P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 2183-2187
© 2019 IJCS
Received: 22-07-2019
Accepted: 24-08-2019

Aarif Khan
Plant Biotechnology Centre, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

NK Gupta
Seed Technology Research, Rajasthan Agricultural Research Institute, Durgapura- Jaipur, Rajasthan, India

M Rizwan
Plant Biotechnology Centre, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

Govind Singh
Plant Biotechnology Centre, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India

Corresponding Author:
NK Gupta
Seed Technology Research, Rajasthan Agricultural Research Institute, Durgapura- Jaipur, Rajasthan, India

Effect of high temperature stress on physiological attributes and anti-oxidative defense mechanism in wheat (*Triticum aestivum* L.)

Aarif Khan, NK Gupta, M Rizwan and Govind Singh

Abstract
Experiment was conducted to study the effect of heat stress on various parameters in five diverse wheat (*Triticum aestivum* L.) genotypes at seedling stage. Seeds were sown in plastic pots in laboratory at 25±2 °C. For heat treatment, 20 days old seedlings were exposed to 36 °C/42 °C for 6 hours. Results showed that the heat stress significantly reduced the dry weight and length of seedlings in all the genotypes. Chlorophyll content and membrane stability index decreased but proline content increased in all the genotypes under stress conditions. Among genotypes, Raj 4083 exhibited better heat tolerance, particularly at 42 °C. Activities of SOD, CAT, APX and POX increased significantly in all the genotypes but per cent enhancement was maximum in Raj 4083 and minimum in PBW 502. The study also provides evidence that antioxidant enzymes like SOD, CAT, GPX, APX have association with heat tolerance in wheat and thus can be used as efficient markers for heat tolerance at seedling stage.

Keywords: Membrane stability, antioxidant, physiology, temperature, wheat

Introduction

High temperature is a major constraint for wheat growth and development, decreasing yield by 3 to 5 per cent per 1 °C rise in temperature (Gibson and Paulsen 1999) [10]. According to a report of intergovernmental panel on climate change (IPCC), global mean temperature is rising at 0.3 °C per decade reaching to approx 1 and 3 °C above the present value by 2025 and 2100 respectively and thus leading to global warming (Jones *et al*. 1999) [15]. Plant responses to high temperatures are mediated by both their inherent ability to survive (basal tolerance) and their ability to acquire tolerance to otherwise lethal temperatures (acclimation). These two mechanisms in cereals are due to the activation of different genetic systems (Maestri *et al*. 2002) [17]. Direct injuries due to high temperature include protein denaturation, aggregation and increased fluidity of membrane lipids. Indirect or slower heat injuries include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Kushwaha *et al*. 2011) [16].

Heat stress induces significant changes in normal physiological processes like photosynthesis, membrane stability and stomatal responses (Gupta *et al*. 2013) [13]. A significant decrease in chlorophyll content due to high temperature has been observed in wheat (Almeselmani *et al*. 2006) [1]. Deshmukh *et al*. (1991) [7] suggested the use of ion leakage as an index for screening genotypes against heat and drought stress in wheat. 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) (Breusegem *et al*. 2001) [4]. Plants get rid of these harmful ROS products by converting them to less reactive chemicals by SOD (Superoxide dimutase) which free O_2^- radicals to H_2O2 and O_2. Catalase (CAT), ascorbate peroxidase (APX) and non- specific peroxidase (POX), in turn, scavenge the higher level of H_2O_2 to non- toxic levels by catabolising it to water and oxygen (Mittler 2002) [18].

Thus, the aim of present investigation was to characterize various morpho-physio-biochemical approaches in wheat genotypes for their ability to acquire thermo-tolerance in contrasting wheat genotypes.

Materials and methods

Crop and experimental conditions

The experiment was conducted in two steps. In first step five wheat genotypes i.e. Raj 4083, Raj 1482, Raj 3765, Lok 1 and PBW 502 were grown in small plastic pots filled with 80g vermiculite potting material supplemented with Hoagland solution. Thirty pots of each genotype were taken for study and each pot was irrigated with 30 ml normal water at alternative day. The experiment was maintained at 25±2 °C temperature, 16/8 day night temperature and 60-70% relative humidity. After 20 days of sowing, the pots were divided into 3 groups as – (i) 1/3 pots were kept at normal room temperature (absolute control), (ii) 1/3 pots were kept in growth chamber at 36 °C for 6 hs (heat treatment – I) and (iii) 1/3 pots were kept in growth chamber at 42 °C for 6 h (heat treatment –II). Observations on various parameters were taken after 5 days of treatment.

Growth and physiological parameters

Chlorophyll content was measured by incubating 50 mg of the leaf material in 10 ml of dimethyl sulphoxide for 4 h at 65 °C. The absorbance of the solvent was recorded at 663 and 645 nm (Hiscox and Isrealstom 1968) [14]. The free proline in leaves was extracted in 3% sulphosalicylic acid and determined using the method of Bates *et al.* (1973) [2]. Membrane injury index was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40 and 100ºC (Sairam *et al.* 2000) [21]. Leaf samples (0.1 g) were cut into discs of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40 °C for 30 min and another set at 100 °C in boiling water bath for 15 min and their respective electric conductivities C_1 and C_2 were measured by conductivity meter. The membrane stability was calculated $MSI = [1-\{C_1/ C_2\}] \times 100$. For growth parameters, the seedlings were rinsed thoroughly with distilled water and the root length was measured from base to the junction. Similarly, the shoot length was measured from tip to the junction of the same plant. The shoot and root were kept separately in oven at 65 °C till constant weight achieved and then dry weight was recorded.

Antioxidants

Superoxide dismutase (SOD; EC1.15.1.1) activity was analyzed by homogenizing 0.5 g leaf tissue in 10 cm^3 chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA (Dhindhsa *et al.* 1981) [9]. The buffer was filtered through cheese cloth and the filtrate was centrifuged in a refrigerated centrifuge (IEC) for 15 minutes at 20,000 x g. The 3.0 cm^3 reaction mixture contained 13 mm methionine; 25 mM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer pH (7.8), 50 mM sodium bicarbonate and 0.1 cm^3 enzyme extract. The reaction was started by adding 2µm riboflavin and placing the tubes below 2x15.00 W fluorescent lamp for 15 min. It was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme develops maximum colour. A non- irradiated complete reaction mixture did not develop colour and served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50 percent in comparison with the tubes lacking enzymes. The enzyme extract for catalase (CAT; EC 1.11.1.6) and guaiacol peroxidase (GPX; EC1.11.1.7) were prepared by grinding 0.5 g fresh leaves in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinyl polypyrrolidone (PVP). The homogenate was filtered through four layers of cheese cloth and then centrifuged at 4 °C for 20 min at 15,000*g*. The supernatant was collected and 2 µl aliquot dilution of the crude extract was used for enzyme assays (Chance & Maehly 1955) [6]. The ascorbate peroxidase (APX; EC 1.11.1.11) was measured following the method of Nakano and Asada (1981) [19] by monitoring the rate of ascorbate oxidation at 290 nm. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 100 mM H_2O_2, 0.25 mM ascorbate (AsA), and the enzyme extract in a final volume of 1 mL. Total soluble protein content was determined according to the method of Bradford (1976) [3] with bovine serum albumin as a calibration standard.

Experimental design and data analysis

All experimental data recorded were averaged for at least three independent assays with three replicates each. The data were subjected to ANOVA for completely randomized factorial design. Differences at $P \leq 0.05$ were considered statistically significant (Gomez and Gomez 1984) [11].

Results and Discussion

Genotypic variation for seedling dry weight was observed at high temperature. Per cent reduction in seedling dry weight was maximum in PBW 502 at 36 °C (9.22%) and at 42 °C (11.26%). It was minimum in Raj 4083 under both the treatments (2.65% at 36 °C and 4.97% at 42 °C). The shoot length was also reduced but the effect was more pronounced at 42 °C treatment. Among genotypes, Raj 1482 (22.7 cm) and Raj 4083 (22.3 cm) exhibited better shoot length over other genotypes (Table 2). There was significant reduction in root length of all the genotypes under high temperature conditions. In all the cases, the per cent decline was higher from 25 °C to 36 °C than from 36 °C to 42 °C. Genetic variability in the reduction of seedling dry weight in wheat genotypes under heat stress indicates the existence of tolerance at early seedling stage in some genotypes. Wahid (2007) [23] reported that high temperature caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and wheat. Goyal and Asthir (2010) [12] suggested that some of the wheat genotypes have the potential to adjust with high temperature conditions by managing their internal plant processes. In our previous studies also, we observed significant variations in growth parameters of high temperature treated wheat seedlings (Gupta *et al.* 2013) [13]. Shoot length and root length also reduced significantly in all the genotypes. Raj 4083 exhibited maximum seedling length in temperature treated plants. It was also noted that the genotypes exhibiting higher root length generally maintained high shoot weight after treating with high temperature (Table 1). It indicates that root length may be one of the important parameters to help the plant in adjusting under heat stress conditions.

The chlorophyll content of wheat leaves also reduced significantly on account of heat stress. The control plants of Raj 3765 exhibited maximum chlorophyll content (2.0 mg g^{-1} fr. wt.) but under heat stress the values were maximum in Raj 4083 (1.42 and 1.11 mg g^{-1} fr. Wt. at 36 °C and 42 °C, respectively). The chlorophyll content was minimum in heat treated leaves of PBW 502 (Table 2). The chlorophyll pigments are important to plants mainly for harvesting light and production of reducing powers. In chloroplast, the chlorophyll is harboured by thyllakoids, a portion of which is

associated with the proteins of PS II. These thyllakoids in PS II are considered the most heat labile cell structures (Vacha *et al*. 2007) [22]. Any damage to thyllakoids caused by heat could therefore be expected to lead to chlorophyll loss. In our investigation also, the heat stress might have damaged the thyllakoid membrane which reduced chlorophyll content in stress treated plants. A critical analysis of this data indicate that wheat genotype Raj 4083 retained high chlorophyll content, the effect was reflected on dry mass and length of seedlings. Gupta *et al*. (2013) [13] reported that chlorophyll content is directly linked with heat susceptibility of wheat genotypes and tolerant genotypes exhibited higher amount of chlorophyll after exposing to high temperature.

Proline serves as a membrane protectant and accumulates in cell cytoplasm under stress conditions without interfering with cellular structure or metabolism (Chakroborty and Tangden 2005) [5]. In present investigation proline content ranged from 7.2 mg g^{-1}fr. Wt. (Raj 1482) to 8.8 mg g^{-1}fr. Wt. (PBW 502) in control plants. In heat treated plants, it was minimum in Raj 1482 (9.7 mg g^{-1}fr. Wt.) and maximum in Raj 4083 (12.9 mg g^{-1} fr. Wt.) at 36 °C) whereas at 42 °C it was maximum in Raj 4083 (15.9 mg g^{-1}fr. Wt.) and minimum in Raj 1482 (10.5 mg g^{-1}fr. Wt.). Thus, enhanced proline might have helped Raj 4083 in combating the high temperature stress more efficiently than in other genotypes (Table 2).

The high temperature conditions disrupt water, ion and organic solutes movement across the plant membrane which interferes with photosynthesis and transpiration. This damage to membranes may be assessed by membrane thermo stability which measures electrolyte leakage from the leaves subjected to high temperature (Sairam *et al*. 2007) [20]. Our results indicate an abrupt decline in membrane stability index at 42 °C, particularly in Lok 1(33.40) and PBW 502 (34.60). Comparatively high membrane stability in Raj 4083 under stress conditions further indicates the presence of tolerance mechanism in this genotype (Table 2). Lower membrane stability reflects the extent of lipid peroxidation which in turn is a consequence of higher oxidative stress due to various environmental stresses (Sairam *et al*. 2000) [21]. However, it is important to evaluate the level of anti-oxidants and lipid peroxidation before supporting this hypothesis.

High temperature or other abiotic stresses are accompanied by the formation of reactive oxygen species such as O_2, H_2O_2 and OH^- which damage membranes and macromolecules (Mittler 2002) [18]. In present investigation, the activity of SOD was enhanced in all the genotypes but per cent increase were maximum in Raj 1482, Raj 4083 and Raj 3765 (Fig 1). The catalase activity ranged from 0.90 - 1.62 units mg^{-1} protein min^{-1} (PBW 502 - Raj 4083) in control plants. The CAT activity varied from 1.28 (PBW 502) to 1.87 (Raj 4083) and 1.42 (PBW 502) to 1.97 (Raj 3765) units mg^{-1} protein min^{-1} when exposed to 36 °C and 42 °C, respectively (Fig 1). Perusal of data indicates that the per cent enhancement in CAT activity was higher in PBW 502 and Lok 1 over other genotypes, but there overall levels were always lower in these genotypes, indicating lack of inherent tolerance to high temperature. Recently Devi *et al*. (2011) [8] also provided evidence that catalase are very efficient tool for removal and control of hydrogen peroxide level in plants. The POX activity was minimum in PBW 502 (259.85 µmol mg^{-1} (protein) $min^{-1)}$ and maximum in Raj 4083 (429.33 µmol mg^{-1}(protein) min^{-1}) in control plants (Fig 1). The heat stress treatment invariably enhanced the POX activity in all the genotypes. At 36 °C it ranged from 307 (PBW 502) to 544.07 (RAJ 4083) µmol mg^{-1} (protein) min^{-1}. At 42 °C, it varied from 373.33 to 628 µmol mg^{-1} (protein) min $^{-1}$ (Lok 1 - RAJ 4083). The APX activity also increased in all the genotypes under heat stress conditions. It was minimum in Lok 1 (197.14 µmol mg^{-1} (protein) min $^{-1}$) and maximum in Raj 4083 (386.00 µmol mg^{-1} (protein) min^{-1}) under control conditions. At 36 °C as well as at 42 °C treatments, maximum APX activities were registered in Raj 4083 (534 µmol mg^{-1} (protein) min^{-1}) (Fig 1). These results further reflected Raj 4083, Raj 3765 and Raj 1482 as temperature tolerant and PBW 502 as susceptible. Catalase and peroxidises are the most important enzymes involved in regulation of intracellular level of H_2O_2. They convert H_2O_2 into H_2O along with the regeneration of NADP+, thus helping the plants under stress conditions (Sairam *et al*. 2007) [20]. Variability in the activities of these antioxidant enzymes across wheat genotypes indicates their differential ability to acquire thermo tolerance.

In conclusion, using a multi parametric approach, Raj 4083 was identified as heat tolerant and PBW 502 as heat susceptible wheat genotypes. It is suggested that seedling dry mass, chlorophyll content, electrolyte leakage and proline content can be used efficiently for assessing heat tolerance at early seedling stage. Enzymes like SOD, CAT, GPX, APX association with heat tolerance in wheat and thus can be used as efficient markers for germplasm screening.

Table 1: Effect of heat stress on seedling dry weight shoot length and root length in wheat seedlings under heat stress conditions

Genotype	**Seedling dry weight (mg plant^{-1})**				**Shoot length (cm)**		**Root length (cm)**		
	25 °C	36 °C	42 °C	25 °C	36 °C	42 °C	25 °C	36 °C	42 °C
Raj 4083	90.5±3.2	88.1±3.4	86.0±4.2	27.8±2.4	24.8±2.1	22.3±1.9	30.0±2.3	27.8±1.8	27.5±2.2
Raj 3765	94.3±4.1	87.8±4.6	82.4±3.4	22.4±1.8	21.2±1.6	17.9±1.4	29.9±1.9	27.0±2.4	26.7±2.2
Raj 1482	91.8±2.9	88.2±4.2	83.1±4.5	29.7±2.7	25.8±2.2	22.7±1.7	32.2±2.7	27.8±2.3	27.0±2.6
LOK 1	84.6±3.5	83.1±3.1	75.8±3.9	23.2±1.9	20.3±1.7	20.2±1.6	28.7±2.1	25.7±2.5	25.3±1.8
PBW502	83.5±3.7	75.8±3.4	74.1±3.5	23.0±2.3	20.8±1.8	18.1±1.6	31.5±2.4	27.0±2.1	25.8±2.1
LSD ($p \leq 0.05$)									
Variety (V)	2.95			1.74				1.99	
Temp. (T)	2.28			1.34				1.54	
V x T	5.11			3.01				3.45	

Table 2: Effect of heat stress on proline content, chlorophyll content and membrane stability index in wheat seedlings under heat stress conditions.

Genotype	Proline content (mg g^{-1} fr. wt.)			Chlorophyll content (mg g^{-1} fr. wt.)			Membrane stability index		
	25 °C	36 °C	42 °C	25 °C	36 °C	42 °C	25 °C	36 °C	42 °C
Raj 4083	8.0±0.74	12.9±1.1	15.9±1.5	1.89±0.54	1.42±0.39	1.11±0.21	73.0±4.6	61.3±3.5	46.7±3.9
Raj 3765	7.7±0.69	10.4±1.3	12.8±1.4	2.00±0.68	1.28±0.32	0.98±0.18	74.7±3.9	59.1±3.9	43.7±4.2
Raj 1482	7.2±0.71	9.7±1.2	10.5±1.6	1.78±0.56	1.07±0.29	0.82±0.18	81.4±5.2	65.0±4.2	41.1±3.2
LOK 1	8.2±0.81	10.2±1.6	11.8±1.2	1.94±0.61	1.09±0.36	1.01±0.22	77.4±4.8	62.1±4.7	33.4±3.2
PBW502	8.8±0.72	9.8±1.1	10.8±1.3	1.67±0.45	1.15±0.32	0.87±0.21	80.7±4.3	56.8±3.9	34.6±3.4
LSD ($p \leq 0.05$)									
Variety (V)	0.78			0.06			3.12		
Temp. (T)	0.60			0.05			2.42		
V x T	1.35			0.11			5.41		

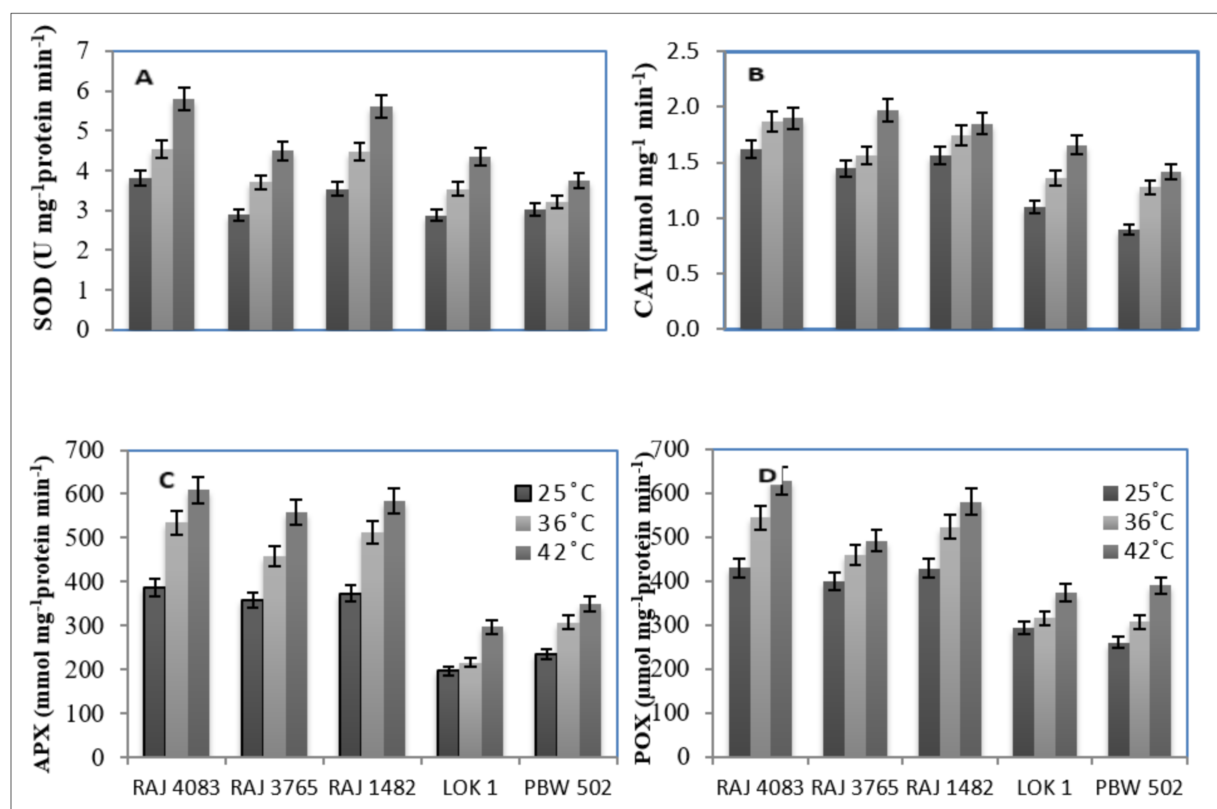

Fig 1: Effect of heat stress on superoxide dismutase (A), catalase (B), ascorbate peroxidase (C) and guiacol peroxidase (D) in wheat seedlings under heat stress conditions. The values are the mean of three replications and the veritical bars represent + SE

References

1. Almeselmani M, Desmukh PS, Sairam RK, Kushwaha SR, Singh TP. Protective role of antioxidant enzyme under high temperature stress. Plant Science. 2006; 17:382-388.
2. Bates LS, Waldrenand RP, Teare ID. Rapid determination of free proline for water stress studies. Plant and Soil. 1973; 39:205-207.
3. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein dye-binding. Annals of Biochemistry. 1976; 38:248-252.
4. Breusegem FV, Vranova E, Dat JF, Inze D. The Role of active oxygen species in plant signal transduction. Plant Science. 2001; 161:405-414.
5. Chakroborty U, Tangden C. Evaluation of heat acclimation and salicylic acid treatments as potent inducers of thermo tolerance in *Cicer arietinum* L. Current Science. 2005; 89:384-389.
6. Chance B, Maehly AC. Assay of catalase and peroxidases. Methods in Enzymology. 1955; 2:764-755.
7. Deshmukh PS, Sairam RK, Shukla DS. Measurement of ion leakage as a screening technique for drought resistance in wheat genotypes. Indian Journal of Plant Physiology. 1991; 34:89-91.
8. Devi VS, Ushanandhini R, Kuppusamy A, Muthuswamy U, Andichettiar TS, Puliyath J. Antioxidant activity of *Cassia auriculata* Linn flowers. Pharmacology Online. 2011; 2:490-498.
9. Dhindhsa RS, Plumb-Dhidsa P, Thorne TA. Leaf senescence: Correlated with increased levels of membrane permeability and lipid per oxidation and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany. 1981; 32:93-101.
10. Gibson LR, Paulsen GM. Yield components of wheat grown under high temperature stress during reproductive growth. Crop Science. 1999; 39:1841-1846.
11. Gomez KA, Gomez AA. Statistical procedures for agricultural research, New York: Wiley, 1984.
12. Goyal M, Asthir B. Polyamine catabolism influences antioxidative defense mechanism in shoots and roots of five wheat genotypes under high temperature stress. Journal of Plant Growth Regulation. 2010; 60:13-25.

13. Gupta NK, Agarwal S, Agarwal VP, Nathawat NS., Gupta S, Singh G. Effect of short-term heat stress on growth, physiology and antioxidative defence system in wheat seedlings. Acta Physiologiae Plantarum. 2013; 35:1837-1842.
14. Hiscox JD, Israelstom GF. A method for extraction of chloroplast from leaf tissue without maceration. Canadian Journal of Botany. 1968; 57:1332-1334.
15. Jones PD, New M, Parker DE, Mortin S, Rigor IG. Surface area temperature and its change over the past 150 years. Geophysics. 1999; 37:173-199.
16. Kushwaha HR, Kumar G, Verma P. Analysis of a salinity induced BjSOS3 protein from *Brassica* indicate it to be structurally and functionally related to its ortholog from Arabidopsis. Plant Physiology and Biochemistry. 2011; 49:996-1004.
17. Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen HT, Marmiroli N. Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Molecular Biology. 2002; 48:667-681.
18. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002; 7:405-410.
19. Nakano YK, Asada A. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 1981; 22:867-880.
20. Sairam RK, Roa KV. Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science. 2007; 163:1037-1046.
21. Sairam RK, Srivastava GS, Saxena DC. Increased antioxidant activity under elevated temperature a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum. 2000; 43:245-251.
22. Vacha F, Adamec F, Valenta J, Vacha M. Special Location of photosystem pigment-protein complexes in thyllakoid membranes of chloroplast of *Pisum sativum* studied by chlorophyll fluroscence. Journal of Luminescence. 2007; 122-123:301-303.
23. Wahid A. Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane sprouts. Journal of Plant Research. 2007; 120:219-228.