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Full Length Research Paper

Protection against heat stress in wheat involves change in cell membrane stability, antioxidant enzymes, osmolyte, H_2O_2 and transcript of heat shock protein

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Terminal heat stress causes an array of physiological, biochemical and morphological changes in plants, which affect plant growth and development. In present investigation, real time quantitative expression analysis of HSP90 gene in root showed a maximum of 2.5 fold increase in the transcript level during seed hardening stage. Similarly, in flag leaf 4.5, 4.3 and 6.5 fold increases in the transcript level were observed during pollination, milky dough and seed hardening stages, respectively. A decrease in the cell membrane stability (from 70 to 65%) was observed with growth and development of wheat. An altered expression of H_2O_2 was observed with highest expression at milky dough stage (0.9 µg/g fresh weight). The highest accumulation of H_2O_2 was observed in response to heat shock of 42°C for 2 h. There was a remarkable decline in proline quantity at different stages of growth with lowest accumulation at seed hardening stage. Under differential heat shock, the highest activity of SOD and CAT were observed in response to heat shock of 40° and 35°C for 2 h. The results from this study suggest a potential role for antioxidant enzymes in the reduction of elevated levels of H_2O_2 in wheat plants grown under heat stress condition.

Key words: Antioxidant enzymes, wheat, heat stress, cell membrane stability, proline, hydrogen peroxide.

INTRODUCTION

Plant growth and yield are adversely affected by abiotic stresses such as high or low temperatures, drought, salinity etc. Among abiotic stresses, heat stress influences photosynthesis, cellular and subcellular membrane components, protein content in cell and antioxidant enzyme activity; thereby significantly limits crop production. Heat stress also induces oxidative stress in plants caused by the generation and the accumulation of super-oxides (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-), which are commonly known as reactive oxygen species (ROS).

Although the daily average temperature for optimal growth conditions of wheat is 22 to 25°C, high temperature reduces the vegetative growth and seed setting in wheat. Changes in ambient temperature occur within hours, unlike drought and salinity stresses. Therefore, plants need to suppress and respond to the adverse effects of heat in a very short time. Gradual temperature increase in a day could cause some alterations in antioxidant metabolism or in other physiological responses and leads to increase in the expression of heat shock proteins (HSPs). HSPs are molecular chaperones which help the plant to tolerate the extreme heat shock

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Abbreviations: HSP, Heat shock protein; **SOD,** superoxide dismutase; **CAT,** catalase; **HS,** heat Stress.

Table 1. List of primers used for quantitative real time PCR (qRT-PCR) amplification, Prime3 software was used to design primer.

Primer	Sequence (5'- 3')	Tm value (°C)
[a]Tub- F_q	TGCATGATCTCCAACTCCACCAGT	62.7
[a]Tub-R_q	TCGTCGAACTCAGCACCAACTTCT	62.7
[b]Hsp-90F_q	TGATGATGGGTGGACTGCCAACAT	62.7
[b]Hsp-90R_q	TCTCGAAGAGCAGCATCACAAGGT	62.7

[a]Tub-Tubulin, [b]HSP-Heat Shock Protein

condition by protecting the native proteins from denaturation. Different HSPs like sHSP, HSP23, HSP70, HSP90, HSP101 etc. have been reported in different plants and non-plant species. HSP90 is an essential heat shock protein found in all eukaryotes studied so far. This protein is known for its role in facilitating maturation of signaling molecules, in managing protein folding, cell cycle control, protein degradation and protein trafficking. The expression of HSP90 has been observed to change with the environmental conditions and has remarkable effect on the total thermotolerance capacity of the plants.

Improving tolerance to heat stress is a major challenge in many C_3 crops given the threat of global warming. Heat stress leads to drastic change in the cell membrane stability and ultimately influences the sensors present in the membrane. Most of the earlier studies on the effects of multitude of abiotic stresses showed changes in the level of several physiological parameters including lipid peroxidation, H_2O_2 production and proline accumulation in wheat. Proline has a role in the protection of plants by acting as a cellular osmotic regulator between cytoplasm and vacuole (Bandurska, 1993; Bohnert and Jensen, 1996). However, it has also been suggested that over accumulation of proline could be toxic to plant cells (Rizhsky et al., 2004). H_2O_2 production is thought to be increased under various abiotic stresses to enhance gene expression of active oxygen scavenging (AOS) enzymes. H_2O_2 was also reported to induce small heat shock proteins (HSP26) in tomato and rice (Liu et al., 1999). H_2O_2 may play an important role in signal transduction for abiotic stress tolerance, although H_2O_2 is toxic at high concentrations. H_2O_2 was implicated as an elicitor of several genes related to stress tolerance.

The dismutation of superoxide radicals into H_2O_2 and oxygen is an important step in protecting the cell and in that conversion SOD and CAT is considered to be the key enzymes. The levels of APX and CAT were observed to be increased in the transgenic rice probably due to generation of H_2O_2 by over expressed SOD (Clement et al., 2011).

The response mechanism of wheat to elevated temperature would help the development of wheat cultivars that perform better under heat stress. However, there is a limited amount of information about the effects of heat in wheat.

The purpose of this study was to analyze the change in cell membrane stability, antioxidant enzyme activity, H_2O_2 production, proline accumulation and transcript level of HSP under differential heat shock in wheat at different growth stages.

MATERIALS AND METHODS

Wheat cultivar (C306) were grown in net house as well as in growth chamber inside Phytotron under controlled conditions with optimum temperature regime of 26/22°C, humidity of 60%, photoperiod of 16 h and light intensity of 350 μmol/m^2/s. C306 cultivar of wheat was selected because of its thermotolerance nature and popularity in North India.

Stress treatments

Plants were randomly divided into four groups (3 groups for HS treatments and 1 group for growth stage specific analysis) at seedling stage (10 days old), and heat stress treatments were applied at 30°C (mild heat stress), 35°C (moderate heat stress) and 40°C (extreme heat stress). For mild heat stress, the plants in the growth chamber were exposed to a gradual temperature increase from 25 to 30°C with an increment of 1°C/10 min. After temperature reached at 30°C, plants were kept at 30°C for 2 h. the same method of stress treatment was applied for moderate and extreme heat stress plants and the treatment was given for 2 h. After each stress treatment, leaf samples were harvested and immediately suspended in liquid nitrogen for subsequent analyses. Samples were also collected from net house at different stages of growth.

RNA extraction and quantitative real time PCR (qRT PCR)

Total RNA was extracted using the Millipore RNA isolation system (Genetix, UK) and quantification was done using QubitTM 2.0 fluorometer (Invitrogen). RNA integrity was verified in 1.2% agarose gels. First strand cDNA synthesis was performed using oligo dT primers and the Superscript II reverse transcriptase (Invitrogen, UK) according to the manufacturer's instructions. First-strand cDNA was diluted to a final concentration of 1.0 ng μl-1. Primers for quantitative Real Time PCR reactions were designed from the deduced sequence corresponding to the wheat hsp90 gene using Prime 3 primers Designing software (Premier Biosoft, USA) (Table 1). For each stress condition as well as for controls, expression measurements were performed using duplicate biological replications and three technical replications. Quantitative PCR was performed in 25 μl reactions using gene specific primers, 1 μl of cDNA as template and the SYBRGreenER qPCR SuperMix Universal (Invitrogen, UK). Reactions were performed on the CFX96 Real- Time PCR system (Biorad, UK). The thermal

profile for qPCR was: 3 min at 95°C, followed by 35 cycles each consisting of 95°C for 15 sec, 60°C for 30 sec and 72°C for 15 sec. The primer specificity and the formation of primer-dimers were monitored by dissociation curve analysis and agarose gel electrophoresis on a 3% agarose gel. The expression levels of wheat tubulin gene were used as internal standards for normalization of cDNA template quantity using tubulin-specific primers (GenBank accession no U76558) as shown in the Table 1. Data analysis was performed using software provided by BioRad, UK. The Comparative Ct (2-ΔΔCt) method was used to calculate the changes in gene transcript as a relative fold difference between an experiment and calibrator sample.

Evaluation of cell membrane stability (CMS)

Samples with similar leaf size were selected at vegetative (8 days old seedlings), pollination, milky dough and seed hardening stages and used to measure cell membrane stability (CMS) using the method of Fokar et al. (1998) with some modifications.

Measurement of H_2O_2 and proline concentration

The amount of H_2O_2 was quantified as described by Loreto and Velikova (2001). Briefly, leaf samples (0.3 g) were homogenized in 3 ml of 1% (w/v) trichloroacetic acid (TCA). The homogenates were centrifuged at 10,000 × g (4°C) for 10 min. Subsequently, 0.75 ml of the supernatants was added to 0.75 ml of 10 mM Potassium phosphate buffer (pH 7.0) and 1.5 ml of 1 M potassium iodide (KI). After that, H_2O_2 content of the supernatant was evaluated by comparison of the absorbance values at 390 nm to a standard calibration curve standard in the range from 10 to 200 nmol/3 ml cuvette. H_2O_2 concentration was expressed as µmol/g FW. Proline contents were determined according to the slightly modified method of Bates et al. (1973). The concentration of proline was calculated using a standard curve.

Antioxidant enzyme activity assay

Leaf materials (1 mg) were ground in 6 ml of ice cold 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM sodium-EDTA and 1% (w/v) polyvinyl–polypirrolidone (PVP). The homogenates were centrifuged at 10,000 × g (4°C) for 10 min. The tissue extracts were either stored at -78°C or immediately used for subsequent analyses of superoxide dismutase and catalase. For the quantification of soluble protein content, coomassie blue dye-binding assay was used (Bradford, 1976). Bovine serum albumin (BSA) was used for the preparation of the standard curve. 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 (Giannopolitis and Ries, 1977). One unit of enzyme activity was determined as the amount of the enzyme needed for the inhibition of 50% NBT reduction rate by monitoring absorbance at 560 nm with spectrophotometer.

Activities of catalase enzyme were measured as described by Chance and Maehly (1955). For assaying CAT activity, the decomposition of H_2O_2 was followed by decline in the absorbance at 240 nm. CAT activity was determined by following the consumption of H_2O_2 (extinction coefficient, 39.4 mm-1 cm^{-1}) at 240 nm over a 3 min interval.

Statistical analysis

The experiment was conducted in a completely randomized design. The uppermost fully expanded leaves were collected randomly as three replicates for each treatment. Data were analyzed using one-way analysis of variance (one-way ANOVA). The standard errors were given in histograms.

RESULTS

Analysis of fold change in expression of HSP90 gene RNA isolated from root as well as flag leaf collected at vegetative, pollination, milky dough and seed hardening stages were subjected to quantitative RT-PCR analysis using HSP90 gene specific primers in order to study the effect of change in H_2O_2 and proline on the transcript level *of HSP90* gene. Expression profiling of *HSP90* gene in root collected from different stages showed 1.5 (polR), 1.2 (MD_R) and 2.5 (SM_R) fold increase in the transcript level at pollination, milky dough and seed hardening stage compare to vegetative stages (Figure 1a). The highest change in fold expression of *HSP90* gene in root was observed during seed hardening stage. However, not much significant difference in the expression was observed in the root during pollination and milky dough stage. Flag leaf collected at different stages of growth showed 4.5 (polL), 4.3 (MD_L) and 6.5 (SM_L) fold increases in the transcript of *HSP90* gene during pollination, milky dough and seed hardening stages (Figure 1b). The maximum expression was observed during seed maturation stage. Since, terminal heat stress is one of the major factors causing severe damage to the total yield of wheat crop and pollination and milky dough stages are more prone to terminal stress. Hence, in order to characterize the expression of *HSP90* gene in these two stages of growth against differential HS, flag leaf was collected from the respective stages for RNA isolation and quantitative RT-PCR analysis. The transcript level was very high in response to differential HS treatment. A 5 fold increase in the transcript of *HSP90* gene was observed in samples collected at pollination stage in response to HS of 35°C for 2h compare to control (22°C). Further, 4 and 5.5 fold increases in the transcript was observed against HS of 35° and 40°C for 2h (Figure 1c). During pollination stage, *HSP90* was more responsive to HS of 40°C for 2 h. Similarly, samples collected from milky dough stage after giving differential HS showed 4.2, 4.4 and 5 fold increases in the transcript of *HSP90* gene against HS of 30, 35 and 40°C for 2 h (Figure 1d). The abundance of *HSP90* transcript from pollination to milky dough showed 0.8 times decrease against HS of 30°C,1.1 times increase against 35°C and 0.9 times decrease against HS of 40°C for 2 h.

Cell membrane stability index

The cell membrane stability (CMS) of C306 cultivar of wheat was estimated at different stages of growth using the method of Fokar et al. (1998). The CMS was observed highest (70%) at vegetative stage when the

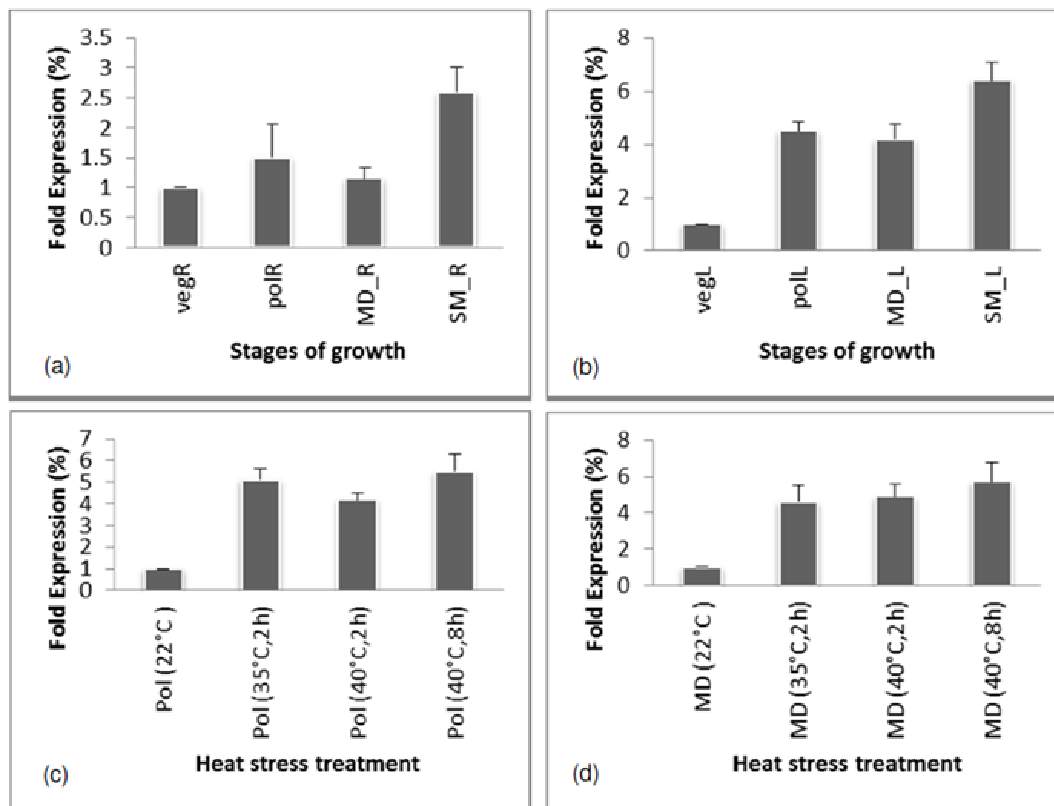

Figure 1. Comparative change in fold expression of HSP90 gene in C-306 (thermo tolerant) cultivar of wheat (a) root samples collected at vegetative (vegR), pollination (polR), milky dough (MD_R) and seed hardening (SD_R) (b) leaf samples collected at vegetative (vegL), pollination (polL), milky dough (MD_L) and seed hardening (SD_L) (c) Flag leaf collected at pollination stage after giving HS of 35° and 40°C for 2 and 8h (d) Flag leaf collected at milky dough stage after giving HS of 35° and 40°C for 2 and 8 h.

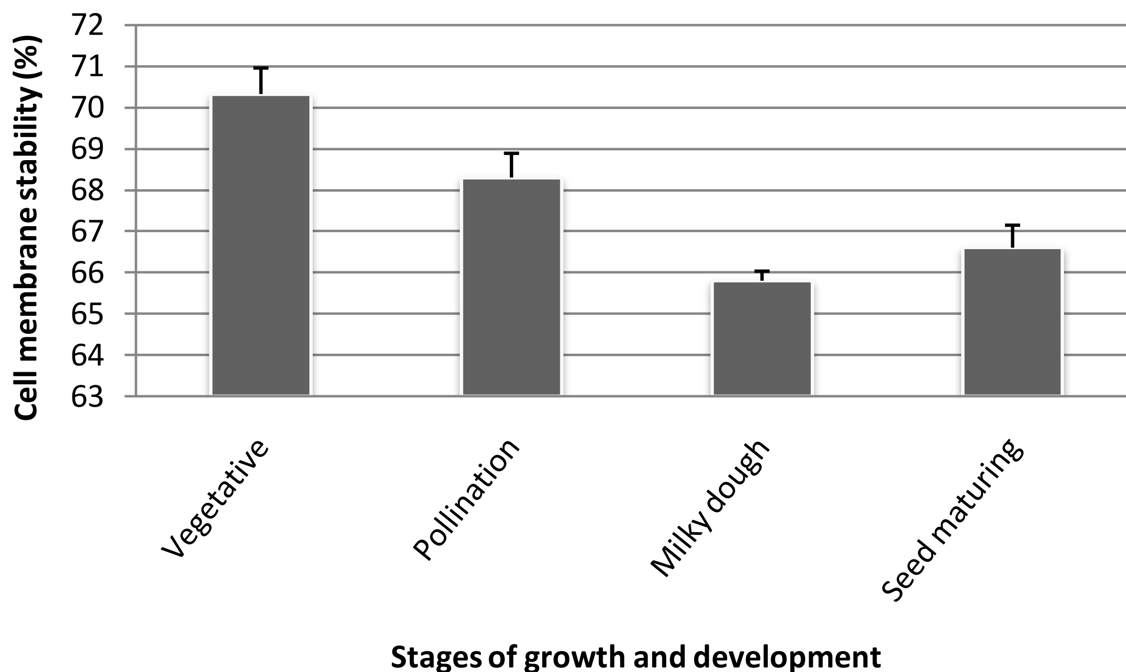

Figure 2. Cell membrane stability index of C306 cultivar of wheat at different stages of growth and development.

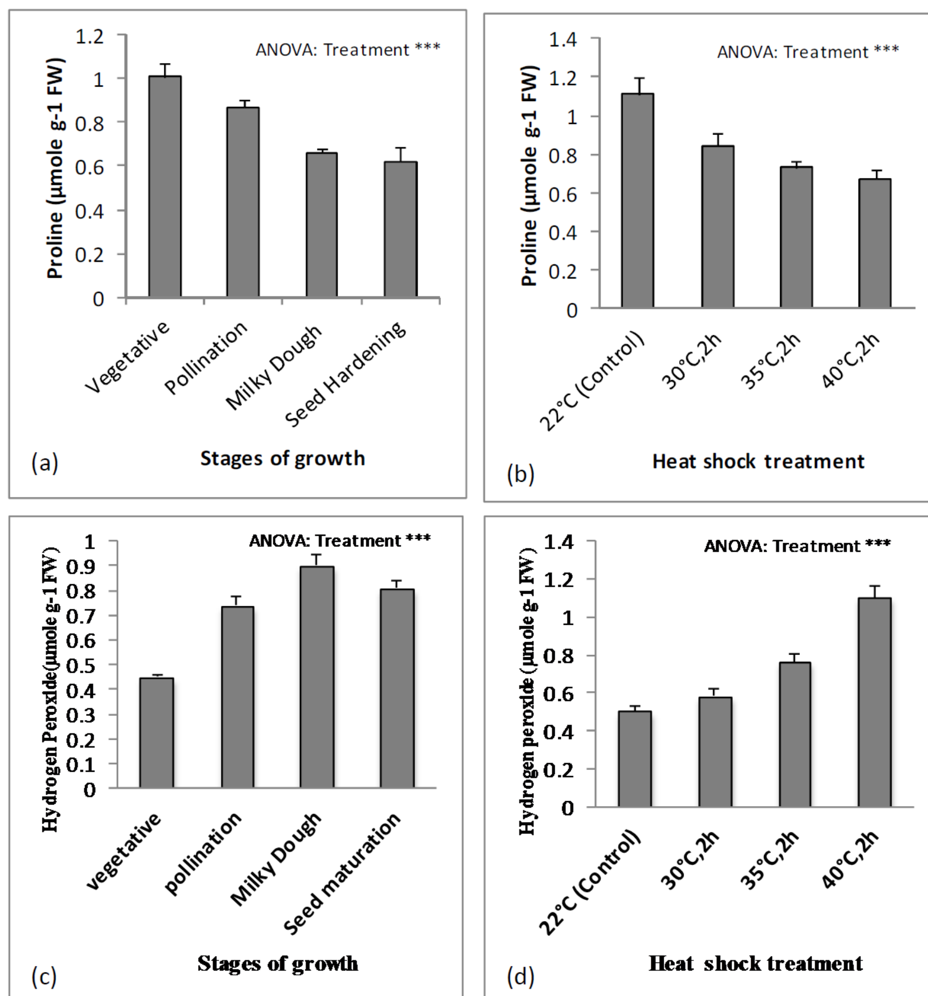

Figure 3. Proline and Hydrogen peroxide accumulation in C-306 cultivar of wheat, (a, c) Proline and Hydrogen peroxide concentration at different stages of growth and (b, d) proline and Hydrogen peroxide accumulation in response to differential HS (30°, 35° & 40°C for 2h) treatment. ANOVA significance levels: *, $P < 0.05$;**, $P < 0.001$;***, vertical bars indicate s.e. (n = 3).

outside temperature was around 25°C (Figure 2). A gradual decrease in the CMS was observed from 68 to 62% at pollination and milky dough stage in response to outside temperature of 33° and 36°C. Further, abrupt increase in CMS (68%) was observed at seed hardening stage when the outside temperature was 39°C. A direct correlation between the outside environmental temperature and CMS has been observed.

Proline accumulation

A very high proline concentration (1.0 µmole/g FW) was observed at vegetative stage when the outside temperature was 25°C (Figure 3a). Further, a decrease in the proline accumulation was observed that is, 0.7, 0.68 and 0.62 µmole/g FW at pollination, milky dough and seed hardening stages when the outside temperature was 33, 36 and 39°C, respectively. A decrease in proline concentration was observed with increase in outside temperature. Proline profiling was also carried out in 8 days old seedling in response to heat shock treatment of 30, 35 and 40°C for 2 h. The proline concentration at 22°C was 1.15 µmole/g FW and further with the differenttial heat shock treatment of 30, 35 and 40°C for 2 H. a gradual decrease in the accumulation of proline (0.9, 0.79 and 0.66 µmole/g FW) was observed (Figure 3b).

Hydrogen peroxide accumulation

A very low concentration of H_2O_2 (0.45 µmole/g FW) was observed at vegetative stage (Figure 3c). A gradual increase in the hydrogen peroxide concentration was

observed from pollination (0.72 µmole/g FW) to milky dough stage (0.9 µmole/g FW). The highest H_2O_2 concentration was observed at milky dough stage which is also considered as one of the most critical stage for wheat grain development. During seed hardening, the hydrogen peroxide concentration was 0.8 µmole/g FW. A decrease in the concentration of the hydrogen peroxide at seed hardening stage may be due to the high activity of peroxide scavenging antioxidant isoenzymes which limits the accumulation of hydrogen peroxide inside the cells. In order to validate the net house experiment regarding the accumulation of hydrogen peroxide at different stages of growth and development, 8 days old seedling grown inside phytotron was given heat shock of 30°, 35° and 40°C for 2 h. A gradual increase in the accumulation of H_2O_2 was observed (0.5, 0.58, 0.78 and 1.1 µmole/g FW) in response to differential heat shock treatment of 22°, 30°, 35° and 40° C for 2 h (Figure 3d). The highest accumulation of H_2O_2 was observed when heat shock of 40°C was given for 2 h.

Superoxide dismutase (SOD) activity assay

The samples collected from C306 variety of wheat after giving differential heat shock treatment inside phytotron were used for SOD enzyme activity assay. A very low specific activity of SOD was observed in control (22°C) which gradually increases from 21 U/mg proteins to 36 U/mg proteins in response to heat shock treatment of 30 and 35°C (Figure 4a). The highest activity was observed when heat shock of 35°C was given for 2 h. A decrease in the activity was observed (31 U/mg protein) in response to heat shock of 40°C for 2 h. The samples collected from net house at different stages of growth and development was subjected to SOD enzyme assay. A continuous increase in the activity of SOD was observed from vegetative (0.05U/h/mg) to milky dough stage (0.1 U/h/mg) and further a decline in the activity was observed during seed hardening stage (0.09 U/h/mg) (Figure 4b).The highest activity was observed during milky dough stage.

Catalase (CAT) activity assay

Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen. In present investigation, an increase in CAT activity was observed in C306 cultivar of wheat collected from net house at different stages of growth and development. The specific activity of CAT at vegetative stage was 4.62 U/min/mg proteins which increased to 9.4 U/min/mg proteins (pollination) and 11.34 U/min/mg proteins (milky dough stage) (Figure 4c). Further, a decrease in CAT activity was observed during seed maturation stage (9.31 U/min/mg proteins). The highest CAT activity was observed during milky dough stage. In order to study the effect of differential heat shock on CAT activity, wheat samples collected from Phytotron was used. A significant increase in CAT activity was observed in response to differential heat shock treatment (Figure 4d). The activity of CAT was 21.35 (22°C), 23.57 (30°C), 31.53 (35°C), and 40.92 U/min/mg proteins (40°C). The highest activity of CAT was observed in response to heat shock treatment of 40°C for 2 h.

DISCUSSION

A change in the level of heat shock protein expression, cell membrane stability, H_2O_2 production, proline accumulation and antioxidant isoenzymes activity in plant cells is an indicator of oxidative stress. An increase in the transcript level of *HSP90* was observed in present investigation in root cells as well as flag leaf (Figure 1); however, abundance of transcript was more in flag leaf compare to root at almost all the stages of growth. Increase in H_2O_2 concentration and decrease in proline accumulation at different stages of growth and development has been reported to have a role in activating the HS transcription factors for inducing the expression of HSPs in response to differential HS. Cell membrane stability, a measure of electrolyte diffusion resulting from heat-induced cell membrane leakage, has been used to screen and evaluate different wheat genotypes for thermal tolerance (Blum and Ebercon, 1981; Saadalla et al., 1990).

Electrical conductivity has been used as an index of membrane stability to identify heat-tolerant genotypes in wheat (Blum and Ebercon, 1981) and for screening of heat-tolerant genotypes in different crops (Blum, 1988). When tissues are subjected to high temperature, electrical conductivity increases due to damage to the cell membrane and consequent solute leakage. In present investigation, a slight decrease in the cell membrane stability was observed with increase in duration of growth and development. The lowest CMS value was observed at milky dough stage. Since, C306 cultivar of wheat is considered as a thermotolerant wheat cultivar; this may be the reason for the little change in the CMS values. Fokar et al. (1998) reported that there was a strong positive association across cultivars between grain weight per ear and cell membrane stability as a measure of heat tolerance, however, membrane stability may be only a small part of the numerous properties and processes that interact to provide tolerance to stress during maturation (Assad and Paulsen, 2002). Since, membrane is the first line of defense having many heat responsive sensors which helps plant to activate its defense mechanism well in advance against heat shock, so the integrity of the membrane is one of the important parameter. The percentage of saturated and un-saturated

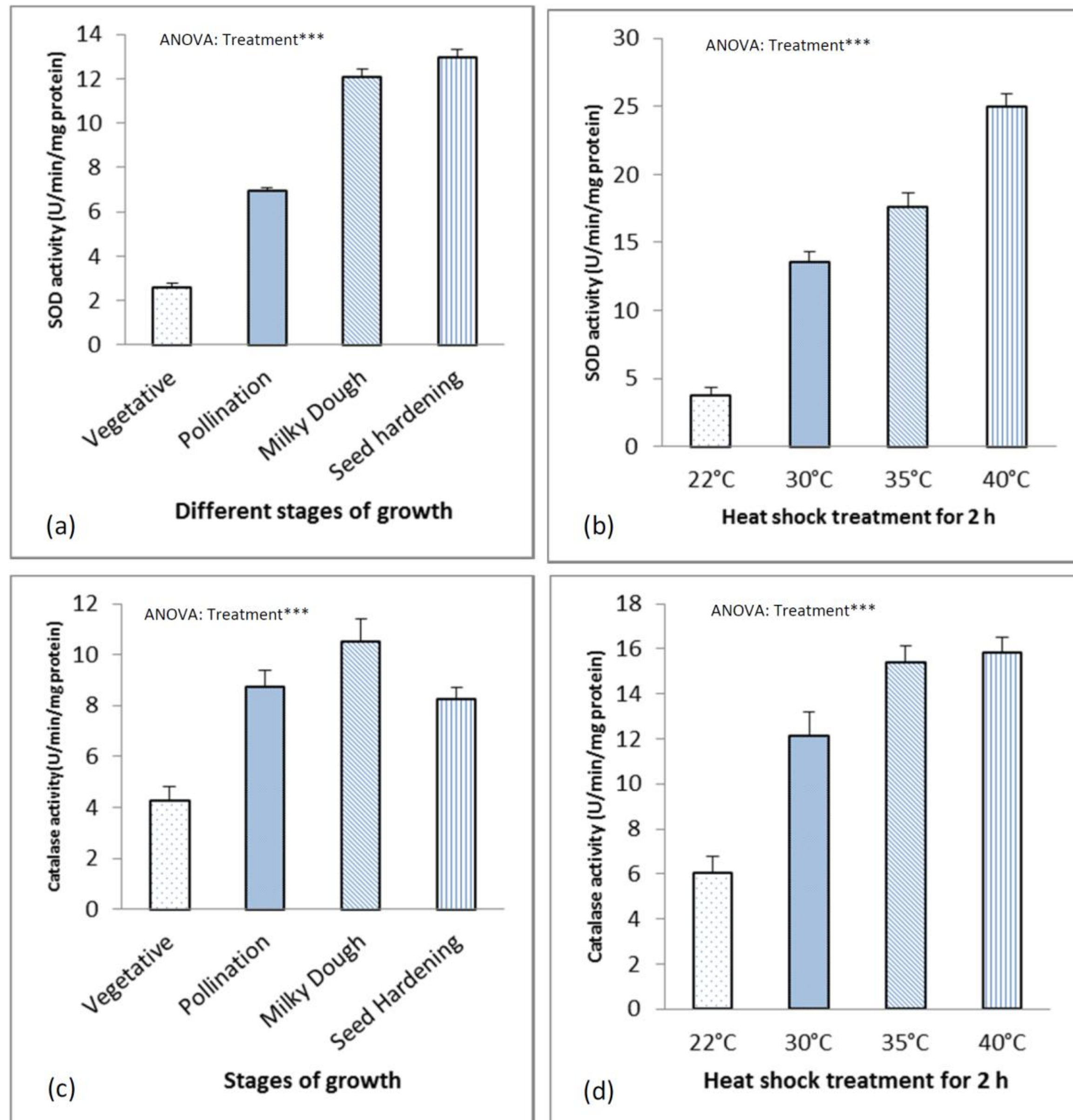

Figure 4. Specific activity of superoxide dismutase (SOD) and catalase (CAT) enzymes in C-306 cultivar of wheat, (a, c) SOD and CAT activity at different stages of growth (vegetative, pollination, milky dough & seed hardening), (b, d) SOD and CAT activity in response to differential HS (30°,35° &40°C for 2h) treatment, ANOVA significance levels:*, $P<0.05$;**, $P<0.001$;***, vertical bars indicate s.e. (n = 3).

fatty acids inside the membrane changes with the type of climate outside. A change in the CMS in response to heat shock at pollination stage is the major issue of concern which needs to be addressed.

Hydrogen peroxide is regularly produced inside the plant system even during their normal metabolic processes, but than the rate of production abruptly increases in response to different abiotic and biotic stresses. Hydrogen peroxide acts as a signaling molecule inside the plant system and it has been considered as the second line of defense in response to heat stress. At the same time the high concentration of hydrogen peroxide is injurious to the plant system and causes the senescence and death of plant cell. An increase in the amount of H_2O_2 is associated with oxidative damage (Sairam and Srivastava, 2000; Larkindale and Knight, 2002; Ozden et al., 2009). In this study, there was an increase in hydrogen peroxide accumulation at high temperatures, which acts as a signaling molecule for the expression of genes which provide tolerance to the plants by either scavenging the free oxygen radicals or by protecting the innate protein from denaturation. H_2O_2 is removed from

cells via a number of antioxidant mechanisms including both enzymatic and non-enzymatic. Cellular responses to H_2O_2 are complex, with considerable cross-talk between responses to several stimuli. The concept of H_2O_2 as a signal is realistic because this molecule is relatively stable and diffusible. The concentrations of H_2O_2 in normal tissues like leaves lies between 0.1 and 5 µmol/g FW (Veljovic-Jovanovic et al., 2002; Cheeseman, 2006) and its turnover is rapid. Populations of perennial ryegrass (*Lolium perenne* L.) tolerant and sensitive to summer stress showed lower contents of hydrogen peroxide (H_2O_2) in leaves against heat stress and the accumulated H_2O_2 content showed a linear relationship with the extent of physiological damage (Soliman et al., 2011). Since, decrease in the H_2O_2 was not observed in present investigation. This may be due to the reason that wheat seedling used in this study might have feeble expression of different isoenzymes responsible for scavenging the H_2O_2. It has also been observed (from our experiment) that at vegetative stage (22°C) the expression of antioxidant isoenzymes is very low.

In many plants, free proline accumulates in response to the imposition of a wide range of biotic and abiotic stresses. Under different abiotic stress conditions and H_2O_2 treatments, proline is accumulated in cells as an osmoprotectant (Hare and Cress, 1997; Ozden et al., 2009). Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species. However, the present results clearly showed that heat stress caused a decline in the level of proline accumulation in wheat. Xuejun Hua et al. (2011) reported that proline accumulation under heat stress decreases the thermotolerance, probably by increased ROS production via Pro/P5C cycle. Proline accumulation may reduce stress-induced cellular acidification or prime oxidative respiration to provide energy needed for recovery. Even a small increase in proline biosynthesis might have a large impact on the level of reduction of the cellular NADP pool (Hare and Cress, 1997).

The activity of antioxidant enzyme SOD, GPX, and CAT increased in all lines after heat treatment, but the increase was more significant in proline overproducing seedlings (Xuejun Hua et al., 2011). Proline pre-treatment to cucumber seedling enhanced eliminative ability to active oxygen and effectively alleviated membrane lipid peroxidative injury caused by high temperature stress by promotion of activities of SOD, POD and CAT (Shuren et al., 2010). Xuejun Hua et al. (2011) showed that proline accumulation during heat stress resulted in elevated level of ROS in mitochondria. The level of proline, thought to be important for plant protection during drought stress, is strongly suppressed in Arabidopsis after heat stress treatments and a combination of drought and heat stresses (Rizhsky et al., 2004). Hare and Cress (1997) suggested that proline degradation upon relief from stress may provide sufficient reducing agents, which, in turn, supports mitochondrial oxidative phosphorylation and generation of ATP required for recovery from stress.

Plants evolved both enzymatic and non-enzymatic antioxidant response systems against ROS injury (Vranova et al., 2002). In enzymatic system, SOD converts free O_2^- radicals to H_2O_2 and O_2 (Breusegem et al., 2001). CAT, APX and POX scavenge the accumulated H_2O_2 to nontoxic levels or form water and oxygen (Mittler, 2002). SOD activity observed in present investigation conforms to the observation made by He et al. (2005), who reported that the activity in wheat significantly increased after 2 h of heat stress application, but decreased to the control levels after 6 h of heat stress application. An increase in SOD activities was observed after 12 d of drought, heat or combined stress of both two cool-season turfgrasses (Jiang and Huang, 2001). Since, SOD are mainly involved in scavenging the free peroxide radicals from the cells in order to protect the important enzymes and organelles involved in various metabolic pathways, its high activity at pollination and milky dough stages is one of the important parameter for enhancing the tolerance capacity of the plant. SOD responds very well to heat stress. SOD activity decreased at 35°C compared to that at 20°C for both of the two different creeping bent grass cultivars. In the present study, the observed diminish activity of SOD in response to 40°C heat shock could be explained by either increased level of enzyme degradation or decline in synthesis of this enzyme. The reduction in SOD activity related genes in the heat stress treated tobacco plants was observed by Rizhsky et al. (2002) which is in accordance with our result.

The present study revealed a significant increase in CAT activity with differential heat shock treatment. Kele and Oncel (2002) reported that heat treatment increased CAT activities in *T. aestivum* genotypes, but decreased in *T. durum* genotypes. CAT activity reached up to the maximum level after ½ and 1 h of heat treatments and thereafter decreased in Kentucky bluegrass (He et al., 2005) as observed in present investigation. A significant increase in CAT activity was also observed at different stages of wheat growth starting from vegetative till milky dough stage. Since, CAT scavenges H_2O_2 to nontoxic levels or catalyzes the formation of water and oxygen, an increase in CAT activity could play a role in the protection of the plants from the damages of upward accumulation of H_2O_2 in wheat leaves in response to heat shock.

Conclusion

Terminal heat stress leads to change in the various parameters like expression of HSPs, cell membrane stability, hydrogen peroxide proline accumulation, and antioxidant enzymes activity etc. in wheat and is important component of thermotolerance capacity. An abundance of *HSP90* transcript was observed in shoot

compare to root at different stages of growth. An altered CMS values, hydrogen peroxide and proline accumulation was also observed with change in antioxidant enzyme activity against differential heat shock. Under heat stress conditions, particularly at reproductive stage, the level gene expression and the upstream regulatory regions of the genes that regulate the antioxidant enzymes such as SOD, CAT, POX and APX needs to be studied in more detail to better understand the mechanism of heat tolerance in wheat.

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