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Roles of super absorbent polymer and ascorbic acid in mitigating deleterious effects of cadmium in wheat

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ABSTRACT

A greenhouse experiment was conducted to explore the role of ascorbic acid foliar application and soil applied super absorbent in mitigating adverse effects of cadmium in terms of some biochemical parameters in wheat. The experimental design was a completely randomized design, arranged in factorial, consisting of three levels of super absorbent polymer (0, 4 and 8 g kg^{-1} soil) and three levels of ascorbic acid (0, 50 and 100 mM) with four replicates. Cadmium contamination caused a significant increase in accumulation of cadmium in the leaves and seeds, antioxidant enzymes activity and lipid peroxidation. By contrast, cadmium contamination decreased seed weight and chlorophyll content in wheat plants. Super absorbent increased seed weight, chlorophyll and ascorbic acid content while it reduced cadmium accumulation in the leaves and seeds, antioxidant enzymes activity and lipid peroxidation. Similar results were found when ascorbic acid was applied.

KEYWORDS

Antioxidant enzymes;
Ascorbic acid;
Lipid peroxidation;
Super absorbent;
Wheat.

INTRODUCTION

Agricultural soils in many parts of the world are slightly to moderately contaminated by heavy metal toxicity such as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As. This could be due to long-term use of phosphate fertilizers, sewage sludge application, dust from smelters and industrial waste as well as bad watering practices in agricultural lands[1, 2,3]. The primary response of plants is the generation of reactive oxygen species (ROS) upon exposure to high levels of heavy metals. Various metals either generate ROS directly through Haber-Weiss reactions or overproduction of ROS and occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity[4,5]. The indirect mechanisms include their interaction with the antioxidant system[6], disrupting the electron transport chain or disturbing the metabolism of essential elements[7]. One of the most deleterious effects induced by heavy metals exposure in plants is lipid peroxidation, which can directly cause bio-membrane deterioration. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane is regarded as a reliable indicator of oxidative stress[7]. Plant cells contain an array of protection mechanisms and repair systems that can minimize the occurrence of oxidative damage caused by reac-

tive oxygen species (ROS)[8]. The induction of ROS scavenging enzymes, such as superoxide dismutase, catalase, peroxidase and ascorbate peroxidase is the most common mechanism for detoxifying ROS synthesized during stress response[9]. One of these systems is the antioxidant system, which involves antioxidant substances such as tocopherols and ascorbic acid (AsA)[10]. Ascorbate functions in coordination with glutathione and several enzymatic antioxidants to counteract the O^{2-} radicals that are produced by the Mehler reaction and photorespiration[11]. Ascorbate has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion and other developmental processes[12]. The regulatory limit of cadmium in agricultural soil is 100 mg kg-1 soil[13]. But this threshold is continuously exceeding because of several human activities. Plants exposed to high levels of cadmium causes reduction in photosynthesis, water uptake, and nutrient uptake. Plants grown in soil containing high levels of cadmium show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips, and finally death[14, 15]. When soils are contaminated with heavy metals, the clean-up is one of the most difficult tasks for environmental engineering. For remediating sites contaminated with inorganic pollutants, several techniques have been developed. Super absorbent polymer application positively influence crop production,improve soil physical propertiesand can be used to reduce heavy metal hazards in plants. Applying sorbents, including zeolite and super absorbent, should result in immobilizing heavy metals and restoring the ionic balance and ratio of nutrients within a soil environment[16,17,18,19, 20].

The aim of this study was to determine cadmium distribution in leaves and seeds of wheat and to understand if soil applied super absorbent and ascorbic acid foliar application could be a strategy for immobilizing cadmium and reducing cadmium deleterious effects in whet.

MATERIALS & METHODS

The experiment was conducted in glasshouse of Agriculture Faculty, Islamic Azad University Varamin, Iran in 2014. The experimental design was a completely randomized design arranged in factorial structure with four replicates. Treatments included three levels of super absorbent polymer (0, 4 and 8 g kg^{-1} soil) and three levels of ascorbic acid foliar application (vitamin C) (0, 50 and 100 mM). Ten seeds (*Triticumaestivum* L. c.vPishtaz) were sown in 30 ×30 cm plastic pots filled with free draining peat-vermiculite (2: 1 volume ratio), contaminated with $CdCl_2$ (80 mg kg^{-1} soil) and mixed with certain amounts of super absorbent polymer (0, 4 and 8 g kg^{-1} soil). The pots were placed in a glasshouse equipped with cool white fluorescent lamps. Room air temperature was 22/20Ú C during the light/dark photoperiod. Photosynthetically active radiation (PAR) at the top of the canopy was 400 µmol m^{-2} s^{-1} while maintaining a 16/8 h day/night photoperiod. Relative humidity in the glasshouse was 70%. The plants were hand watered daily until saturated with freshly prepared nutrient solution (Full strange Hoagland pH 6). Ascorbic acid foliar application was performed twice at stem elongation and booting stages, using a manually operated hand sprayers. Distilled water was used as control. At seed filling stage leaf samples were collected from the same leaves and immediately frozen in liquid nitrogen and stored at -80°C.

Seed yield

At maturity stage, plants were harvested at the soil surface and seeds were collected and weighted. Seed yield per pot was determined. Above ground parts were dried for 48 h at 85Ú C in a laboratory oven.

Cadmium content

Leaves and seed samples were separately digested by HNO_3 and $HCIO_4$ in tubes placed on an A1 block brought gradually to 205°C. Cadmium was determined by atomic absorption spectrophotometry, using ICP-AES atomic absorption spectrophotometer (Inductively coupled plasma atomic emission spectroscopy, SPS 1200VR, Seiko, Japan).

Chlorophyll assay

Chlorophyll was extracted in 80% acetone from the leaf samples according to Arnon[21]. Extracts were

filtrated and total chlorophyll content was determined by spectrophotometer at 645 and 663 nm. The content of chlorophyll was expressed as mg g-1 fresh weight.

Antioxidant enzyme activity assay

Catalase activity was estimated by the method of Cakmak and Horst[22]. The reaction mixture contained 100 µl crude extract, 500 µl 10mm H_2O_2 and 1400 µl 25mm sodium phosphate buffer. The decrease in the absorbance recorded at 240 nm for 1 min by a spectrophotometer. Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitrobluetetrazolium according to the method of Giannopolitis and Ries[23]. The reaction mixture contained 100 µl l µM riboflavin, 100 µl 12 mM L-methionine, 100 µl 0.1 mM EDTA (pH 7.8), 100 µl 50 mM Na2CO_3 (pH 10.2), 100 µl 75 µM nitrobluetetrazolium in 2300 nitrobluetetrazolium 25mM sodium phosphate buffer (pH 6.8) and 200 µl crude enzyme extract, in a final volume of 3 ml. Glass test tubes that contained the reaction mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. One unit of Superoxide dismutase activity was defined as the amount of enzyme which caused 50 % inhibition of photochemical reduction of nitrobluetetrazolium.

Ascorbic acid assay

Ascorbic acid was extracted from 2 g shoot fresh material by 4% oxalic, then made up to known volume (100 mL) and centrifuged at 2000 rpm for 5 min. then added 10 10 mL of 4% oxalic acid then titrate using 2,6-dichlorphenol-indophenol as described by Sadasivam and Manickamm[24].

Lipid peroxidation assay

The level of membrane damage was determined by measuring MDA as the end product of peroxidation of membrane lipids[25]. In brief, samples were homogenized in an aqueous solution of trichloroacetic acid (10% w/v), and aliquots of filtrates were heated in 0.25% trichloroacetic acid. The amount of MDA was determined from the absorbance at 532 nm, followed by correction for the non-specific absorbance at 600 nm. The content of MDA was determined using the extinction coefficient of MDA ($\varepsilon = 155\ \mu M^{-1}\ cm^{-1}$).

Statistical analysis

All data were analyzed from analysis of variance (ANOVA) using the GLM procedure in SAS[26]. The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. Duncan's multiple range tests was used to measure statistical differences between treatment methods and controls.

RESULT AND DISCUSSION

Analysis of variance revealed that the main effects of super absorbent polymer and ascorbic acid foliar application were significant on all measured traits (TABLE 1). However the interaction between super absorbent polymer and ascorbic acid was significant on none of the traits. As can be seen from

TABLE 1 : Analysis of variance on wheat attributes affected by super absorbent polymer and ascorbic acid foliar application exposed to cadmium stress*, ** and ns indicate significant at 5%, significant at 1% and no significant, respectively

Sources of variation	df	Seed yield	Grain weight	Seed cadmium	Leaves cadmium	Total chlorophyll	Superoxide dismutase	Catalase	Ascorbic acid	Malondialdehyde
Replication	3	ns	ns	ns	ns	ns	ns	ns	ns	ns
Super absorbent polymer	2	**	**	**	**	**	**	**	**	**
Ascorbic acid	2	**	**	**	**	**	**	**	**	*
Interaction	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
C.V		2.56	1.31	1.42	4.40	1.38	2.43	6.58	6.84	1.17

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TABLE 2 : Comparison of main means wheat attributes affected by super absorbent polymer and ascorbic acid foliar application exposed to cadmium stress

Treatments	Seed yield (g per pot)	100-Seed weight (g)	Seed Cd (mg kg^{-1})	Leaf Cd (mg kg^{-1})	Chlorophyll (mg.lit^{-1})	SOD (ΔA/mg pro min^{-1})	CAT (ΔA/mg pro min^{-1})	Ascorbic acid (mg g^{-1}FW)	Malondialdehyde (nmol g^{-1} FW)
Super absorbent									
0 g.kg^{-1} soil	102.15c	17.19c	4.64a	37.35a	21.09c	817.95a	232.77a	0.294c	13.98a
4 g.kg^{-1} soil	112.65b	19.09b	3.75b	29.52b	24.25b	733.38b	177.81b	0.376b	11.13b
8 g.kg^{-1} soil	125.32a	20.51a	3.11c	24.55c	26.99a	657.17c	128.04c	0.489a	7.86c
Ascorbic acid (mM)									
0	110.23c	16.47c	4.01a	30.68a	22.70c	765.40a	198.87a	0.333c	11.45a
50	114.23b	15.94b	3.83b	30.55a	24.01b	747.80b	188.6b	0.386b	11.09b
100	124.15a	19.38a	3.67c	30.20b	25.69a	695.30c	151.09c	0.440a	10.44c

Treatment means followed by the same letter within each common are not significantly different (p <0.05) according to duncan's multiple range test

TABLE 2, the lowest seed weight was obtained when no super absorbent polymer was applied, while the highest seed weight was related to those plants which were treated with 8 g per kg soil super absorbent polymer. Plants exposed to high levels of cadmium causes reduction in photosynthesis, water uptake, nutrient uptake and seed weight. Plants grown in soil containing high levels of cadmium show visible symptoms of injury reflected in terms of chlorosis, growth inhibition and browning of root tips[14, 15]. On the other hand, super absorbent polymer application improves soil physical propertiesand can be used to reduce heavy metal hazards in plants. Applying sorbents, including zeolite and super absorbent, should result in immobilizing heavy metals[16,17,18,19,20].

The primitive effect of ascorbic acid on stem and root length may be the result of increasing of cell division in apical meristem or increased cell division and cell enlargement[27] due to water uptake caused by a decrease in the osmotic potential by increasing soluble sugars. The effect of ascorbic acid on dry mater may be due to an increase leaf area, stabilizing the enzymes involved in amino acid metabolism[28], increased potassium content in plant material which leads to an increase in leaf area, enhanced the production of photosynthesis and their subsequent translocation resulting in dry mater accumulation. In addition, the result demonstrated that the highest cadmium content in the leaves and seeds was observed when wheat plants not treated with super absorbent polymer, while the lowest cadmium content was related to those plants which were treated with super absorbent polymer (TABLE 2). Super absorbent polymer application improves soil physical propertiesand can be used to reduce cadmium in the leaves or seeds. Applying sorbents, including zeolite and super absorbent, should result in immobilizing heavy metals and restoring the ionic balance and ratio of nutrients within a soil environment[16, 17, 18, 19, 20]. Moreover, the result showed that the highest cadmium content in the leaves and seeds was obtained when no ascorbic acid was applied on the plants. In addition, the result indicated that the lowest cadmium content in the leaves and seeds was related to those plants which were sprayed with ascorbic acid at 100 mM (TABLE 2). Zhao and Mo[29] exposed garlic in cadmium solution and showed that ascorbic acid could reduce the toxicity of cadmium to the root tips and shoot plants. It has been reported that ascorbic acid plays a role in cadmium detoxification in plants[30]. As can be seen from TABLE 2, the lowest chlorophyll content was found in plants which were not treated with super absorbent polymer, while the highest chlorophyll content was related to those plants which were treated with super absorbent polymer. It has been confirmed that heavy metals affect PSI and PSII function[31]. The chlorophyll proteins, which took protons for photosynthesis in PSII, were decomposed and decreased under cadmium stress[32]. When soil is contaminated with heavy metals this leads to an increase in free radi-

cals in chloroplasts and destruction of chlorophyll molecules by ROS, which results in reduction of photosynthesis and growth. In a study, Ouzounidou[33] has reported that chlorophyll synthesis can significantly be reduced in plants when cultivated in soils contaminated by heavy metals. Super absorbent polymer application can be used to reduce cadmium absorption by wheat plants. Furthermore, the result showed that the highest chlorophyll was related to those plants which were treated with 100 mM ascorbic acid (TABLE 2). Failure of chlorophyll degradation can increase the amount of ROS produced to an extent where the detoxification capacity of the antioxidant systems may be overridden. Inhibition of chlorophyll biosynthesis has been reported in plants under metal and salt stress[34]. Ascorbic acid is a detoxifier and neutralizer of superoxide radicals and other singlet oxygen species; by prevention of the activity of free radicals it can enhance the chlorophyll content. According to the results wheat plants grown in cadmium contaminated soil without super absorbent polymer treatment showed a significant increase in SOD and CAT activity in the leaves (TABLE 2). The primary response of plants to heavy metal stress is the generation of reactive oxygen species (ROS) upon exposure to high levels of heavy metals. Various metals either generate ROS directly through Haber-Weiss reactions or overproduction of ROS and occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity[4, 5]. The indirect mechanisms include their interaction with the antioxidant system[6]. The enzymes assayed are scavengers of free radical species. Superoxide dismutase converts one form of ROS (O^{2-}) to another equally toxic one (H_2O_2). Hydrogen peroxide is converted to oxygen and water by catalase and peroxidase, which use ascorbate as the hydrogen donor[35]. The activities of superoxide dismutase and catalase are usually enhanced by heavy metal stress such as Cd, Hg, Ni, Pb and Fe[36,32,37]. Application of ascorbic acid decreased superoxide dismutase and catalase activity in plants (TABLE 2). This might be due to elimination of free radicals by ascorbic acid. Ascorbic acid has been found to be loaded in the phloem of source leaves and is then transported to other tissues[38]. When ascorbic acid was applied to the leaves of plants in our study, there was an obvious decrease in superoxide dismutase and catalase activities in the leaves. In addition, the result showed that the lowest ascorbic acid content in leaves was obtained when wheat plants were not treated with super absorbent polymer, while the highest ascorbic acid content in leaves was related to those plants which were treated with super absorbent polymer. Super absorbent polymer can be used to reduce cadmium uptake and diminish reactive oxygen species generation in wheat plants. The result also showed that the highest ascorbic acid content in leaves was related to those plants which were treated with 100 mM ascorbic acid (TABLE 2). Ascorbic acid plays a protective role against ROS which are formed during biotic and abiotic stresses[11]. Ascorbate is oxidized by oxygen free radicals and dehydroascorbate is generated[11]. This leads to a decline in antioxidant activities, which is followed by an increase in oxidative damage. One of the most deleterious effects induced by heavy metals exposure in plants is lipid peroxidation, which can directly cause bio membrane deterioration. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane is regarded as a reliable indicator of oxidative stress[7]. The highest level of MDA was observed when wheat plants were not treated with super absorbent polymer, while the lowest MDA in the leaves was related to those plants which were treated with super absorbent polymer. Super absorbent polymer application can be used to reduce cadmium uptake and diminish reactive oxygen species generation in wheat. It was observed that 100 mM ascorbic acid decreased the MDA content in the leaves compared with untreated plants (TABLE 2). One of the best known toxic effects of ROS is damage to cellular membranes and lipids. Plasma membranes are oxidized by ROS to generate MDA. Exogenous ascorbic acid partially inhibits these increases because ascorbic acid is a scavenger of ROS[11]. Zhang and Kirkham[39] reported similar inhibitory effects of exogenous ascorbic acid on lipid peroxidation in sunflower seedlings exposed to osmotic stress.

CONCLUSION

In conclusion, super absorbent polymer positively

influenced wheat responses to cadmium contamination, thus can be used to reduce cadmium hazards in contaminated area. Applying super absorbent should result in immobilizing heavy metals and restoring the ionic balance and ratio of nutrients within a soil environment. In addition, this study has shown that ascorbic acid foliar application can increase the survival capacity of wheat plants under cadmium stress. The increase in resistance to cadmium stress is associated with the antioxidant activity of ascorbic acid, a partial inhibition of cadmium stress induced increases in lipid peroxidation by ROS, and a decrease in antioxidant activity. According to the results it can be suggested that application of ascorbic acid can reduce the harmful effects of ROS and improves wheat resistance to cadmium contamination.

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