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Full Length Research Paper

Influence of sulfur on cadmium (Cd) stress tolerance in *Triticum aestivum* L.

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A pot experiment was conducted to investigate the influence of sulfur on cadmium (Cd) stress tolerance in *Triticum aestivum* L. (cv. Samma). The treatments were given as follows: 0 mM S + 0 mM Cd (control), 1 mM S + 0 mM Cd (S_1), 5 mM S + 0 mM Cd (S_2), 10 mM S + 0 mM Cd (S_3), 0 mM S + 1 mM Cd (Cd), 1 mM S + 1 mM Cd (S_1+Cd), 5 mM S + 1 mM Cd (S_2+Cd),) and 10 mM S + 1 mM Cd (S_3+Cd). Plants fed with Cd showed reduced plant growth characteristics and increased malondialdehyde (MDA) accumulation. However, S-exposed plants clearly exhibited enhanced antioxidant enzymes activity (catalase, peroxidase and superoxide dismutase), chlorophyll *a* and *b* content and total soluble carbohydrates (TSC) accumulation, and decreased MDA content in wheat plants. The results indicate that application of S mitigated the adverse effects of Cd stress by enhancing TSC, photosynthetic pigments and antioxidant enzymes.

Key words: Antioxidant, cadmium stress, carbohydrates, photosynthetic pigments, *Triticum aestivum*.

INTRODUCTION

Environmental stresses such as salinity, drought, heat, cold, flooding and heavy metal are major threat to the agricultural productivity worldwide. The heavy metal, cadmium (Cd) is very toxic to plants as well as humans health (Prasad, 1995). Cd is commonly released into the arable soil from industries, energy, municipal sources and farming practices, and has been ranked no. 7 among the top 20 toxins (Yang et al., 2004). Cd is easily taken up by plant roots and transported to the leaves through xylem. Most plants are sensitive to low concentrations of Cd, which disturb the physiological and molecular mechanisms by which plants carry out adaptive response to environmental stresses. Therefore, it is important to understand the physiological mechanism by which plants perform normally under abiotic stress and to also improve the tolerance of plant to abiotic stress. Cd causes inhibition of physiological process such as photosynthesis, respiration, cell elongation, plant water relationship and assimilation of nitrogen, sulphur and phosphate, thus resulting in poor growth and development of plants (Sanitá di Toppi and Gabbrielli, 1999; Mishra et al., 2006; Mobin and Khan, 2007). However, the mechanisms involved in its toxicity as well as the cell response against the metal have not been well established (Sandalio et al., 2001; Rodríguez-Serrano et al., 2009).

Sulphur (S) is required at 0.1 to 1.0% (on a dry weight basis) for growth and development (Marschner, 2002). Up to 90% of the total S is present in most plants as cysteine and methionine that are predominantly bound to protein (Giovanelli, 1990). Reduced S is required in the function of cofactors, such as acetyl coenzyme A, thiamine, biotin and lipoic acid. The S-containing tripeptide, glutathione, is involved in the regulation of protein synthesis and in the compensation of various forms of stress (Rennenberg and Brunold, 1994; Kranner and Grill, 1996). Both functions of glutathione are highly significant for the survival of plants in a stressful environment. Literatures are full of reports that show the use of different strategies to combat the inhibitory effects of Cd in crop plants, but information on the involvement of mineral nutrition for Cd stress

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Abbreviations: MDA, Malondialdehyde; **TSC,** total soluble carbohydrates.

tolerance is limited (Gill and Tuteja, 2011).

Wheat (*Triticum aestivum* L.) is a very important crop, as it is one of world's leading grains producing crops together with rice and corn. With this consideration in mind, this experiment was planned to investigate the effect of S on Cd stress tolerance in wheat plant.

MATERIALS AND METHODS

Plant materials and cultivation conditions

This experiment was carried out under glasshouse condition using wheat (*T. aestivum* L. cv. Samma) obtained from a local market in Riyadh, Saudi Arabia. Healthy seeds were surface sterilized with 1% sodium hypochlorite for 10 min, and then vigorously rinsed with sterilized double distilled water (DDW) before sowing. The seeds were sown in plastic pots (25 cm diameter, 25 cm height) filled with perlite and supplied with Raukura's nutrient solution (Smith et al., 1983). The pots were arranged in a simple randomized design in the greenhouse with a single factor and four replicates. One week after sowing, seedlings were thinned so that each pot contained healthy plants of uniform size. Pots were irrigated every two days with DDW (100 ml) to keep the perlite moist.

When the plants were at the stage of two to three true leaves, cadmium (Cd) solution was added to the pots with experimental wheat plants to attain the final concentration from 0 to 1 mM. The treatments were given as follows: 0 mM S + 0 mM Cd (control), 1 mM S + 0 mM Cd (S_1), 5 mM S + 0 mM Cd (S_2), 10 mM S + 0 mM Cd (S_3), 0 mM S + 1 mM Cd (Cd), 1 mM S + 1 mM Cd (S_1 + Cd), 5 mM S + 1 mM Cd (S_2+Cd),) and 10 mM S + 1 mM Cd (S_3+Cd). The sources of S and Cd were ammonium sulphate $(NH_4)_2SO_4$ and cadmium chloride ($CdCl_2$), respectively. The plants were sampled at 25 days after sowing to assess their growth characteristics [plant height, shoot fresh plant^{-1} (shoot FW), dry weight plant^{-1} (shoot DW)] and area leaf^{-1} and physio-biochemical attributes [chlorophyll (Chl) *a* and b, total soluble carbohydrates (TSC) content, malondialdehyde (MDA) content, and activity of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD)].

Plant growth characteristics

Plant height was measured using a meter scale after removal from the pots. After recording fresh weight (FW) with balance, plants were placed in a 60°C oven for 48 h and then were weighed for dry weight (DW). Leaf area (LA) was measured using a LI-3000 Portable Leaf Area Meter (LI-COR, Lincoln, NE, USA).

Physiological and biochemical parameters

Chl was extracted from fresh leaves using the dimethylsulphoxide (DMSO) method of Barnes et al. (1992). Chl *a* and Chl *b* concentrations were calculated based on the absorbance of the extract at 663.8 and 646.8 nm. On the other hand, MDA content was determined according to the method of Heath and Packer (1968). Leaves were weighed and homogenates containing 10% trichloroacetic acid (TCA) and 0.65% 2-thiobarbituric acid were heated at 95°C for 60 min, cooled to room temperature and then centrifuged at 10,000 × g for 10 min. The absorbance of the supernatant was read at 532 and 600 nm against a reagent blank.

To determine the enzymatic activities of the antioxidant proteins, a crude enzyme extract was prepared by homogenizing 500 mg of leaf tissue in extraction buffer containing 0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000 × g for 20 min at 4°C. The supernatant was used for the enzymatic assays described below. We used the method of Chance and Maehly (1955) to determine POD (E.C. 1.11.1.7) activity using 5 ml of an assay mixture containing phosphate buffer (pH 6.8), 50 M of pyrogallol, 50 mM of H_2O_2, and 1 ml of the enzyme extract diluted 20X. This was incubated for 5 min at 25°C, after which the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by measuring absorbance at 420 nm. A unit of peroxidase activity was the amount of purpurogallin formed per mg protein per minute.

Furthermore, Aebi (1984) method was used to measure CAT (EC 1.11.1.6) activity. The decomposition of H_2O_2 was monitored by the decrease in absorbance at 240 nm. For the assay, a 50 mM phosphate buffer (pH 7.8) and 10 mM H_2O_2 were used. The activity of SOD (EC 1.15.1.1) was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) according to the methods of Giannopolitis and Ries (1977). The reaction solution (3 ml) contained 50 µmol NBT, 1.3 µmol riboflavin, 13 mmol methionine, 75 nmol ethylenediaminetetraacetic acid (EDTA), 50 mmol phosphate buffer (pH 7.8), and 20 to 50 µL enzyme extract. The reaction solution was irradiated under a bank of fluorescent lights at 75 µmol m^{-2} s^{-1} for 15 min. The absorbance at 560 nm was read against a blank (non-irradiated reaction solution). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

Statistical analysis

Each pot was treated as one replicate and all the treatments were repeated three times. The data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's multiple-range test at $p<0.05$% level.

RESULTS

The application of S significantly enhanced all growth traits relative to the control. Among the S applications, 1 mM of S proved best by enhancing maximum growth characteristics such as plant height, shoot FW, shoot DW and LA (Figures 1 A, B and C). However, at Cd, growth parameters were significantly reduced. Application of S enhanced all four growth attributes under Cd stress, and maximum enhancement was recorded at 1 mM of S. Under Cd stress, application of 1 mM of S increased plant height by 46.00, shoot FW by 62.25, shoot DW by 128.18 and LA by 51.86% over the Cd application (Figures 1A to C).

In addition, application of S significantly improved total soluble carbohydrates, and concentration of Chl *a* and *b* in leaves relative to the control, although treatment with 1 mM of S gave higher value for total soluble carbohydrates, and photosynthetic pigments (Figures 2A and B). However, application of Cd decreased the concentration of total soluble carbohydrates, Chl *a* and *b* as compared to controls. Application of S significantly ameliorated total soluble carbohydrates and photosynthetic pigments over the Cd treatment. Moreover,

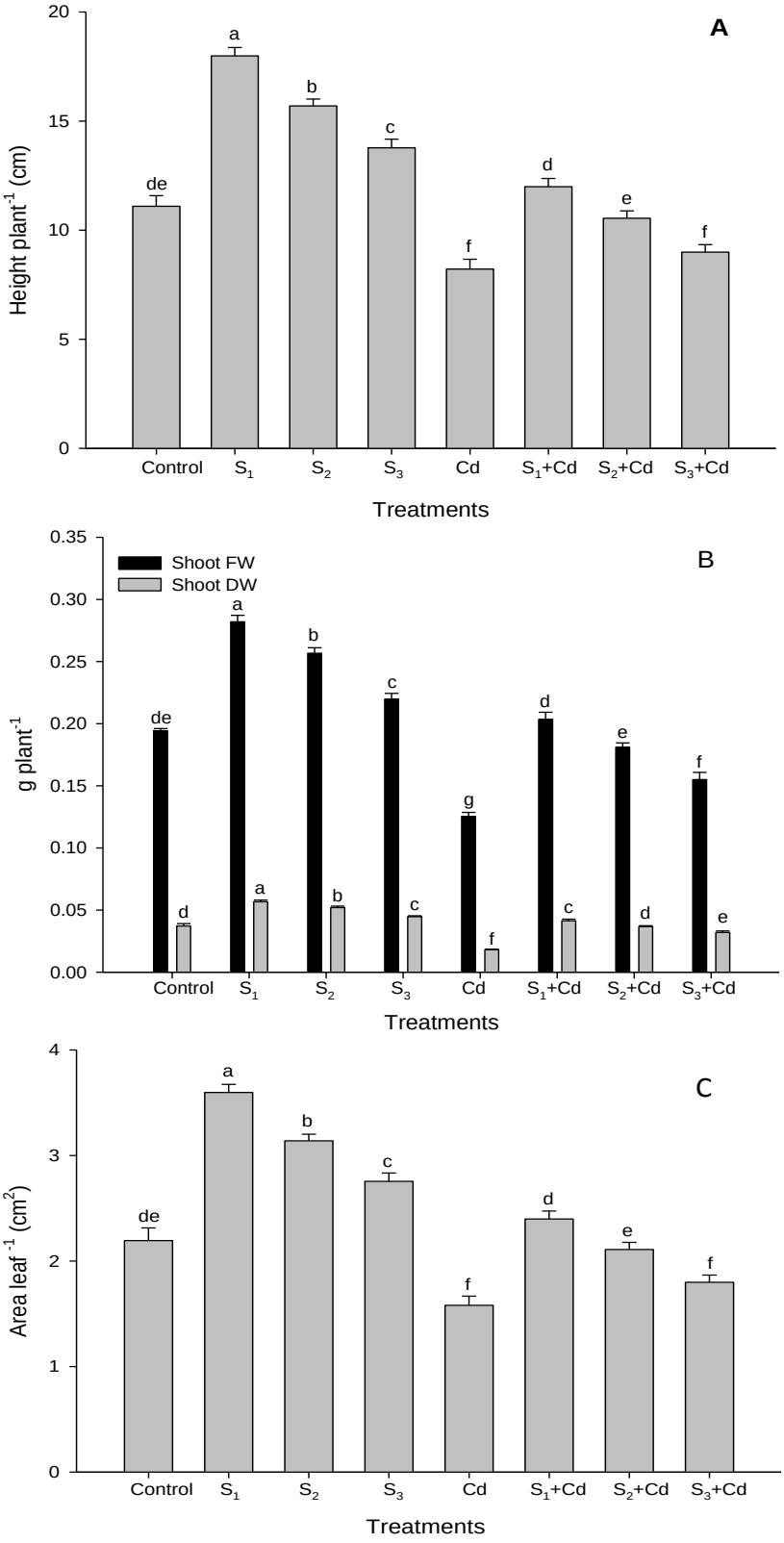

Figure 1. Effect of sulphur on height plant^{-1} (A), shoot FW and shoot plant^{-1} DW (B) and area leaf^{-1} (C) of wheat under cadmium stress. Bars followed by the same letters show no statistical difference at P<0.05 (Duncan's multiple range test). Average of four determinations are presented with bars indicating SE. FW, fresh weight, DW, dry weight, SE, standard error.

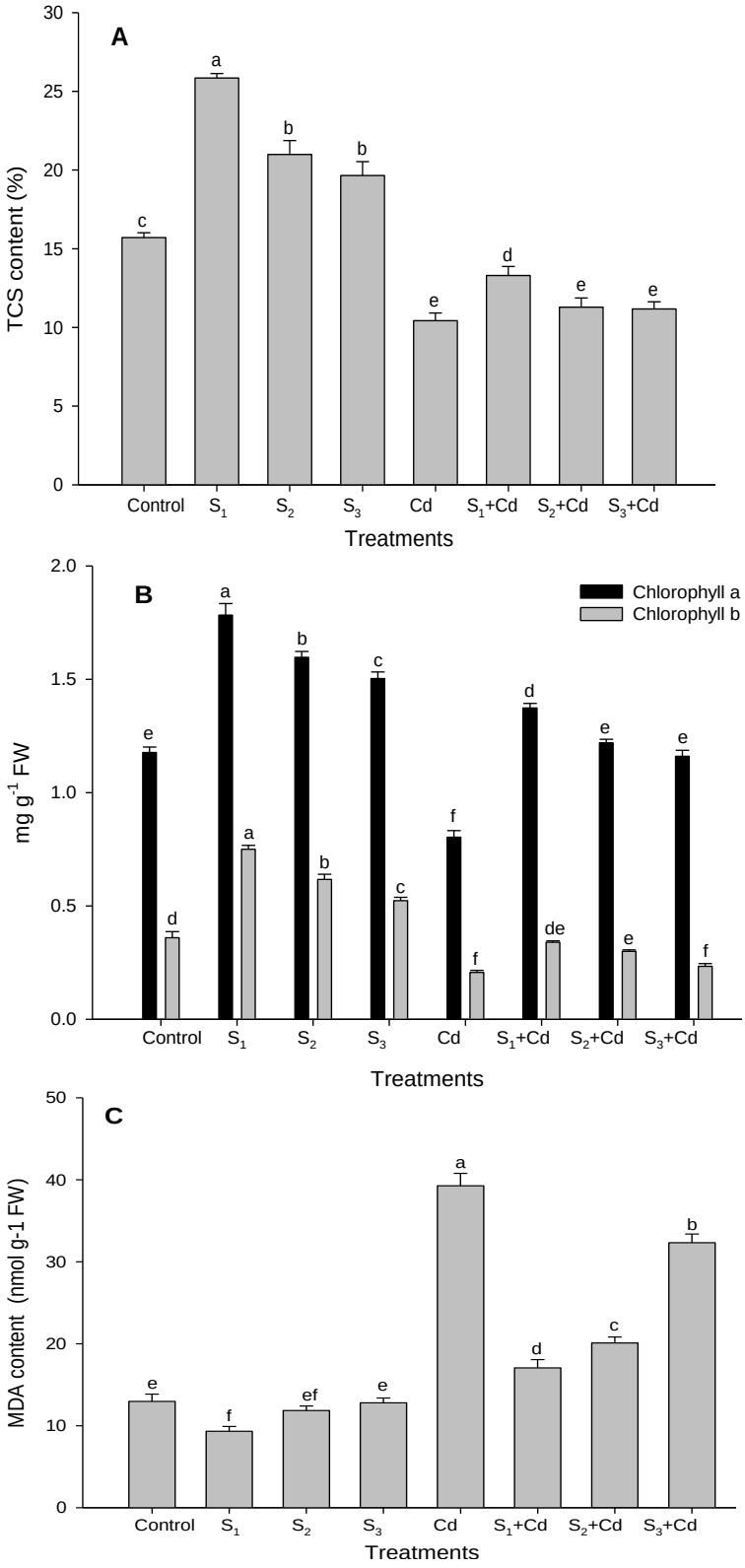

Figure 2. Effect of sulphur on TSC content (A), Chl *a* and *b* (B), and malondialdehyde content (C) in wheat under cadmium stress. Bars followed by the same letters show no statistical difference at P<0.05 (Duncan's multiple range test). Average of four determinations are presented with bars indicating SE. TSC, Total soluble carbohydrates; SE, standard error.

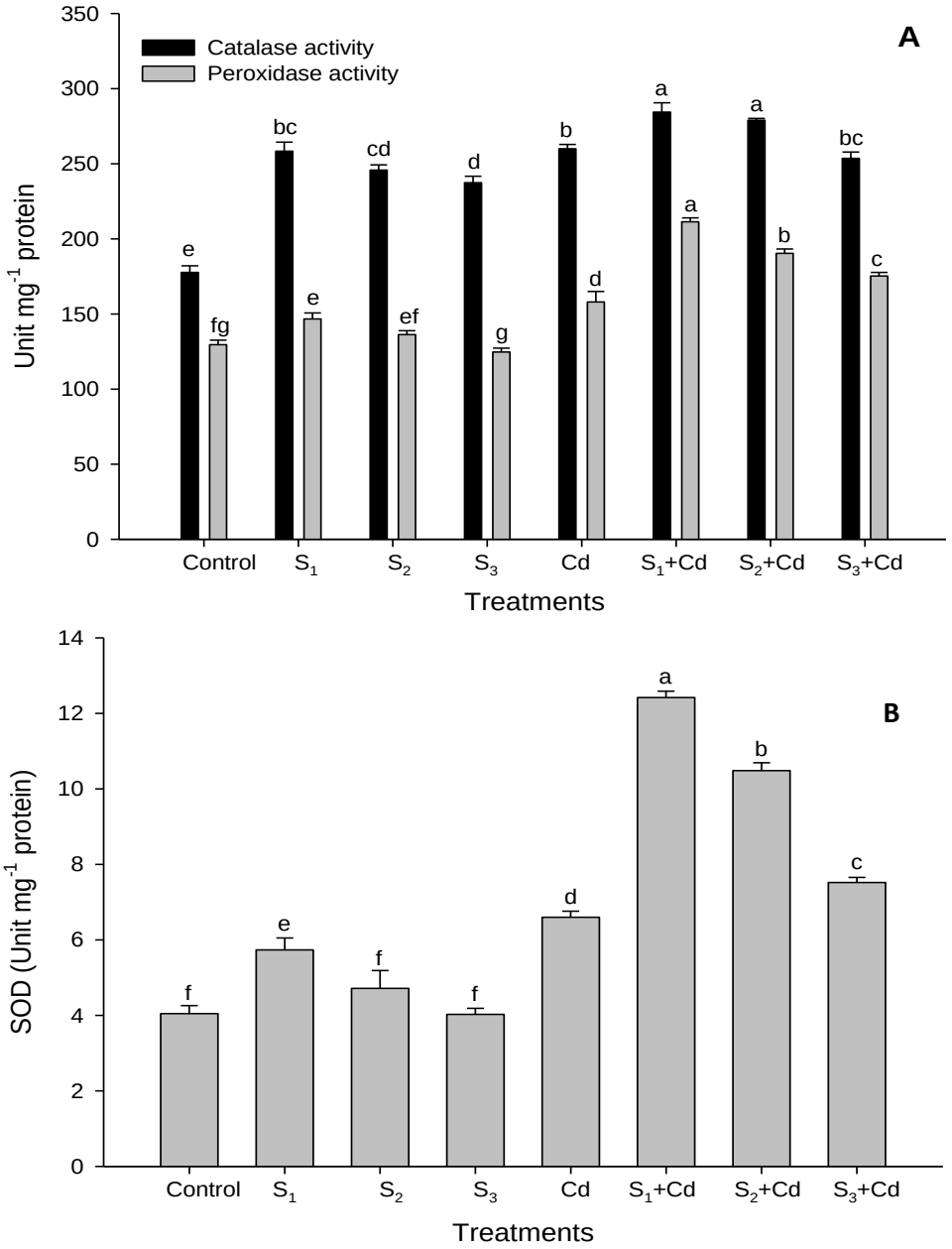

Figure 3. Effect of sulphur on CAT and POD (A) and superoxide dismutase (SOD) (B) activity in wheat under cadmium stress. Bars followed by the same letters show no statistical difference at P<0.05 (Duncan's multiple range test). Average of four determinations are presented with bars indicating SE. SE, standard error. CAT, catalase; POD, peroxidase; SOD, superoxide dismutase.

the application of 1 mM of S was more effective in alleviating the adverse effect of Cd stress. Also, the application of 1 mM of S improved total soluble carbohydrates by 27.42, Chl *a* by 70.96 and Chl *b* by 64.49% over the Cd (Figures 2A and B).

Furthermore, the application of Cd increased MDA content as compared to the other treatments. However, application of S significantly reduced MDA content in leaf of wheat (Figure 2C) with the lowest content of MDA was recorded in plants treated with 1 mM of S. Application of 1 mM of S reduced MDA content by 56.59% over the Cd. Our results show that under non-stress as well as stress conditions, application of S significantly induced the activity of antioxidant enzymes such as POD, CAT and SOD (Figures 3A and B). However, application of 1 mM of S improved maximum activity of these enzymes. Treatment with 1 mM of S enhanced activity of CAT by 9.36, POD by 33.81 and SOD by 88.28% over the Cd

application (Figures 3A and B).

DISCUSSION

The individual Cd treatment significantly decreased growth parameters of wheat plant (Figures 1A to C) when compared to control and others treatments. Many researchers reported in their study that Cd is extremely phytotoxic and causes inhibition of plant growth (Vassilev et al., 1995; Sandalio et al., 2001; Dong et al., 2005; Kurtyka et al., 2008). The growth reduction produced by Cd could be due to the degradation of chlorophyll molecules (Sandalio et al., 2001). However, application of S significantly increased growth traits of wheat plants. The growth-promoting effect of S can be traced on the basis of its role in plants to form several metabolically active compounds, including adenosine-5-phosphosulphate, coenzyme A, cysteine, enzymes, glutathione, methionine, and proteins (Nason and McElroy, 1963; Clarkson and Hanson, 1980; Marschner, 2002). These observations strengthen the findings of Siddiqui et al. (2012) who reported that exogenous application of S enhanced the growth characteristics of *Brassica juncea* L. plants under salt stress. Thus, we may postulate that application of S helped the plant to restore the altered growth traits induced by Cd stress.

Total soluble carbohydrates are the major soluble constituent that helps the plants in osmotic adjustment. Also, carbohydrates provide rapidly growing cells with energy and with the carbon skeletons required to synthesize organic compounds (Taiz and Zeiger, 2010). In the present experiment, accumulation of TSC was found to be decreased in plants grown in medium containing Cd. This may be due to suppression of the activity of enzymes of carbohydrate metabolism under Cd stress (Sanitá di Toppi and Gabbrielli, 1999; Verma and Dubey, 2001). However, application of S induced the accumulation of TSC (Figure 2A). Lunde et al. (2008) also reported that carbohydrate metabolism was affected under sulphur deficiency and antioxidants. In the presence of S nutrition, ozone had no effect on total soluble carbohydrates content (Adedipe et al., 1972).

Moreover, it is well established that photosynthetic pigments play vital role in the synthesis of carbohydrates. Cd-suffering plants exhibited a significant reduction in Chl *a* and *b* (Figure 2 B). The decreased Chl *a* and *b* might be due to alteration of chloroplast (Vassilev et al., 1995) and reduction of photo system (PS) II and I, and also the light associated light-harvesting antenna (Lunde et al., 2008), leading to the poor dry matter production. However, plants fed with S exhibited higher value for photosynthetic pigments. The efficiency of S in enhancement of different metabolic activities can be attributed to its role in the formation of S containing compounds such as cysteines, methionine, glutathione and several coenzymes (Giovanelli, 1990). The tripeptide glutathione is involved in the regulation of protein synthesis (Kranner and Grill, 1996). Ascorbate and glutathione help to stop the degradation of photosynthetic pigments rate with oxidized glutathione (GSSG) and nicotinamide adenine dinucleotide phosphate (NADPH) (Kato and Shimizu, 1985). Therefore, S could be responsible for enhancing the tolerance of plant to Cd stress by improving photosynthetic pigments.

Cd-exposed plants showed increased value for MDA concentration in leaf of wheat plants (Figure 2 C) because Cd produces excessive reactive oxygen species including superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) causing cell death due to oxidative stress (Shah et al., 2001). However, application of S was very effective in reduction of the MDA accumulation under Cd stress (Figure 2C). It may be due to the improvement of antioxidant enzymes activity such as CAT, POD and SOD (Figures 3A and B). An increase in the activity of POD, CAT and SOD was observed upon S treatment. We inferred that the maximum CAT, POD and SOD activity in wheat plants under Cd stress could increase the ability to scavenge reactive oxygen species (ROS) which could cause membrane damage (Agarwal and Pandey, 2004).

The improved antioxidant system in plants by the application of S that may be explained on the basis of its role in the formation of macromolecules, have been proven to provide tolerance of plant to Cd stress in plants by increasing enzyme's activity. Domínguez-Solís et al. (2004) reported that cysteine biosynthesis is associated with Cd tolerance. Glutathione plays vital role in protein synthesis and nucleic acid, works as an activator of enzyme activity and regulates the S nutrition (Chalapathi et al., 2008). GSH plays a central role in plant defense against oxidative stress, and has the ability to directly scavenge the metal induced ROS such as $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$ (Noctor and Foyer, 1998; Kopriva and Rennenberg, 2004). Therefore, S could be responsible for enhancing the tolerance of plant to Cd stress by scavenging the ROS.

Conclusion

On the basis of the results obtained in the present experiment, it can be concluded that application of S decreased the MDA content and enhanced the activity of antioxidant enzymes, Chl *a,* Chl *b* and total soluble carbohydrates, suggesting its tolerance capacity to protect the plant from oxidative damage induced by Cd stress. The increased photosynthetic pigments, accumulation of TSC and activity of CAT, POD and SOD in wheat plants fed with S could be responsible for improved LA and dry matter production under Cd stress. The results indicate that S application mitigated the ill effects of Cd stress and adjusted the plants to perform normally.

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