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Effect of heat stress on growth, physiological and biochemical activities of wheat (*Triticum aestivum* L.)

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Abstract

Globalization and modern processes of industrialization and race for energy, food, economy and mainly technology producing havoc for living things in the environment. Such processes also increasing the temperature and heat of earth, which has a hazardous effect on different economic crop plants. Our focus was to investigate the effect of heat stress on wheat crop as it is the most important daily food part. The present investigations were carried out to determine the influence of different concentrations of heat stress on physiological, biochemical and growth parameters of wheat (*Triticum aestivum* L.). The heat stresses C- Control (25°C), T1- 45°C, T2- 40°C, T3- 35°C were applied in separate growth chambers to the plants after 24 days of their germination. The high temperature stress significantly reduced the chlorophyll *a* and chlorophyll *b* contents of wheat plants. High temperature stress reduced the growth of wheat and resulted in significant reduction in leaf relative water content, leaf fresh weight and leaf dry weight. Antioxidant enzymes such as Ascorbate peroxidase (APX), Peroxidase (POD) and Glutathione reductase (GR) also showed a significant increase in their activity under high temperature stress. However, CAT enzyme showed a significant reduction under higher concentration (T1- 45 °C) of heat stress, while CAT activity was significantly increased by moderately high temperature stress. Malondialdehyde (MDA) level was significantly increased by both the higher concentration (T1- 45 °C, T2- 40°C,) of heat. It showed that plants exposed to severe heat stress under goes enhanced lipid peroxidation as a result of which MDA level is increased in wheat plants, however low temperature stress did not show significant effect on MDA level.

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Introduction

In modern world the flora of many countries is facing many stresses and hazards due to severe climatic and environmental conditions. In this regard, human intervention is more severe too. Especially the economically important and food crops are also severely affected from such stresses. Modern industrialization and developmental processes have increased the release of toxic substances like heavy metals, carbon dioxide, carbon mono oxide and other harmful gasses into the environment. Release of harmful and hot gasses increased the temperature of the world, thus creating a havoc for the survival of economically important plants. Therefore, an extensive attention is needed to reduce and control such stresses for different economically important crops.

Abiotic stresses like metal toxicity, salinity, drought and high and low temperature are very hazardous to all the agricultural and economically important plants. Such extreme stresses adversely affect the physiological and biochemical activities and result in an over production of highly reactive oxygen species (ROS). These toxic species directly react with nucleic acid, proteins and lipids, which causes the peroxidation of these lipids and start the injury of membranes, protein destruction, inhibition of enzymes and disturbing the pigments of plants bodies under severe heat stress (Davis, 1987). Much increase in membrane destruction is experienced due to lipid peroxidation under heat stress. Lipid peroxidation is the most determined consequences of reactive oxygen species activity on function and structure of cell membranes content (Blokhina *et al.*, 2003). MDA content represents the lipid peroxidation under heat stress (Davidson and Schiestl., 2001; Larkindale and Knight. 2002; Vacca *et al.*, 2004). Antioxidant defence is a multiple enzymes system contribute in the scavenging of ROS in plants. SOD scavenge the O_2 in the first step of ROS metabolism.

The activity of SOD shows the amount of O_2 and H_2O_2 and play an important and central role in the defence system of plants (Bowler *et al.* 1992).

Temperature stress is one of the contributing factor in limiting of economically important plants growth and their yield. Stress caused by heat also adversely affect the plant growth, photosynthetic activities, destroy the cell membranes and even causes the death of cells of plants (Xu *et al.*, 2006). Under high temperature stress the cell and membrane injuries occur due to the formation and over production of reactive oxygen species commonly called ROS, like radicals of hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and supper oxides (O_2^-) (Mittler, 2002). High amount of different types of antioxidant enzymes involve in the detoxification of these reactive oxygen species. The regulation and control of ROS is carried out by many enzymes, which performs the activity of scavenging of these reactive oxygen species. These enzymes are SOD (Superoxide dismutase), POD (Peroxidases), APX (Ascorbate peroxidases), CAT (Catalases), GPX (Glutathione peroxidases) and GR (Glutathione reductases). Similarly, glutathione, carotenoids and ascorbate act also as non-enzymatic antioxidants (Yin *et al.*, 2008).

High temperature stress results in destruction of chlorophyll *a* and *b*. Such adverse effect of high temperature on photosynthetic activities are due to the production of toxic oxygen specie (Camejo *et al.*, 2006). The chlorophyll contents and stomatal performance are adversely affected by high temperature stress in different plant species due to reduction in rubisco activity Morales *et al.*(2003). Severe heat stress generally reduces the time of developmental processes which results in decrease carbon assimilation activities such as transpiration and photosynthetic activities. Physiological activities of photosynthetic pigments such as chlorophyll *a* and *b* are sensitive to high temperature stress (Stone,2001). High temperature stress affects the photosynthesis of C3 plants as compare ton C4 plants. It changes the activities of carbon metabolic enzymes specially rubisco and also change the rate of RUBP production disturbance of electron transfer and inhibition of the enzymes which release oxygen in PSII (Salvucci and Crafts-Brandner, 2004b).

Heat shock is produce by severe heat stress which reduces the photosynthetic activity of chlorophyll pigments (Todorov*etal.*,2003).Shoot dry weight, fresh weight, relative water content and total absorption rate in maize plant are significantly decreased however the leaf area of sugarcane is affected in small amount under high heat stress (Ashraf and Hafeez, 2004; Wahid *et al.*,2007).

The present study was carried out to determine the effect of heat stress on growth, physiology and biochemistry of wheat.

Material and methods

Uniformly sized seeds of *Triticum aestivum* L. were obtained from Agricultural Research Centre, Sarai Naurang District Lakki Marwat Pakistan. Sterilization of seeds was performed by rinsing in 0.2% mercuric chloride solution. Sowing of seeds was made in plastic pots (8 × 12 cm^2) filled with a mixture of clay and sand (1:1) in a glass house in the Department of Botany, University of Science and Technology, Bannu. After 24 days of germination all the pots of wheat plants were kept in growth chamber on four different temperatures Control 25°C, T1- 45°C, T2- 40°C, T3- 35°C). After 24 hours all the plants were harvested and analysed for growth, physiology and biochemistry.

Plant growth analysis

The growth of plants was analysed by determining the fresh weight (FW) and dry weight (DW) of plant leaf tissues using a digital balance. The DW was observed by heating the samples in a hot air oven at 60 °Cfor 48 h.

Determination of leaf relative water content (LRWC %)

First the leaf fresh weight, dry weight and turgid weight were determined using a digital balance. After that the following formula was used to determine the leaf relative water content % according to method of Gao (2000).

LRWC = FW-DW/TW-DW × 100

Where,

FW=Fresh weight

DW=Dry weight

TW-Turgid weight

Determination of chlorophyll content

Chlorophyll was extracted using80% acetone, the absorbance of chlorophyll extract was recorded at 663 and 645 nm and chlorophyll content was calculated according to Arnon (1949). Leaf tissue (0.1 g) was homogenized in 80% acetone using mortar and pestle. Homogenate was centrifuged at 10,000 x g for 10 min. Pellet was re-homogenized in 80% acetone.

The process was repeated till colourless pellet will be obtained. Absorbance of supernatant was recorded at 663 and 645 nm on a UV-visible spectrophotometer.

Assays for ROS scavenging and antioxidant enzymes

For the determination of various antioxidant enzymes' activities, 0.5 g leaves were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.8) with mortar and pestle. The crude enzyme extract was twice centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatants were analyzed for enzyme activities.

Peroxidase (POD) activity was determined according to Zhou and Leul (1998) using guaiacol as the substrate in a total volume of 3 ml. The reaction mixture consists of enzyme extract, 50 mM potassium phosphate buffer (pH 6.1), 1 % guaiacol, 0.4 % H_2O_2 rise in the absorbance due to oxidation of guaiacol was measured at 470 nm. Enzyme activity was calculated in terms of absorbance on 470 nm per g FW per min at 25 ± 2 °C.

Ascorbate peroxidase (APX) activity was determined using the method of Nakano and Asada (1981). The reaction mixture made as 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 2% H2O2, and 0.1 mL enzyme extract in a final volume of 3 mL. The reduction in absorbance at 290 nm for 1 min was noted and the extent of ascorbate oxidized was calculated using extinction coefficient (ε = 2.8 mM-1 APX was defined as 1 mmol mL-1 per min at 25°C. cm-1). One unit of ascorbate oxidized as 1 mmol mL-1 ascorbate oxidized per min at 25°C.

Catalase (CAT) (EC 1.11.1.6) activity was determined by the method of Daud *et al*., (2014). Concisely, the loss of H2O2 was observed by assessing the reduction in absorbance at 240 nm (E = 0.036mM−1 cm−1) of a reaction mixture consisting of 25mM potassium phosphate buffer (pH 7.0), 10mMH2O2, and enzyme extract. The final activity was stated as Ug−1 FW.

Determination of oxidative stress markers (MDA)

Lipid peroxidation in the form of MDA contents were determined by a standard protocol. MDA contents were determined by the methods of Daud *et al*., (2014). 1 ml of protein extract was added to 2 ml of a reaction mixture containing 10 % (v/v) trichloroacetic acid and 0.5% (v/v) thiobarbituric acid. Whole mixture was retained in a water bath at 95 °C for 30 min and then directly shifted to an ice water bath. After 15 minutes, the mixture was centrifuged at 10,000 g for 10 min, the absorbance of the supernatant was read at 532 and 600 nm. Non-specific absorbance at 600 nm was deducted from that at 532 nm. The following formula was applied to calculate malondialdehyde content using its absorption coefficient (ε) and expressed as nmolmalondialdehyde g−1 fresh mass following the formula: MDA (nmol g-1 FM)= [(A532-A600) ×V×1000/ε] ×W where ε is the specific extinction coefficient (=155 mM cm−1), V is the volume of crushing medium, W is the fresh weight of leaf, A600 is the absorbance at 600 nm wavelength and A532 is the absorbance at 532nm wavelength.

Statistical analyses

Analysis of variance technique (one-way ANOVA) was used for statistical analysis and the least significant difference (LSD) test (Steel and Torrie, 1984) was applied for comparison of all the treatments means. Similarly, students Statistics (version 8.1 USA) was used for determination of Coefficient of correlation.

Results and discussion

Current progress in manufacturing sector and globalization has resulted in environmental pollution and raised the temperature of the earth as well as air environment which is very harmful and severely affect the physiology of plants like photosynthesis, respiration, plant biochemistry, plant water relationship and therefore causes a reduction in yield of economically important plants.

Table 1. Effect of heat stress on physiological Growth attributes wheat (*Triticum aestivum* L.).

Treatments	LRWC	Leaf fresh weight(g)	Leaf dry weight(g)
C	44.423±0.331^{a}	0.27±0.033^{a}	0.09±0.023^{a}
T1	19.447±0.033^{d}	0.11±0.032^{c}	0.02±0.026^{d}
T2	25.653±0.328^{c}	0.16±0.033^{b}	0.05±0.057^{c}
T3	29.220±0.057^{b}	0.21±0.033^{b}	0.07±0.023^{b}

English Alphabets represents statistical difference among treatments. Treatments: C- Control (25°C), T1- 45 °C, T2- 40°C, T3- 35°C.

Therefore, it is necessary to determine the solution to avoid economically important plants from toxic effects of stress caused by temperature and heat.

In our present findings leaf fresh and dry weight of wheat were much decreased by the high temperature stress (Table 1). It showed that wheat plants are sensitive to high temperature stress. These results agree with previous findings of Wahid (2007), who have reported that high temperature stress reduce the fresh weight and dry weight of plants.

Relative water content in plants was significantly reduced by severe drought and temperature stresses (Xu and Huang, 2012). Drought and heat stress are said to be have a key role in water deficit of plants. In our investigation, the adverse effect of heat stress at all the treatments was clear on the leaf relative water content of wheat plants and was significantly decreased by heat stress (Table 1).

It is evident from our findings that wheat leaves are sensitive to a very high heat stress.

These results are like the previous findings of Ivanov *et al.* (2001) who have reported that severe temperature stress causes water deficit due to decrease absorption of water by roots of plants as stressed plants have strong water shortage. Results presented in Fig.1and 2 showed that chlorophyll *a* and *b* contents were significantly reduced by heat stress at higher concentration (T1- 45 °C) and moderately higher concentration (T2- 40°C) as compared to control. However lowest concentration (T3- 35°C) has no significant effect on chlorophyll *a* content of wheat as compared to control

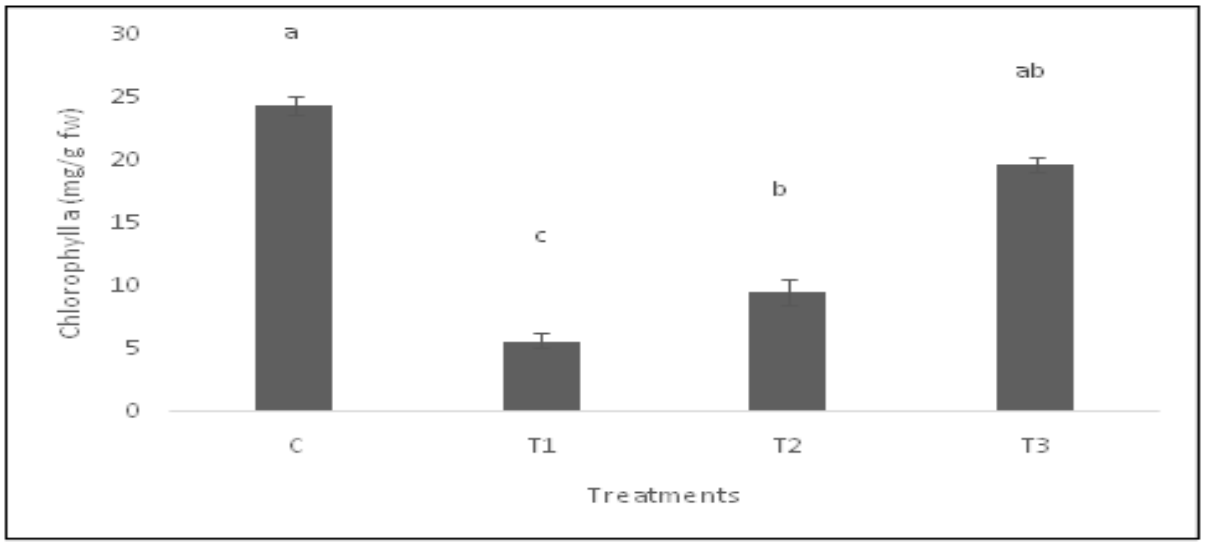

Fig. 1. Effect of heat stress on Chlorophyll *a* of wheat.

The harmful effect of higher concentration of heat stress was evident on both the chlorophyll *a* and chlorophyll *b* contents of wheat plants.

This decline may be due to the damage of chloroplast structure. Kumar *et al.*,(2012) reported that high temperature stress resulted in chlorophyll *a* and *b* contents of rice, when they were treated with high temperature stress.

In our findings, high temperature stress (both T1 and T2) resulted in decrease amount of chlorophyll *a* and *b* contents of wheat. However, the lowest temperature stress (T3) has no adverse effect on chlorophyll contents of wheat plants.

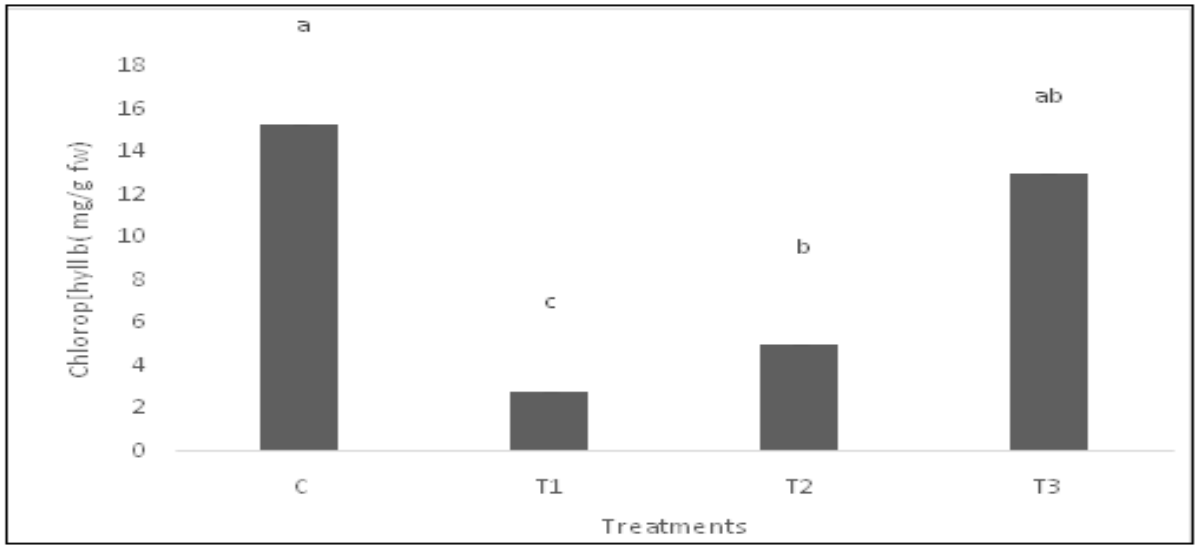

Fig. 2. Effect of heat stress on chlorophyll *b* of wheat.

These results clearly show that chlorophyll damage is directly linked with the sensitivity of wheat plants to a high temperature stress with respect to their chlorophyll contents. These results are similar to the previous findings of Jagtap *et al.* (1998), who have reported that chlorophyll content was declined in *Sorghum biolor* L when it was exposed to a higher temperature stress.

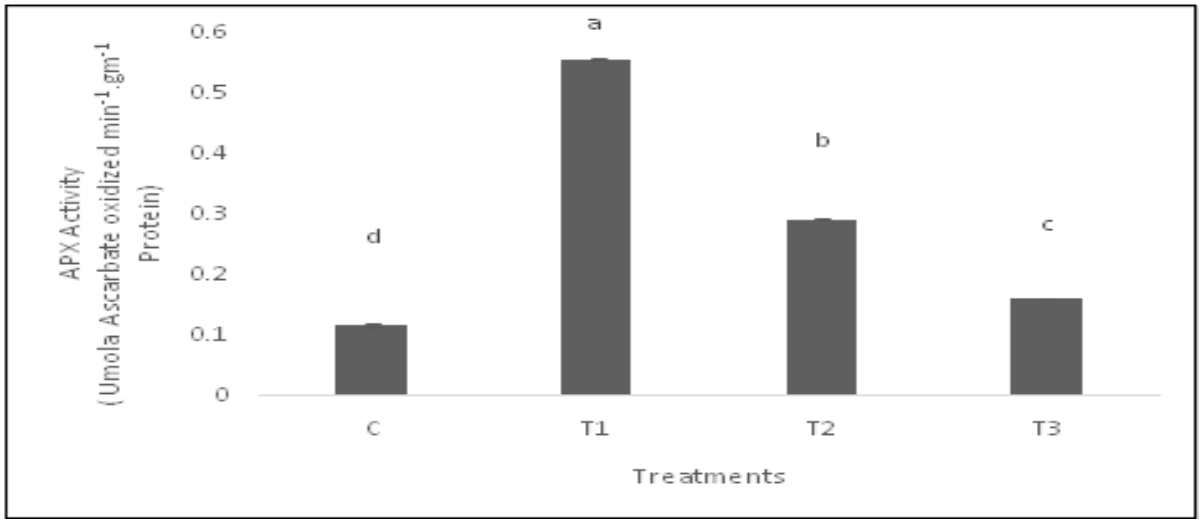

Fig. 3. Effect of heat stress on APX activity of wheat.

Fig. 3 represents that APX activity is directly proportional to increase in temperature. APX activity is significantly increased by all the temperature concentrations as compared to control. The lowest heat stress has also increased the APX activity of wheat over control. Which shows that increase in temperature stress up to a specific level results in higher APX activity. In our findings, it was showed that there was a gradual increase in APX activity with increase in heat stress. He (2010) reported that in chloroplast of plants enhanced H2O2 scavenging reactions causes the production of higher amount of APX enzymes.

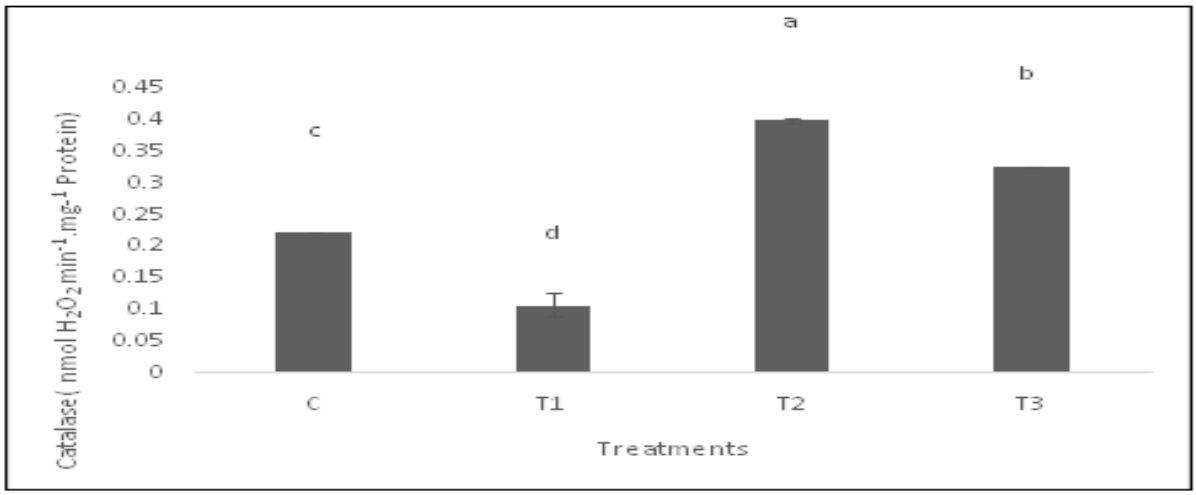

Fig. 4. Effect of heat stress on (CAT) catalase activity of wheat.

Production of APX enzymes against heat stress was lowest at 35 Ċ, but it was much increased at temperature of 45 Ċ. Which showed that APX activity become increased to avoid the plants from damage by ROS, when plants are facing severe stress. These results are like the previous findings of Almeselamni *et al.* (2006) who have reported that high amount of APX is produced in wheat plants when they are exposed to a high temperature stress.

Fig. 4 represented that catalase activity is significantly decreased at higher concentration of heat as compared to control. However, T2 and T3 significantly increased the catalase activity of wheat as compared to both control and T1. It showed that higher temperature stress may results in inhibition of catalase activity of wheat plants. Present study showed that the CAT activity was prominently supressed at high temperature stress, which may be due the accumulation of higher amount of MDA at higher temperature stress.

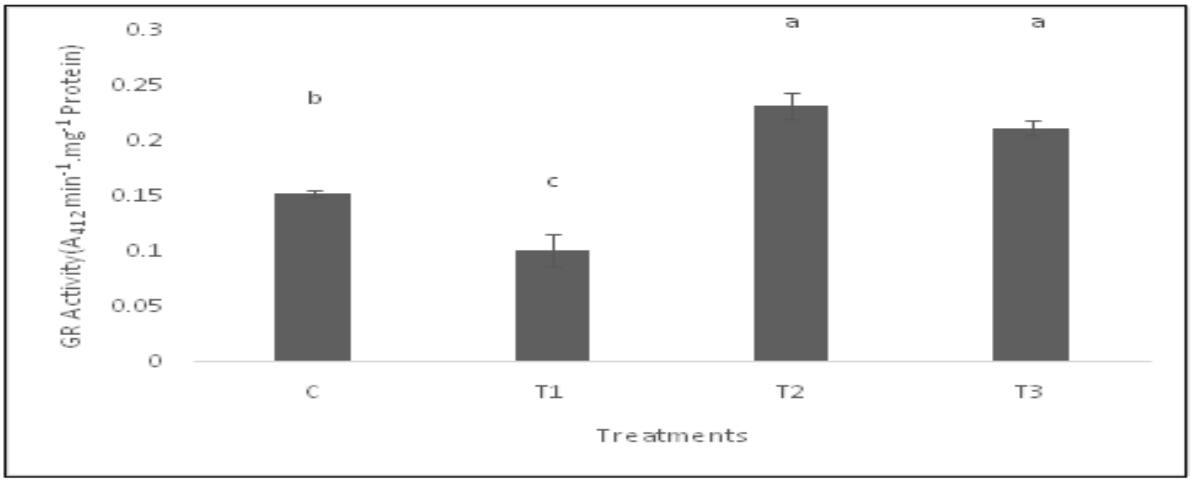

Fig. 5. Effect of heat stress on GR activity of wheat.

These results are in agreement with previous findings of Zhao *et al.* (2010) who have reported that decreased activities of CAT were closely related to MDA accumulation. In our findings, it was also clear that CAT activity was much increased at moderately high temperature stresses (T2- 40 °C, T3- 35 °C). These results are similar to previous findings of Kumar *et al.* (2012) who have reported that CAT activity was increased in wheat plants, when they were exposed to moderately high temperature stress.

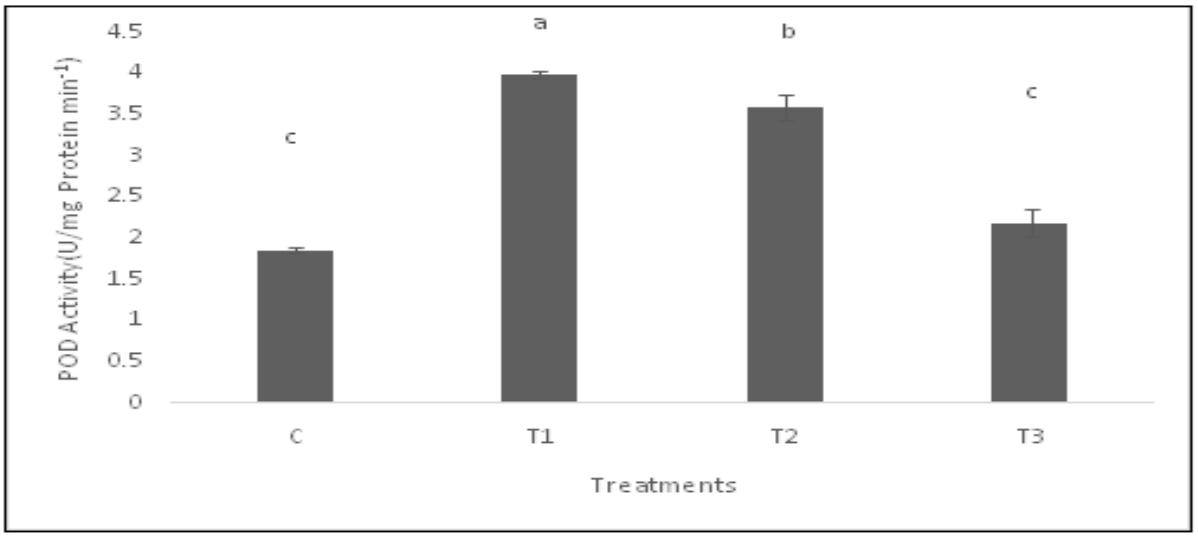

Fig. 6. Effect of heat stress on POD of wheat.

Results presented in Fig.5 showed that GR activity of wheat plants is significantly decreased by higher concentration of temperature (T1-45 Ċ) as compared to control. However, the effect of both the T2 (40Ċ) and T3 (35 Ċ) was prominent and significantly increased the GR of wheat plants as compared to control and higher concentration of heat stress (T1-45 Ċ).

On exposure to a high temperature stress the increase and then reduction of GR activity in apple leaves was reported by Ma *et al.* (2008). In our findings, the activity of GR was significantly reduced at very high temperature stress (T1- 45 °C), which may be due to the sensitivity of GR to a very high temperature stress. However, the effect of moderately high temperature stresses (T2- 40 °C, T3- 35 °C) exhibited an increase in GR activity. These results are similar to previous findings of Locatto *et al.* (2009) who have reported that GR activity increased in the cells of tobacco when they were exposed to moderately high temperature stress.

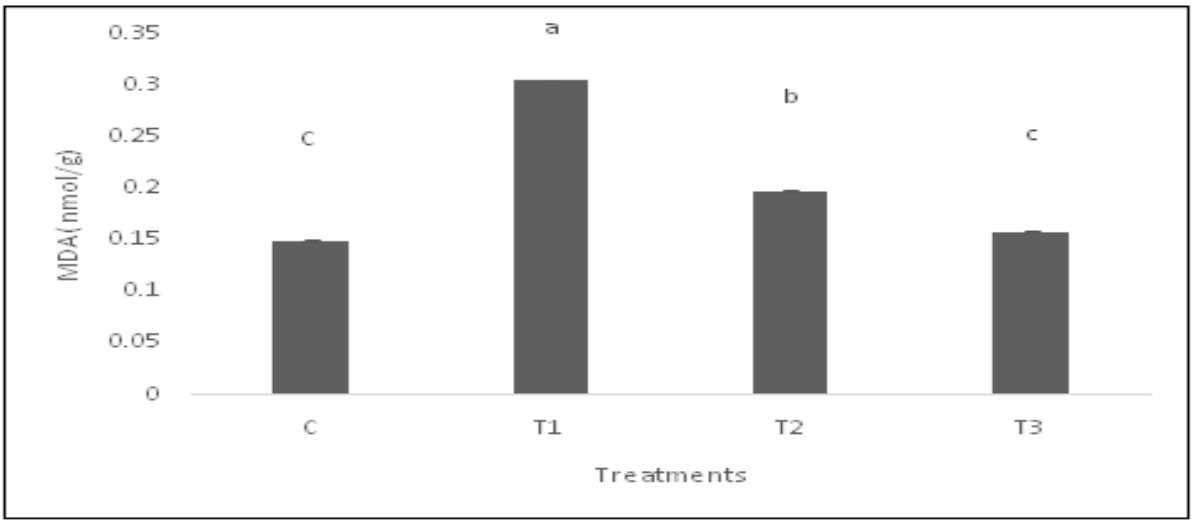

Fig. 7. Effect of heat stress on MDA of wheat.

Results presented in Fig.6 showed that all the treatments of temperature have significantly increased the POD activity of wheat as compared to control. It means that increase in temperature not above the lethal range results in increase POD activity of wheat plants Antioxidant enzymes like POD, SOD and APX play an important role in avoiding the cells from injury and detoxification of reactive oxygen species, which are produced because of severe stresses Ge *et al.*, (2005). Present study showed the higher production of POD in response to increase in temperature stress. These results are in agreement with previous findings of Liu and Huang (2000) who have reported increase activity of POD in response to heat stress in plants.

Results presented in Fig.7 showed that higher temperature (T1- 45 °C, T2- 40°C) significantly increased the MDA level of wheat as compared to control. It also showed that increase in temperature up to higher concentration results in significant increase in MDA level. However, the lower concentration of heat has no significant effect on the MDA level as compared to control.

Lipid per oxidation results in release of MDA and causes cellular damage, such activities are then controlled by the action of antioxidant enzymes like APX and POD, which regulates and reduce the ROS level Ge *et al.*,(2005).

Present study showed that higher temperature stress resulted in much enhancement of MDA production in wheat leaves.

These are in agreement with previous findings of Nagi and Devarai (2011) who have reported that higher amount of MDA is produced in horse gram when they are subjected to high temperature stress.

Conclusion

Present investigation resulted in the conclusion that a very high temperature and severe stress adversely affect the growth indices such as fresh weight, dry weight and relative water content of wheat plants. Chlorophyll *a* and chlorophyll *b* contents of wheat were observed to be more sensitive at higher temperature induced stress.

However, enzymes responsible for scavenging of ROS (APX, POD, CAT and GR) exhibited increase production against lower and moderately high temperature stresses, but all the above antioxidant enzymes showed significant decrease under severe temperature stress (T1- 45 °C, T2- 40°C). Lipid peroxidation in the form of MDA level was significantly increased under higher temperature stresses, however lower temperature stress had no significant effect on MDA level of wheat.

Further studies are required to determine the basic molecular mechanism behind the stress tolerance and sensitivity of wheat against different heat stresses.

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