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Assessment of the impact of thiourea on biochemical and physiological characteristics of wheat under heat stress

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Abstract
We assessed thiourea spray treatment of three different concentrations 0.5mM, 0.6m M and 0.7mM was given only once at 40 days after sowing to variety HUW 468 at timely and late sowing conditions. Observations were taken on upper fully expanded leaves for recording different physiological, biochemical observations at 10, 20 and 30 days after thiourea spray i.e. 50, 60 and 70 days after sowing and yield parameters were recorded at harvest. Highest percent decrease in membrane stability index (MSI) of HUW468 was noticed at 70 DAS under third date of sowing (Dec 25) as compared to the normal sowing. The highest increase in chlorophyll (23.08%) was noticed with 0.6mM thiourea at 70 DAS in HUW 468. There was slight decrease in protein content at different days after sowing OF HUW-468 under normal as well as late sowings. There was not much change in phenol content at different days after sowing of HUW-468 under normal as well as late sowings in all the three genotypes. No change in the phenol content significantly in all the three genotypes under normal as well as very late sowing. Spray of thiourea resulted in small increase in the peroxidase activity in all the three genotypes under normal, late and very late sowing, and the maximum increase was noticed with 0.6mM thiourea. Highest increase was seen under normal sowing at 70 DAS.

Keywords: Membrane stability index, phenol, protein, thiourea, peroxidase

1. Introduction
In India, the whole wheat growing area falls under the tropical and subtropical environment. The optimum sowing time for wheat in the major wheat growing area in India (North-west and central India) is first three weeks of November. A yield reduction of 0.7% per day occurs when sowing is delayed. Delayed planting in wheat causes poor grain filling that results in shrivelled grains of below standard (Kaur and Behl, 2010) [16]. Heat causes more than 30% yield loss in wheat. Heat stress influences various processes including physiological, growth, developmental, yield and quality of crop. High temperatures, above 30^{o}C affect final grain weight by reducing the duration of grain filling, because of the suppression of current photosynthesis and by inhibition of starch production in the endosperm. According to Levitt (1980) [19] abiotic stress is, any change in environmental conditions that reduces or adversely affects plant growth and development. This high temperature at the time of grain development limits the yield and quality of wheat (Alkhatib and Plauson, 1984) [2]. It is therefore, proposed to study the response of the wheat crop grown under normal and late sowing conditions (Gupta *et al*., 2002) [13] leading to heat stress particularly at the grain filling stage and the effect of thiourea on these responses. This study is important in view of present scenario of climate change.

Thiourea has been reported to play a vital role in the physiology of plants both as a sulphydryl compound and as an amino compound like urea (Mayer, 1956; Mayer and Poljakoff-Mayber, 1958) [23, 24]. It promotes growth in cytokinin requiring callus tissues in absence of kinetin in various crops (Erez, 1978) [11]. Application of thiourea especially under late sown condition may enhance photosynthetic efficiency with greater translocation and partitioning of metabolites towards reproductive sink, which ultimately leads to greater seed yield. In wheat, 500 ppm foliar spray of thiourea at tillering and flowering increased the grain yield by 24 per cent (Sahu and Singh, 1995) [28]. The heat tolerant wheat variety cv. PBW-343 exhibited higher content of antioxidants and activates anti-oxidative enzymes, while lower content of lipid peroxides as compared to the heat sensitive cv. HD-2329 (Asthir *et al.*, 2012) [5]. Analysis of biochemical traits at heading stage in wheat leaves sprayed with 2.5-5.0 mmol thiourea showed increase in caroteniods, antioxidant enzyme activity, metabolites inducing growth promoters and photosynthetic pigments and carbohydrates (Abdlkader *et al.*, 2012) [1].

Yadav (2005) [36] found that application of 500 ppm thiourea in wheat (seed soaking + foliar spray) significantly increased the growth parameters such as plant height, number of tillers m^{-1} row length, total dry matter accumulation, dry matter accumulation in leaf and panicle, and chlorophyll content in leaves over control. Similarly, 500 ppm spray of thiourea in oat at tillering and flowering gave very encouraging results with respect to growth parameters (Yadav, 2000) [37]. Likewise, Lakhana (2002) [18] observed that foliar spray of thiourea (500 ppm) brought a significant increase in plant height, dry matter accumulation, dry matter partitioning and leaf area index (LAI) over control.

Mahavar (1989) [22] in a study while assessing the role of thiourea in mustard production, observed that foliar spray of 0.2 per cent thiourea significantly increased the LAI, dry matter accumulation per plant as compared to control. Opium poppy seeds soaked with thiourea solution showed significant improvement in dry matter accumulation, LAI and crop growth rate (CGR) over the control (Saini 1991) [29]. Khafi (1991) [17] recorded that foliar spray of 0.1 per cent thiourea significantly increased the plant height, LAI, dry matter per plant and dry matter accumulation by leaves, stem and siliqua in mustard.

Foliar spray of 500 ppm thiourea increased dry matter accumulation per plant, LAI and number of leaves per plant at physiological maturity of maize (Sachan 1991, Sahu *et al*., 1993, Rathore 1996 and Sharma 1997) [26, 27, 25, 32]. Intodia and Tomar (1994) [14] noted that application of 500 ppm thiourea (both seed soaking and foliar application) on foxtail millet significantly increased the plant height, dry matter distribution in spikes and LAI over water sprayed control. Sahu and Singh (1995) [28], while assessing the role of thiourea on wheat productivity via soil and foliar application, observed that foliar application of thiourea brought about a significant increase in dry matter accumulation and its distribution to leaves, stems and spikes.

Wheat variety HUW 468 is an important variety in Rajasthan covering large area and is recommended for normal sowing time. However, the early growth of this variety is very slow, and the maturity generally coincides with high temperature. Therefore, it is necessary to look for the use of chemicals like thiourea to break the yield stagnation of these important varieties under normal and late sown conditions.

Thus, for exploitation of genetic yield potential of the crop to the economic maxima with low cost technologies, time of planting, varieties and application of thiourea are the important deciding factors. However, work on the physiological manipulation of the crop involving these factors is lacking. It was, therefore, considered necessary to study the effect of thiourea on physio-biochemical attributes of heat tolerance in wheat genotypes objective to study the effect of thiourea on biochemical parameters of wheat under heat stress.

2. Materials and method

The research work was carried out at the Experimental Farm, Institute of Agricultural sciences, Banaras Hindu University, Varanasi during Rabi season 2016-18. Geographically, Varanasi is situated in the agro-climatic zone of eastern plain region of Uttar Pradesh, the urban agglomeration is stretched between 82° 56'E - 83° 03'E and 25° 14'N - 25° 23.5'N. Experiment was conducted at 25018/N and 83003/E Longitude.

2.1 Environmental conditions

The climate of the region is typically semi-arid, characterized by aridity of atmosphere, with extremity of temperature fluctuations in summer and winter. During summer, maximum temperature ranges between 35-45 ^{0}C while in winter it may fall down to as low as 4 ^{0}C. The average rainfall of this area is approximately 1140 mm, most of which is received during rainy season from July to September. The soil was sandy loam having a bulk density of 1.5 g cm^{-1}, pH 8.2, ECe 1.1 dSm^{-1}, SAR 12.5, field capacity 11.8%, permanent wilting point 2.8%, particle density 2.65 mg m^{-3}, organic carbon 0.17%. Laboratory work was conducted in Department of Plant Physiology, Institute of Agriculture Sciences BHU, Varanasi.

2.2. Experimental Design:

The experiment was laid in Randomized Block Design (RBD). The genotype was planted in four lines in the one-row fashion of a 1m row length and row spacing was maintained at 22.5cm. The experiment was done with two sowing dates one is timely sown in 3rd week of November and 2nd is late sown in the 3rd week of December. Plots were maintained in each date of sowing. The line sowing method was used for sowing. The field was irrigated at all critical stages of wheat and followed by a recommended standard of cultural and agronomic practices to raise the healthy crop. Fertilizers NPK were applied @ 120:60:40 kg/ ha during total crop cycle.

2.3. Treatment Details

Thiourea was sprayed at 40 days after sowing. Three different concentrations of thiourea 0.5 Molar, 0.6 Molar and 0.7 Molar were used. Thus, for the genotype, the treatments were:

T0: Control, T1: 0.5 M Thiourea, T2: 0.6 M Thiourea and T3: 0.7 M Thiourea

2.4. Biochemical analysis

2.4.1. Membrane stability index (%)

It was determined by the method of Sullivan (1972) [34]. A paired set of plant material (1 g leaves each) under different treatments were washed thoroughly with three changes of distilled water to remove electrolytes adhering to plat material and taken into test tubes. To each test tube 25 ml of double distilled water (ddH_2O) was added. Now one set of test tubes was covered with tuber cork and incubated in a water bath at 45 ^{0}C for 1 hour and the other set at room temperature. The set kept at room temperature was denoted as control and the other kept at 45 ^{0}C was denoted as treatment. Both the control and treatment set were kept at 10 ^{0}C for 24 hours to allow diffusion of electrolytes from leaf segments. The test tubes were brought to 25 ^{0}C and shaken to mix the contents initial conductance of the test tube contents was determined with conductivity meter (systronics, India). Now tubes of both the sets were boiled for 30 minutes in boiling water bath. After cooling to room temperature, the volume was made to 25 ml with double distilled water and the final conductance was measured. The membrane stability was determined by the following equation:

Per cent membrane stability (MS) = $[(1-T_1/T_2) / (1-C_1/C_2)] \times 100$ Where, T and C refers the EC of treatment and controls and subscripts 1 and 2 to initial and final conductance respectively.

2.4.2. Chlorophyll content

Total chlorophyll content (mg g^{-1} fresh weight) (The sum of chlorophyll "a" and chlorophyll "b") were estimated according to the method of Arnon (1949) [4]. Sample extract was prepared from 100 mg of leaf samples in 10 ml of 85% acetone and the homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was transferred to a 25 ml measuring cylinder. The residue was re-extracted with 5 ml of acetone, centrifuged and the supernatant was transferred to the measuring cylinder.

Final volume of the supernatant was made to 20 ml with 85% acetone. Finally, the optical density (OD) of chlorophyll "a" and "b" was measured at 663 nm and 645 nm. The total chlorophyll content was calculated by the formula:

Total chlorophyll (mg/l extract) was calculated as:

Total chlorophyll (mg/l) = $20.02\ A_{645} + 8.02\ A_{663}$

2.4.3 Soluble protein content

Protein (mg/g fr.wt.) was measured by method of (Lowry *et al.*, 1951) [20]. 100 mg plant leaves were homogenized in 25 mM phosphate buffer (pH 7.5). The supernatant was collected after centrifugation and final volume was made to 10 ml with the buffer. Aliquot (0.2 ml) was taken in test tube and to this 0.8 ml distilled water added, followed by 5 ml alkaline copper solution. The mixture was mixed well and kept at room temperature for 10 minutes. Then 0.5 ml of normal Folin's reagent was added rapidly with immediate mixing and kept at room temperature for 30 min under dark. Absorbance of blue colour was measured at 660 nm. The standard curve was prepared by taking known amount of bovine albumin serum (BSA).

2.4.4 Total phenols

Total phenol content (µg/g) in leaves was estimated by folin-ciocalteu method as described by Mahadevan and Sridhar (1974) [21]. Estimation of phenols with folin-Ciocalteu reagent is based on the reaction between phenols and an oxidizing agent phosphomolybdate which results in the formation of a blue complex (Bray and Thrope, 1954) [7]. A blank containing all the reagents minus plant extract was used to adjust zero absorbance. The intensity of the colour of the samples containing plant extract was measured at 650 nm. Total phenol content was worked out from the readings with the help of standard curve made from different concentrations of catechol.

2.4.5 Estimation of peroxidase activity (enzyme unit) x 10^3

Peroxidase activity was estimated by using method of Shannon *et al*., (1971) [31] To estimates peroxidase activity, 100 mg leaf sample was grinded in 10 ml of 0.1 M phosphate buffer (pH 7.5) and the homogenate was centrifuged at 5000 rpm for 10 minutes. Supernatant was collected and the volume of this enzymes extract was made to 10 ml with phosphate buffer. Enzyme extract (0.2 ml) was added to the 5.6 ml of working solution in cuvette, mixed well and absorbance was adjusted to zero at 460 nm. Reaction was started by adding 0.2 ml of 1M H_2O_2 in the cuvette and mixing it. Absorbance was taken at 15 seconds intervals upto 120 seconds. Enzyme activity was calculated as enzyme units. One enzyme unit was defined as increase in absorbance of 0.1 OD per minute per gram fresh weight of the samples.

2.4.6 Superoxide Dismutase Activity (EU mg^{-1} protein min^{-1})

Superoxide dismutase (SOD) activity was assayed in fully expanded leaves at active tillering, flowering and grain filling stage under normal and delayed sown plants by the method of Dhindsa *et al* (1982) [8]. 3.0 ml of the reaction mixture containing 0.1 ml of 1.5 M sodium carbonate, 0.2 ml of 200 mM NBT,0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml of distilled water and 0.1 ml of enzyme extract were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml riboflavin (60 µM) and placing the tubes below a light source of two 15W fluorescent lamps for 15 minutes. Reaction was stopped by switching off the light and covering the tubes by black cloth. A non-irradiated complete mixture that did not develop colour served as blank. Absorbance was recorded at 560 nm by using spectrophotometer (ELICO SL- 196). Enzyme units were calculated as follows:

$$\text{Enzyme Unit (EU)} = \left[\frac{\text{Enzyme}^{*}_{(\text{light})} - (\text{Enzyme}^{\#}_{(\text{light})} - \text{Enzyme}^{*}_{(\text{dark})})}{\text{Enzyme}^{*}_{(\text{light}/2)}}\right]$$

* Without enzyme and # with enzyme

The EU was expressed on per g fresh weight basis as well as on the basis of per mg protein (specific activity).

2.5 Statistical analysis

All the observations were taken in triplicates and data were analysed statistically using randomized block design in field experiment. Standard error of mean ($SE_{m\pm}$) and critical difference (CD) were calculated at 5 per cent level of significance with error degree of freedom.

3. Results

3.1 Membrane stability index (MSI) (%)

Effect of thiourea on MSI is shown in table 4.4 in variety HUW 468. Highest MSI 70.67 was recorded at 0.6mM at timely sown condition and lowest 54.55 were recorded at control condition. While in late sown condition Highest MSI 65.01 was recorded at 0.6mM at late sown condition and lowest 50.78 was recorded at control condition.

Table 1: Effect of heat stress in association with thiourea on Membrane Stability Index (MSI) of wheat (%)

MSI																							
control						0.5M						0.6M						0.7M					
50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS	
T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
59.85	55.85	56.59	53.12	54.55	50.78	64.67	60.51	61.78	57.81	59.23	55.58	70.07	65.01	67.04	62.2	64.49	59.83	69.56	64.45	66.63	61.74	64.07	59.37

Where DAS = Days after sowing, M = Molarity, T = Timely, L =Late sowing *SE(m) = 0.092 *C.V. = 0.29

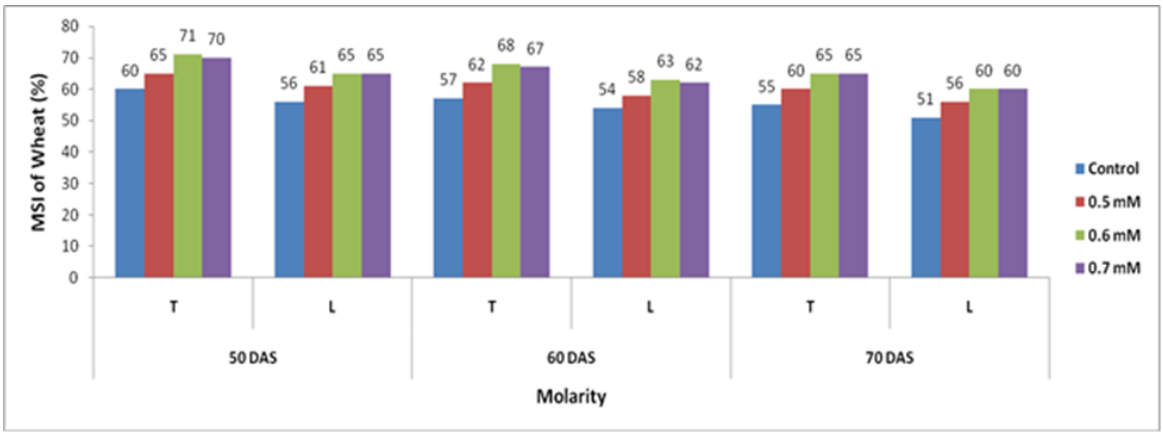

Fig 1: Effect of heat stress in association with thiourea on Membrane Stability Index (MSI) of wheat (%)

3.2. Chlorophyll (mg g^{-1} fresh weight)

Effect of thiourea on chlorophyll is shown in table in 4.4.2 variety HUW 468. Highest chlorophyll 1.63 was recorded at 0.6mM at timely sown condition and lowest 1.0 were recorded at control condition. While in late sown condition Highest chlorophyll 1.29 was recorded at 0.6mM at late sown condition and lowest 0.78 was recorded at control condition.

Table 2: Effect of heat stress in association with thiourea on Chlorophyll of wheat (mg g^{-1} fresh weight)

Chlorophyll																							
Control						0.5M						0.6M						0.7M					
50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS	
T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
1.38	1.1	1.19	0.9	1	0.78	1.52	1.21	1.31	1.05	1.13	0.9	1.63	1.29	1.41	1.12	1.21	0.96	1.59	1.26	1.39	1.1	1.18	0.93

Where DAS = Days after sowing, M = Molarity, T = Timely, L =Late sowing *SE(m) =0.313 *C.V. = 0.219

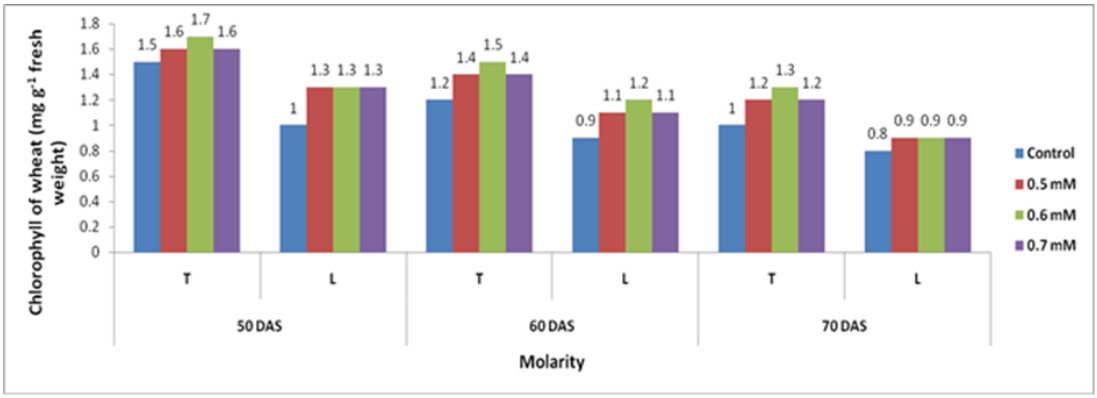

Fig 2: Effect of heat stress in association with thiourea on Chlorophyll of wheat (mg g^{-1} fresh weight)

3.3. Total phenols (µg g^{-1})

Effect of thiourea on phenol is shown in table 4.6 in variety HUW 468. Highest phenol 4.20 was recorded at 0.6mM at timely sown condition and lowest 4.11 were recorded at 0.7mM. While in late sown condition Highest phenol 4.16 was recorded at 0.6mM at late sown condition and lowest 4.09 was recorded at control condition.

Table 3: Effect of heat stress in association with thiourea on phenol content in wheat (µg/g)

Phenol																									
control						0.5M						0.6M							0.7M						
50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS			50 DAS		60DAS		70DAS	
T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
4.16	4.12	4.15	4.1	4.14	4.09	4.18	4.14	4.17	4.12	4.16	4.11	4.2	4.16	4.19	4.14	4.18	4.12	4.11	4.15	4.18	4.14	4.19	4.13

Where DAS = Days after sowing, M = Molarity, T = Timely, L =Late sowing *SE(m) = 0.064 *C.V. = 0.168

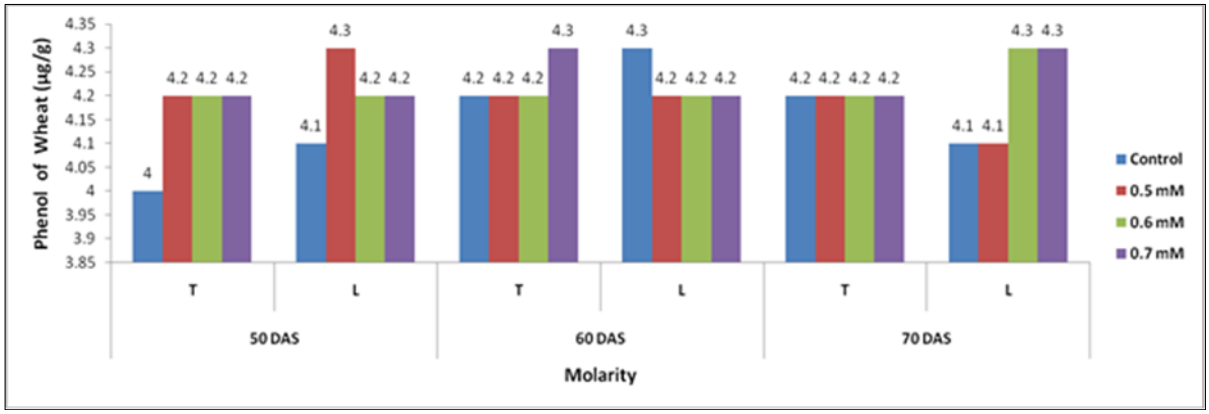

Fig 3: Effect of heat stress in association with thiourea on phenol content in wheat (µg/g)

3.4 Soluble protein content (mg g^{-1} fresh weight)

Effect of thiourea on soluble protein is shown in table 4.7 in variety HUW 468. Highest protein content is 23.50 was recorded at 0.6mM at timely sown condition and lowest 20.90 were recorded at Controlled condition. While in late sown condition highest protein 22.80 was recorded at 0.6mM at late sown condition and lowest 21.05 was recorded at Control (untreated).

Table 4: Effect of heat stress in association with thiourea on soluble protein content (mg g^{-1})

Soluble protein content (mg g^{-1} fr.wt.)																							
Control						0.5 mM						0.6 mM						0.7 mM					
50 DAS		70 DAS		90 DAS		50 DAS		70 DAS		90 DAS		50 DAS		70 DAS		90 DAS		50 DAS		70 DAS		90 DAS	
T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
20.9	21.4	21.6	22.1	21.3	21.0	22.1	22.1	21.1	21.2	22.1	21.0	22.4	22.8	22.5	22.6	23.5	22.3	21.5	21.3	21.4	21.5	22.4	21.2

Where DAS = Days after sowing, M = Molarity, T = Timely, L =Late sowing *SE(m) = 0.054 *C.V. = 0.284

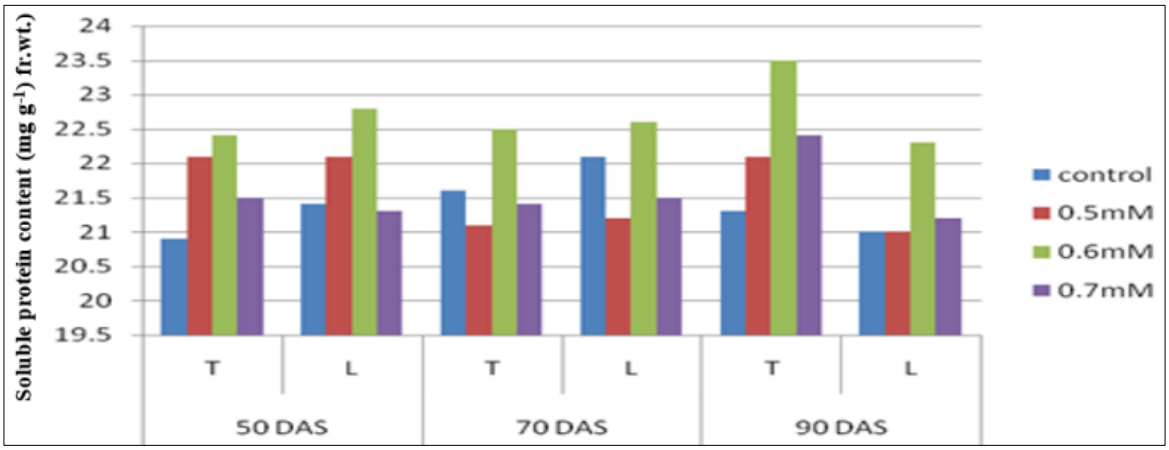

Fig 4: Effect of heat stress in association with thiourea on soluble protein content (mg g^{-1})

3.5 Peroxidase (enzyme unit) x 10^3

Effect of thiourea on peroxidase is shown in table 4.8 in variety HUW 468. Highest peroxidase 0.60 was recorded at 0.6mM at timely sown condition and lowest 0.46 were recorded at control condition. While in late sown condition highest peroxidase 0.53 was recorded at 0.6mM at late sown condition and lowest 0.39 was recorded at control condition.

Table 5: Effect of heat stress in association with thiourea on peroxidase of wheat (enzyme unit) x 10^3

Peroxidase of Wheat (enzyme unit) x 10^3																							
Control						0.5 mM						0.6 mM						0.7 mM					
50 DAS		60 DAS		70 DAS		50 DAS		60 DAS		70 DAS		50 DAS		60 DAS		70 DAS		50 DAS		60 DAS		70 DAS	
T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
0.5	0.4	0.3	0.4	0.5	0.4	0.6	0.5	0.6	0.5	0.6	0.4	0.6	0.5	0.6	0.5	0.6	0.4	0.5	0.5	0.6	0.5	0.5	0.4

Where DAS = Days After Sown, m = milli, M = Molar, T = Timely Sown, L = Late Sown. *SE(m) = 0.082 *C.V. = 0.074

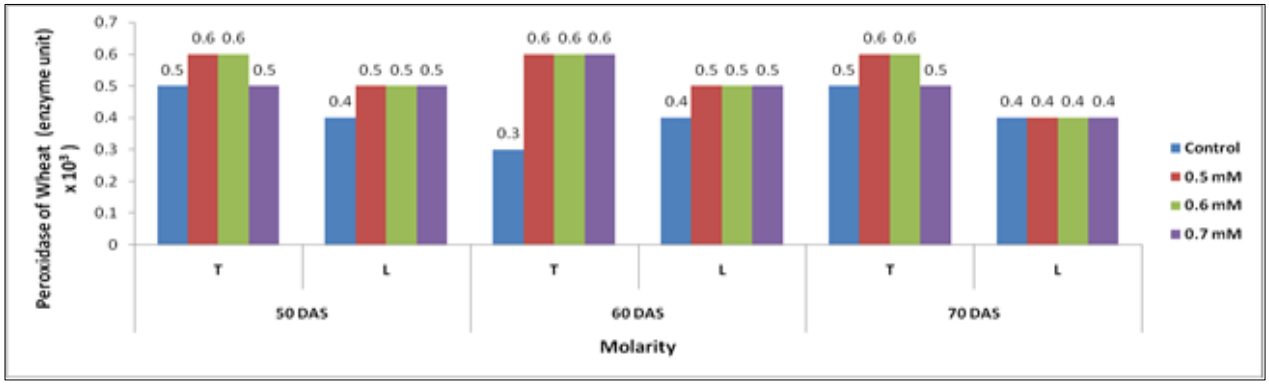

Fig 5: Effect of heat stress in association with thiourea on peroxidase of wheat (enzyme unit) x 10^3

3.6 Superoxide Dismutase Activity (EU mg^{-1} protein min^{-1})

Effect of thiourea on SOD is shown in table 6 in variety HUW 468. Highest SOD 0.54 was recorded at Controlled condition at timely sown condition and lowest 0.37 were recorded at 0.6mM. While in late sown condition Highest SOD 0.69 was recorded at controlled condition at late sown condition and lowest 0.52 was recorded at 0.6mM.

Table 6: Effect of heat stress in association with thiourea on SOD of wheat (EU mg^{-1} protein min^{-1})

SOD																							
control						0.5M						0.6M						0.7M					
50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS		50 DAS		60DAS		70DAS	
T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L	T	L
0.42	0.59	0.49	0.63	0.54	0.69	0.41	0.54	0.45	0.6	0.49	0.65	0.37	0.52	0.41	0.57	0.46	0.63	0.38	0.53	0.42	0.58	0.47	0.64

Where DAS = Days after sowing, M = Molarity, T = Timely, L =Late sowing*SE(m) = 0.053 *C.V = 0.062

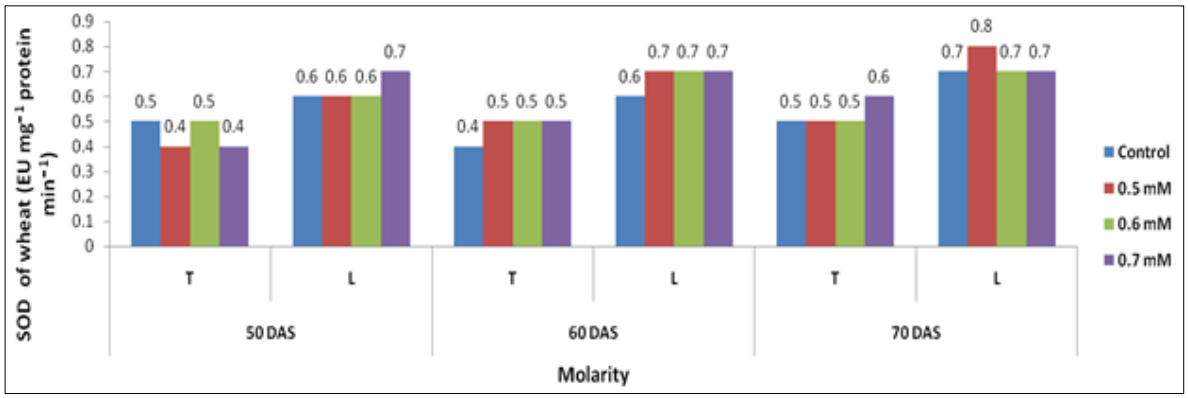

Fig 6: Effect of heat stress in association with thiourea on SOD of wheat (EU mg^{-1} protein min^{-1})

4. Discussion

The physiological parameters studied in the present investigation are per cent seed germination, Membrane stability index (MSI), total chlorophyll content, and leaf area. Among the biochemical parameters, total protein content, total phenols, peroxidase activity were investigated in fully expanded leaves. Membrane stability index (MSI) decreased at different days after sowing under all the two dates of sowing of the genotypes. Highest percent decrease in HUW468 was noticed at 70 DAS under third date of sowing (Dec 25) as compared to the normal sowing. Spray of thiourea could restore the MSI to some extent in all the three genotypes under different dates of sowing. Maximum restoration was observed with 0.6mM thiourea in all the three genotypes, however, highest MSI was noticed in HUW468 under very late. Decrease in MSI could be due to heat stress which makes the lipid bilayer of biological membrane more fluid by either denaturation of proteins or an increase in unsaturated fatty acids (Savchenko *et al*., 2002) [30]. Such alteration enhances permeability of membrane and results in loss of electrolytes. In another study, significant decrease in MSI with delay in sowing as well as different days after sowing under higher temperature as compared to the ambient (as during normal sowing date) stress has also been reported in Cowpea (Ismail & Hall, 1999) [15]. Since the function of cellular membrane under heat stress (Late sowing) is critical for the processes such as photosynthesis and respiration (Blum, 1988) [6].High temperature and salinity have a common facet of oxidative damage (Wahid *et al*., 2007a) [35] but foliar spray of thiourea (it has imino and thiol functional groups) provides a ready source of nitrogen and thiol which has great role in alleviating oxidative stress damage in physiologically more important leaf tissues. Chlorophyll content decreased at different days after sowing under all the three dates of sowing in all the three genotypes. It has also been experimentally seen under controlled conditions that heat stress inhibit Chlorophyll accumulation in wheat as reported by Efeoglu and Terzioglu (2009) [9]. Spray of thiourea could restore the chlorophyll content to some extent in all the three genotypes under different dates of sowing. The highest increase in chlorophyll (23.08%) was noticed with 0.6mM thiourea at 70 DAS in HUW 468. Our results are supported by results of Sharma *et al*. (2008) [33] which showed Increase in Chlorophyll content along with other yield

attributes in wheat with use of bio-regulators and thiourea foliar spray. Emmanuel *et al.* (2010) [10] has also reported increase in Chlorophyll content and amylase, SOD enzymes due to rare earth elements treatment in wheat. Higher temperature stress, witnessed as a result of delayed sowing induces degradative action on cell membrane including chloroplast membrane and chlorophyll. Spray of thiourea is found to increase the leaf area in this variety under normal, late sowing but maximum increase was noted with 0.6mM thiourea. Highest increase was seen in HUW468 under late sowing at 50 DAS. Thiourea is reported to delay leaf ageing and senescence and enhances photosynthetic efficiency leading to increased growth and yield of plants. The response of crop species to temperature depends upon the temperature optima of photosynthesis, growth and yield which is crucial for stress tolerance and also reported by Anjum *et al*. (2011) [3] in wheat. This contention is further supported by the results of the present study. Our results are in agreement with the results reported by Sahu *et al*. (1993) [27], Garg *et al.* (2003) [12] who have shown that photosynthetic efficiency of thiourea applied plants was better in mungbean as net photosynthetic rate was higher along with the higher contents of total chlorophyll as compared to the control.

There was slight decrease in protein content at different days after sowing of HUW-468 under normal as well as late sowings. However, total protein content under control was lower in late sowing as compared to normal sowing in all the three genotypes. Spray of thiourea did not influence the protein content in the three genotypes under normal, late and very late sowing. There was not much change in phenol content at different days after sowing of HUW-468 under normal as well as late sowings in all the three genotypes. Spray of thiourea also did not change the phenol content significantly in all the three genotypes under normal as well as very late sowing. There was no significant change in peroxidase activity at different days after sowing of HUW-468 under normal, late as well as very late sowing. Spray of thiourea resulted in small increase in the peroxidase activity in all the three genotypes under normal, late and very late sowing, and the maximum increase was noticed with 0.6mM thiourea. Highest increase was seen under normal sowing at 70 DAS. Increased activity of peroxidase due to thiourea suggests that there is need to scavange free radicals, however Yonova and Zozikova (2001) [38] reported decrease in peroxidase activity and catalase activity due to thiourea treatment but increase in guaiacol peroxidase activity in barley.

5. Conclusions

This preliminary study on effect of thiourea concentrations on mitigating heat stress effect is encouraging and if this economic source is utilized for maintaining wheat plant tolerance at the threshold level of 0.6 mM of thiourea, there is possibility in alleviation of terminal heat stress in terms of physiological processes in plants. The increased antioxidant enzymes activity further strengthens this fact. On the basis of interesting findings, as presented scientifically in the preceding pages, this study needs further clarifications by planning extensive physiological, biochemical and molecular research necessary in the present scenario. The temperature sensitive wheat plants need transformation to overcome the terminal heat stress by developing tolerance through any strategy and this research is just a ray of hope in this direction.

6. References

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