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Alleviation of Oxidative-Stress Induced by Drought Through Application of Compost in Wheat (*Triticum aestivum* L.) Plants

Kamel A.H. Tartoura

Botany Department, Faculty of Agriculture,
Suez Canal University, Ismailia, Egypt

Abstract: Water deficit is one of the main environmental factors that adversely affect plant growth and productivity. The present study aimed to investigate the effect of compost (Co) addition to soil on total biomass production (TBP), reactive oxygen species (ROS) and activities of the antioxidant enzymes [superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR)], as well as non-antioxidant enzymes [ascorbate (ASC), glutathione (GSH)] in wheat plants under well-watered and drought stress conditions. Seeds were germinated in plastic pots containing either sandy soil or sandy soil amended with the compost under natural conditions. At the beginning of blooming stage, two levels of soil moisture (80% of the soil field capacity for well-watered control and 40% for drought stress treatment) were applied for 28 days. Drought stress significantly decreased TBP; however application of compost increased this parameter. Plant biomass production was higher in compost-treated plants than that in untreated ones. Data also showed that there were lower superoxide ($O_2^{\cdot-}$) anion radical and hydrogen peroxide (H_2O_2) and the by-product of lipid peroxidation [malondialdehyde (MDA)] concentrations in leaves of compost-treated plants grown under well-watered (WW) and drought stress (DS) conditions. Compost application led to increasing the activities of the antioxidant enzymes SOD, GPX, CAT, APX and GR of wheat grown under drought conditions, compared to the control plants. Levels of ASC and GSH in leaves were higher in compost-treated than that in untreated plants under WW and DS conditions. All these changes contributed to protecting wheat leaves from oxidative stress damage induced by drought as indicated by low levels of ROS. Data suggested that compost alleviates oxidative damage in wheat plants grown under drought by improving plant growth and activating antioxidant defense systems, thereby improving stability of membranes in plant cells. This study provides evidence for a beneficial effect of compost application in enhancing drought tolerance of wheat under water deficit conditions.

Key words: Compost · Drought stress · Lipid peroxidation · ROS-scavenging anitioxidants · *Triticum aestivum* L.

INTRODUCTION

Drought stress is one of the main environmental factors that adversely affect plant growth and productivity. Drought usually induces the accumulation of reactive oxygen species (ROS), which cause oxidative damage to plants [1, 2]. If not effectively and rapidly removed from plants, ROS can damage a wide range of cellular macromolecules such as lipids, enzymes and DNA [3]. Plants can protect themselves against oxidative stress damage by antioxidant defense systems including enzymatic and non-enzymatic antioxidant compounds [4].

A major challenge in agricultural practice and research today is how to cope with the environmental stresses including drought in an economical and an environmental sustainable approach. Compost derived from agricultural wastes can improve crop tolerance and increase plant growth via providing better soil structure, supply of nutrients and building up antagonistic microorganisms [5-10]. Therefore, addition of compost to soils influences a wide array of agronomic and physiological characteristics and is, therefore, a crucial component for any sustainability of agriculture system [11].

Corresponding Author: Kamel A.H. Tartoura, Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, E-mail: tartoura@msu.edu.

Consequently, it has been hypothesized that compost addition to agricultural soils could result in improving protection capacity of plants under stressful environmental conditions such as water deficit. To date, little information is available on the metabolic and physiological changes under drought stress by compost application [12-15].

The aim of this study was to address the effects o f compost derived from cow manure, chicken manure and wheat straw on protection of wheat (*Triticum aestivum* L. cv. Sids 1) plants against water deficit by measuring TBP, ROS and ROS-scavenging systems.

MATERIALS AND METHODS

Plant Material and Treatments: Sandy soil was collected from the soil layer 0-25 cm depth at Faculty of Agriculture Farm, Suez Canal University. The soil was air-dried and ground to pass a 2 mm sieve. The soil pH was 7.9, EC (dS/m) was 1.1, Total organic matter (TOM, %) was 0.5, total C, N, K in g/ Kg are 2.9, 0.3 and 0.025, respectively. The used compost was produced from a mixture of cow manure, chicken manure and wheat straw at ratio of 3:1:1 (v/v). The compost pH and EC (dSm^{-1}) were 6.8 and 4.4, respectively. TOM (%) was 75.6; total C, N, P and K (g/Kg) dried samples were 450, 27.0, 9.40 and 25.2, respectively [13].

Wheat (*Triticum aestivum* L. cv Sids) seeds were planted in plastic pots (12 seeds /pot) containing sandy soil amended with the compost at rate of 100 g /Kg soil and grown along with control that received no compost under natural conditions. The environmental conditions were as follows: 11-h photoperiod, temperature of 25/20°C day/night and a relative humidity of 65/70%. The pre-planting irrigation was applied 15 days before planting. After germination, seedlings were watered at intervals of 72 h. During the growing period, the water content of the soil in all pots was maintained near 80% of the field capacity (FC). At the beginning of blooming stage, two levels of soil moisture (80% soil field capacity for well-watered (WW) control and 40% for drought stress (DS) treatment) were applied for 28 days following the methods of Desclaux and Roumet [15]. The water deficit was initiated by withholding water. The pots were weighed daily to maintain the desired soil water levels by adding appropriate volumes of water. The experimental treatments consisted of: control [sandy soil without compost addition (T1)] and three treatments [sandy soil amended with compost (T2); sandy soil under DS (T3) and sandy soil amended with compost under DS (T4)] arranged in a randomized complete blocks design with four replicates, giving a total of 16 pots. Simultaneously, total 3 sets of independent experiments were performed. Data are the mean values of three independent experiments. The youngest fully developed leaves were taken after 7 days of treatment for biochemical assays.

Total Biomass Production Measurement: Plants were harvested on the 28th day of exposure to the drought when plants were 90 days old. The total dry weight (DW) of shoots plus root systems was measured after drying samples in an oven at 70°C for 2 days.

Assay of ROS and MDA: Superoxide radical was determined according to Elstner and Heupel [16]. Flag leaves (1.0g) were homogenized in ml of 50 mM potassium phosphate buffer (pH7.8) and centrifuged at 12,000 g for 20 min. The incubation mixture contained 1 ml of supernatant, 1 ml of 50 mM potassium phosphate buffer (pH7.8) and 1 ml of 1mM hydroxylammonium chloride and the mixture was incubated at 25 °C for 20 min. The mixture was subsequently incubated with 2 ml of 17mM sulphanilic acid and 2 ml of 7 mM α-naphthyl amine at 25°C for 20 min. The final solution was mixed with an equal volume of ethylether and the absorbance of the pink phase was read at 530 nm. The production rate of $O_2^{\cdot-}$ was calculated based on a standard curve.

The H_2O_2 content was measured by monitoring the absorbance of the titanium-peroxide complex at 415 nm, following the method of Patterson *et al.* [17].

MDA of extracts was determined by the thiobarbituric acid reaction as described by Sudhakar *et al.* [18].

Determination of Antioxidant Enzymes: Soluble proteins in flag leaves of treated and untreated wheat were extracted with 50 mM ice cold potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 1 mM ethylenediaminetetraacetic acid (EDTA). The activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) were determined as described by Jiang and Zhang [19]. Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was determined according to the method described by Hammerschmidt [20]. Protein content was estimated according to Bradford [21] using bovine serum albumin as a standard.

ASC and GSH Assays: ASC and GSH were determined by extracting a half gram of fresh leaf tissues with 5 ml of 5% (w/v) trichloroacetic acid and centrifuging at 15,000g for 15 min at 4 °C. The supernatant was used for ASC and GSH assays. ASC was determined using the method described by Chen and Wang [22]. The ASC assay mixture (5 ml) contained 1.0 ml supernatant, 100 mM phosphate buffer (pH 7.7), 10% (w/v) trichloroacetic acid, 44% (v/v) H_3PO_4, 4% (w/v) 2, 2'-bipyridyl and 3% (w/v) $FeCl_3$. The final mixture was incubated in 37°C for 60 min and cooled to room temperature. Absorbance of the colored solution was recorded at 525 nm. A modified method of Wang and Jiao [23] was employed for the assay of GSH. The GSH assay mixture (3.7 ml) contained 1.0 ml supernatant, 100 mM phosphate buffer (pH 7.7) and 0.60 mM 5,5'-dithio-bis (2-nitrobenzoic acid). Absorbance was recorded at 412 nm.

Statistical Analysis: The data were subjected to statistical analysis using COSTAT computer software (CoHort Computer Software, Berkeley, CA, USA). Least significant differences (LSD) test was applied to compare the treatment means. Graphical presentation of data was carried out using MICROSOFT EXCEL program (Microsoft Corporation, Los Angeles, CA, USA).

RESULTS AND DISCUSSION

Effect of compost on TBP: Figure 1 shows that drought stress significantly decreased total biomass production in wheat plants. However, under the same conditions, Co markedly increased the TBP to become similar to that of the control plants. Further, it is obvious that compost greatly increased TBP of wheat plants grown under WW and DS conditions.

Effect of compost on $O_2^{\cdot -}$, H_2O_2 and MDA: Compared to the control plants, DS significantly increased $O_2^{\cdot}$-level in flag leaves. However, compost treatment significantly decreased leaf $O_2^{\cdot}$-level in Co-treated and Co + DS-treated plants (Fig. 2A).

Fig. 2B shows that DS considerably increased the H_2O_2 concentration in flag leaves, relative to the control. However, adding compost to sandy soil significantly decreased leaf H_2O_2 concentrations in Co-treated plants under WW and DS conditions.

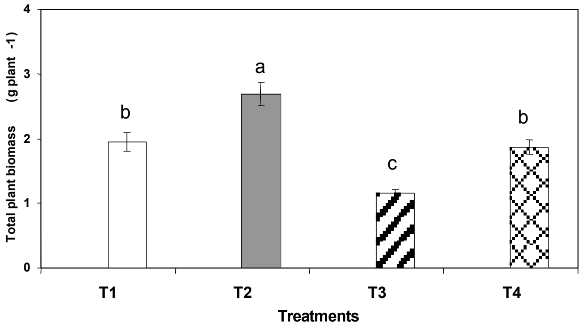

Fig. 1: Total plant biomass production in compost-treated and untreated *wheat (Triticum aestivum L.)* plants subjected to well-watered and drought stress conditions. Data are the mean values ± SD of four replicates from three independent experiments. T1, control without any treatments; T2, compost-treated plants; T3, drought stress (DS)-treated plants; and T4, Co + DS-treated plants. Vertical bars represent ± SD. Different letters indicate statistically significant differences between treatments at the 0.05 level.

Malondialdehyde was measured as an index of lipid peroxidation and its level is an indicator of the extent of oxidative damage. Changes in MDA levels were similar to those of H_2O_2. Drought stress treatment significantly increased flag leaf MDA concentration, whereas application of Co significantly decreased the accumulation of MDA (Fig. 2C). Thus, it is evident that application of Co markedly decreased the concentration of $O_2^{\cdot -}$, H_2O_2 and MDA in leaves of compost-treated plants, as compared with the untreated ones.

Effect of Compost on Antioxidant Enzymatic Activities: Compost-treated plants had higher level of leaf SOD than that of untreated ones under DS conditions (Fig. 3A). Under WW conditions, there is a slight increase in SOD activity in compost-treated plants, relative to the control, as shown in Fig. 3A.

Drought stress markedly increased leaf GPX activity (Fig. 3B). This increase was dramatically elevated by Co under water deficit conditions. Application of compost greatly increased GPX activity under WW and DS conditions by 28.5% and 30.5%, respectively, in comparison with the untreated plants.

Drought stress caused a significant increase in the leaf CAT activity (Fig. 3C). Application of Co markedly increased leaf CAT activity under DS conditions.

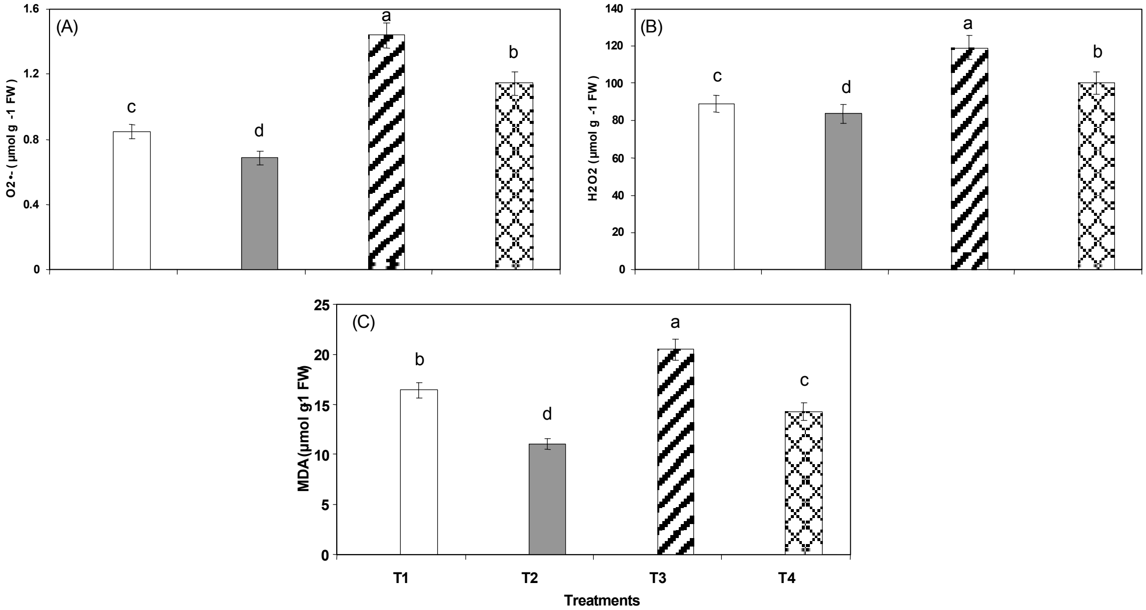

Fig. 2: Concentrations of $O2^{-}$-(A), H_2O_2 (B) and MDA (C) in leaves of compost-treated and untreated wheat plants subjected to WW and DS conditions. Rest of the legend is the same as Fig. 1.

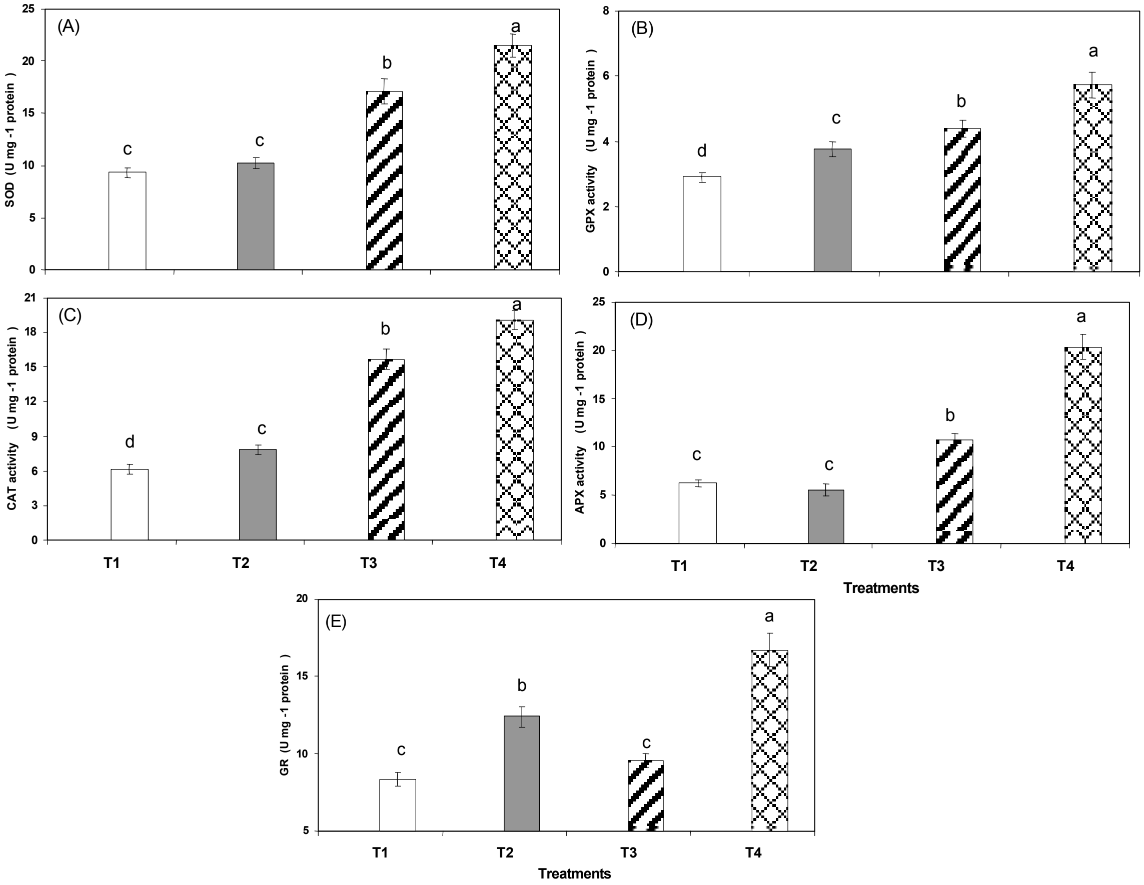

Fig. 3: Activities of SOD (A), GPX (B), CAT (C) and GR (D) in leaves of compost-treated and untreated wheat plants subjected to WW and DS conditions. Rest of the legend is the same as Fig. 1.

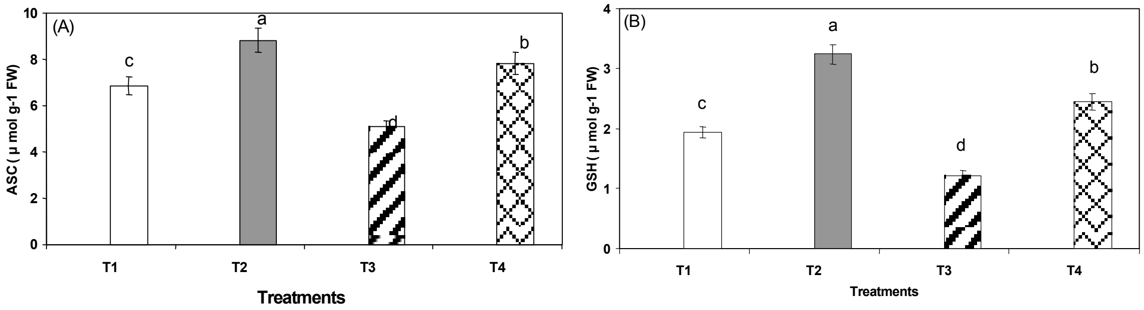

Fig. 4: Ascorbate (A), and glutathione (B), in leaves of compost-treated and untreated wheat plants subjected to well-watered and drought stress conditions. Rest of the legend is the same as Fig. 1.

Treatments with Co under DS increased CAT activity by 22.1 in comparison with the untreated plants. CAT activity was higher in Co-treated plants than in untreated ones under WW conditions.

Fig. 3D showed that drought stress markedly increased leaf APX activity. This increase was also higher in Co-treated plants than that in untreated under DS conditions. Treatment with Co under DS conditions increased APX activity by 89.18% in comparison with the untreated plants. There is no significant difference in APX activity between compost-treated and untreated plants under WW conditions.

Fig. 3 E showed that under WW and DS conditions, compost-treated plants had higher levels of leaf GR activities than those of untreated ones. Treatment with compost under WW and DS increased GR activity by 37.6 and 75.0%, respectively, compared with the untreated plants.

Effect of compost on ASC and GSH levels: Drought considerably decreased ASC content of wheat flag leaves (Fig. 4). Application of Co increased ASC under DS conditions; compost-treated plants had 56% higher in the ASC content than that in untreated plants under DS conditions. Compost treated plants had 100% higher in the GSH than that in untreated plants under WW conditions. Under DS conditions, compost-treated plants had higher levels of ASC and GSH than those in untreated ones, as shown in Fig. 4A, B. It is evident that Co-treated plants showed notably higher levels of ASC and GSH under WW and DS conditions.

DISCUSSION

There is an evidence that composted organic matter has a great number of beneficial effects on plant growth and development under environmental stresses [12-15, 25-27]. In this study, application of Co increased TBP in wheat plants grown under WW and DS conditions (Fig. 1), which in agreement with earlier findings reported by Mata-González *et al.* [12], Tartoura [13], Antolín *et al.* [15]. Stimulatory effects of Co on plant growth have often been related to alteration of the chemical and physical properties of the soil, increasing organic matter content, water holding capacity, microbes diversity, providing macro-and micronutrients essential for plant growth and suppressing plant diseases which collectively contributes to plant growth enhancement [8-10,28-30]. However, there is no information at the biochemical level on the response of compost-treated plants to DS conditions. The wheat plants cultivated in sandy soil amended with the compost derived from agricultural wastes was studied under drought conditions as an approach to understanding the effect of compost on plant defense mechanisms that cope with environmental stresses.

In higher plants, ROS are continuously produced in chloroplasts, mitochondria and peroxisomes [1]. Production and removal of ROS are strictly controlled under WW conditions. When higher plants are subjected to water deficit, the equilibrium between ROS production and scavenging is broken, resulting in oxidative damage to proteins, DNA and lipids. MDA is one of the byproducts of lipid peroxidation and could reflect the degree of the peroxidation of membrane lipids [31]. In the present study, drought stress increased MDA in wheat leaves, with increases related to the magnitude of $O_2^{\cdot}$-and H_2O_2. These free radicals were higher in DS than in WW plants, causing the membrane lipid peroxidation (Fig. 2C). Application of Co markedly decreased $O_2^{\cdot-}$and H_2O_2 in WW and DS leaves, indicating a lower ROS accumulation in Co-treated wheat plants, as shown in Fig. 2A, B. It seemed that a decrease in TBP in Co-untreated wheat plants (Fig. 1) was associated with increasing $O_2^{\cdot-}$anion, H_2O_2 levels, resulting in high concentration of MDA (Fig. 2). It is clear that Co application markedly alleviated oxidative stress damage in wheat induced by drought as indicated by improving TBP and decreasing activated oxygen species, thereby reducing lipid peroxidation. It is interesting to note that the levels of O_2^--and H_2O_2 along with MDA level were significantly lower in Co-treated plants than in untreated ones (Fig. 2A, B, C), suggesting involvement of the compost in the ROS metabolism in wheat plants that are not exposed to environmental stresses.

Drought stress elicits other biochemical responses in higher plants that minimize its deleterious effects. One important component of protective systems is enzymatic antioxidants. SOD catalyses the dismutation of O_2^--to H_2O_2. The H_2O_2 generated can be eliminated via peroxidases, which are found throughout the cell and have high affinity for H_2O_2 [32]. CAT also dismutates H_2O_2 to oxygen and water. SOD and CAT are considered key players in the antioxidant response system as they regulate the cellular concentration of O_2^{--} and H_2O_2 [33]. These two enzymes, together with APX, GR and other, constitute the major defense system against ROS in the plant cell [4, 34-36]. In this study, activities of leaf GPX, CAT, APX and GR were higher in compost-treated wheat plants than in non-compost treated ones (Fig. 3B, C, D, E). The higher activities of such antioxidant enzymes in Co-treated plants would partly explain the lower H_2O_2 concentration in Co-treated plants, protecting the wheat plants against oxidative stress damage induced by drought, in turn enhancing drought tolerance. Similar results have been reported by many researchers in other studies under abiotic stresses[4,13,37,38]. Total SOD activity in leaves of wheat plants significantly increased as a result of Co application under DS conditions. Compost treated plants had a slight increase of SOD activity, relative to the control, under WW conditions (Fig. 3A). Increasing SOD activity has been correlated with induced resistance of plants to salinity [39] and drought stress [40-44]. In fact, Pastori and Trippi [40,41] found that drought-resistant maize strains had higher levels of antioxidant enzymes (APX, GR and SOD) than did drought-sensitive strains, allowing the plants to cope with several oxidative stresses produced by senescence or by incubating plants with paraquat and/or H_2O_2. On the other hand, the increases observed in the activities of SOD and GR in response to drought stress are probably due to the *de novo* synthesis of enzymatic proteins [42]. This indicates that the main differences in SOD activity in compost treated plants appeared when plants were subjected to drought stress.

Ascorbate and glutathione are two important antioxidants of the antioxidant defense systems, which scavenge ROS under oxidative conditions and in turn protect the biomembranes from oxidation [45-48]. ASC and GSH, which are the crucial antioxidants in ASC-GSH cycle [1, 49]. This cycle, occurring in higher plants, serves the removal of H_2O_2 and is an important antioxidant defense. Up to 30% of the photosynthetically produced electrons are dissipated by the ASC-GSH cycle under DS conditions and 2-5% under normal conditions [50]. In this study, compost markedly enhanced the ASC and GSH contents of wheat leaves under WW and DS conditions.

The higher APX activity and ASC content in compost-treated plants would result in faster removal of H_2O_2 via the ASC-GSH cycle, helping to alleviate oxidative damage. The greater GSH content of Co-treated plants was related to GR activity, because the ASC-GSH cycle closed with GR converting glutathione disulphide back into GSH. In addition, GSH occurs in the glutathione peroxidase cycle, converting H_2O_2 into water using reducing equivalents from GSH. Therefore, the higher ASC and GSH contents of leaves in compost-treated plants would help the wheat plants grown under DS in dissipating the photosynthetically produced electrons and in alleviating oxidative damage.

A correlation between the intracellular antioxidant capability to reduce ROS and drought stress tolerance has been demonstrated in a number of plant species [34,46,51]. The increase in antioxidant compounds and related enzyme activities under drought stress can help plants to better survive under environmental stress. The present results suggested that compost alleviates drought induced oxidative damage by increasing the expression of genes related to production of antioxidant molecules and antioxidant enzymes activities. The modes of compost action in protecting wheat against drought-induced oxidative stress are mainly attributed to the beneficial effects of compost application on both soil and plant. Compost components could synergistically act in affecting numerous physiological and biochemical functions, including water and mineral uptake and transport, photosynthesis, enzyme activation and osmotic potential. In addition, compost represents a good source of all macro-and micronutrients, organic matters, hormone-like substances, biotic agents, carbon dioxide, nitric oxide and many others, which play important roles in stimulating metabolic processes, promoting growth and increasing the synthesis and accumulation of more metabolites in plant tissues [6-10,52,53].

Apart from nutrients and organic matter, compost contains a substantial amount of humic substances (HS) [54]. HS are natural organic polyelectrolytes [55]. Among the different functional actions of HS, their ability to improve plant growth has been well established in diverse plant species and growth conditions [55,56]. However, the mechanism responsible for this HS biological action is poorly understood. Whereas some authors propose that HS promote plant growth by improving the soil bioavailability of certain nutrients, principally iron and zinc [55,57], others suggest that HS can also directly affect plant metabolism [58]. Based on the foregoing results, compost increased TBP under DS conditions. This indicates that compost helps the plants to cope with DS, probably maintaining its photosynthetic processes intact, or little altered in response to Co application. Compost also resulted in efficient antioxidant machinery systems allowed the Co-treated plants to cope better with oxidative stress damage induced by drought. In fact, the prevention of oxidative stress and the elimination of AOS are the most effective approaches used by plants to gain tolerance against several abiotic stresses, including drought.

In conclusion, application of compost had positive effects on reactive oxygen metabolism of wheat plants including decreasing the concentrations of ROS and increasing both enzymatic and non-enzymatic antioxidants in leaves of wheat plants grown under WW and DS conditions. The lower oxidative damage in Co-treated plants appeared due to higher activities of enzymatic-and non-enzymatic antioxidants.

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