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Exogenous Application of Thiourea Improves the Performance of Late Sown Wheat by Inducing Terminal Heat Resistance

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Abstract

This study was conducted to evaluate the potential of thiourea in improving the terminal heat resistance in bread wheat. Four wheat genotypes PBW 550 and PBW 343 (heat sensitive) and C 306 and C 273 (heat resistant) were field sown at normal time (November) or late (December) to expose the crop to heat stress during grain-filling. Temperature during grain filling was ~25.6^{o}C in normal and 29.4^{o}C in late sown wheat crop. Prior to sowing, wheat seeds were soaked in 6.6 mM thiourea solution) for 6 h. Thiourea was also applied as foliar spray (6.6 mM) at anthesis. Heat stress, during grain filling, led to disruption of cellular membrane by increasing membrane injury index, lipid peroxide and H_2O_2 contents. Nonetheless, heat resistant (C 306 and C 273) genotypes had lower lipid peroxidation and membrane injury owing to better expression of antioxidants. However, thiourea application ameliorated the heat-induced damages by stimulating the total antioxidant activity through decrease in lipid peroxidation and membrane injury. Thiourea application also increased the total soluble proteins, amino acids and chlorophyll contents in all the tested genotypes. This all caused substantial increase in plant height, peduncle length, peduncle weight and grain weight. Genotypes PBW 550 and PBW 343 showed higher grain weight in spite of greater injury to membranes over genotypes C 306 and C 273. Combined application of thiourea as seed treatment and foliar spray was more effective in improving the wheat performance by enhancing membrane stability, antioxidant potential and yield components.

Keywords: Foliar spray; Grain weight; Heat stress; Plant defense system; Thiourea

Introduction

Many crops are exposed to heat stress during some stage of their life cycle, which can occur before a crop has emerged or during maturation. In wheat, the frequency and severity of exposure to heat stress increases during the post anthesis period (terminal heat) in many arable areas of the world. Wheat yield losses due to heat stress average 10–15% per annum (Wardlaw and Wrigley, 1994). In India, particularly Punjab, farmers by and large delay wheat sowing due to intensive cropping system, which pushes the grain filling stage to high temperature stress. This increased temperature not only hastens the phenological stages of crop development but also reduces duration of grain filling stages thereby lowering the grain yield and its quality (Tewari and Tripathy, 1999; Farooq *et al*., 2011).

The adverse effects of heat stress can be mitigated by developing crop plants with improved resistance against heat. Many stress alleviating agents including thiols are crucial for enhancing the crop productivity as these improves the metabolic imbalances produced in a cell during stress. Thiols are well-known to maintain the redox state (–SH/-S-S- ratio) of the cell and its proper functioning under stress conditions (Sahu *et al*., 2005; Nathawat *et al*., 2007). Since, thiourea has been identified as an effective bioregulator imparting stress tolerance to crops; it is quite possible that seed treatment with external thiols in form of thiourea might result in up-regulation of antioxidant defense system. Improvement in plant growth and development under different stresses due to application of thiourea has been observed in crops like maize (Sahu *et al*., 1993), wheat (Sahu and Singh, 1995; Sahu *et al*., 2006), pearl millet (Parihar *et al*., 1998) and clusterbean (Garg *et al*., 2006). Available reports show that pre–conditioning of seeds with thiourea up-regulates the antioxidant defense mechanism under water deficit conditions in wheat (Nathawat *et al*., 2007) and pearl millet (D'Souza *et al*., 2009). It also relieves salinity induced seed dormancy in *Allenrolfea occidentalis* at lower concentration (Gul and Weber, 1998) and photosynthesis and nitrogen metabolism under rainfed conditions in clusterbean (Garg *et al*., 2006). Despite numerous studies on thiourea acting as effective bioregulator imparting resistance to crop plants against abiotic stresses (Srivastava *et al*., 2009; Anjum *et al*., 2011; Perveen *et al*., 2013); to the best of our knowledge, no study has been conducted to elucidate the influence of

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exogenously applied thiourea on membrane stability and antioxidant defense system of wheat under heat stress.

In this study, wheat seedlings were pretreated with 6.6mM thiourea and then were exposed to heat stress by delaying the sowing for one month to experience heat stress. As stress-related signals, proline, amino acid and proteins accumulate in large quantities in response to heat, and this entails great implications for heat tolerance of plants. We hypothesized differential effect of thiourea application on tall traditional and heat resistant genotypes but low yielding (C 306, C 273) over temperature sensitive but high yielding genotypes (PBW 343 and PBW 550). The obtained results would generate useful information for creating heat tolerant genotypes from crosses of tall traditional varieties with modern semi-dwarf wheat (C 306/PBW 534//PBW 534 and C 273/ PBW 343// PBW 343).

Materials and Methods

Four wheat genotypes C 306, C 273 (heat resistant) and PBW 550, PBW 343 (heat susceptible) were raised in the experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India. The crop was sown on November 16, 2010 (normal sowing) and December 11, 2010 (late sowing) in plots consisting of 4 rows of 1 m each. The experiment was conducted with three replications. Row to row spacing was maintained at 23 cm. The mean temperature during grain development for two sowing period varied between 4-6^{o}C and was recorded by a field meteorological laboratory fitted with microprocessor controlled data logger, which recorded daily maximum/minimum temperature, sunlight duration, relative humidity, rainfall, wind velocity, etc. Irrigation and fertilizer application were as per the standard practices of the region. At the time of sowing seeds were surface sterilized with 0.1% $HgCl_2$ for 1 min, rinsed thoroughly with distilled water and imbibed in solution of thiourea (6.6 mM) for 6 h followed by foliar spray at anthesis (90 days after sowing, DAS). Seeds pre-soaked in water were taken as control. All enzymatic and other estimation like membrane injury index, chlorophyll, lipid peroxide, H_2O_2, protein and amino acid contents were estimated from the first fully expanded leaf (third from top) at vegetative stage (30 and 60 days after sowing) and flag leaf at anthesis and post-anthesis stages (90 and 120 days after sowing).

Membrane injury index (MII) was determined following Asthir *et al*. (2012). Flag leaf (0.5 g) was excised and washed with distilled water to remove adhering electrolytes. The tissue was then immersed in test tubes containing 20 mL of distilled water. After 24 h, the sample was then boiled for 30 min and conductivity was measured again. Membrane injury index was calculated as a ratio of electrical conductivity before and after boiling and was expressed in percentage.

Lipid peroxidation was determined as contents of thiobarbituric acid reactive substance (TBARs) as described by Larkindale and Knight (2002). Briefly, 0.5 g grains were homogenized with 3 mL 20% (w/v) trichloroacetic acid and 0.5% (v/v) thiobarbituric acid (2:1 ratio) and incubated at 95°C for 30 min. The reaction was stopped by placing the reaction tubes in an ice bucket. The lipid peroxide contents were determined using coefficient of absorbance of 155 mM^{-1} cm^{-1}.

Hydrogen peroxide was quantified following Loreto and Velikova (2001). Briefly, leaf samples (0.3 g) were homogenized with 3 mL of 1% (w/v) trichloroacetic acid. The homogenates were centrifuged at 10,000 × *g* (4°C) for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1 *M* KI. Hydrogen peroxide content of the supernatant was evaluated by comparison of the absorbance values at 390 nm to a standard calibration curve in the range of 10 to 200 nmol.

Chlorophyll contents were determined following the method of Hiscox and Israelstam (1979). Soluble proteins were extracted in 0.1 M NaOH and precipitated with trichloroacetic acid and estimated by the method Lee and Takahashi (1966). Total free amino acids were extracted and determined following the method of Singh *et al*. (1978). Total antioxidant activity was measured as described by Prieto *et al*. (1999). Frozen leaves (1 g) were homogenized with 10 mL distilled H_2O followed by boiling for 1 h. To 2 mL of extract, 1 mL of TAA reagent was added, and incubated for 90 min at 95^{o}C and then kept for cooling. Color developed was read at 695 nm. The antioxidant activity was expressed as ascorbic acid equivalent. Five random plants were harvested randomly from 1 m row length to record plant height, peduncle length, peduncle weight and 1000-grain weight. There were three replications for each measurement. Experimental data recorded were statistically analyzed by multifactor ANOVA (Statgraphics plus version 2.1). Values are presented as means ± SD.

Results

Heat stress experienced in the late sown wheat reduced the total chlorophyll content**s** in all the four genotypes (Fig. 1a), while combined treatment of thiourea i.e., seeds treatment and foliar spray at anthesis stage substantially increased the chlorophyll contents (Fig. 1a). In both normal and late sown crop, membrane injury index (MII) increased till 90 days after sowing (DAS) but a decrease was seen towards maturity (Fig. 1b); whereas thiobarbituric acid reactive substances (TBARs) showed continuous decline with growth stages (Fig. 1c). The magnitude of MII and TBARs were higher in sensitive genotypes (PBW 343 and PBW 550) than resistant ones (C 306 and C 273) especially in the late sown crop (Fig. 1b, c); Genotypes PBW 343 and PBW 550 showed comparatively higher MII and TBARs followed by genotypes C 306 and C 273 at vegetative and at anthesis stages. A significant decrease in the MII and TBARs was noted from thiourea seed treatment than respective control at all stages in four tested genotypes.

With crop advancement, significant decrease in H_2O_2 contents was observed, which was further decreased by exogenous application of thiourea in all the tested genotypes (Fig. 2a). Maximum H_2O_2 contents were found in genotypes PBW 550 and PBW 343 in both timely and late sown crop. High temperature also led to a significant increase in total antioxidant activity throughout the crop duration in all the tested genotypes (Fig. 2b). Genotypes C 306 and C 273 had more total antioxidant activity than genotypes PBW 550 and PBW 343. However, thiourea application further increased total antioxidant activity in all tested genotypes (Fig. 2b).

Highest protein contents were noted in genotype PBW 550 followed by genotype C 273 (Fig. 2c). Thiourea application further increased the protein contents in the normal and late sown crop. However, combined application of thiourea (seed pretreatment and foliar spray) caused maximum increase in total protein contents. Whereas amino acids contents (Fig. 2d) increased up to 90 DAS and then decreased till maturity. However, significant increase in amino acids contents was noted in late sown crop. Genotypes C 306 and C 273 showed more accumulation of amino acid than genotypes PBW 550 and PBW 343. Thiourea application further enhanced the amount of amino acid contents in all the genotypes (Fig. 2d).

Plant height was affected the most under heat stress (in late sown wheat) as reflected in short stature of plants in all the tested genotypes. Genotypes C 306 and C 273 were generally taller than the PBW genotypes, which otherwise are high yielding genotypes (Table 1). Genotype C 273 showed maximum reduction of 25% in plant height (120.4 cm under control to 89.7 cm under stress) compared to PBW 550, in which decreased was by 22% (81.0 cm under control to 63.0 cm under stress). Similarly decrease in peduncle length and peduncle weight was observed in the late sown crop (Table 1). Genotypes C 306 and C 273, being heat resistant, showed maximum peduncle length and peduncle weight in both normal and late sown crop. In genotype C 306, an increase of 14% in peduncle length was noted from combined application of thiourea as compared to normal sown crop. Maximum grain weight was observed in genotype PBW 550, which was decreased by 10% by heat stress; whereas in genotype C 273, heat stress decreased the grain weight by 8% (Table 1). Thiourea application significantly increased the grain weight in all tested genotypes at both normal and late sowing (Table 1).

Discussion

This study indicated that performance of late sown wheat is strongly influenced primarily due to high temperature during grain filling (terminal heat stress) (Figs. 1, 2; Table 1). However, the response of tested genotypes was differential; genotypes C 306 and C 273 were better able to perform well under the heat stress than the genotypes PBW 550 and PBW 343.

Although many studies have shown a wide range of physiological and biochemical responses by plants to heat stress (Wahid *et al*., 2007), few have explored how these processes are linked to heat resistance of the whole plant under field conditions. This study demonstrated that both thiobarbituric acid reactive substances (TBARs) and H_2O_2 contents increased upon exposure to heat stress (Fig. 1c, 2a). Genotypes C 306 and C 273 showed minimum membrane injury index (MII) (Fig. 1b) and TBARs (Fig. 1c) indicating thereby, that these genotypes have a better protection mechanism to withstand oxidative stress caused by heat stress.

Thiourea was externally applied as seed treatment and foliar spray to mitigate heat-induced damages. To determine the possible mechanism of thiourea action in this regard, membrane integrity, chlorophyll contents and oxidative stress indicators, which are adversely influenced by heat stress (Mohammed and Tarpley, 2009; Bala *et al*., 2010; Yin and Huang, 2011) and can be used as reliable markers of stress injury (Ahmadizadeh *et al*., 2011), were monitored. Membrane damage as indicated by MII increased in the late sown crop; however, that damage was significantly decreased with thiourea application (Fig. 1b). Thiourea plays a vital role in maintaining redox state of membrane proteins as it can quench reactive oxygen species (ROS) generated during heat stress. The oxidized form of sulphydryl groups under heat stress may be responsible for membrane damage, which was reduced significantly with the exogenous thiourea application resulting in improved grain growth.

Oxidative stress, measured as TBARs indicates a product of lipid peroxidation and H_2O_2 content, increased with an increase in temperature. Thiourea treated plants significantly lowered the oxidative stress, which is in agreement with its role in quenching ROS and protect the cells from lipid peroxidation (Mahatma *et al*., 2009). This was also associated with greater total antioxidant activity under heat stress. Increase in TBARs content in late sown wheat has also been observed by Bala *et al*. (2010). During early vegetative stages maximum decrease in TBARs content with thiourea treatment was observed in the genotype PBW 550 (Fig. 1c). Thiourea stabilizes the lipoprotein structure as indicated by producing less TBARs content. Degradation of chlorophyll in leaves in the late sown crop might be associated with production of ROS leading to decreased photosynthetic efficiency. Adverse effects on photosynthesis and reduced accumulation of chlorophyll under HT stress was also reported earlier (Ghobadi *et al*., 2011). In this study, chlorophyll contents decreased in late sown crop as compared to timely sown at all growth stages as shown in Fig. 1a. Total chlorophyll content in genotype PBW 550 was higher than other three genotypes tested, which were further improved by thiourea application (Fig. 1a). The deficiency of reduced sulphydryl groups in stressed plants leads to chlorosis

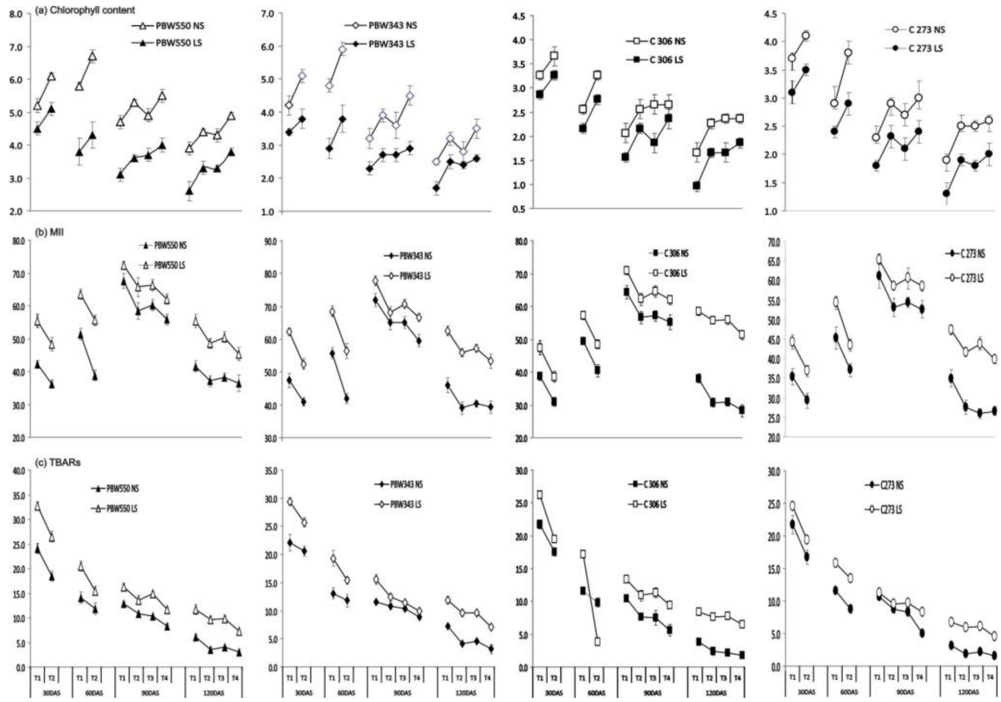

Fig. 1: Effect of thiourea (TU) application on (a) Chlorophyll content (mg g^{-1} FW), (b) membrane index injury (MII, % injury) and (c) thiobarbituric acid reactive substance content (TBARs, umolg^{-1} FW) in leaves of four genotypes in the normal sown (NS) and late sown (LS) crop at 30, 60, 90 and 120 days after sowing (DAS). T1= Control, T2 = Seeds dip with TU (6.6mM) at the time of sowing, T3 = Foliar spray of TU at anthesis, T4 = combined treatment of TU (T3+T4). Analysis of variance is significant at $p \leq 0.05$; Chlorophyll – G=0.69, Tr=0.49, T=0.12; MII – G=0.59, Tr=0.42, T=1.02; TBARs – G=0.30, Tr=0.21, T=0.52. G= genotypes, Tr= presence or absence of TU, T= sowing time

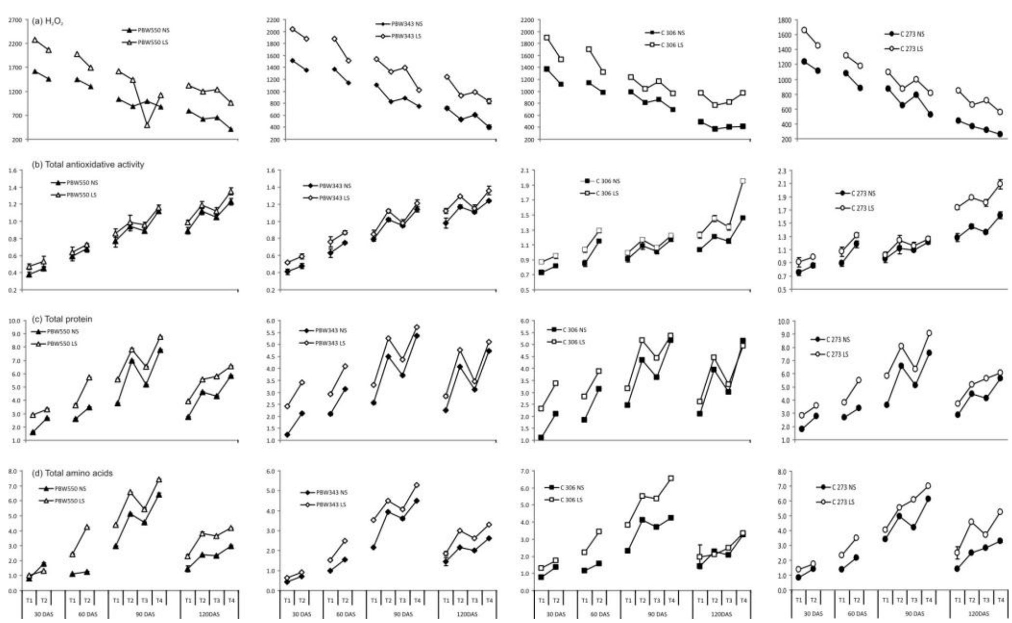

Fig. 2: Effect of thiourea (TU) application on (a) hydrogen peroxide content (H_2O_2, nmolg^{-1} FW), (b) total antioxidant activity (TAA, mg AAE g^{-1} FW), (c) total protein (TP, mg g^{-1} DW) and (d) total free amino acid (TFA, mg g^{-1} DW) in leaves of four genotypes in the normal sown (NS) and late sown (LS) crop at 30, 60, 90 and 120 days after sowing (DAS). T1= Control, T2 = Seeds dip with TU (6.6mM) at the time of sowing, T3 = Foliar spray of TU at anthesis, T4 = combined treatment of TU (T3+T4). Analysis of variance is significant at $p \leq 0.05$; H_2O_2 – G=0.30, Tr=0.21, T=0.52; TAA – G=0.87, Tr=0.62, T=0.15; TP – G=0.87, Tr=0.62, T=0.15; TFA – G=0.62, Tr=0.42, T=0.11

Table 1: Effect of thiourea application on plant height, peduncle length, 1000-grain weight and peduncle weight in four genotypes of wheat under normal sown (NS) and late sown (LS) conditions

Cultivars	Sowing Time	Plant height (cm)				Peduncle Length (cm)				1000 grain weight (g)				Peduncle Weight (g)			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
PBW	NS	81.0	85.6^{a}	84.6^{b}	89.6^{c}	35.3	36.8^{a}	36.6^{b}	37.0^{c}	40.1	41.8	41.0	43.6^{a}	0.35	0.41^{a}	0.38	0.45^{b}
550	LS	63.0^{x}	68.8^{ax}	67.0^{bx}	75.0^{cx}	28.8^{x}	31.0^{ax}	29.5^{bx}	33.6^{cx}	36.1^{x}	38.4	38.1	40.4^{ax}	0.18^{x}	0.22^{ax}	0.19	0.25^{bx}
PBW	NS	84.7	89.8^{a}	86.3^{b}	92.0^{c}	29.3	31.6^{a}	29.8^{b}	34.0^{c}	37.4	40.1^{a}	39.4	42.3^{b}	0.30	0.32	0.32	0.35^{a}
343	LS	74.4^{x}	79.7^{ax}	77.6^{bx}	86.6^{cx}	24.5^{x}	26.3^{ax}	25.5^{bx}	30.0^{cx}	34.1^{x}	36.2^{x}	36.2^{x}	39.4^{bx}	0.19^{x}	0.22^{x}	0.21^{x}	0.24^{ax}
C 306	NS	119.3	124.7^{a}	122.6^{b}	134.3^{c}	44.0	46.3^{a}	45.1^{b}	48.2^{c}	36.3	39.6^{a}	38.1^{b}	42.1^{c}	0.42	0.46^{a}	0.45^{b}	0.47^{c}
	LS	90.0^{x}	102.7^{ax}	100.0^{bx}	110.0^{cx}	35.3^{x}	37.4^{ax}	36.6^{bx}	41.6^{cx}	35.0^{x}	38.7^{ax}	37.7^{bx}	40.1^{cx}	0.22^{x}	0.25^{ax}	0.23^{x}	0.28^{bx}
C 273	NS	120.4	124.0^{a}	123.0^{b}	133.0^{c}	42.6	45.6^{a}	44.0^{b}	47.6^{c}	37.2	39.8^{a}	38.6	40.1^{b}	0.45	0.48^{a}	0.48^{b}	0.51^{c}
	LS	89.7^{x}	96.0^{ax}	100.0^{bx}	110.3^{cx}	35.3^{x}	40.6^{ax}	39.1^{bx}	43.1^{cx}	34.1^{x}	36.0^{ax}	35.0	38.6^{bx}	0.24	0.27^{ax}	0.27^{ax}	0.32^{cx}

T1= Control, T2 = Seeds dip with TU (6.6mM) at the time of sowing, T3 = Foliar spray of TU at anthesis, T4 = combined treatment of TU (T3+T4).
Analysis of variance is significant at $p \leq 0.05$; abc denotes differences between control and thiourea treatment and x denotes differences between NS and LS conditions

(Garg *et al*., 2006) and seems to be a key factor in affecting chlorophyll levels as observed in this study. This was also apparent from the results of thiourea application wherein significant loss of chlorophyll in PBW 550 was prevented especially in the LS crop compared to NS crop (Fig. 1a).

Terminal heat stress experienced by genotypes under late planting condition caused increase in total proteins up to 90 days after sowing followed by steady decrease till maturity (Fig. 2c). Increase in soluble protein in pollen of rice under heat stress was also observed by Tang *et al*. (2008), which contribute to maintain cell structure and function under stress conditions. Mahatma *et al*. (2009) also reported increase in amino acid contents with the exogenous application of thiourea. Significant increase in amino acid contents with both seed treatment and foliar spray of TU has also been reported by Garg *et al*. (2006).

The various morphological parameters studied revealed significant variation under stress and non-stress environmental conditions among different genotypes. Heat stress significantly affected yield related parameters. Decrease in plant height due to heat stress might be due to carbon limitation via photosynthesis or reserve mobilization/transport from the photosynthetic tissues that is an important area of future research. The role of peduncle in heat stress was well demonstrated due to its role in photosynthesis and stem reserve mobilization (Villages *et al*., 2007). Ahmadi *et al*. (2008) also observed similar decrease in plant height in cereals. Plant height of all the genotypes decreased in the late sown crop, however a significant increase in the height was observed with thiourea pretreatment in both normal and late sown crops (Table 1).

Grain weight is an important yield-contributing factor. It depicts the boldness and density of yield. Brief exposure of plant to heat during grain filling can decrease the seed weight (Wahid *et al*., 2007; Farooq *et al*., 2011). Heat stress during grain filling period leads to shortening of grain filling duration, which ultimately results in decreased yield by decreasing kernel weight (Tahir and Nakata, 2005; Farooq *et al*., 2011). Grain filling encompasses translocation of assimilates before and after anthesis and remobilization of vegetative reserves hence affecting yield. Delay of wheat sowing date reduced wheat yield as a result of exposure to heat stress. Reduction of grain weight in wheat under heat stress could be caused by accelerated phases of crop development, accelerated senescence, increased respiration, reduced photosynthesis and inhibition of starch synthesis in developing kernels (Hamam and Khaled, 2009).

This study demonstrates that low H_2O_2 production and high antioxidant activity contribute to greater heat tolerance. Foliar spray and seed pretreatment with TU significantly improved leaf membrane stability and enzyme performance leading to increased yield components.

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