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Full Length Article

Potential of Thiourea in Modifying Membrane Stability, Osmoprotectants, Vitamins and Antioxidants Levels under Cadmium Stress in Maize

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Abstract

To assess the effect of medium supplementation of thiourea (TU) on maize in cadmium (Cd) contaminated soil, a pot experiment was performed in spring (February) and autumn (July) during 2009. Seeds of two maize cultivars, Pak-Afgoi (Cd tolerant) and EV-20 (Cd sensitive) were sown in pots containing sand. After 10 days of growth, plants were stressed with two Cd treatments (0 and 1000 μM) and grown for 20 days until the appearance of Cd toxicity symptoms. TU levels (0 and 0.25 m*M*) were applied to 30 day old plants in nutrient solution and grown further for 20 days. After TU application, changes in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) relative membrane permeability (RMP), total free amino acids, anthocyanins (ANT), soluble phenolics (SP), soluble sugars (SS), glycinebetaine (GB),free proline (FP) contents, levels of ascorbic acid (AsA), niacin (NIA) riboflavin (RIB) and reducing power assay (RPA) were measured. Cd stress significantly decreased the levels of AsA, NIA, RIB and RPA whilst enhanced the levels of MDA, H_2O_2 and RMP, SP, ANT,SS, total free amino acid, GB and FP contents in both cultivars under Cd stress conditions in both seasons. TU enhanced the synthesis of osmoprotectants, SP and ANT,AsA, NIA and RIB, which seemed to act as antioxidants to scavenge the H_2O_2 and reduce oxidative damage. The difference in cultivars for different parameters was significant in both the seasons; Pak-Afgoi showed the lower and EV-20 the higher damage due to Cd stress. The autumn season was more adverse than spring. In crux, medium supplementation of TU exhibited a great potential to mitigate the adverse effects of Cd stress on maize. The applied TU level is suggested for field use in the areas where marginally Cd contaminated soils limit the agriculture production.

Keywords: Cd toxicity; Oxidative stress; Thiourea; Vitamins; Maize; Autumn season

Introduction

Cadmium (Cd) is responsible for causing multifarious damages in crop plants (Ahmadee *et al.*, 2016). A number of studies report the oxidative stress, lipid peroxidation and variation in antioxidative enzymatic activities in plants caused by Cd. The activity of membrane enzyme like H^+-ATPase is adversely affected by Cd (Fodor *et al*., 1995; Naz *et al.*, 2016). Among the many disruptions caused by Cd, a more elaborative one is oxidative damage, leading to enhanced membrane lipids peroxidation and RNA modifications (Chmielowska-Bąk *et al.*, 2018). It is reported that extent of oxidative damage is concomitant with tissue Cd concentration (Gill *et al.*, 2011; Leng *et al.*, 2018). Sandalio *et al.* (2001) reported that direct exposure of pea plants to Cd stress caused substantial changes in the activities of antioxidants and other metabolites. Cd is also responsible for increasing the permeability of membrane by damaging the proteins making them inactive. Several other enzymes like CO_2 fixation, mitochondrial oxidative phosphorylation etc. are also inactivated by Cd stress (De Filippis and Zeigler, 1993; Wahid *et al*., 2009).

Increased Cd contents seriously affect activity of enzymes, where it reacts with –SH group in the peptides and change it structures (Gouia *et al*., 2000; Hall, 2002). It is also responsible for changes in the enzymatic antioxidant activities and concentration of non-enzymatic antioxidants (Adhikari *et al*., 2006; Hussain *et al.*, 2019). Further harmful effects are lipid peroxidation and membrane damage, disturbed functions of ATPase, blockage of channels and transporters, production of reactive oxygen species (ROS) etc. (Wahid *et al*., 2009; Andresen and Küpper, 2013). In addition to the already discussed Cd damages, it also reduces the mitochondrial and chloroplastic electron transport (Shah *et al.*, 2001; Zhang *et al.,* 2005). An increased in the amount of MDA, H_2O_2 contents is reported in Egyptian grass and clover (Aly, 2012).

Thiourea (TU), a biochemical compound, contains

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sulfur and nitrogen, and has two functional groups, –imino and –thiol. These groups are involved in plant growth especially under normal and stress conditions (Wahid *et al.*, 2017). Thiol is involved in the formation of proteins, chlorophyll, glutathione and vitamins, while imino provides as a nitrogen source (Noctor *et al.*, 2011; Iqbal *et al.*, 2013; Perveen *et al*., 2015). TU has capacity to improve the plant growth under favorable and non-favorable conditions, and induced the regulation of multiple metabolic processes in plants. Being water soluble, the TU can be absorbed easily in the living tissues. Furthermore, it can enhance seed germination, seedling growth and grain yield in economic crops (Sivritepes *et al*., 2005; Anjum *et al*., 2008; Seleiman and Kheir, 2018).Other prominent roles of TU include relieving the salinity induced seed dormancy in *Allenrolfea occidentalis* seeds under salt stress (Gul and Weber, 1998); improved net photosynthesis and chlorophyll contents in cluster bean (*Cyamposis tetragonoloba*) under drought stress (Garg*et al.,* 2006), and improved photosynthesis in bread wheat under salt and heat stresses (Anjum *et al.,* 2011).

Maize (*Zea mays* L.) is an important commercial crop. It has short life cycle and is suited to cultivate in those areas of the world where climatic conditions are conducive. Normally, it is grown in both spring and autumn seasons in Pakistan. However, environmental stresses limit the maize production to a great extent. It is reported that maize shows high sensitivity to Cd intoxication, which is among the major reasons for reduced grain yield in maize (Anjum *et al*., 2015; Retamal-Salgado *et al.*, 2017). Nonetheless, maize productivity can be increased if it is improved for tolerance to abiotic stresses. Different approaches are used to increase crop productivity under Cd stress. Of these, plant selection, production of transgens, exogenous use of hormones and nitrogen containing compounds, and stress signaling molecules are of great significance (Ahmad *et al.*, 2015; Farooq *et al.*, 2016; Wahid *et al.*, 2017).

Studies are meager that show the role of medium supplementation of TU in improving metabolites (osmoprotectants, phenolics and vitamins) biosynthesis under Cd stress in maize. We predict that medium supply of TU may profoundly modify the metabolites synthesis and improve Cd tolerance in maize over different seasons. The objectives of this study was to assess the ameliorative action of medium supplemented TU on membrane lipid peroxidation, hydrogen peroxide, osmoprotectants, phenolics and vitamins in maize plants grown under Cd stress.

Materials and Methods

Experimental Details

The study was conducted in spring (Feb–Apr) and autumn (Aug–Oct) seasons with similar setup. Seeds of Pak-Afgoi and EV-20 (Cd-tolerant and sensitive varieties, respectively) were sown in plastic pots containing washed sand. After 6 days of germination, four seedlings with similar size were kept in each replicate. Thirty days old plants were supplemented with 1000 μM Cd level in the medium and grown for 10 days and let the signs of Cd toxicity on leaves appear. After that 0.25 m*M* aqueous TU solution was supplemented in the medium. The plants were grown for another 15 days after the TU supplementation.

Membrane Characteristics and Oxidative Stress

Relative membrane permeability (RMP): For the estimation of RMP, the method of Yang *et al*. (1996) was used. Leaf material (0.5 g) was transferred to test tubes containing 10 mL of distill water, and test tubes shaken vigorously for 5 sec, and initial electrical conductivity (EC_0) was measured. The test tubes were wrapped with aluminum foil and kept overnight at 4ºC. Next morning EC of the solution was taken to represent EC_1. The test tubes were autoclaved for 15 min at 121ºC, the leachate filtered and measured for EC_2. RMP (%) was estimated with the equation = $(EC_1 – EC_0 / (EC_2 - EC_0) \times 100$.

Hydrogen peroxide (H_2O_2): The method of Velikova *et al.* (2000) was used for estimation of H_2O_2. A 0.5 g of fresh leaf tissue was grinded in an ice bath using 1 mL of 0.1% (w/v) trichloroacetic acid (TCA). The slurry was centrifuged at 12000 × g for 15 min. A 0.5 mL of supernatant was added to 10 mL of phosphate buffer (pH 7.0) and 1 mL of 1M potassium iodide; vortexed and absorbance taken at 390 nm. The unknown sample values were derived from standard curve prepared using 35% H_2O_2 stock solution.

Malondialdehyde (MDA): To measure MDA with the method of Heath and Packer (1968), 0.1 g of fresh leaf was extracted in 1 mL of 0.5% TCA and centrifuged at 12000 × g for 15 min. Supernatant (1 mL) was mixed with 1 mL of TBA (prepared by dissolving thiobarbaturic acid to 0.5% in 20% (w/v) TCA]. The mixture was heated in a water bath at 95^{o}C, cooled and centrifuged at 7500 × g for 15 min and a clear solution was obtained. The absorbance of mixture was read at 532 and 600 nm, using 5% TCA as blank. For correction of non-specific turbidity was subtracted from the values at 600 nm from 532 nm. The MDA amount was computed as: (nmol/mL) = $(A_{532}-A_{600})/155000)\times10^6$

Osmoprotectants

Free proline (FP): The FP was analyzed by using method of Bates *et al.* (1973) after grinding fresh leaf material in aqueous sulphosalicylic acid. Acid ninhydrin and glacial acetic acid were used for the reaction of the extract at 100ºC in water bath for 1h. The reaction was terminated in an ice bath and then 4 mL of toluene was added; vortexed for 15–20 sec. The chromophore containing proline was aspirated warmed to room temperature and measured for the amount of proline at 520 nm by using spectrophotometer (Hitachi U-2001, Japan).

Glycinebetaine (GB): Grieve and Grattan (1983) method was used to measure GB in fresh material. Fresh leaf samples were extracted in test tube containing 2N H_2SO_4; vigorously shaken. Mixed 2 mL of the extract with an equal volume of periodide (prepare by dissolving excess of iodine in potassium iodide solution) and shifted in a refrigerator at 4ºC for 16 h. Centrifuged the mixture at 10,000×*g*, kept at 4ºC for 15 min and supernatant discarded. Periodide crystals in the bottom of the tube were dissolved in 10 mL of 1, 2-dichloroethane, vortexed and left at room temperature. After 15 min, absorbance of the colored solution was taken at 365 nm. Standard curve was constructed using pure GB in the range 10–50 μmol/L and actual amount determined in the unknown samples.

Soluble sugars (SS): The SS were quantified in the fresh sample by extracting 0.1 g of tissue in 5 mL of 0.2 M phosphate buffer (pH 7.0). An aliquot (0.1 mL) was taken in the test tube and added 3 mL anthrone reagent, briefly vortexed and heated the mixture in boiling water for 15 min at 95ºC. The test tubes were cooled in running tap water, briefly vortexed and the absorbance of colored complex was read at 625 nm (Yoshida *et al.,* 1976).

Total free amino acids (TFAs): The TFAs were measured by Hamilton and van Slyke (1943) method after homogenize in leaf material (0.5 g) with 1 mL phosphate buffer (pH 7.0). To the homogenate, added 1 mL of 10% pyridine + 1 mL of 2% ninhydrin solution. Heated the mixture for 30 min in boiling water and made up to 50 mL. The absorbance was read at 570 nm.

Vitamins

Ascorbic acid (AsA): The AsA was analyzed following AOAC (1990). Extraction was made by dipping 5 g of fresh sample in 100 mL H_2O for 30 min. The mixture was filtered and 10 mL of the filtrate was added to 25 mL of 20% glacial acetic acid titrated against standardized 2, 6-dichloroindophenol (0.05 g/100 mL) solution. The standard curve constructed with increased concentrations of AsA to compare the values.

Niacin (NIA): The NIA was measured with the method of Okwu and Josiah (2006). For this purpose, 0.5 g fresh material was taken and vigorously shaken with 50 mL of $1NH_2SO_4$ for 30 min. Later 3 drops of concentrated ammonia solution were carefully added to the sample mixture and filtered with Whatman No. I filter paper. Ten mL of filtrate was pipette into a 50 mL conical flask and 5 mL each of 10% potassium cyanide solution and 0.02N H_2SO_4. Absorbance of colored mixture was read at 470 nm using spectrophotometer. For the exact concentration of NIA, standard curve was constructed using a range of standards.

Riboflavin (RIB): For RIB estimation, 5 g leaf tissue was extracted with 100 mL of 50% ethanol by vigorously shaking for 1h, and filtered. Filtrate (100 mL) was mixed with 10 mL of 5% potassium permanganate and 10 mL of 30% H_2O_2 and kept the mixture to at 50ºC in a water bath for 30 min followed by addition of 2 mL of 40% sodium sulfate, and final volume made to 50 mL. After keeping at room temperature for 15 min, the absorbance was taken at 510 nm.

Reducing power assay (RPA): Preparation of samples to estimate RPA was done with Sofowora (1993) method. The air dried fresh samples were macerated to make paste. Paste (0.5 g) was soaked in 20 mL of 98% methanol and left the mixture for 48 h and filtered. The filtrate was concentrated to 2 mL using rotatory evaporator and stored at 4ºC. For estimation of RPA with the method of Perumal and Becker (2003), the preserved extract was re-dissolved in 80% methanol and phosphate buffer (5 mL pH 6.0) was added. Then added 5 mL of 1% potassium ferricyanide, incubated at 50ºC for 20 min and then 5 mL of 10% TCA was added. The mixture was centrifuged at 3000 × g for 10 min. The upper 5 mL layer of the supernatant was mixed with 5 mL distilled water 1 mL of 0.1% ferric chloride, and absorbance of colored mixture was taken at 700 nm.

Phenolics Accumulation

Anthocyanins (ANT): Analyzed according to the method of Stark and Wray (1989). For their extraction, 0.1 g of fresh leaf material was macerated with 1 mL of 1% acidified (with HCl v/v) methanol and then heated at 50ºC for 1 h. After centrifugation, the absorbance of supernatant was taken at 532 nm using acidified methanol as blank. The ANT was expressed as $A_{535.}$

Soluble phenolics (SP): The SP were estimated using method of Julkenin-Titto (1985). Extract prepared in 1 mL of 80% acetone was centrifuge at 12000×*g* for 15 min. A 0.5 mL of the supernatant was diluted with equal amount of distilled water; added 0.5 mL of Folin-Ciocalteus reagent and vortexed. Then added 2.5 mL of 20% Na_2CO_3 and volume maintained up to 5 mL; vortex for 15–20 sec and measured the absorbance of blue colored mixture at 765 nm after incubation in dark for 20 min.

Statistical Analysis of Data

Completely randomized factorial design with three replicates was used. The data for all variables recorded during both the seasons were pooled to perform analysis of variance for both the seasons separately MSTAT-C software. Duncan's Multiple Range Test was applied to determine the differences among the factors and their interactions (Steel *et al*., 1996). Treatment means were marked with letters when the interactions were significant.

Results

Oxidative Damage and RMP

H_2O_2: In spring season, no significant (P>0.05) difference in the varieties, but a significant one ($P<0.01$) in the treatments with no interaction ($P>0.05$) of these factors was noted. However, in autumn season the varieties and treatments indicated significant ($P<0.01$) difference with significant ($P<0.01$) V×T interaction. H_2O_2 contents were low in control plants, which further reduced with TU in both the varieties. Plants treated with 1000 μM Cd indicated a substantial increase in H_2O_2 contents although the level was relatively low in Pak-Afgoi (tolerant maize) whilst medium supplied TU to these plants was highly effective in reducing H_2O_2 (Fig. 1a). In autumn season, although the varieties and treatments followed the same pattern as seen in spring season, the production of H_2O_2 was higher across the treatments and EV-20 (sensitive maize) was less responsive to TU in reducing H_2O_2contents (Fig. 1a).

MDA: In both spring and autumn seasons, the difference in the varieties and treatments was significant ($P<0.01$) with a significant ($P<0.01$) interaction of these two factors. In both spring and autumn seasons, although low in control plants, the MDA contents were further decreased with TU supply while EV-20 displayed a greater reduction in spring and Pak-Afgoi in autumn. Cd stress exponentially increased MDA contents in both varieties, but more increased in EV-20, while medium supplemented TU reduced it bringing virtually at par with control plants in both the seasons. Of the two, autumn season was more adverse than spring (Fig. 1b).

RMP: Data showed significant ($P<0.01$) difference in varieties and treatments with significant ($P<0.01$) interaction of both these factors in both the seasons. RMP was low in untreated plants of both the varieties, which further reduced (improved) with medium supplemented TU and this reduction was greater in EV-20. Medium supplemented Cd increased RMP in both the varieties while Pak-Afgoi indicated a much lesser increase. Application of TU to Cd treated plants was highly effective in improving the RMP and bringing it to the control levels in both the varieties. However, the difference in the seasons was non-significant ($P>0.05$) for RMP (Fig. 1c).

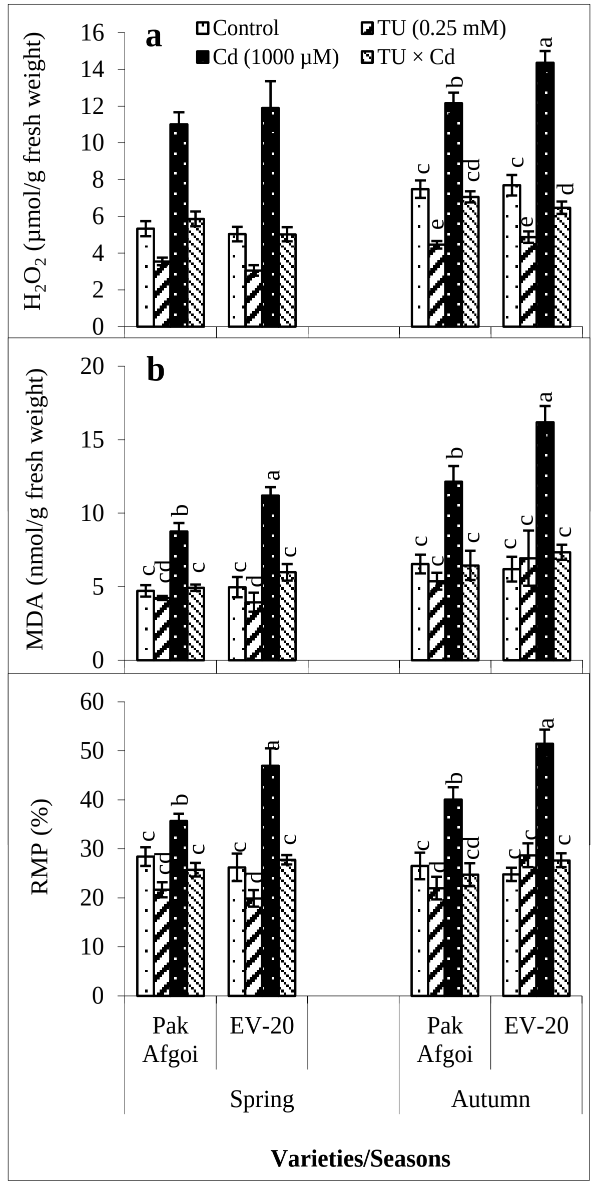

Fig. 1: Changes in some membrane characteristics and oxidative damage in maize varieties under Cd stress and role of thiourea in alleviating Cd toxicity in spring and autumn seasons. The alphabets on the data columns show significant interaction of varieties and treatments

Osmoprotectants

FP: As regards FP accumulation in both the seasons, data indicated significant ($P<0.01$) difference in the varieties and treatments with significant ($P<0.01$) interaction of these factors. Under control condition, in both the seasons, there was no difference in the FP accumulation in control and TU treated plants. Cd treatment led to a substantial FP accumulation being the highest in Pak-Afgoi while application of TU to Cd treated plants brought the FP down to the level of control plants in both the seasons. However, FP accumulation in both the varieties was higher in autumn grown plants than in spring season plants (Fig. 2a).

GB: In both seasons, the data for GB indicated significant difference in the varieties ($P<0.05$) and treatments ($P<0.01$) along with significant ($P<0.01$) interaction of these factors. Spring grown plants of both the varieties indicated greater GB accumulation than autumn season plants. In spring season, GB was the lowest in Pak-Afgoi, which increased with TU application. Cd treatment produced a greater increase in GB in EV-20 as compared to Pak-Afgoi, while medium supplemented TU reduced the GB accumulation in both the varieties (Fig. 2b). In autumn, the GB accumulation was much lower than that observed in spring season. In this season, normally grown plants indicated an increase in GB

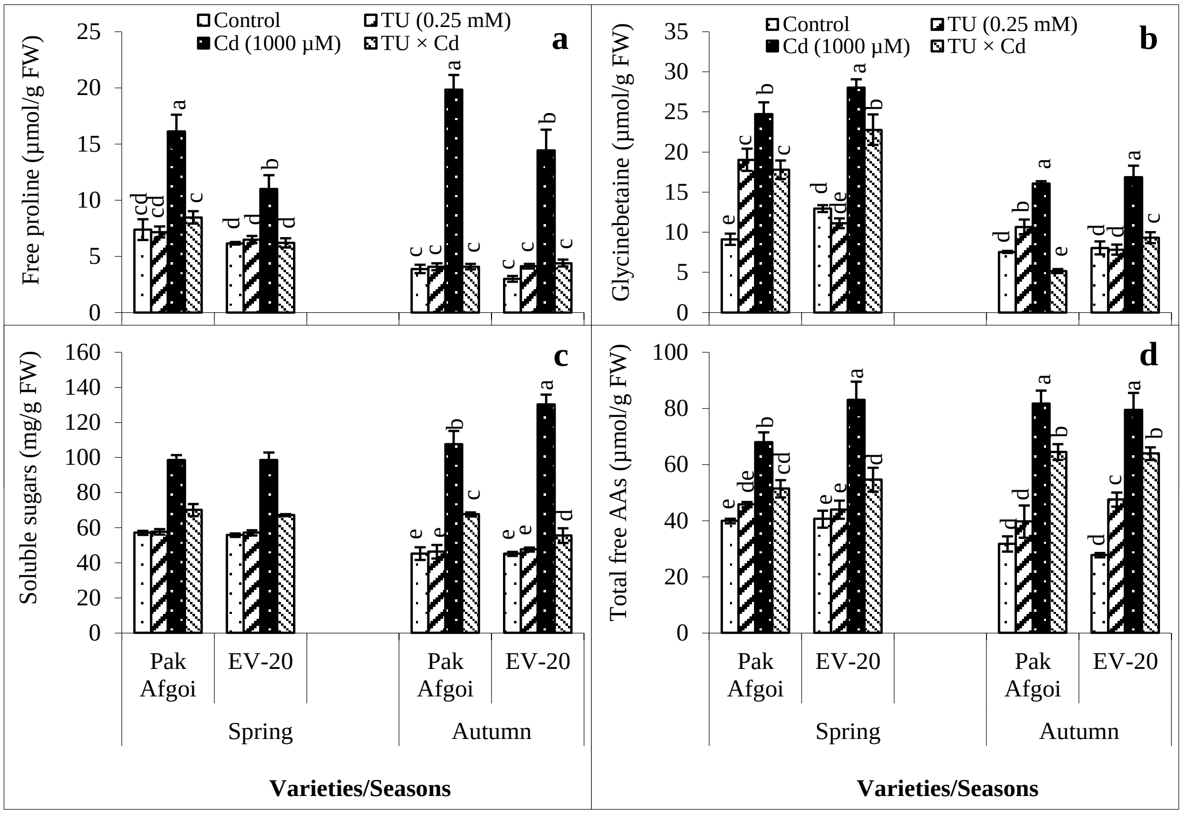

Fig. 2: Changes in some osmoprotectants levels of maize varieties under Cd stress and the role of thiourea in mitigation of Cd effect in spring and autumn seasons. The alphabets on the data columns show significant interaction of varieties and treatments

over control in Pak-Afgoi but not in EV-20. Cd treatment increased GB equally in both the varieties; while TU applied to Cd stressed plants reduced GB greater in EV-20 than Pak-Afgoi (Fig. 2b).

SS: In spring season data revealed no significant (P>0.05) differences in the varieties, but a significant (P<0.01) in the treatments but there was no interaction (P>0.05) of both the factors. In autumn season, however, the varieties, treatments and their interaction were significant (P<0.01). In both seasons, TU treated or untreated plants of both the varieties indicated similar SS contents. Cd stress increased SS contents equally in both the varieties in spring season while this increase was greater in EV-20 in autumn season. Root application of TU to Cd treated plants lowered the SS contents but was significantly higher than control in both the seasons and varieties (Fig. 2c).

Total free amino acids (FAAs): In both the seasons significant (P<0.01) differences in varieties, treatments and their interaction were found. In spring season, the FAAs were the lowest in control plants of both the varieties; while medium supplemented TU produced a little increase. Cd treatment produced a greater increase in this character in EV-20 than Pak-Afgoi, while TU applied to Cd stressed plants was effective in lowering the FAAs (Fig. 2d). In autumn grown plants of both the varieties, untreated plants showed the lowest FAAs contents while TU increased these values. Cd treated plants manifested substantial increase in FAAs but this increase was greater in Pak-Afgoi, while use of TU was effective in reducing the FAAs to a considerable extent in both the varieties (Fig. 2d).

Phenolics Contents

SP: Statistical analysis of data revealed non-significant (P>0.05) difference in the varieties but significant (P<0.01) difference in the treatments with significant (P<0.01) interaction of these factors in both the seasons. In spring season, the SP contents were the lowest in control (untreated) plants, while TU application enhanced them significantly. The root application of Cd substantially increased them equally in both the varieties, while application of TU to Cd treated plants reduced SP (Fig. 3a). In autumn season, the SP accumulation was lower than that observed in spring season. Being lowest in control plants, SP were increased with TU application to both the varieties; being higher in Pak-Afgoi. Cd treated plants of Pak-Afgoi indicated greater SP than EV-20 and TU applied to Cd treated plants reduced SP in the control levels (Fig. 3a).

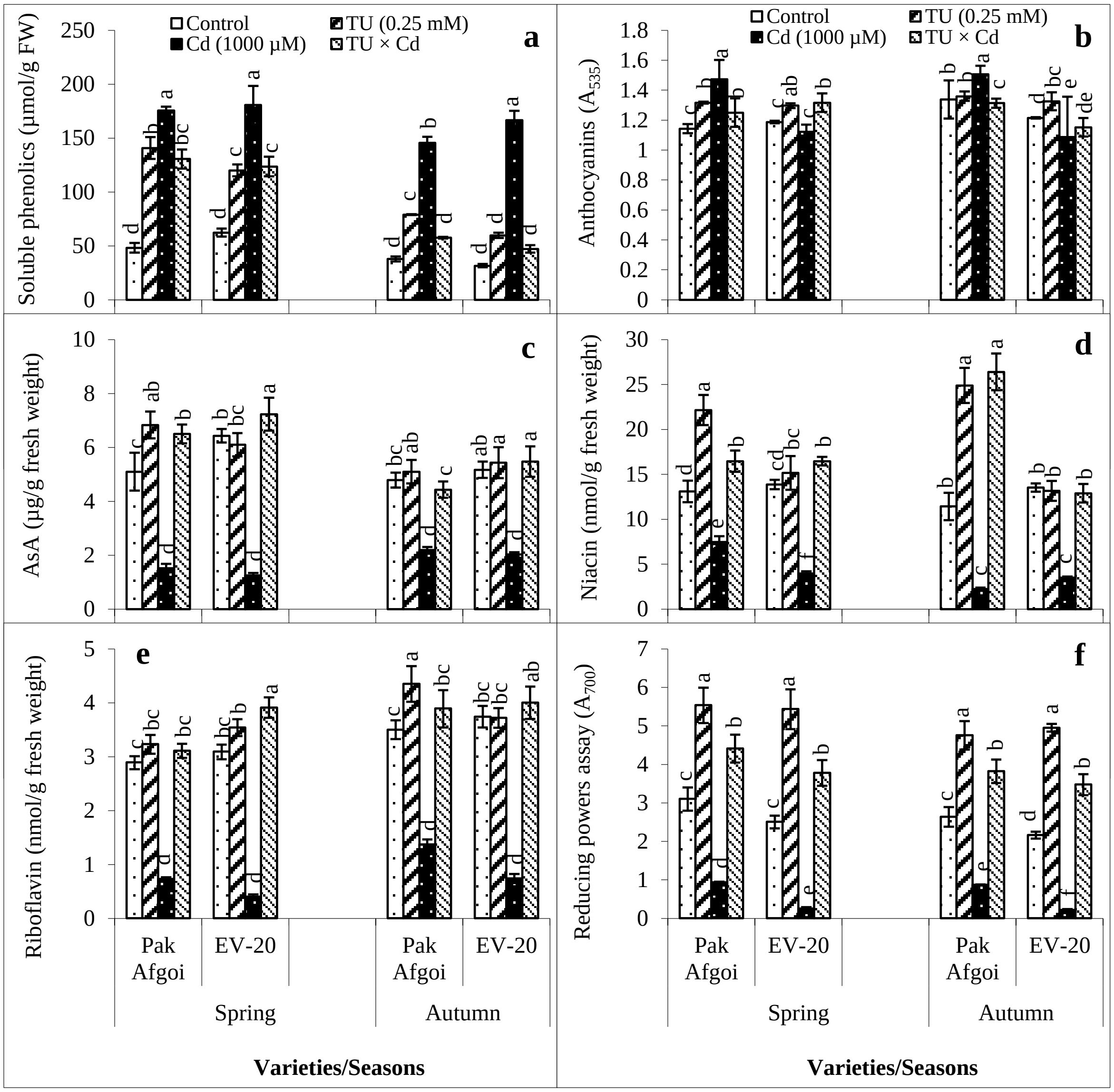

Fig. 3: Variations in phenolics and vitamins in the leaves of maize varieties under Cd stress and the role of thiourea in alleviating the Cd contents in spring and autumn seasons. The alphabets on the data columns show significant interaction of varieties and treatments

ANT: For both the seasons, data revealed significant difference in the varieties and treatments ($P<0.01$) with ($P<0.01$) significant interaction of both these factors for ANT. In spring season, the ANT was low in control plants while medium supplemented TU increased it in both the varieties. The Cd contamination further increased ANT in Pak-Afgoi but decreased in EV-20, while medium supplemented TU to Cd treated plants decreased ANT in Pak-Afgoi while brought down to the control level in EV-20 (Fig. 3b). In autumn season, a similar trend of ANT synthesis was notable across the treatments in both the varieties, but its accumulation was relatively greater in Pak-Afgoi as compared to EV-20 (Fig. 3b).

Vitamins Accumulation

AsA: In spring season, data indicated no difference ($P>0.05$) in the varieties but a significant one in the treatments ($P<0.01$) with significant ($P<0.05$) varieties × treatments interaction, while in autumn, there was significant difference in the varieties and treatments ($P<0.01$) with significant ($P<0.05$) interaction of these factors for AsA. In spring season under control condition, Pak-Afgoi exhibited low AsA contents than EV-20, while medium supplemented TU substantially increase it in both the varieties. Root treatment with Cd declined AsA in both the varieties while TU applied to Cd treated plants increased AsA more in Pak-Afgoi than

EV-20. In autumn season the trend of AsA accumulation across the treatments was similar to that observed in spring grown maize except AsA was relatively more accumulated in Cd treated plants (Fig. 3c).

NIA: The data for NIA revealed significant (P<0.01) difference in the varieties and treatments with significant (P<0.01) V×T interaction. In both the seasons, the NIA was similar in control plants of both the varieties while medium supplied TU increased it much more in Pak-Afgoi than EV-20. Cd treatment produced a substantial decline in NIA while TU application was highly effective in enhancing NIA under Cd stress (Fig. 3d).

RIB: In both the seasons, data revealed significant (P<0.01) difference in the varieties and treatments (P<0.01) with significant (P<0.05) varieties × treatments interaction, while in autumn, there was significant difference in the varieties (P<0.05) and treatments (P<0.01) with significant (P<0.01) interaction of these factors for RIB. In spring season under control condition, both the cultivars had similar RIB while medium supplemented TU enhanced it almost equally in both the varieties. Cd treatment was detrimental to RIB in both the varieties, while TU was more effective to this attribute in EV-20 than Pak-Afgoi. In autumn season plants of both the varieties, the RIB accumulation trend was similar to spring season with the difference that over all RIB contents were greater excepting Cd treated plants which showed low RIB (Fig. 3e).

RPA: In both autumn and spring seasons, data indicated no significant (P>0.05) difference in the varieties but a significant one in the treatments (P<0.01) with significant (P<0.05) interaction of varieties and treatments for RPA. Results suggested that in both the seasons, RPA was similar in untreated (control) plants but TU made a substantial increase in both the varieties. Cd treatment was highly damaging to this character; the damage being greater in EV-20 than Pak-Afgoi, while TU applied to Cd treated plants was quite efficacious in improving PRA in both the varieties (Fig. 3f).

Discussion

Among the multifarious effects of Cd, membrane damage is perhaps the most important one (Azevedo *et al.*, 2005; Wahid *et al*., 2009; Vassilev and Lidon, 2011). The plants have employed various mechanisms to repair the damage caused by Cd to the cellular membranes. These mechanisms include enhancing the synthesis of osmoprotectants such as GB, FP and SS and enzymatic and non-enzymatic antioxidants (Adhikari *et al*., 2006; Guo *et al*., 2009). In the present case it was noted that root applied Cd was damaging to the membrane lipids, as revealed from the data for RMP. The data further showed that in Cd treated plants, there was a high generation of H_2O_2 in both the seasons although tolerant maize was more resistant (Fig. 1a and b). Medium supplied TU was quite effective in reducing the generation of H_2O_2, production of MDA as well as reducing RMP, and bringing their levels closer to the control in both the varieties and seasons (Fig. 1c), which provides clear evidence for the involvement of TU in offsetting the oxidative stress. Some studies showed the beneficial use of TU as foliar spray or seed dressing in various plant species in offsetting the oxidative damage caused by salinity (Anjum *et al*., 2008) and drought (Garg*et al*., 2006), while no study to date has thus far documented the effectiveness of medium supplementation of TU in reducing Cd toxicity and the mechanisms involved.

It has been established that osmoprotectants accumulate as an adaptive mechanism under abiotic stresses (Wahid *et al*., 2009). The accumulation of amino acid especially proline in free form, GB and SS has been well established in the alleviation of stress toxicity by enhanced activity of antioxidants, reduced oxidative stress, improved membrane properties and signaling sugar metabolism (Chen and Murata, 2008; Rosa *et al*., 2009). In this study, Cd treatment led to a high accumulation of TFAs, FP, GB and SS as a toxicity effect in both the cultivars, while medium supplemented TU curtailed this toxicity and reduced their levels significantly (Fig. 2). This indicated that an indiscriminate accumulation of osmolytes in both the varieties is an adaptive response to maintain the membrane integrity and TU appeared to help maize in better metabolites adjustments under Cd stress.

Plants show the expression and biosynthesis of an array of antioxidants which may be enzymatic and non-enzymatic in nature (Racchi, 2013). Here data were recorded to find out possible involvement of SP, ANT, AsA, NIA and RIB as non-enzymatic antioxidants (Fig. 3). Results showed that although there was increased synthesis of SP and ANT in both the varieties treated with Cd, and medium supplemented with TU reduced the SP synthesis about equally in both the varieties, while declined the anthocyanins contents more in EV-20 (sensitive maize) than Pk-Afgoi (tolerant maize) during both the seasons. This appeared as an important metabolic adjustment as antioxidant. The ANT synthesis has a definite role as antioxidant in Cd tolerance of maize (Hussain *et al.*, 2013) and lettuce (Kosyk *et al.*, 2017).

Vitamins are important metabolites, which act as cofactors in metabolic phenomena and act as developmental cues in plants exposed to environmental stresses (Smith *et al*., 2007). Thus, the metabolic efficiency of cell is determined by optimum levels of vitamins. Important vitamins in plant metabolism are AsA, NIA, RIB, thiamine etc., while the RPA is a measure of how much energy is available for operation of metabolic functions of cells (Duh, 1998; Kramer and Evans, 2011). Prevailing stress conditions usually result in the reversion of normal metabolic function which also disturbs the energy balance of the cell (Loix *et al.*, 2017; Zemanová *et al.*, 2017). However, such information is lacking for plants grown under Cd intoxication. Results of this experiment showed that although Cd treatment hampered the AsA, NIA and RIB concentration, as well as reduced RPA, in both the varieties, the tolerant maize not indicated greater NIA and RIB in both the seasons but it also manifested greater RPA. Nevertheless, TU successfully reversed the adverse effect of Cd in most instances in the two seasons. Contrarily, AsA was almost equally influenced by Cd and profound action of TU was also similar in both the varieties. A number of studies show that vitamins in addition to their normal function as cofactors in metabolism (Smith *et al*., 2007) also act as stimulants of enzymatic antioxidant system and mitigate the oxidative damage by dousing ROS and protecting cytoplasmic membranes form such effects (Guo *et al*., 2009; Asensi-Fabado and Munné-Bosch, 2010). These data suggested that Cd tolerance of maize is exclusively related to the levels of NIA and RIB in the maintenance of metabolic functions. Nonetheless, action of TU was well explicit in the mitigation of Cd effect and restoration of metabolic function by providing greater reducing powers in both the varieties.

Conclusion

Cadmium stress stimulated the oxidative stress on maize, while TU nullified the ROS production by metabolic adjustments. Of these metabolites, accumulation of FP, ANT, NIA and RIB seemingly acted as antioxidants, and high generation of reducing powers was also pivotal. Medium supply of TU to roots improvised the leaf growth indicating its Cd binding role at root level. Notwithstanding, this is the first comprehensive report of alleviation of Cd phytotoxicity by root application of TU, while this role needs to be ascertained with the use of broad range of treatments and at multiple time intervals.

References

Adhikari, T., E. Tel-Or, Y. Libal and M. Shenker, 2006. Effect of cadmium and iron on rice (*Oryza sativa* L.) plant in chelator-buffered nutrient solution. *J. Plant Nutr.*, 29: 1919–1940

Ahmad, P., M. Sarwat, N.A. Bhat, M.R. Wani, A. Gul Kazi and L.S. Phan Tran, 2015. Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. *PLoS One*, 10: e0114571

Ahmadee, M., M.B. Nasab, A. Naseri and A. Kardani, 2016. Assessment of heavy metals in Zohreh River, Iran. *Soil Environ.*, 35: 161–170

Aly,A.A., 2012.Application of DNA (RAPD) and ultrastructure to detect theeffect of cadmium stress in Egyptian clover and Sudan grassplantlets. *J. Stress Physiol., Biochem*., 8: 214–257

Andresen, E. and H. Küpper, 2013. Cadmium toxicity in plants. *Met. Ions Life Sci.*, 11: 395–413

Anjum, F., A. Wahid, F. Javed and M. Arshad, 2008. Influence of foliar applied thiourea on flag leaf gas exchange and yield parameters of bread wheat (*Triticum aestivum* L.) cultivars under salinity and heat stress. *Int. J. Agric. Biol.*, 10: 619–626

Anjum, F., A. Wahid, M. Farooq and F. Javed, 2011. Potential of foliar applied thiourea in improving salt and high temperature tolerance of bread wheat (*Triticum aestivum*L.). *Int. J. Agric. Biol.*, 13: 251–256

Anjum, S.A., M. Tanveer, S. Hussain, M. Bao, L. Wang, I. Khan, E. Ullah, S.A. Tung, R.A. Samad and B. Shahzad, 2015. Cadmium toxicity in maize (*Zea mays* L.): Consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. *Environ. Sci. Pollut. Res.*, 22: 17022–17030

AOAC, 1990. *Official methods of analysis of the Association of Official Analytical Chemists*, 15th edition, pp: 1058–1059. Association of Official Analytical Chemists, Arlington, Virginia, USA

Asensi-Fabado, M.A. and S. Munné-Bosch, 2010. Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends Plant Sci.*, 15: 582–592

Azevedo, H., C. Gloria Pinto and C. Santos, 2005. Cadmium effects in sunflower: membrane permeability and changes in catalase and peroxidase activity in leaves and calluses. *J. Plant Nutr.*, 28: 2233–224

Bates, I.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205–207

Chen, T.H. and N. Murata, 2008. Glycinebetaine: An effective protectant against abiotic stress in plants. *Trends Plant Sci.*, 13: 499–505

Chmielowska-Bąk, J., K. Izbiańska, A. Ekner-Grzyb, M. Bayar and J. Deckert, 2018. Cadmium stress leads to rapid increase in RNA oxidative modifications in soybean seedlings. *Front. Plant Sci.*, 8, Article 2219

De Filippis, L.F. and H. Zeigler, 1993. Effect of sub lethal concentrations of zinc, cadmium and mercury on the photosyntlietic carbon reduction cycle of *Euglena*. *J. Plant Physiol.*, 142: 167–172

Duh, P.D., 1998. Antioxidant activity of burdock (*Arctium lappa* Linné): Its scavenging effect on free-radical and active oxygen. *J. Amer. Oil Chem. Soc*., 75: 455–461

Farooq, M.A., S. Ali, A. Hameed, S.A. Bharwana, M. Rizwan, W. Ishaque, M. Farid, K. Mahmood and Z.Iqbal, 2016. Cadmium stress in cotton seedlings: Physiological, photosynthesis and oxidative damages alleviated by glycinebetaine. *S. Afr. J. Bot.*, 104: 61–68

Fodor, E., A. Szabo-Nagy and I. Erdei, 1995. The effects of cadmium on the fluidity and H^+-ATPase activity ol plasma membrane from sunflower and wheat roots. *J. Plant Physiol.*, 147: 87–92

Garg, B.K., U. Burman and S. Kathju, 2006. Influence of thiourea on photosynthesis, nitrogen metabolism and yield of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) under rainfed conditions of Indian arid zone. *Plant Growth Regul.*, 48: 237–245

Gill, S.S., N.A. Khan and N. Tuteja, 2011. Differential cadmium stress tolerance in five indian mustard (*Brassica juncea* L.) cultivars. An evaluation of the role of antioxidant machinery.*Plant Signal. Behav.*, 6: 293–300

Gouia, H., M.H. Ghorbal and C. Meyer, 2000. Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiol. Biochem*., 38: 629–638

Grieve, C.M. and S.R. Grattan, 1983. Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70: 303–307

Gul, B. and D.J. Weber, 1998. Effect of dormancy relieving compounds on the seed germination of non-dormant *Allenrolfea occidentalis* under salinity stress. *Ann. Bot.*, 82: 555–560

Guo, B., Y. Liang and Y. Zhu, 2009. Does salicylic acid regulate antioxidant defense system, cell death, cadmium uptake and partitioning to acquire cadmium tolerance in rice? *J. Plant Physiol.*, 166: 20–31

Hall, J.L., 2002.Cellular mechanisms for heavy metal detoxification and tolerance. *J. Exp. Bot.*, 53: 1–11

Hamilton, P.B. and D.D. Van Slyke, 1943. Amino acid determination with ninhydrin. *J. Biol. Chem.*, 150: 231–233

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts. *Arch. Biochem. Biophys.*, 125: 189–198

Hussain, I., S. Akhtar, M.A. Ashraf, R. Rasheed, E.H. Siddiqi and M. Ibrahim, 2013. Response of maize seedlings to cadmium application after different time intervals. *ISRN Agron.*, 2013, Article ID 169610

Hussain, I., M.A. Ashraf, R. Rasheed, M. Iqbal, M. Ibrahim, T. Zahid, S. Thind and F. Saeed, 2017. Cadmium-induced perturbations in growth, oxidative defense system, catalase gene expression and fruit quality in tomato. *Int. J. Agric. Biol.,* 19: 61–68

Iqbal, N., A. Masood, M.I.R. Khan, M. Asgher, M. Fatma and N.A. Khan, 2013. Cross-talk between sulfur assimilation and ethylene signaling in plants. *Plant Signal. Behav.*, 8:e22478

Julkenin-Titto, R., 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Agric. Food Chem.*, 33: 213–217

Kosyk, O.I., I.M. Khomenko, L.M. Batsmanova and N.Y. Taran, 2017. Phenylalanine ammonia-lyase activity and anthocyanin content in different varieties of lettuce under the cadmium influence. *Ukr. Biochem. J.*, 89: 85–91

Kramer, D.M. and J.R. Evans, 2011. The importance of energy balance in improving photosynthetic productivity. *Plant Physiol.*, 155: 70–78

Leng, B.Y., W.J. Jia, X. Yan, F. Yuan, X.X. Dong and B.S. Wang, 2018. Cadmium stress in halophyte *Thellungiella halophila*: Consequences on growth, cadmium accumulation, reactive oxygen species and antioxidative systems. *Earth Environ. Sci.*, 153: 062002

Loix,C., M. Huybrechts, J. Vangronsveld, M. Gielen, E. Keunen and A. Cuypers, 2017. Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. *Front. Plant Sci.*, 8: 1867

Naz, S., M.A. Anjum and S. Akhtar, 2016. Monitoring of growth, yield, biomass and heavy metals accumulation in spinach grown under different irrigation sources. *Int. J. Agric. Biol*., 18: 689–697

Noctor, G., G. Queval, A. Mhamdi, S. Chaouch and C.H. Foyer, 2011. Glutathione. *Arabidopsis Book*, 9: e0142

Okwu, D.E. and C. Joshia, 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr. J. Biotechnol.*, 5:357–361

Perveen, A., A. Wahid, S. Mahmood, I. Hussain and R. Rasheed, 2015. Possible mechanism of medium-supplemented thiourea in improving growth, gas exchange, and photosynthetic pigments in cadmium-stressed maize (*Zea mays*). *Braz. J. Bot.*, 38: 71–79

Perumal, S. and K. Becker, 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three agroclimatic origins of Drumstick tree leaves. *J. Agric. Food Chem.*, 51: 2144–2155

Racchi, M.L., 2013. Antioxidant defenses in plants with attention to *Prunus* and *Citrus* spp. *Antioxidants*, 2: 340–369

Retamal-Salgado, J., I. Matus, I. Walter and J. Hirzel, 2017. Absorption and distribution of cadmium of three maize hybrids in three environments. *J. Soil Sci. Plant Nutr.*, 17: 266–278

Rosa, M., C. Prado, G. Podazza, R. Interdonato, J.A. González, M. Hilal and F.E. Prado, 2009. Soluble sugars–metabolism, sensing and abiotic stress: a complex network in the life of plants. *Plant Signal. Behav.*, 4: 388–393

Sandalio, L.M., H.C. Dalurzo, M. Gomez, M.C. Romero-Puertas and L.A. Rio, 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.*, 52: 2115–2126

Seleiman, M.F. and A.M.S. Kheir, 2018. Saline soil properties, quality and productivity of wheat grown with bagasse ash and thiourea in different climatic zones. *Chemosphere*, 193: 538–546

Shah, K., R.G. Kumar, S. Verma and R.S. Dubey, 2001.Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Sci.*, 161: 1135–1144

Sivritepes, H.O., N. Sivritepes, A. Eris and E. Turhan, 2005. The effects of NaCl pre-treatment on salt tolerance of melons grown under long term salinity. *Sci. Hortic.*, 106: 568–581

Smith, A.G., M.T. Croft, M. Moulin and M.E. Webb, 2007. Plants need their vitamins too. *Curr. Opin. Plant Biol.*, 10: 266–275

Sofowora, A., 1993.*Medicinal Plants and Traditional Medicine in Africa*, pp. 289–291. Spectrum Book Ltd. Ibadan, Nigeria

Stark, D. and V. Wray, 1989. Anthocyanins. *In*: *Methods in Plant Biology*; *Plant Phenolics*, Vol. 1, pp. 325–356. Academic Press, London

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1996. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition. McGraw Hill Co., New York, USA

Vassilev, A. and F. Lidon, 2011.Cd-induced membrane damages and changes in soluble protein and free amino acid contents in young barley plants. *Emirates J. Food Agric*., 23: 130–136

Velikova, V., I. Yordanov and A. Edriva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. *Plant Sci.*, 151: 59–66

Wahid, A., M. Arshad and M. Farooq, 2009. Cadmium phytotoxicity: Responses, mechanisms and mitigation strategies. *In*: *Advances in Sustainable Agriculture-Book Series*, Vol, 1, pp: 371–403.Lichtfouse, E. (ed.). Springer, The Netherlands

Wahid, A., S.M.A. Basra and M. Farooq, 2017. Thiourea: a molecule with immense biological significance for plants. *Int. J. Agric. Biol.,* 19: 911–920

Yang, G., D. Rhodes and R.J. Jolly, 1996. Effect of high temperature on membrane stability and chlorophyll fluorescence in glycine betaine containing maize lines. *Aust. J. Plant Physiol.*, 23: 437–443

Yoshida, S., D.A. Forna, 1.Cock and K.A. Gomez, 1976. *Laboratory Manual for Physiological Studies of Rice*, IRRI. Los Banos, Philippines

Zhang, H.Y., Y.N. Jiang and Z.Y. He, 2005. Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). *J. Plant Physiol.*, 162: 977–984

Zemanová, Z., M. Pavlík and D. Pavlíková, 2017. Cadmium toxicity induced contrasting patterns of concentrations of free sarcosine, specific amino acids and selected microelements in two *Noccaea* species. *PLoS One*, 12: e0177963

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