. A mutual positive correlation between SOD and CAT activity was established under both sowing condition but it was supplemented under elevated temperatures (r=0.74) as compared to normal (r=0.35) (table 1). At grain filling, RWC was negatively correlated with SOD (r= -0.60) and CAT (r= -0.16) while positive with MSI (r=0.24) which shows when plant have an appropriate water status and membrane stability in leaves, increment in SOD and CAT activity is not required. While under elevated temperatures, RWC found positively correlated SOD (r= 0.46) and CAT (r=0.47) and negatively correlated with MSI (r= -0.35). Because of the delayed sowing, elevated temperature disturbed membrane stability resulting increased enzyme activity so that plant could maintain water loss under elevated temperatures. MSI found negatively correlated with SOD under both conditions but under elevated temperatures, the correlation was more negative which shows that more the cell membrane is hampered, more will be the enzyme activity to cope up with the losses due to elevation in temperatures. Under both conditions SOD and CAT activity positively correlated with each other but under normal sowing, the correlation was found less (r=0.80) as compared to the correlation under elevated temperatures (r=0.84) shows improved relationship between SOD and CAT due to heat stress (table 2).

Relative water content in flag leaves

Relative water content (RWC) was found reduced from 17.43 % to 48.46 % and 2.77% to 48.49% during anthesis and grain filling (15DAA) stage under elevated temperatures as compared to normal sowing conditions. Least reduction in RWC during both stages was observed in UP2967 while highest in UP2526 (at anthesis) and UP2628 (at grain filling). RWC found higher during anthesis and reduced as varieties moves towards grain filling (table 3 and 4). The results of analysis of variance showed significant difference in RWC between the varieties and between the two sowing conditions at 0.01 probability level of significance during both stages. Interaction between treatment and variety also found significant at 0.01 probability level during both stages (anthesis and grain filling).

Membrane stability Index in flag leaves

MSI was found reduced from 28.58 % to 63.96% and 31.24% to69.03% during anthesis and grain filling (15DAA) stage, under elevated temperatures as compared to normal sowing conditions. Least reduction in membrane stability index during anthesis stage recorded in UP2967 while highest in UP2526.However, during grain filling, least reduction in HD3086 while highest in UP2565. MSI found higher during anthesis as compared to grain filling stage (table 3 and 4). The results of analysis of variance showed significant difference in MSI between the varieties and between the two sowing conditions at 0.01 probability level of significance during both stages. Interaction between treatment and variety also found significant at 0.01 probability level during both stages (anthesis and grain filling).

Lipid peroxidation in flag leaves

MDA content was found increased from 11.54% to 79.66% at anthesis and 8.43% to 69.62% at grain filling (15DAA) stage, under elevated temperatures as compared to normal sowing conditions. Least increment in MDA content during anthesis stage recorded in HD3059 while highest in UP2628. However, during grain filling, least increment recorded in UP2748 while highest in UP2784.MDA content found lower during anthesis and increased during grain filling stage (table 3 and 4). The results of analysis of variance showed significant difference in MDA content between the varieties and between the two sowing conditions at 0.01 probability level of significance during both stages. Interaction between treatment and variety also found significant at 0.01 probability level of significance during both stages (anthesis and grain filling).

Superoxide dis-mutase and catalase activity in flag leaves

SOD and CAT activity was significantly increased by elevated temperatures as compared to normal sowing conditions during both sensitive stages (at anthesis and at grain filling stage).An increment of 7.32% to 150.00 % in SOD activity was noted at anthesis while at grain filling, an increment of 2.13% to 72.73% was analyzed due to elevated temperatures. Least increment in SOD activity during anthesis stage and grain filling was shown by HD3086 while highest in UP2526 (table 3 and 4). The results of analysis of variance showed significant difference in SOD activity between the varieties and between the two sowing conditions at 0.01 probability level during both stages. Interaction between treatment and variety also found significant at 0.01 probability level of significance during both stages (anthesis and grain filling). Similarly, an increment of 2.86 % to 340.00 % in CAT activity was recorded during anthesis while at grain filling, an increment of 1.31% to 108.81% was noted due to elevated temperatures. Least increment in CAT activity during anthesis stage studied in UP2967 while highest in UP2565. During grain filling, least increment was recorded for UP2784 while highest in UP2748 (table 3 and 4). The results of analysis of variance showed significant difference in CAT activity between the varieties and between the two sowing conditions at 0.01 probability level of significance during both stages. Interaction between treatment and variety also found significant at 0.01 probability level of significance during both stages (anthesis and grain filling). On comparing between two sensitive stages, antioxidant activity found higher at the time of grain filling in comparison to anthesis stage.

Discussion

Water relations and stability of cell membrane are one of the important factors that influences yield potential of the crop (Mazorra *et al.* 2002) [18]. Current study indicates that the relative water content and membrane stability index in flag leaves of all wheat varieties were negatively affected under elevated temperatures (late sowing) as compared to non-stress condition (timely sowing condition). Low level of RWC and MSI was observed under grain filling as compared to anthesis stage in flag leaves of all wheat varieties. Under heat stress, transpiration rate in leaves increased resulting excess water loss, changes in water potential along with its components and water availability decreases as a result relative water content also deceases (Wahid and Close, 2007) [32]. Similar observation was observed in a study in which transpiration rate was found increased in leaves of late sown wheat genotypes and the photosynthetic rate was reduces due to the acceleration of leaf senescence process (Xie *et al*. 2016) [33]. Reduced water related parameters were reported in two wheat varieties i.e., Sehar 2006 and Faislabad 2008 sown under different sowing dates. Highest RWC in both wheat varieties was recorded in early sown condition. Water potential and osmotic potential also found negatively affected by elevated temperatures in wheat (Sattar *et al*., 2017) [28]. A reduction in relative water content (upto 80%) was observed by various wheat genotypes (Salt-6, Lu-26s, TJ-83, RN-09111, KIRAN-95, NRL-1236 and NIA-AS-14-10 under late sowing condition while under timely sowing conditions UP TO 90% RWC was reported in all genotypes (Mahboob *et al*., 2018) [17]. A 25% reduction in RWC observed by wheat variety (Faisalabad-2008) under late sowing as compared to normal sowing conditions (Sattar *et al*., 2020) [29].

Higher temperature stress (due to late sowing) could increase membrane injury upto 72 % and could reduce MSI values with same percentage, together which is responsible for limiting the productivity in winter wheat (Potter *et al.*, 1999) [22]. Similar results were also observed during an experiment in which wheat genotypes under timely sown conditions showed lower membrane injury i.e only upto 7.48% (higher MSI) while under delayed sowing the membrane injury increased upto 49.15% (lower MSI) during temperature sensitive stage (anthesis and 15DAA) of wheat life cycle. High temperature (late sowing) disturbs the conformation of proteins in cell membrane, integrity and functioning of biological membrane system also disturbed which ultimately leads to reduced stability of membrane (Chandra, 2006) [7]. High MSI is positively related to tolerance behavior of the wheat plant asPBW452×LoK 54 cross was found the most tolerant one by performing least relative injury of 26% as compared to other wheat cultivars. Under high temperature, leakage of ions causes disturbance in cell membrane which is identified by measuring MSI (Reddy *et al*., 2008) [23]. MSI values was found lower in heat stress treated PBW343 and C306 genotypes of wheat during anthesis and 15 days after anthesis and a greater reduction was observed in PBW343 (heat sensitive) than in C306 (heat tolerant) (Almeselmani, 2009) [2]. Maximum values of MSI were found for the varieties which are thermo-tolerant i.e. PBW574 (84.82%), Raj3765 (68.23%) Lok54 (66.10%) K0307 (66.09%) DBW14 (61.18%) and minimum MSI were found for the varieties which are sensitive towards heat stress i.e. HS240 (30.54%) and WH1020 (56.79%) (Dhyani 2010) [8]. During an experiment, wheat genotypes Halna, NW 1014, DBW 16, K 911, and AAI 11 had high MSI with less percent reduction in yield and yield components as compared to genotypes PBW 343 and DBW 16. So higher MSI can be considered as heat tolerance trait because tolerant plant showed less leakage due to accumulation of high saturated fatty acids and monounsaturated fatty (Jaiswal *et al*., 2017) [14]. A significant decrease in cell membrane thermo stability index observed in different wheat genotype under delayed sowing (upto 9.1%) as compared to normal sowing (ranged from 29 to 70.6%). Maximum MSI upto70.6% observed in genotype SRN-09111and Dani under optimal sowing condition while under late sowing condition they showed decreased MSI

upto87.11%. Elevation in temperature beyond the threshold cause an increase in kinetic energy of molecules across membranes resulting loosening in cell membranes causes decrease in MSI values under delayed sowing condition (Khan *et al*., 2020) [15].

In plant system, antioxidants enzymes productions are triggered by the increased level of ROS and when the lipid peroxidation disturbs cellular homeostasis. In current research high lipid peroxidation (MDA content), SOD and CAT activity was increased under delayed sowing as compared to timely. While under grain filling lipid peroxidation and antioxidants enzymes activity found much higher as compared to the same parameters under anthesis stage. Elevated temperatures under delayed sowing during anthesis and grain filling period generates reactive oxygen species (ROS) (singlet oxygen, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH) and heat stress induce oxidative stress along with tissue dehydration in plant system. Generation and reactions of ROS are very common during cellular injury due to high temperature (Liu and Huang 2000) [16]. Autocatalytic peroxidation of lipid membranes and pigments due to ROS leads to loss of membrane semi-permeability (Xu and others 2006). In response plants shows increment in both enzymatic and nonenzymatic mechanisms to scavenge the rapidly evolving ROS. To detoxify ROS. Removal of O^2 by (SOD) which generates H_2O_2, which is further removed by catalase into water and oxygen as byproducts. Both becomes non-toxic to plant system however H_2O_2 and OH- also a non-toxic product to plants but hydroxyl radical (OH-) could damage chlorophyll pigments, proteins, DNA structure, lipids, and other important macromolecules and thus lethally affects plant metabolism growth and yield (Sairam and Tyagi, 2004) [25]. From previous researches it is also observed that high SOD and CAT activity indicates the ability of variety or genotypes can tolerate high temperature as well as increased SOD and CAT activity shows oxidative damage due to high temperature when crop is under late sown condition (Yildiz *et al.* 2008) [35]. SOD activity was also found higher in tolerant wheat cultivar in comparison to susceptible cultivars and found increased in case of late sowing and very late plantings. Highest SOD activity was recorded at 15 days after anthesis in very late planting followed by anthesis stage and lowest activity was recorded at vegetative stage (Almenselmania *et al*., 2009) [2]. 6 different wheat genotypes were evaluated for heat tolerance behavior during their different growth stages. On the basis of heat susceptibility index wheat genotypes Inqilab-91 considered as heat tolerant, Sitta as heat sensitive and Nesser &Sarsabz as moderately tolerant while Fareed & FD-83 as sensitive towards elevated temperatures. MDA content was found increased above twofold in most of genotypes under heat condition. Least increment in MDA content observed for heat-tolerant genotype (Nesser). SOD activities also found increased under heat stress (Hameed *et al*., 2012) [11]. Winter wheat (*cv*. Yangmai 16) was grown under different treatments. A significant increment in MDA concentration in wheat leaf was observed when the plant treated with heat stress of 32/28°C for 2 days in comparison to controlled condition of 24/20°C). Lipid peroxidation due to ROS responsible for reduced permeability of biological membrane (Xin *et al*., 2016) [34]. Accumulation of MDA was found higher under elevated temperatures in heat sensitive cultivar of Brassica *(Campestris)* as compared to heat tolerant (wucai). Sensitive cultivar showed severe damage to photosynthetic apparatus and membrane system under heat stress (Zou *et al*., 2017) [36]. The SOD activity was found significantly increased upto 54% in wheat variety (Faisalabad-2008) with exposure of heat stresses as compared to same variety under non stress condition. ROS generated due to heat stress induces the production of abscisic acid that act as a signal molecule under stressed conditions and regulate the gene expressions that control the production of enzymatic antioxidants such as superoxide dismutase (Sattar *et al*., 2020) [29]. A wheat variety when subjected to various combinations of heat treatment in comparison to control conclude that under elevated temperatures the catalase activity (CAT) in plant was increased (61%) to compensate the bad effect of ROS and convert harmful active species to inactive condition. (Sattar *et al*., 2020) [39]. Tolerant variety maintained its thermo-stability under heat stress condition by accumulation more antioxidant activity (SOD and CAT) or vis least reduction in SOD and CAT activity (15 to 20%) as compared to the heat-susceptible (Mohi-ud-Din *et al*., 2021) [19].

Environmental stresses lead to the generation of reactive oxygen species (ROS). Heat stress can induce oxidative stress along with tissue dehydration. Generation and reactions of ROS, that is, singlet oxygen, superoxide radical (O 2-), hydrogen peroxide (H2O2), and hydroxyl radical (•OH), are common events during cellular injury by high temperature (Liu and Huang 2000) [16] and drought (Farooq and others 2009) [9]. Autocatalytic peroxidation of membrane lipids and pigments by ROS leads to loss of membrane semipermeability (Xu and others 2006). Plants have evolved both enzymatic and nonenzymatic mechanisms to scavenge the rapidly evolving ROS. Enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Zhang and others 1995; Lee and Lee 2000), and nonenzymatic antioxidants such as tocopherols, ascorbic acid (AsA), and glutathione (GSH) (Wingsle and Hallgren 1993; Kocsy and others 1996; Noctor and Foyer 1998; Noctor and others 1998) work in concert to detoxify ROS. The removal of O 2 - by superoxide dismutase (SOD) generates H 2 O 2, which is removed by ascorbate peroxidase and catalase. However, both O2- and H2O2 are not as toxic as the OH-, which is formed by the combination of O2- and H2O2. The hydroxyl radical OH- can damage chlorophyll, protein, DNA, lipids, and other important macromolecules, thus fatally affecting plant metabolism and limiting growth and yield (Sairam and Tyagi 2004) [25] Environmental stresses lead to the generation of reactive oxygen species (ROS). Heat stress can induce oxidative stress along with tissue dehydration. Generation and reactions of ROS, that is, singlet oxygen, superoxide radical (O2-), hydrogen peroxide (H2O2), and hydroxyl radical (•OH), are common events during cellular injury by high temperature (Liu and Huang 2000) [16] and drought (Farooq and others 2009) [9]. Autocatalytic peroxidation of membrane lipids and pigments by ROS leads to loss of membrane semipermeability (Xu and others 2006). Plants have evolved both enzymatic and nonenzymatic mechanisms to scavenge the rapidly evolving ROS. Enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Zhang and others 1995; Lee and Lee 2000), and nonenzymatic antioxidants

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Conclusion

Delayed sowing of wheat increases the air temperature during anthesis and grain filling both by 4.07ºC & 3.71ºC which affects overall flag leaf performance of cultivars and reduced yield. Tolerant varieties that maintain water status, membrane integrity and it's functioning even under heat stress conditions may ameliorate the adverse effects of high temperatures under late sowing. Higher antioxidants level also protect plants from negative affect of heat stress, reduce forced senescence and promotes stay green traits in leaves. During investigation, UP2565 and UP2748 showed better yield performance under elevated temperatures, could be recommended for late sowing and future breeding program to develop more heat tolerant lines. More efforts are required to identify heat tolerant cultivars with stay green traits by using such multiparametric approach involving physiological and biochemical assays.

Table 1: Pearson's correlation between parameters at anthesis under normal sowing and elevated temperature condition.

	Normal Sowing						Elevated Temperatures				
	RWC	*MSI*	*MDA*	*SOD*	*CAT*		*RWC*	*MSI*	*MDA*	*SOD*	*CAT*
RWC	1						1				
MSI	0.321	1					0.576	1			
MDA	-0.415	-0.632	1				0.060	-0.260	1		
SOD	0.044	-0.060	-0.100	1			0.299	-0.130	-0.475	1	
CAT	-0.117	-0.659	0.595	0.357	1		-0.321	-0.259	-0.631	0.741	1

**. Correlation is significant at the 0.01 level (2-tailed),

*.Correlation is significant at the 0.05 level (2-tailed).

Note; Relative water content (RWC), Membrane stability Index (MSI), Malondialdehyde Assay (MDA), Superoxide dis mutase (SOD) and Catalase (CAT) activity.

Table 2: Table 1b Pearson's correlation between parameters at grain filling under normal sowing and elevated temperature conditions.

	Normal Sowing						Elevated Temperatures				
	RWC	*MSI*	*MDA*	*SOD*	*CAT*		*RWC*	*MSI*	*MDA*	*SOD*	*CAT*
RWC	1					RWC	1				
MSI	0.245	1				MSI	-0.354	1			
MDA	0.389	-0.414	1			MDA	-0.203	0.854*	1		
SOD	-0.606	-0.104	-0.297	1		SOD	0.466	-0.588	-0.708	1	
CAT	-0.168	0.095	-0.237	0.805*	1	CAT	0.471	-0.885	-0.911	0.845*	1

**. Correlation is significant at the 0.01 level (2-tailed),

*. Correlation is significant at the 0.05 level (2-tailed).

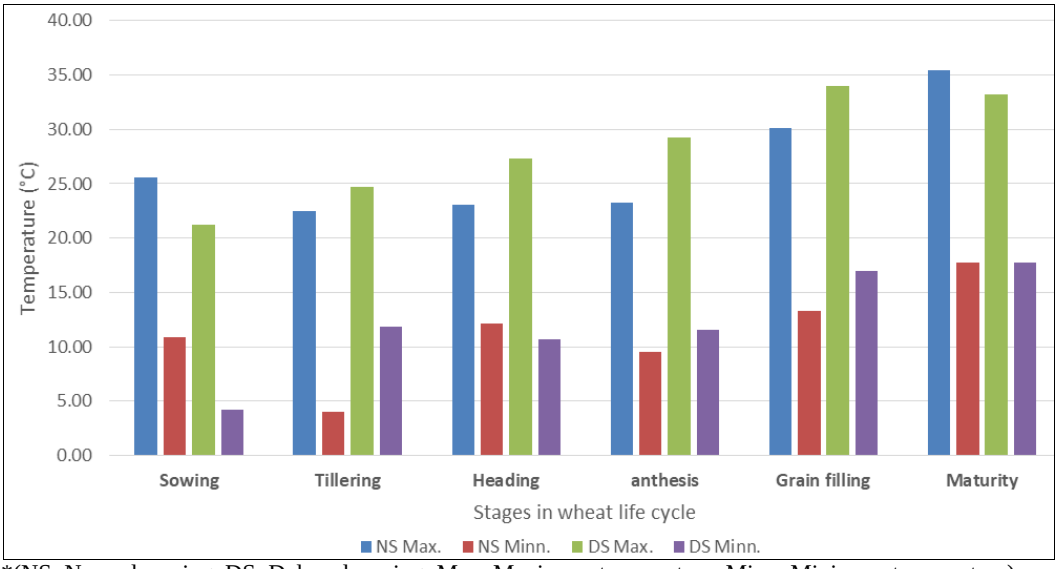
Note; Relative water content (RWC), Membrane stability Index (MSI), Malondialdehyde Assay (MDA), Superoxide dis mutase (SOD) and Catalase (CAT) activity.

Table 3: Effect of normal (NS) and delayed sowing (DS) on relative water content, membrane stability index, lipid peroxidation, superoxide dis mutase and catalase enzyme activity in flag leaves of different wheat varieties during anthesis stage.

	RWC		MSI		MDA Content		SOD		CAT	
Varieties	(%)		(%)		µmole/g FW		Units/gFW		µmole O.D H_2O_2 dismuted	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
UP2628	82.11c	56.35c	42.32d	20.43b	27.59e	49.56a	0.18c	0.23d	0.28e	0.38e
HD3086	89.70a	63.95a	41.02e	20.44b	31.95c	46.49b	0.41a	0.44b	0.63c	0.65d
UP2967	77.81e	64.24a	34.65f	24.74a	32.62c	46.98b	0.28b	0.35c	0.70b	0.72c
UP2784	82.77c	55.89c	55.91b	20.30b	30.00d	41.95c	0.20c	0.22d	0.30e	0.44e
UP2526	87.25b	44.97e	50.49c	18.20c	28.53d	40.51c	0.12d	0.30c	0.53d	1.09b
UP2565	82.98c	62.17b	57.54a	24.83a	20.21f	27.00d	0.45a	0.50a	0.25e	1.10b
UP2784	80.18d	53.29d	40.75e	14.99d	36.48b	40.89c	0.43a	0.54a	0.96a	1.24a
HD3059	77.66e	61.78b	42.67d	17.71c	40.34a	45.00b	0.23b	0.41b	0.46e	0.76c
Mean	**82.56**	**57.83**	**45.67**	**20.02**	**30.97**	**42.30**	**0.29**	**0.37**	**0.52**	**0.80**
	SEm±	**CD5%**	**SEm±**	**CD5%**	**SEm±**	**CD5%**	**SEm±**	**CD5%**	**SEm±**	**CD5%**
Treatment	0.22	0.63	0.14	0.39	0.34	0.99	0.08	0.02	0.01	0.03
Variety	0.44	1.25	0.28	0.78	0.69	1.98	0.02	0.05	0.02	0.06
T×V	0.63	1.77	0.39	1.10	0.97	2.80	0.02	0.07	0.03	0.08

Table 4: Effect of normal (NS) and delayed sowing (DS) on relative water content, membrane stability index, lipid peroxidation, superoxide dis mutase and catalase enzyme activity in flag leaves of different wheat varieties during grain filling stage (15DAA).

	RWC		MSI		MDA Content		SOD		CAT	
Varieties	(%)		(%)		µmole/g FW		Units/gFW		µmole O.D H_2O_2 dismuted	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
UP2628	72.66a	37.43h	33.77d	15.28b	43.01a	56.83c	0.27d	0.41d	0.53d	0.95e
HD3086	55.88e	41.99f	25.02g	17.20a	41.86a	57.04c	0.47b	0.48c	0.85c	1.10e
UP2967	55.33e	53.80b	32.57d	15.77b	42.73a	60.39b	0.49b	0.61a	0.96c	1.38d
UP2784	58.66d	50.63c	42.24a	16.57a	40.27b	68.32a	0.35c	0.37d	0.68d	0.68f
UP2526	66.33b	39.70g	31.81e	16.51a	42.39a	58.52b	0.33c	0.57b	1.08b	1.26d
UP2565	63.66c	56.03a	40.83b	12.64c	41.39a	44.94e	0.60a	0.63a	1.64a	2.25b
UP2784	55.33e	49.89d	28.21f	10.08d	41.29a	44.77e	0.58a	0.64a	1.26b	2.63a
HD3059	56.57e	45.22e	35.55c	11.75c	42.23a	49.24d	0.45b	0.55b	0.88c	1.72c
Mean	**60.56**	**46.85**	**33.76**	**14.48**	**41.90**	**55.01**	**0.44**	**0.53**	**0.90**	**1.50**
	SEm±	**CD5%**	**SEm±**	**CD5%**	**SEm±**	**CD5%**	**SEm±**	**CD5%**	**SEm±**	**CD5%**
Treatment	0.226	0.639	0.209	0.590	0.391	1.127	0.093	0.027	0.413	0.119
Variety	0.452	1.279	0.417	1.180	0.782	2.253	0.019	0.054	0.826	0.238
T×V	0.640	1.809	0.590	1.669	1.106	3.186	0.026	0.054	0.117	0.337



*(NS; Normal sowing, DS; Delayed sowing; Max; Maximum temperature, Minn; Minimum temperature)

Fig 1: Maximum and minimum air temperature (ºC) during different phenological stages of wheat in year 2018-19.

Conflict of Interest

The authors declare that there is no conflict of interest.

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