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Application of super absorbent polymer and ascorbic acid to mitigate deleterious effects of cadmium in wheat[1]

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ABSTRACT

The growing use of chemical fertilizers, insecticides and pesticides can cause potential contamination with heavy metals to soil and groundwater, posing environmental and health threats. Heavy metals can also affect crop yield. A greenhouse experiment was conducted to explore the role of ascorbic acid foliar application and soil-applied super absorbent to mitigate adverse effects of cadmium (Cd), in terms of biochemical parameters in wheat. The experiment was installed in a completely randomized design, with treatments arranged in a factorial scheme with three levels of super absorbent polymer (0 g kg^{-1}, 4 g kg^{-1} and 8 g kg^{-1} of soil) by three levels of ascorbic acid (0 mM, 50 mM and 100 mM), with four replicates. The Cd contamination caused a significant increase in the accumulation of Cd in leaves and seeds, as well as in antioxidant enzymes activity and lipid peroxidation. It also decreased seed weight and chlorophyll content in wheat plants. The super absorbent increased seed yield (22.68 %), seed weight (19.31 %), chlorophyll (27.97 %) and ascorbic acid content (65.51 %), while it reduced the Cd accumulation in leaves (34.27 %) and seeds (32.97 %), as well as antioxidant enzymes activity and lipid peroxidation (43.77 %). Similar results were found when ascorbic acid was applied. Ascorbic acid increased seed yield, seed weight and chlorophyll content by 12.62 %, 17.66 % and 13.17 %, respectively. As a result, the super absorbent polymer and ascorbic acid could improve the survival capacity and yield of wheat plants in response to Cd contamination in the soil.

KEY-WORDS: Antioxidant enzymes; heavy metals contamination; lipid peroxidation.

RESUMO

Uso de polímero superabsorvente e ácido ascórbico para mitigar os efeitos deletérios de cádmio em trigo

O uso crescente de fertilizantes químicos, inseticidas e pesticidas apresenta elevado potencial de contaminação do solo e de lençóis freáticos com metais pesados, constituindo-se em ameaça ao meio ambiente e à saúde. Metais pesados podem afetar a produtividade das culturas. Um experimento em casa-de-vegetação foi efetuado para avaliar o papel da aplicação foliar de ácido ascórbico e da utilização de um polímero superabsorvente para proteção do solo, para mitigar os efeitos adversos de cádmio (Cd) em parâmetros bioquímicos, na cultura do trigo. O delineamento experimental utilizado foi o inteiramente casualizado, em arranjo fatorial consistindo de três níveis de polímero superabsorvente (0 g kg^{-1}, 4 g kg^{-1} e 8 g kg^{-1} de solo) e três níveis de ácido ascórbico (0 mM, 50 mM e 100 mM), com quatro repetições. A contaminação com Cd resultou em significativo acúmulo de Cd em folhas e grãos, além de elevar a atividade de enzimas antioxidantes e a peroxidação lipídica. Também reduziu o peso de grãos e o teor de clorofila nas plantas de trigo. O polímero superabsorvente aumentou o rendimento de grãos (22,68 %), peso de grãos (19,31 %), clorofila (27,97 %) e teor de ácido ascórbico (65,51 %), enquanto reduziu o acúmulo de Cd nas folhas (34,27 %) e grãos (32,97 %), além de diminuir a atividade de enzimas antioxidantes e a peroxidação lipídica (43,77 %). Resultados semelhantes foram encontrados quando o ácido ascórbico foi aplicado. O ácido ascórbico aumentou o rendimento e peso de grãos e o teor de clorofila em 12,62 %, 17,66 % e 13,17 %, respectivamente. Como resultado, o polímero superabsorvente e o ácido ascórbico podem melhorar a capacidade de sobrevivência e a produtividade de plantas de trigo, em resposta à contaminação de Cd no solo.

PALAVRAS-CHAVE: Enzimas antioxidantes; contaminação por metais pesados; peroxidação lipídica.

INTRODUCTION

Agricultural soils in many parts of the world are slightly to moderately contaminated by heavy metals such as cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), chrome (Cr), lead (Pb), and arsenic (As) (Yadav 2010). This could be due to long-term use of chemical fertilizers, sewage sludge application, dust from smelters and industrial waste, as well as bad watering practices in agricultural lands (Bell et al. 2001, Schwartz et al. 2001, Passariello et al. 2002).

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Like other environmental stresses, the primary response of plants to high levels of heavy metals is the generation of reactive oxygen species (ROS). Various metals generate ROS directly through Haber-Weiss reactions or the overproduction of ROS could be an indirect consequence of heavy metal toxicity (Wojtaszek 1997, Mithofer et al. 2004). The indirect mechanisms include their interaction with the antioxidant system (Srivastava et al. 2004), disrupting the electron transport chain (Qadir et al. 2004) or disturbing the metabolism of essential elements (Dong et al. 2006). One of the most deleterious effects induced by heavy metals in plants is lipid peroxidation, which can directly cause bio-membrane deterioration. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membranes, is regarded as a reliable indicator of oxidative stress (Demiral & Turkan 2005).

Plant cells contain an array of protection mechanisms and repair systems that can minimize the occurrence of oxidative damage caused by ROS (Latef 2010). 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 (Gressel & Galun 1994). One of these systems is the antioxidant system, which involves antioxidant substances such as tocopherols and ascorbic acid (Foyer et al. 1994).

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 (Noctor & Foyer 1998). Ascorbate has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion and other developmental processes (Pignocchi & Foyer 2003).

The regulatory limit of Cd in agricultural soil is 100 mg kg^{-1} of soil (Salt et al. 1995). But this threshold has continuously being exceeded because of several human activities. The exposure of plants to high levels of Cd causes reduction in photosynthesis, as well as water and nutrient uptake. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death (Wojcik & Tukiendorf 2004, Mohanpuria et al. 2007). Remediation of soils contaminated with heavy metals is one of the most difficult tasks for environmental engineers. For remediating sites contaminated with inorganic pollutants, several techniques have been developed.

The super absorbent polymer application positively influences crop production, improves soil physical properties and can be used to reduce heavy metal hazards in plants growing in soils contaminated with heavy metals (Prasad & Freitas 1999). The application of sorbents, such as zeolite and super absorbents, immobilize heavy metals and restore the ionic balance and ratio of nutrients in the soil (Kinraide 2007, Kozlowskak & Badora 2007, Gambus & Rak 2005, Zielazinska & Wyszkowska 2005, Pyrzynska 2007).

Super absorbent polymers hold a large amount of polyfunctional groups (amino and imino groups) that can effectively adsorb heavy metal ions (Huang et al. 2011). In such polymers, chelating functionalities are present in the polymeric side chains or are embedded in the backbone. The choice of the type of ligand, ligand density, structure and solubility of the polymer, as well as pH, governs the metal ion affinity, retention efficiency and selectivity. The number of surface adsorbing groups limits the sorption process. In the case of super absorbent polymers, metal ions can easily enter the polymeric network and, hence, these polymers are expected to exhibit a higher sorption capacity (Roy et al. 2011).

Wheat is the first major and staple food crop in the world (Shaimaa et al. 2012). The crop has high potential to accumulate Cd (Bose & Bhattacharyya 2008). Even small increases in the Cd content of grains could have long-lasting and widespread harmful impacts on the well-being of consumers (Singh et al. 2010). The Cd accumulation in wheat is a consequence of selective Cd uptake and high Cd bioavailability in soil, which usually results from anthropogenic activities, such as mining, smelting and atmospheric deposition (Wang et al. 2010).

Ascorbic acid is one of the most effective compounds to improve the plants tolerance to oxidative stresses (Noctor & Foyer 1998). A wealth of information suggests that ascorbic acid plays a significant role in protecting plants against several environmental stresses (Noctor & Foyer 1998, Ullah et al. 2016).

The effects of exogenous application of ascorbic acid on carbohydrates, proteins, proline, other free amino acids, glycolipids, phospholipids, sterols, total lipids and cell wall fractions have not been examined in plants under heavy metal stress. Therefore, the aim of this study was to determine Cd distribution in leaves and seeds of wheat grown under contaminated soil, and also to understand if soil-applied super absorbent and ascorbic acid foliar application could be a strategy for immobilizing Cd, thus reducing its deleterious effects in wheat.

MATERIAL AND METHODS

The experiment was conducted in a glasshouse of the Agriculture Faculty of the Islamic Azad University Varamin, Iran, in 2014. It was installed in a completely randomized design with treatments arranged in a 3 × 3 factorial scheme, with four replicates. Treatments included three levels of super absorbent polymer (0 g kg^{-1}, 4 g kg^{-1} and 8 g kg^{-1} of soil) by three levels of ascorbic acid foliar application (vitamin C) (0 mM, 50 mM and 100 mM).

Ten seeds of wheat (*Triticum aestivum* L. c.v Pishtaz) were sown in 30 cm × 30 cm plastic pots filled with free draining peat-vermiculite (2:1 volume ratio). The $CdCl_2$ (80 mg kg^{-1} of soil) was mixed into the soil prior to potting. In addition, the different concentrations of super absorbent polymer (0 g kg^{-1}, 4 g kg^{-1} and 8 g kg^{-1} of soil) were incorporated into the soil at the same time. The pots were placed in a glasshouse equipped with cool white fluorescent lamps. Room air temperature was 22/20 ºC, during the 16/8 h light/dark photoperiod. Photosynthetically active radiation (PAR) at the top of the canopy was 400 µmol m^{-2} s^{-1}, during the light photoperiod. Relative humidity in the glasshouse was 70 %. Plants were hand watered daily until saturated with freshly prepared nutrient solution (100 % Hoagland solution, pH 6). Ascorbic acid foliar application was performed twice at stem elongation and booting stages, using a manually operated hand sprayer. Distilled water was used as a control. At the seed filling stage, flag leaves were collected and immediately frozen in liquid nitrogen and stored at -80 ºC until laboratory analyses. At the maturity stage, plants were harvested at the soil surface and seeds were collected and weighted. Seed yield per pot was determined.

All leaves were dried for 48 h, at 85 ºC, in a laboratory oven, for determining cadmium contents. Leaves and seed samples were separately digested by HNO_3 and $HClO_4$ in tubes placed on an A1 block brought gradually to 205 ºC. Cd was determined by atomic absorption spectrophotometry, using an ICP-AES atomic absorption spectrophotometer (Inductively Coupled Plasma Atomic Emission Spectroscopy, SPS 1200VR, Seiko, Japan).

Chlorophyll was extracted in 80 % acetone from the leaf samples (Arnon 1949). Extracts were filtrated and total chlorophyll content was determined by spectrophotometer at 645 nm and 663 nm. The content of chlorophyll was expressed as mg g^{-1} of fresh weight.

Catalase activity was estimated by the Cakmak & Horst (1991) method. The reaction mixture contained 100 µl of crude extract, 500 µl of 10 mM H_2O_2 and 1,400 µl of 25 mM sodium phosphate buffer. Catalase activity was estimated by recording the absorbance reduction at 240 nm, for 1 min, using a spectrophotometer. Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium (Giannopolitis & Ries 1977). The reaction mixture contained 100 µl of 1 µM riboflavin, 100 µl of 12 mM L-methionine, 100 µl of 0.1 mM EDTA (pH 7.8), 100 µl of 50 mM Na_2CO_3 (pH 10.2), 100 µl of 75 µM nitroblue tetrazolium, 2,300 µl of 25 mM sodium phosphate buffer (pH 6.8) and 200 µl of 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 that caused 50 % inhibition of photochemical reduction of nitroblue tetrazolium.

Ascorbic acid was extracted from 2 g shoot fresh material by 4 % oxalic acid in a final volume of 100 ml. The tube was centrifuged at 2,000 rpm for 5 min and, afterwards, 100 ml of 4 % oxalic acid was added. The solution was, then, titrated using 2,6-dichlorphenol-indophenol (Sadasivam & Manickamm 1996).

The level of membrane damage was determined by measuring MDA as the end product of peroxidation of membrane lipids (De Vos et al. 1991). 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 to 100 ºC, for 30 min. 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 ($\varepsilon = 155\ \mu M^{-1}\ cm^{-1}$).

All data were analyzed from analysis of variance (Anova) using the GLM procedure in SAS (SAS Institute 2002). The assumptions of the variance analyses were tested by checking if the residuals were random, homogenous, with a normal distribution and a mean of about zero. Linear regression analyses were performed and plotted in graphs to show the dispersion of the data along the regression line.

RESULTS AND DISCUSSION

The main effects of super absorbent polymer and ascorbic acid foliar application were significant for all measured traits (Table 1). However, the interaction between super absorbent polymer and ascorbic acid was not significant for any of the evaluated traits.

The maximum seed yield was obtained when 8 g kg^{-1} of super absorbent or 100 mM of ascorbic acid were applied (Table 2, Figures 1 and 2). The lowest seed weight was observed when no super absorbent polymer was applied, while the highest seed weight was obtained by treating the soil with 8 g of super absorbent polymer per kg (Table 2, Figure 1). Increased water and nutrients absorption may explain the increased seed weight after the application of super absorbent polymer.

Similar results were reported by Ullah et al. (2016). Plants exposed to high levels of Cd showed reduced photosynthesis, water uptake, nutrient uptake and seed weight. Plants grown in Cd contaminated soil also showed visible symptoms of injury reflected in terms of chlorosis, growth inhibition and browning of root tips (Wojcik & Tukiendorf 2004, Mohanpuria et al. 2007). On the other hand, the super absorbent polymer application improves soil chemical and physical properties (Bai et al. 2010), as it reduces heavy metal hazards in plants. It has been reported that the application of sorbents, such as zeolite and super absorbent, immobilizes heavy metals (Gambus & Rak 2005, Zielazinska & Wyszkowska 2005, Kinraide 2007, Kozlowskak & Badora 2007, Pyrzynska 2007).

Ascorbic acid plays a role in Cd detoxification in plants (Huang et al. 2000). The effect of ascorbic

Table 1. Analysis of variance for wheat attributes affected by super absorbent polymer and ascorbic acid foliar application exposed to cadmium (Cd) stress (Varamin, Iran, 2014).

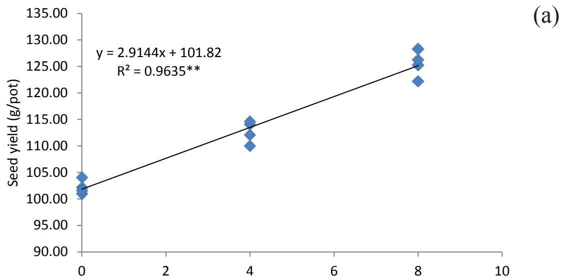
Sources of variation	df	Seed yield	Grain weight	Seed Cd	Leaf Cd	Total chlorophyll	Superoxide dismutase	Catalase	Ascorbic acid	Malondialdehyde
Super absorbent polymer	2	**	**	**	**	**	**	**	**	**
Ascorbic acid	2	**	**	**	**	**	**	**	**	*
Interaction	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)		3.44	5.24	4.22	6.63	4.19	5.23	8.27	8.16	4.21

*, ** and ns: significant at 5 %, 1 % and not significant, respectively.

Table 2. Comparison of means of some wheat traits affected by super absorbent polymer and ascorbic acid foliar application exposed to cadmium (Cd) stress (Varamin, Iran, 2014).

Treatments	Seed yield	100-seed weight	Seed Cd	Leaf Cd	Chlorophyll	SOD	CAT	Ascorbic acid	Malondialdehyde
	g pot^{-1}	g	mg kg^{-1}		mg lit^{-1}	ΔA/mg pro min^{-1}		mg g^{-1} FW	nmol g^{-1} FW
Super absorbent									
0 g kg^{-1} of soil	102.15 c	17.19 c	4.64 a	37.35 a	21.09 c	817.95 a	232.77 a	0.294 c	13.98 a
4 g kg^{-1} of soil	112.65 b	19.09 b	3.75 b	29.52 b	24.25 b	733.38 b	177.81 b	0.376 b	11.13 b
8 g kg^{-1} of soil	125.32 a	20.51 a	3.11 c	24.55 c	26.99 a	657.17 c	128.04 c	0.489 a	7.86 c
Ascorbic acid (mM)									
0	110.23 c	16.47 c	4.01 a	30.68 a	22.70 c	765.40 a	198.87 a	0.333 c	11.45 a
50	114.23 b	15.94 b	3.83 b	30.55 a	24.01 b	747.80 b	188.60 b	0.386 b	11.09 b
100	124.15 a	19.38 a	3.67 c	30.20 b	25.69 a	695.30 c	151.09 c	0.440 a	10.44 c

Treatment means followed by the same letter within each column were not significantly different ($p < 0.05$), according to the Duncan's Multiple Range test. SOD: superoxide dismutase activity; CAT: catalase activity.



acid on stem and root length may be the result of an increasing cell division in the apical meristem or of increased cell size (Arteca 1996) due to water uptake caused by a decrease in the osmotic potential by increasing soluble sugars. In addition, the results demonstrated that the highest Cd contents in leaves and seeds were observed on wheat plants not treated with super absorbent polymer.

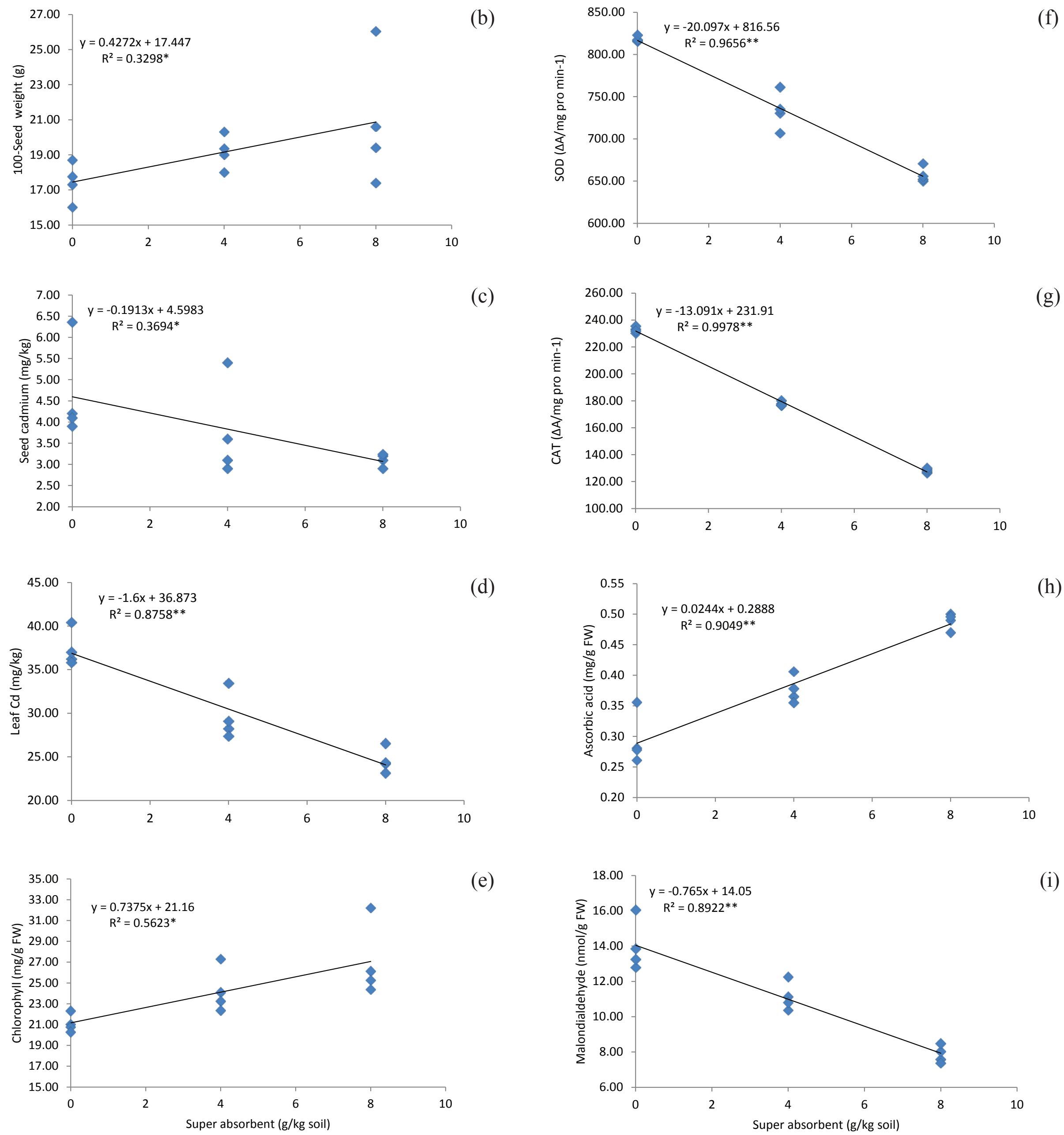
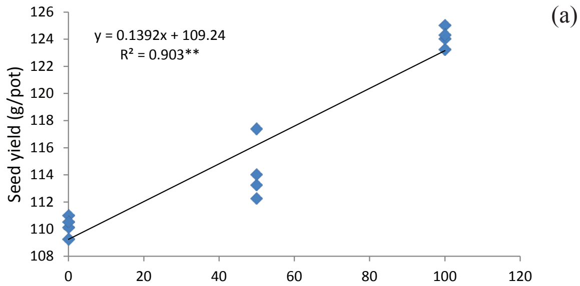


Figure 1. Linear regression demonstrating the relationship among super absorbent polymer concentrations with the following wheat traits: seed yield (a), 100-seeds weight (b), seed cadmium content (c), leaf cadmium content (d), chlorophyll content (e), superoxide dismutase (SOD) activity (f), catalase (CAT) activity (g), ascorbic acid content (h) and malondialdehyde content (i) (Varamin, Iran, 2014).

On the other hand, the lowest Cd content was observed in plants treated with super absorbent polymer (Table 2, Figure 1). The super absorbent polymer application improves soil physical and chemical properties (Bai et al. 2010), and can be used to reduce the Cd concentration in leaves and seeds. Moreover, our results showed that the highest



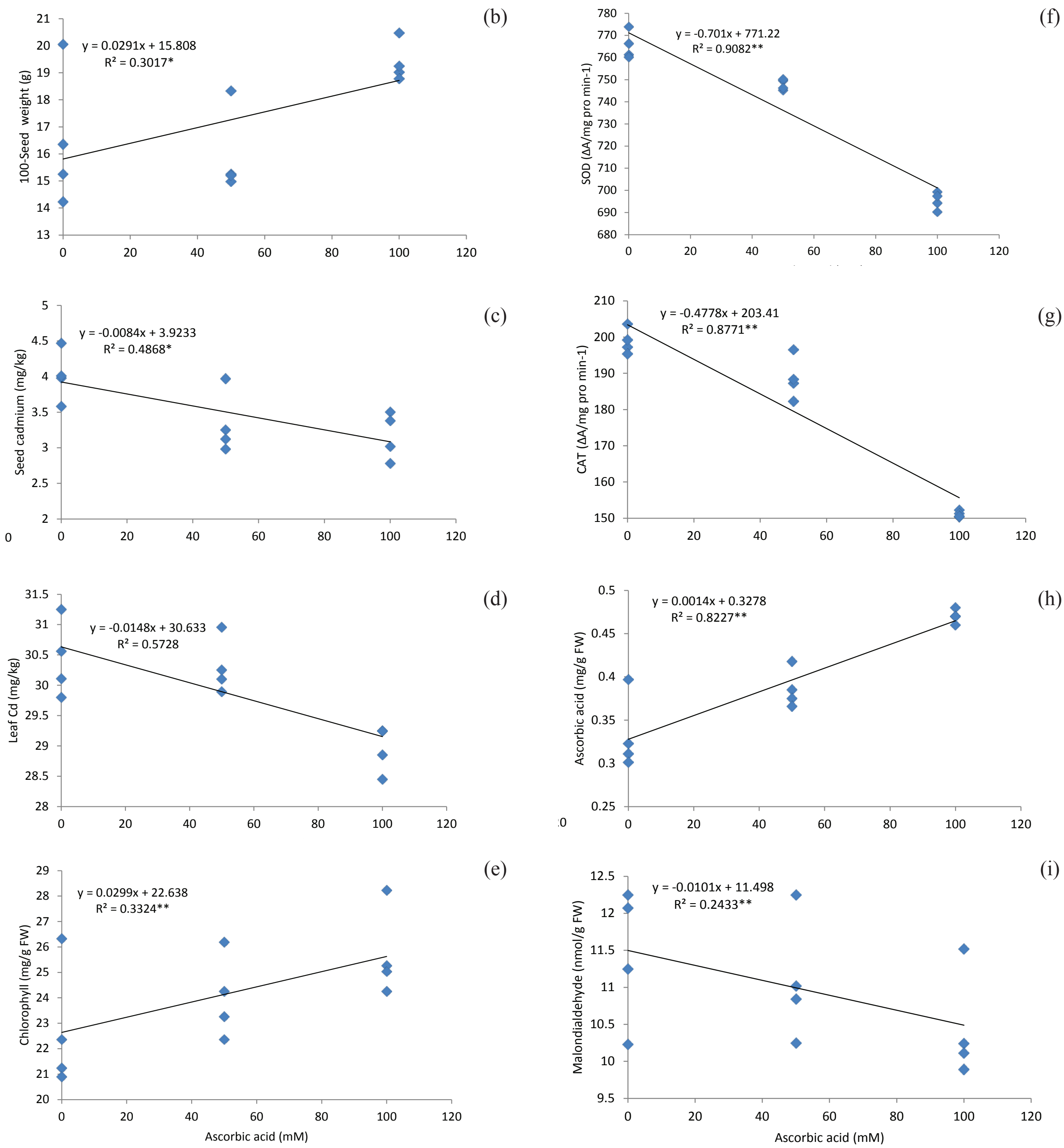


Figure 2. Linear regression demonstrating the relationship among ascorbic acid concentrations with the following wheat traits: seed yield (a), 100-seeds weight (b), seed cadmium content (c), leaf cadmium content (d), chlorophyll content (e), superoxide dismutase (SOD) activity (f), catalase (CAT) activity (g), ascorbic acid content (h) and malondialdehyde content (i) (Varamin, Iran, 2014).

Cd content in the leaves and seeds was obtained when no ascorbic acid was applied to the plants. Conversely, the lowest Cd content in leaves and seeds was obtained in plants sprayed with ascorbic acid at 100 mM (Table 2, Figure 2). Similar results were reported by Zhao & Mo (1997), who exposed garlic plants to a Cd solution and showed that ascorbic acid could reduce the toxicity of Cd to the root tips and plant shoots.

The lowest chlorophyll content was found in plants which were not treated with super absorbent polymer, while the highest chlorophyll content was observed in plants treated with super absorbent polymer (Table 2, Figure 1). It has been confirmed that heavy metals affect PSI and PSII functions (Yang et al. 1989). It has been shown that chlorophyll proteins, which transfer protons for photosynthesis in PSII, were decomposed and decreased under Cd stress (Peng & Wang 1991). When soil is contaminated with heavy metals, this leads to an increase in free radicals in chloroplasts, which destroys chlorophyll molecules by ROS, reducing photosynthesis and growth.

Ouzounidou (1995) concluded that the chlorophyll synthesis can be significantly reduced in plants cultivated in soils contaminated by heavy metals. Furthermore, it has been reported that the super absorbent polymer application in sunflower grown under drought stress conditions increased the chlorophyll content (Nazarli et al. 2010). Thus, the super absorbent polymer application can be used to reduce the Cd absorption by wheat plants.

Furthermore, our results showed that the highest chlorophyll content was observed in plants treated with 100 mM of ascorbic acid (Table 2, Figure 2). The chloroplast is probably the ascorbate richest site of plants, with concentrations in the chloroplast matrix reaching up to 50 mM (Halliwell 1987).

Ascorbic acid application to leaves has been reported to increase the concentration of ascorbic acid in plants (Mozafar & Oertli 1993). Therefore, the ascorbic acid content in the chloroplasts could be increased by exogenous ascorbic acid application, which in turn could result in protection of the chloroplast membrane integrity and chlorophyll, especially under environmental stresses. Inhibition of chlorophyll biosynthesis has been reported in plants under metal stress (Sinha et al. 2003). Ascorbic acid is a detoxifier and neutralizer of superoxide radicals and other singlet oxygen species. Ascorbic acid prevents the activity of free radicals, reducing chlorophyll degradation and, therefore, increasing chlorophyll content.

According to our results, wheat plants grown in Cd contaminated soil without super absorbent polymer treatment showed a significant increase in superoxide dismutase (SOD) and catalase (CAT) activity in the leaves (Table 2, Figure 1). This result probably reflects the fact that super absorbent reduces defense mechanisms against reactive oxygen species (ROS). SOD and CAT activity are higher because these enzymes participate in the defense mechanism of plants against oxidative stress. Other researchers have reported an increase in SOD and CAT activities under heavy metal stress, such as Cd, Hg, Ni, Pb and Fe (Ma 2000, Pang et al. 2001, Yang et al. 2001).

The primary response of plants to heavy metal stress is the generation of ROS upon exposure to high levels of heavy metals. Various metals either generate ROS directly through Haber-Weiss reactions or indirectly as a consequence of heavy metal toxicity (Wojtaszek 1997, Mithofer et al. 2004). Similar results were found by Sharma & Dubey (2005), who reported a significant reduction in antioxidant enzymes (CAT and SOD) activity in barley seedlings by using super absorbent.

The application of ascorbic acid decreased superoxide dismutase and catalase activity in plants (Table 2, Figure 2). This might be due to the reduction of free radicals caused by ascorbic acid. Ascorbic acid is oxidized to dehydroascorbate by oxygen free radicals, reducing the amount of ROS (Noctor & Foyer 1998). Ascorbic acid has been found to be concentrated in the phloem of source leaves, from where it is then transported to other tissues (Tedone et al. 2004). When ascorbic acid was applied to the leaves of plants in our study, there was a significant decrease in superoxide dismutase and catalase activities in the leaves.

In addition, 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 observed in plants treated with super absorbent polymer (Table 2, Figure 1). Super absorbent polymer can be used to reduce Cd uptake and diminish ROS generation in wheat plants. Our results also showed that the highest ascorbic acid content in leaves was obtained in plants treated with 100 mM of ascorbic acid (Table 2, Figure 2). Ascorbic acid plays a protective role against ROS, which are formed during biotic and abiotic stresses (Noctor & Foyer 1998). Ascorbate is oxidized by oxygen free radicals, generating dehydroascorbate (Noctor & Foyer 1998). This leads to a decline in the antioxidant enzymes activity.

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 membranes, is regarded as a reliable indicator of oxidative stress (Demiral & Turkan 2005). 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 obtained in plants treated with super absorbent polymer. Super absorbent polymer application can be used to reduce Cd uptake and diminish ROS generation in wheat.

It was also observed that 100 mM of ascorbic acid decreased the MDA content in leaves, when compared with untreated plants (Table 2, Figure 2). One of the best-known toxic effects of ROS is the damage on cellular membranes and lipids. Plasma membranes are oxidized by ROS, generating MDA. Exogenous ascorbic acid application partially inhibits oxidative stress, evaluated by MDA increases, because ascorbic acid is a scavenger of ROS (Noctor & Foyer 1998). Zhang & Kirkham (1996) have reported similar inhibitory effects of exogenous ascorbic acid on lipid peroxidation in sunflower seedlings exposed to osmotic stress.

CONCLUSIONS

1. Super absorbent polymer can be used to remediate Cd contaminated soils, as it positively influences wheat responses to Cd contamination.
2. Ascorbic acid foliar application increases the yield of wheat plants under Cd stress.
3. The application of ascorbic acid could reduce the harmful effects of ROS and improves wheat resistance to Cd contamination.

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