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Full Length Article

Medium Supplementation of Thiourea Orchestrates the Metabolites Levels to Diminish the Oxidative Damage in Hybrid Maize

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Abstract

Worldwide heat stress has become a main issue in crop production and adverse effects of heat stress can be minimized with the exogenous application of stress alleviating compounds. The aim of this work was to find out a possible role of heat tolerance with the medium supplementation of a selected level of thiourea (0.25 m*M*) in reducing heat stress using five maize (*Zea mays* L.) hybrids grown in autumn and spring seasons. Heat stress (7–10ºC higher than ambient) was imposed by keeping the plants in open door plexiglass fitted canopies for 20 days. Heat stress reduced the shoot and root dry weigh while medium supplemental thiourea recuperated the heat stress effects. Among the physiological parameters there was a reduction in the leaf chlorophyll a, b carotenoids and chlorophyll/carotenoids ratio, while thiourea supply reduced the chlorophyll/ carotenoids ratio indicating a greater role of carotenoids in heat tolerance. Heat stress increased the degree of oxidative stress revealed from enhanced production of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) more in the shoot than root while medium supplementation of thiourea nullified this tendency especially in the spring season. Thiourea caused an enhanced accumulation of free proline, anthocyanins and ascorbic acid as indicators of stress tolerance more in the spring than autumn season. Correlations established between shoot and root dry weight with respective metabolites revealed that under thiourea medium supplementation H_2O_2 and MDA were negatively while free protime, anthocyanins and ascorbic acid were positively correlated under heat stress. Results revealed that effectiveness of thiourea was much greater in spring than in winter season, while differences in the hybrids were well marked. Medium supplementation of thiourea appeared to improve the shoot physiological attributes by eliciting root to shoot signaling relating to enhanced carotenoids synthesis and reduced the oxidative damage. © 2019 Friends Science Publishers

Keywords: Oxidative damage; Carotenoids; Thiourea; Seasons; Correlations; Signaling

Introduction

Climate change is looming issue and major concern for the plant scientists. Global temperature is rising by 0.2ºC per decade due to which average temperature is expected to increase 1.8–4.0ºC than the existing level by 2100. It is apprehended that high global temperature will alter the temperature zones and conditions for production of crops (IPCC, 2007). Adverse changes occur in physiology, growth and development of plants, ultimately there is considerable yield loss occur under temperature stress (Porter, 2005; Barnabas *et al.*, 2008; Farooq *et al.*, 2011). Enzymes involved in different metabolic pathways are sensitive to various levels of heat stress. Excessive production of reactive oxygen species occurs under heat stress that leads to oxidative damage (Hasanuzzaman *et al*., 2013). Heat stress affects the function of enzymes and metabolic pathways enhances the production of reactive oxygen species (ROS) including singlet oxygen, superoxide radical, hydroxyl radical and hydrogen peroxide that causes oxidative damage (Asada, 2006). Proteins oxidation, peroxidation of lipids and damage to DNA occur due to ROS that are strong oxidizing agent that are responsible for cell death (Hasanuzzaman *et al*., 2013).

It is known that malondialdehyde (MDA) increased in rice (*Oryza sativa*) and maize (*Zea mays*) genotypes due to heat stress as a result of oxidative damage caused by escalated production of ROS (Chalanika De Silva and Asaeda, 2017). Savicka and Skute (2010) reported that ROS produced at 33ºC that disrupt membrane properties, proteins destruction and deactivation of enzymes as a result cell sustainability decreases. High temperature increased the hydrogen peroxide (H_2O_2) and MDA production, thereby damaging the membranes (Mohammed and Tarpley, 2010). However, the production of ROS and damage to cellular structures can be prevented with the use of stress alleviating molecules including hormones, osmoprotectants and plant extracts used in whatever the application mode (Wahid *et al.*, 2017; Hussain *et al.*, 2018; Tabassum *et al.*, 2018).

During stressful conditions, such as heat stress, the redox homeostasis in plants is managed by both enzymatic and non-enzymatic antioxidants (Gill and Tujeta, 2011; Miller *et al*., 2010) and osmoprotectants (Wahid *et al.*, 2007;

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Asthir, 2015). Among the various stress alleviating compounds, thiourea is being increasingly used in a number of studies due to its profound effects on plants during stressful conditions. The major roles of thiourea include the reduced peroxidation of lipids and H_2O_2 production by inducing the antioxidant machinery, improving the carotenoids contents and production of such metabolites that rescue the plants from different stresses when used as seed pretreatment, foliar spray and medium supplementation (Wahid *et al.*, 2017). Thiourea application induced tolerance in maize against salt stress, it decreased Na but enhanced the uptake of nitrogen (N), potassium (K), calcium (Ca) and phosphorus (P). Growth of maize is regulated by thiourea because it reduced contents of MDA and H_2O_2 and changes the activities of antioxidants, in addition improves the uptake of photosynthetic pigments (Abdelkader *et al*., 2012; Wahid *et al*., 2017).

Over time, novel roles of thiourea are emerging. As yet there is no consensus on a single mechanism of thiourea action. Instead the roles of thiourea range from whole plant growth improvement to improved nutrient uptake and modulation of gene expression. We surmise that prevailing climatic conditions and different seasons may have differential effects of thiourea in modulating the plant metabolism. The objective of this study was to underline the role of medium supplementation of thiourea on the changes in photosynthetic pigments, oxidative damage, osmolytes accumulation and non-enzymatic antioxidants in five maize hybrids grown in spring and autumn season.

Materials and Methods

The seeds of five maize hybrids named as DK6789, 30Y87, 32B33, 31R88 and 30R50 were obtained from the Maize and Millet Research Institute (MMRI), Yousafwala, Sahiwal, Pakistan. Separate experiments were conducted in two growth seasons *i.e*., spring (February–March) and autumn (July–September) during 2015. Seeds (10 in number) of each hybrid were grown in plastic pots containing 10 kg of thoroughly washed sand. A hole was made in the bottom for leaching during replacement of sand solution. All the pots were applied with 1 L of the half strength nutrient solution (Hoagland and Arnon, 1950), which was replaced after every four days. The experiment design was completely randomized with three replications. Four healthy and uniform plants were retained in each pot after germination and thinning.

Fifteen days old plants were subjected to different treatments namely control (no thiourea or heat stress applied), medium supplemented thiourea (0.25 m*M*), heat stress and combined thiourea and heat stress application. Thiourea was applied by dissolving in nutrient solution while heat stress was applied by shifting the potted plants to open door plexiglass fitted canopies that had about 7–8ºC higher temperature than ambient. After 15 days of treatment application plants were harvested and the data were taken for growth and physiological attributes. Plants were uprooted carefully, washed with tap water and blotted dry. A portion of the shoot and root was put in zip bags and immediately transferred to a freezer at -35–40ºC. Remaining portion was put in paper bags for drying in an oven at 70ºC for one week to get shoot and root dry weight.

Immediately after harvesting the plants, the concentration of chlorophylls a and b measured following the method of Arnon (1949) whereas carotenoids were estimated following the method of Davis (1976). Fresh leaves (0.5 g) were grind well and extracted in 20 mL of 80% acetone. The extract was vacuum filtered through Whatman No. 2 filter paper. The absorbance of the supernatant was measured at 645, 663 and 480 nm on spectrophotometer (Hitachi-220 Japan). The H_2O_2 was determined using the procedure of Velikova *et al*. (2000). Plant material separated into shoot and root (0.1 g) was mixed with 0.1% (w/v) trichloroacetic acid (TCA) in an ice bath. Mixture was centrifuged at 12,000 × *g* for 15 min and supernatant was separated. A 0.5 mL each of 10 m*M* potassium phosphate buffer (pH 7.0) and 1M KI were added to 0.5 mL of supernatant, while water was used as blank. The absorbance of supernatant was taken at 390 nm. Following the above procedure, a range of the standards of H_2O_2 (0, 0.2, 0.4, 0.6, 0.8 and 1.0 μM) were made and the absorbance was measured to construct a standard curve. The malondialdehyde (MDA) concentration was ascertained as proposed by Heath and Packer (1968). One mL of 5% TCA (w/v) was used to homogenize 0.1 g fresh leaf tissue. Mixture centrifuged at 12,000 g for 15 min. Supernatant was removed and TBA was added in equal volume. This solution was then allowed to boil at 95ºC for 30 min. After cooling the mixture it was again centrifuged for 5 min at 7500. Absorbance of the solution was taken at 532 nm and 600 nm. Absorbance reading at 600 nm was subtracted from that taken at 532 nm to correct the non-specific turbidity. For the calculation of MDA contents, an extinction coefficient of 155 m/cm was used and 5% TCA solution was used to run as blank.

Free proline was measured with the method of Bates *et al*. (1973). A 0.5 g of fresh shoot and root material was grinded in 3% of aqueous sulphosalicylic acid and filtered. One mL of the filtrate was mixed with 1 mL of acid ninhydrin (1.25 g ninhydrine in 30 mL glacial acetic acid) and 1 mL of glacial acetic acid in a test tube and vortexed. The mixture was kept at 100ºC in water bath for 1 h and then transferred in ice bath to terminate the reaction. Mixture was cooled and added 4 mL of toluene, vortexed for 15–20 sec. Chromophore containing the free proline was aspirated in a test tube, warmed at room temperature and absorbance was measured at 520 nm. The same procedure was followed for blank using 2 mL of toluene. The standard curve was developed by using proline (10 to 50 μg/2 mL). Anthocyanins contents were examined by the method of Stark and Wray (1989). A 0.1 g of fresh leaf and root material was mixed with 1 mL of acidified methanol (1% HCl v/v) in a microfuge tube. After crushing with glass rod it was allowed to heat at 50ºC for 1 h followed by centrifuge.

The supernatant was separated and absorbance was measured at 535 nm on spectrophotometer. Acidified methanol was used as blank. The quantity of anthocyanins was expressed as A535. Ascorbic Acid was analyzed using the method of Mukherjee and Choudhuri (1983). Ten mL solution of 6% TCA was used to extract 0.25 g of leaf fresh material as described. Four mL of the extract was mixed with 2 mL of 2% dinitrophenyl hydrazine solution (by dissolving 2 g of compound in 100 mL of HCl). Then one drop of 10% thiourea solution (prepared in 70% ethanol) was added and boiled for 20 min in water bath, cooled and 5 mL of 80% H_2SO_4 (v/v) was added to the mixture at 0°C. The absorbance was read at 530 nm. A standard curve was prepared by using varying AsA standards to estimate the amount of AsA in extract.

The data were subjected to statistical analysis using STATISTIX v.8.0 software. The analysis of variance was performed and means were compared using LSD test. The correlations were drawn of shoot and root attributes with respective biochemical attributes of the hybrids.

Results

Growth Characteristics

For shoot dry weight, in spring season there was significant overall interaction ($P<0.01$) of the hybrids, thiourea levels and heat stress treatments for shoot and root dry weight. However, in autumn season no interaction was noted ($P>0.05$) in the hybrids, thiourea levels and heat treatments for this attribute (Table 1). Data showed that in spring season dry mass of shoot was the highest in 30Y87 and the lowest in 32B33 under control condition. Heat stress reduced the shoot dry weight in all the hybrids; reduction was the highest in 30R50 (~34%) but the lowest in 31R88 (~20%). Thiourea application enhanced this attribute in all hybrids but a highest improvement was recorded by ~7% in DK6799 under control condition while by 66% in 30Y87 under heat stress condition. In autumn season, however, shoot dry weight was lower as compared to Spring season. Nonetheless, a highest shoot dry weight was recorded in DK6789 under control and in 30Y87 under heat stress. Medium supplementation of thiourea enhanced this attribute maximally by 11% in 30Y87 under control while by 35% in 30R50 under heat stress (Fig. 1a).

In case of root dry mass, there was significant overall interaction ($P<0.01$) of the hybrids, thiourea levels and heat stress treatments for shoot and root dry weight in spring season. However, no interaction of these factors was notable ($P>0.05$) in the hybrids, thiourea levels and heat treatments for this attribute in autumn season (Table 1). Data showed that in spring season a highest root dry weight was produced by 32B33 and a lowest one in 31R88 under control condition. Heat stress reduced this parameter in all the hybrids; reduction being the highest in DK6789 (22%), while the lowest in 31R88 (~8%). Medium supply of thiourea enhanced this attribute in all hybrids and a highest improvement was found by ~54% in 31R87 under control condition and by 66% in 30Y87 under heat stress. In autumn season, on the other hand, root dry weight was quite lower than the spring season but it was highest in DK6789 under control and in 30Y87 under heat stress. Heat stress maximally reduced the root dry mass by ~34% in 31R88. Medium supplementation of thiourea enhanced this attribute maximally in 30Y87 (49%) under control while in 31R88 (89%) under heat stress (Fig. 1b).

Table 1: Least significant difference (LSD) values and significance of three way interactions of some growth and physiological attributes of maize hybrids with medium supplementation of thiourea under control and heat stress conditions in two seasons

Parameters	Spring season		Autumn season	
	Shoot LSD	Root LSD	Shoot LSD	Root LSD
Dry weight	0.244*	0.120**	0.220ns	0.090ns
Chlorophyll a	0.107ns	NA	0.107ns	NA
Chlorophyll b	0.079ns	NA	0.085ns	NA
Carotenoids	0.040*	NA	0.040*	NA
Chlorophyll/carotenoids ratio	0.182*	NA	0.141**	NA
Hydrogen peroxide	0.271**	0.482**	0.440*	0.388*
Malondialdehyde	0.654*	0.478*	0.797*	0.511*
Free proline	1.528**	0.564**	2.145ns	0.680ns
Shoot anthocyanins	0.023*	0.019*	0.025ns	0.015ns
Shoot ascorbic acid	0.730*	0.376*	0.968ns	0.455ns

Photosynthetic Pigments

Non-significant interaction ($P<0.01$) was observed in maize hybrids, thiourea levels and heat stress ($P>0.05$) for chlorophyll a and b in spring and autumn seasons. However, carotenoids and chlorophylls/carotenoids ratio indicated significant ($P<0.05$) interaction of these variables in both the seasons (Table 1). Data showed that in spring season chlorophyll a content was the greatest in 30Y87 and the lowest in 32B33 under control condition. Heat stress reduced this parameter in all the hybrids but 31R88 (~23%) indicated a highest decline. Thiourea application improvised this attribute in all hybrids but a highest improvement (52%) under control while by 95% under heat stress in 32B33. In autumn season, a highest chlorophyll a was estimated in 30Y87 under control and in DK6789 under heat treatment. Heat stress declined the chlorophyll a in all hybrids with a maximum decline in 30Y87 (19%). Medium supplementation of thiourea diminished this attribute maximally by 19% in 30Y87 under control while improved it by 11% in 32B33 under heat stress (Fig. 2a). In spring season, chlorophyll b content was the highest in DK6789 and lowest in 32B33 under control condition, which was reduced in all hybrids under heat stress with a maximum reduction of 43% in 30R50. Medium supply of thiourea improved this attribute in all hybrids but maximally by 27% in 31R88 under control condition.

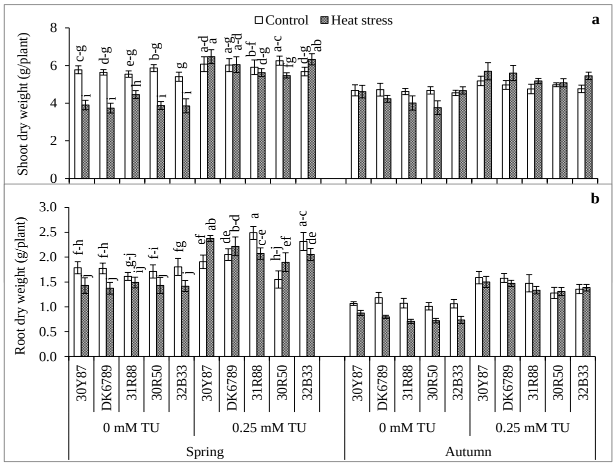

Fig. 1: Variations in some growth parameters of maize hybrids under thiourea and heat stress treatment in spring and autumn seasons

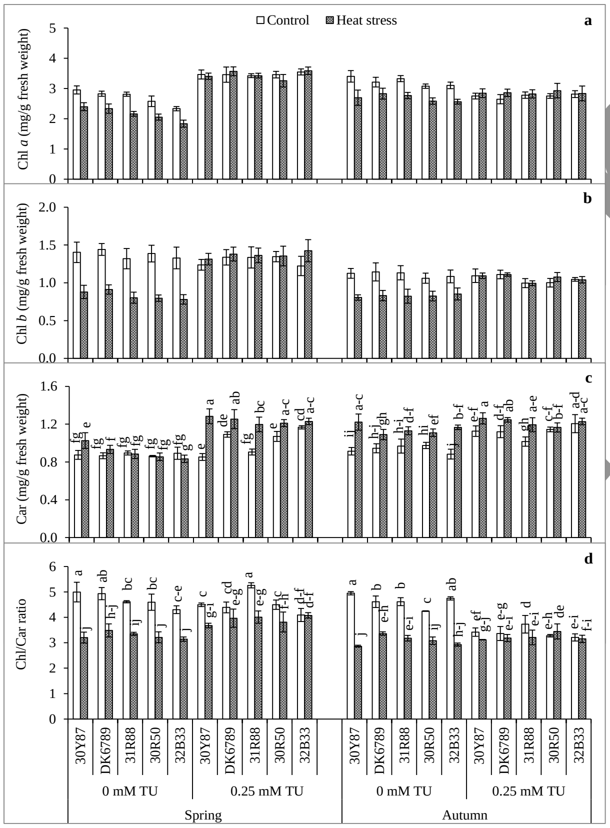

Fig. 2: Variations in leaf area, chlorophyll a, chlorophyll b and carotenoids under thiourea and heat stress treatment in spring and autumn seasons

Under heat stress too, medium supplementation of thiourea improved this attribute in all hybrids, an increase being the maximum in 32B33 (~82%). In autumn season, a maximum chlorophyll b was detected in DK6789 under control and thiourea supplementation. Medium supply of thiourea improved the chlorophyll b by 7% both in 30Y87 and DK6789 while under heat stress a maximum improvement of 36 was noticed in 30Y87 (Fig. 2b).

Data showed that in spring season leaf carotenoids content was the highest in 31R88 under control condition. Medium supplementation of thiourea effectively enhanced (up to 31%) the carotenoids content in all hybrids except in 30Y87 (reduced by 3%) under control condition. However, under heat stress thiourea treatment enhanced the carotenoids in all hybrids being the maximum 32B33 (47%). In autumn season, maximum carotenoids content was observed in 30R50 under control condition, which was enhanced due to heat stress in all the hybrids by 5% (31R88) to 37% (32B33). Medium supply of thiourea improved this character maximum by 47% in 32B33 under control condition while by 14% in DK6789 (Fig. 2c). As regards total chlorophylls/carotenoids ratio (CCR) in spring season, this ratio was the highest in 30Y87 under control condition. Heat stress declined CCR in all hybrids (maximum by 25% in 30R50). Medium supply of thiourea decreased the CCR in 30Y87 and DK6789 but increased in rest of the hybrids under control but increased in all hybrids over respective controls being maximum in 32B33 (30%). In autumn season, the CCR, being highest in 30Y87 under control, was reduced in all hybrids under heat stress with a maximum reduction in 30Y87 (42%). Medium supply of thiourea to control plants reduced CCR in all hybrids while under heat this ratio was increased in all hybrids except 5% reduction noted in DK6789 (Fig. 2d).

Oxidative Stress

H_2O_2 contents: Significant differences and interaction of stress treatments, thiourea levels and maize hybrids was observed in the shoot and root tissue in spring ($P<0.01$) and autumn ($P<0.05$) seasons (Table 1). In spring season shoot H_2O_2 content in all hybrids kept uniformly low in control plants which increased under heat stress in all hybrids from 26% (32B33) to 50% (DK6789). Medium supplementation of thiourea reduced the shoot H_2O_2 contents both under control (6–13%) and heat stress (2–15%) conditions. In the autumn season the shoot H_2O_2 content in all hybrids was higher than those noted in spring season while heat stress led to an increase in this character by 25% (31R88) to 53% (30Y87). Medium supply of thiourea reduced the shoot H_2O_2 both under control (3–19%) and heat stress (38–47%) in different hybrids (Fig. 3a). As for root tissue the spring grown hybrids displayed similar H_2O_2 contents which increased substantially (50–86%) upon exposure to heat stress. The medium supplementation of thiourea effectively reduced this attribute both under control (23–41%) and heat stress (17–40%). In autumn season, like shoot tissue, the root H_2O_2 content was greater, while heat stress further increased it by 50–142%. However, medium supplementation of thiourea reduced the root H_2O_2 up to 12% under control and up to 47% under heat stress (Fig. 3b).

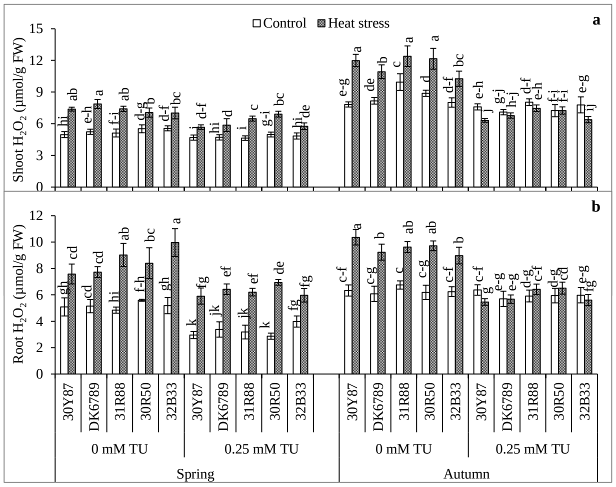

Fig. 3: Variations in H_2O_2 contents of shoot (a) and root (b) under thiourea and heat stress treatments in spring and autumn seasons

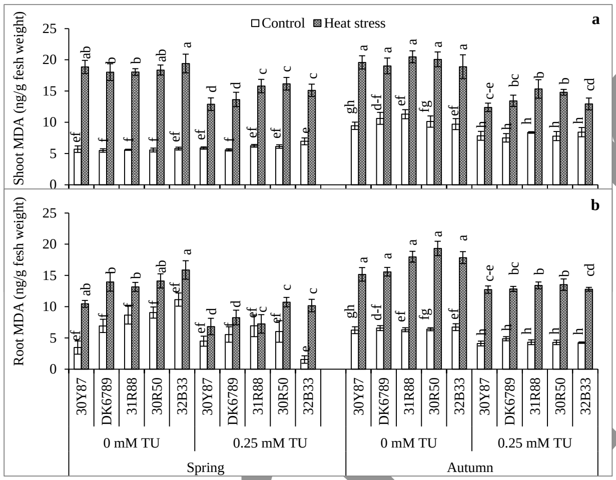

Fig. 4: Variations in the MDA contents of shoot (a) and root (b) in maize hybrids under thiourea and heat stress treatments in spring and autumn seasons

MDA contents: Significant ($P<0.01$) difference in heat treatments, thiourea levels and hybrids were noted with significant ($P<0.05$) overall interaction of these factors was noted in spring ($P<0.05$) and autumn ($P<0.01$) seasons both in the shoot and root (Table 1). In spring season the shoot MDA content was similar in all the hybrids while imposition of heat stress increased the shoot MDA content in all hybrids (169–187% over control). Medium supplementation of thiourea reduced the accumulation of shoot MDA in all hybrids ranging from 2% (32B33) to 13% (30Y87) under control and 12% (31R88 and 30R50) to 32% (30Y87) under heat stress. In autumn season the accumulation of shoot MDA in all hybrids was greater than that measured in spring season while heat stress further exaggerated its contents from as low as 81% (31R88) to 107% (30Y87). Medium supplementation of thiourea reduced the shoot MDA content from 13% (32B33) to 29% (DK6789) under control while from 25% (31R88) to 37% (30Y87) under heat stress (Fig. 4a). As regards root tissue in spring season, the MDA content was quite variable among the hybrids, which was increased with the heat stress treatment from as low as 52% (32B33) to as high as 92% (DK6789). Medium supplementation of thiourea declined root MDA in all the hybrids from 16% (DK6789) to 29% (30R50) under control while from 35% (30Y87) to 45% (in DK6789 and 31R88). In autumn season the root MDA content remained lower than those noted in spring season but heat stress substantially increased it from as low as 137% (DK6789) to as high as 202% (30R50). Medium supply of thiourea reduced the root MDA from 16% (DK6789) to 29% (30R50) under control condition while from 16% (30Y87) to 30% (30R50) under heat stress (Fig. 4b).

Stress Protectants and Antioxidants

Free proline: Significant difference ($P<0.01$) was observed in heat stress, thiourea levels and maize hybrids with significant ($P<0.01$) interaction of all these factors for shoot and root in spring season but no such interaction was evident in autumn season (Table 1). In spring season 30Y87 accumulated greatest free proline in shoot of all other hybrids, whilst heat stress led to substantially exaggerated accumulation of this metabolite in all hybrids (139–195% over control). Medium supplementation of thiourea attenuated the shoot free proline content both under control (2–10% in most hybrids) and heat stress (10–24%) conditions. As regards autumn season, shoot free proline accumulation was about twofold greater than that observed in spring season while heat stress led to its further accumulation by 37–48% in the hybrids. Medium supply of thiourea minimized the shoot free proline contents under control (2–17%) and under heat stress (12–21%) in the hybrids (Fig. 5a). As regards root tissue in spring season, the free proline content kept low while heat stress led to its twofold accumulation in various hybrids. Medium supply of thiourea led to reduced accumulation of root free proline by 4–16% under control while by 8–33% under heat stress. In autumn season under control condition, the root free proline accumulation was relatively higher than that observed in spring season, while the heat stress further enhanced its synthesis by 25–73% in different hybrids. Medium supply of thiourea enhanced the root free proline level in 31R88 (2%) and 32B33 (3%) while in other hybrids there was about 16% decline in it under control condition. Under heat stress medium supplementation of thiourea reduced the free proline contents by 19–25% in different hybrids (Fig. 5b).

Anthocyanins: Data showed significant difference ($P<0.01$) in heat stress, thiourea levels and maize hybrids with significant ($P<0.01$) interaction of all these factors for shoot and root in spring season ($P<0.05$) but no such interaction was evident in autumn season (Table 1).

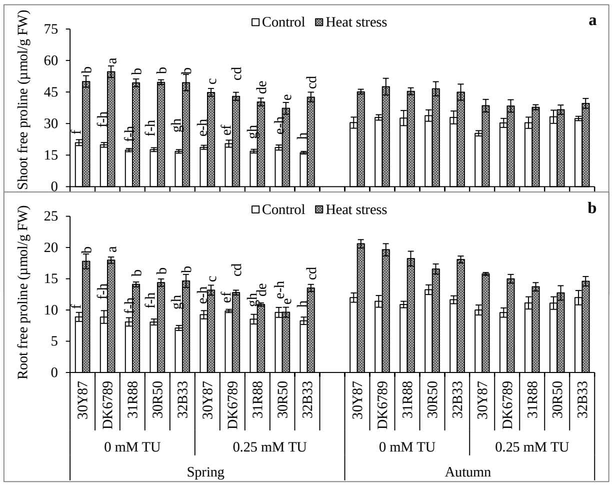

Fig. 5: Variations in free proline accumulation pattern in shoot (a) and root (b) in maize hybrids under thiourea and heat stress treatments in spring and autumn seasons

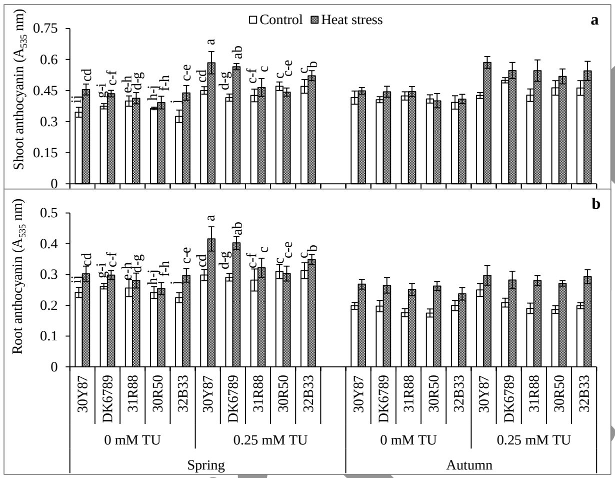

Fig. 6: Differences in anthocyanins contents of shoot (a) and root (b) in maize hybrids under thiourea and heat stress treatments in spring and autumn seasons

In spring season 31R88 showed greater anthocyanins contents in shoot among all the hybrids, whilst heat stress led to substantially enhanced accumulation of this metabolite in the hybrids from 3% (31R88) to 35% (32B33). Medium supplementation of thiourea further improved the anthocynins accumulation both under control (7–44%) and heat stress (13–30%). Data for autumn season showed that shoot anthocyanins accumulation were relatively greater than in spring season. Heat stress led to decline in its accumulation by 2% in 30R50 while enhanced by 4–10% in other hybrids. Medium supplementation of thiourea further improved the level of this metabolite under control (1–23%) and heat stress (23–33%) in different hybrids (Fig. 6a). As for root anthocyanins contents in spring season, it was accumulated the highest in DK6789 while heat stress led to its variable accumulation in different hybrids. Medium supply of thiourea enhanced anthocyanins accumulation from 10% (31R88) to 32B33 (39%) under control whereas by 15–38% under heat stress. In autumn season under control condition, the root anthocyanins level was relatively lesser than that observed in spring season, while heat stress markedly enhanced its contents (34–50%) in different hybrids. Medium supply of thiourea enhanced the root anthocyanins by 0–26% under control condition whilst by 3–23% under heat stress in different hybrids (Fig. 6b).

Ascorbic acid (AsA): There was significant difference ($P<0.01$) in heat stress, thiourea levels and maize hybrids with significant ($P<0.01$) interaction of all these factors for shoot and root in spring season ($P<0.05$) but no interaction of all these factors was noted in autumn season (Table 1). In spring season for shoot tissue, a highest AsA accumulation was observed in 32B33, whilst heat stress led to marked decline in its level by 4% (31R88) to 26% (32B33). Medium supply of thiourea substantially accumulated the shoot AsA by 47–73% under control while by 109–198% under heat stress. As regards autumn season, shoot AsA accumulation was similar to that observed in spring season while heat stress led to its decline by 6–12% in different hybrids. Medium supply of thiourea minimized the shoot AsA contents under control (4–10%) and under heat stress (102–118%) in the hybrids (Fig. 7a). Taking into account root tissue in spring season, the AsA contents was similar in all hybrids while heat stress led to its reduction in reduction in 32B33 (13%) and 30R50 (16%) but increased in other hybrids. Medium supply of thiourea led to an enhancement in root AsA by 47–57% under control while by 56–123% under heat stress. In autumn season under control condition, the root AsA accumulation was similar to that noted in spring season, while the heat stress reduced its synthesis by 12–24% in different hybrids. Medium supplementation of thiourea enhanced the root AsA level by 24–28% under control condition while by 25–43% under heat stress in different hybrids (Fig. 7b).

Correlations

Correlations were established separately of shoot and root dry weight with the respective biochemical attributes revealed that in spring season under control condition the shoot and root dry weight was not correlated with any of the biochemical attribute. Furthermore, under medium supplementation of thiourea no correlation of any biochemical attribute of shoot and root dry weight under control condition while under heat stress H_2O_2 was negatively correlated while free proline, anthocyaqnins and AsA were positively correlated with shoot dry weight and anthocyanins and AsA were positively correlated with root dry weight (Table 2).

In autumn season no correlation was found of the shoot and root dry weight with any physiological attribute in the absence or presence of medium supplementation of thiourea under control condition. However, under heat stress without

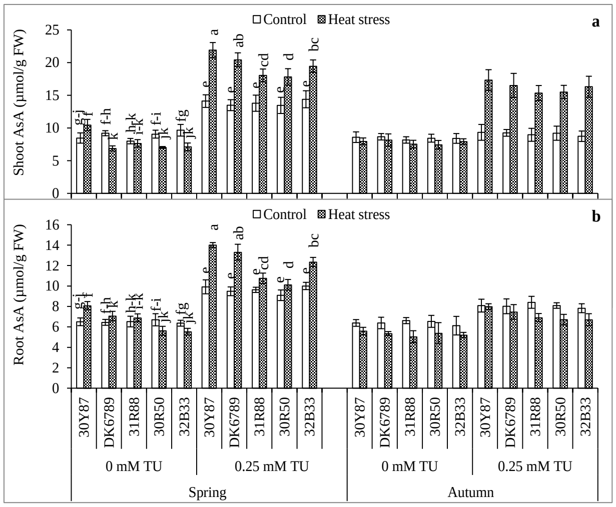

Fig. 7: Changes in ascorbic acid contents of shoot (a) and root (b) in maize hybrids under thiourea and heat stress treatments in spring and autumn seasons

medium supplementation of thiourea, shoot dry weight exhibited no correlation of with any of the biochemical attribute while root dry weight was negatively correlated with MDA and positively with free proline. Medium supply of thiourea indicated no relationship of any biochemical attribute with shoot and root dry weight under control condition except a positive correlation of AsA with shoot dry weight. However, under heat stress with medium supply of thiourea there was a positive correlation of carotenoids and AsA and a negative one of MDA with shoot dry weight. Likewise, root dry weight was negatively correlated with H_2O_2 but positively with free proline and AsA contents (Table 2).

Discussion

Results of growth parameters showed that heat stress decreased the dry weight of both shoot and root in both the seasons, while medium supplementation of thiourea effectively nullified the adverse effect of heat stress, although more effectively in spring season while marked differences were noticed among the hybrids (Table 1 and Fig. 1). It has been shown that in various plant species under stress conditions, the exogenous supply of thiourea brought in significant improvement in growth and yield attributes (Anjum *et al.*, 2011; Asthir, 2015). However, quite significant improvement in the shoot growth with the medium supplementation of thiourea alludes to the specific roles of thiourea in maize hybrids under this investigation.

Maintenance of optimal leaf photosynthetic pigment contents is pivotal for normal plant growth. Heat stress enhances activity of the labiality of photosynthetic membranes due to excessive production of ROS, leading to disruption of reaction centers thus enabling chlorophyllase to digest the chlorophyll molecules (Farooq *et al.*, 2011). The carotenoids, in addition to harness light, act as scavengers of ROS (Wahid, 2007) and thus provide a degree of stability to the photosynthetic membranes (Bonente *et al.*, 2008). In this study, the medium supplementation of thiourea recuperated the heat damage on the chlorophyll a and b and enhanced the carotenoids contents more in spring than in summer season eventually leading to an increased chlorophylls/carotenoids ratio in all the hybrids, which seems to be a specific role of thiourea in inducing heat tolerance in maize hybrids in this study (Fig. 2).

Oxidative damage is considered as one of the main consequence of heat stress in cereals that disturbed metabolic events either by enhancing the ROS or by decreasing the cell ability to scavenge the oxygen radicals (Wahid *et al.*, 2007; Rai *et al.*, 2018). In this study content of H_2O_2 and MDA were increased due to heat stress while medium supplementation of thiourea significantly declined the levels of both these metabolites with clear differences among the hybrids in both the seasons. Furthermore, there was a greater reduction in the H_2O_2 and MDA contents of shoot than root (Fig. 3 and 4). Various other reports show the reduction in the production of stress induced ROS with the medium supply of thiourea (Kaya *et al.*, 2015), which further supports the metabolic role of thiourea in the alleviation of oxidative damage (Zhu *et al.*, 2002).

From the above it is clear that mediums supply of selected level of thiourea effectively reduced the oxidative damage on maize hybrids. Some endogenously synthesized biomolecules such as osmoprotectants, phenolics and vitamins are also known to reduce the stress effects on the biomembranes (Hayat *et al.*, 2012; Mahmood *et al.*, 2014; Hussain *et al.*, 2016). However, it is not known that whether thiourea supply can improve their levels or not. To explore this, we measured the levels of free proline (an osmoprotectant), anthocyanins (a phenolic compound) and AsA (as a vitamin) in the shoot and root of maize hybrids in both the seasons. Results showed that thiourea supplementation increased the levels of all these metabolites under heat stress and more tangible in the spring season. Of these metabolites, the accumulation of free proline and anthocyanins was confined to root tissue than shoot whilst AsA was more accumulated in the shoot tissue and that also in the spring season (Fig. 5, 6 and 7). The correlations data revealed that under heat stress close negative association with H_2O_2 and MDA and positive with free proline and anthocyanins for root and AsA for shoot, being evident in both the seasons (Table 2). This revealed that both free proline and anthocyanins accumulation were involved in the improved of root structure and function under heat stress, while ascorbic, as antioxidant, was more crucial to shoot, which seems to be a novel role of medium supplemental thiourea in the heat tolerance in the hybrid maize. These findings are even more important in view of few reports that root temperature is more crucial determinant of plant growth under heat stress than shoot temperature (Martin *et al.*, 1989;

Table 2: Correlation coefficient (*r*) of some growth attributes with some physiological attributes of maize hybrids under medium supplementation of thiourea subjected to control and heat stress conditions

Treatments		Spring season				Autumn season			
		0 m*M* Thiourea		25 m*M* thiourea		0 m*M* Thiourea		25 m*M* thiourea	
	Variable	SDW	RDW	SDW	RDW	SDW	RDW	SDW	RDW
Control	Chl a	0.449ns	-	-0.613ns	-	0.238ns	-	-0.439ns	-
	Chl b	0.608ns	-	0.572ns	-	0.312ns	-	0.572ns	-
	Car	-0.827ns	-	-0.300ns	-	0.550ns	-	0.145ns	-
	Chl/Car	0.565ns	-	0.162ns	-	-0.220ns	-	-0.192ns	-
	H_2O_2	-0.206ns	-0.438ns	0.314ns	-0.076ns	-0.114ns	-0.261ns	-0.532ns	0.173ns
	MDA	-0.054ns	0.650ns	-0.612ns	-0.543ns	0.092ns	0.296ns	-0.744ns	0.334ns
	FP	0.486ns	-0.584ns	0.666ns	0.655ns	-0.116ns	-0.620ns	-0.677ns	-0.801ns
	Ant	0.146ns	0.021ns	0.004ns	-0.369ns	0.390ns	0.514ns	-0.068ns	0.726ns
	AsA	-0.228ns	0.306ns	-0.749ns	0.589ns	0.611ns	-0.159ns	0.893*	-0.191ns
Heat stress	Chl a	-0.040ns	-	0.600ns	-	-0.119ns	-	-0.435ns	-
	Chl b	-0.417ns	-	0.025ns	-	0.190ns	-	0.577ns	-
	Car	-0.144ns	-	0.814*	-	0.726ns	-	0.991**	-
	Chl/Car	0.094ns	-	-0.141ns	-	-0.550ns	-	-0.795ns	-
	H_2O_2	-0.072ns	0.353ns	-0.962**	-0.675ns	-0.621ns	0.574ns	-0.848ns	-0.884*
	MDA	-0.347ns	0.188ns	-0.801ns	-0.772ns	-0.707ns	-0.889*	-0.904*	-0.869ns
	FP	-0.509ns	-0.656ns	0.935*	0.706ns	-0.512ns	-0.878*	-0.680ns	0.954*
	Ant	-0.318ns	-0.345ns	0.884*	0.942*	0.236ns	0.625ns	0.804ns	0.729ns
	AsA	-0.063ns	0.032ns	0.881*	0.914*	0.733ns	0.833ns	0.950*	0.905*

Significant at *, $P<0.05$; **, $P<0.01$ and ns, $P>0.05$

Arai-Sano *et al.*, 2010). The heat tolerance in thiourea can be attributed to the amino, imino and sulfhydryl functional group as established in previous studies (Perveen *et al.*, 2015; Wahid *et al.*, 2017).

Conclusion

Heat stress reduced the growth and physiological attributes of all the maize hybrids, although autumn season was more adverse. The maize hybrids 30Y87, DK6789 and 32B33 responded more positively to thiourea supplementation and thus displayed better heat tolerance. Medium supplemented thiourea minimized the loss of chlorophyll species but enhanced the carotenoids contents and showed reduced chlorophyll/carotenoids ratio. Thiourea supplementation declined the production of H_2O_2 and MDA, and improved the accumulation of free proline and anthocyanins (more in root) and AsA (more in shoot), which were distinctly correlated to heat tolerance by maize hybrids in both the growing seasons. Presence of functional groups (amino, imino and sulfhydryl) in thiourea molecule likely involved in root to shoot signaling were plausibly pivotal for improved maize growth under heat stress.

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