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Full Length Article

Alleviating Effect of Exogenous Application of Ascorbic Acid on Growth and Mineral Nutrients in Cadmium Stressed Barley (*Hordeum vulgare*) Seedlings

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Abstract

An experiment was carried out in sand-filled pots under normal temperature (28±2ºC) to assess the role of exogenously applied ascorbic acid in alleviating the effect of cadmium (Cd) stress on four barley (*Hordeum vulgare* L.) genotypes (Jau-83, Jau-87, Paidar 91 and Haider 93). After germination, seedlings were exposed to different Cd concentrations **(**0, 100, 300, 500 and 700 µM $CdCl_2$) along with AsA (200 mg L^{-1}) and grown for 15 days. The results suggested that exposure to increased Cd levels caused a significant reduction in growth and mineral nutrients contents of barley seedlings. However, there was a noticeable difference in the effect of Cd on mineral concentrations among genotypes and the difference mainly coincided with differential accumulation of Cd in the shoot and root tissues. When AsA was applied to Cd-stressed plants, it decreased Cd accumulation in shoots and roots and also showed partial reversal of Cd stress effects. It was also observed that at the same Cd concentrations Cd tolerance index of Jau-83 was the highest among the four barley genotypes, indicating that Jau-83 had lower Cd contents in roots may be more tolerant to Cd stress. The application of AsA was effective in reducing the toxicity of increased Cd by reducing the root or shoots Cd contents, as well as by improving the seedling growth attributes and the mineral nutrients in barley.

Keywords: Barley; Cadmium; Growth; Mineral nutrition

Introduction

Cadmium (Cd) is highly toxic, non-essential environmental pollutant found in air, water and soil (Sandalio *et al*., 2001; Benavides *et al*., 2005). One of the major sources of addition of Cd into agricultural soils is the application of phosphate fertilizers (Grant and Sheppard, 2008). The other sources for increasing Cd pollution in the soil are industrial effluents such as manufacture of plastics, paint pigments, batteries, alloy making, electroplating and manures (Devkota and Schmidt, 2000; Nedelkoska and Doran, 2000; Yang *et al*., 2004).

Cadmium can be taken up by the plants along with water and nutrients, when cultivated in Cd-polluted soil (Sheppard *et al*., 2007). Although Cd has been identified as a reason for various morphological, physiological, biochemical and structural alterations in plants, water disproportion and decreasing in rate of seed germination are the specific mechanisms in this regard (Mishra *et al*., 2006; Wahid and Khaliq, 2015). Moreover, Cd causes various phytotoxic symptoms such as browning of root tips, reduction in root length, thus resultantly directed to diminish development and less biomass accretion and finally plant death (Sanita di Toppi and Gabbrielli, 1999; Wahid *et al*., 2008).

A noticeable effect of Cd addition in growth medium is a marked reduction in shoot length of wheat (Veselov *et al*., 2003), *Phaseolus vulgaris* (Bhardwaj *et al*., 2009) and barley (Gubrelay *et al*., 2013). Similarly, Cd inhibits root growth more promptly than shoot growth (Vitoria *et al*., 2001). Hence, Cd addition reduced the shoot fresh and dry weight also have been studied previously in different plant species such as wheat (Mane *et al*., 2010) and barley (Zaltauskaite and Sliumpaite, 2013). Cadmium may impede with nutrient uptake due to competition for the same trans-membrane carriers, thereby leading to the altered tissue nutrient contents (Connolly *et al*., 2002). These interactions between Cd and other essential nutrients may lead to physiological disarrays as well as a reduction in growth (El-Beltagi *et al*., 2010). Cadmium exposure significantly affected essential ions in roots and shoots has been explored in rice (Liu *et al*., 2003), wheat (Zhang *et al*., 2002), barley (Wu and Zhang, 2002) and lettuce (Monteiro *et al*., 2009). These mineral nutrients also have specific of defensive role against lethal effects of Cd (Khan *et al*., 2007).

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Ascorbic acid (AsA) is a strong antioxidant and abundantly occurs in plants (Smirnoff, 2000). It plays many important roles in various cellular processes. It is involved in cell division and cell wall expansion, and regulates the plant growth and development (Pignococchi and Foyer, 2003). Exogenous application of AsA stimulates total leaf area, photosynthetic pigments and growth of plants under drought stress (Amin *et al.*, 2009). AsA is one of the most effective compounds, which improve the tolerance of the plants to oxidative stresses. A wealth of information suggests that AsA plays significant role in protection of plant against several environmental circumstances (Paital and Chainy, 2010), such as salt stress (Shalata and Neumann, 2001), ozone (Sanmartin *et al*., 2003), UV-B and pathogenesis (Fotopoulos *et al*., 2006), drought (Fotopoulos *et al*., 2008), and in heavy metal stress (Vwioko *et al*., 2008).

Barley is ranked fourth-largest cereal crop, rich in carbohydrate along with moderate amount of protein, phosphorus, calcium and minor amount of vitamin B (Daniel and Hopf, 2000). It is constituent of many foods. Barley is commonly used for beer production. In Pakistan, barley is extensively used as green fodder for livestock feed and for small ruminant animals in winter (Khan *et al*., 1999). We hypothesize that Cd-toxicity to barley can be alleviated by exogenous AsA application due to its antioxidative role. The objective of the present study was to assess the role of ascorbic acid in the alleviation of toxic effects on seedling growth parameters, mineral nutrients and the genotypic responses to Cd toxicity.

Materials and Methods

Experimental Details

The pot experiment was conducted in the growth chamber under controlled conditions of light and temperature (28±2ºC) in the Dept. of Botany, University of Agriculture, Faisalabad, Pakistan. Seeds of barley genotypes (Jau-83, Jau-87, Paidar 91 and Haider 93) were obtained from Ayub Agricultural Research Institute, Faisalabad. Cadmium levels were prepared using cadmium chloride ($CdCl_2$). Ten seeds of each genotype were sown in small plastic pots containing washed river sand under five cadmium treatments (0, 100, 300, 500 and 700 µM) and 200 mg L^{-1} of AsA. Treatment combinations used during the course of study were: 0 µM Cd + 0 mg AsA, 100, 300, 500 and 700 µM Cd with or without the application of AsA. After germination, the seedlings were irrigated at alternate day intervals with half strength of Hoagland's solution (Hoagland and Arnon, 1950) along with the corresponding Cd treatment combination for 15 days. After harvesting the plants, different seedling growth parameters and contents of mineral nutrients were measured.

Growth Determination

Shoot and root lengths and their fresh weights were measured immediately after harvesting. For taking their dry weights, both the parts were put in paper bags and dried in an oven for 7 days.

Determination of Mineral Nutrients

The dried ground material (0.5 g) of shoots, and roots were digested in concentrated HNO_3 (5 mL) at 100ºC temperature and then raised the temperature gradually to 250ºC until the samples became clear. Then made volume of the extracted up to 50 mL using a volumetric flask. Filtered the extract and used it for the determination of mineral nutrients concentrations. The dissolved amount of potassium (K) and calcium (Ca) were determined by using flame photometer (Model: PFPI-7, Jenway, UK), while magnesium (Mg) and cadmium (Cd) were determined with atomic absorption spectrometer (Model: AAnalyst-3100 Perklin Elmer, USA). Cd content was calculated by multiplying the dry weight of root or shoot with their Cd concentration.

The phosphorus (P) content was determined according to (Yoshida *et al.*, 1972). One gram of dried and ground plant tissue was digested with 10 mL of acid mixture (nitric acid, 750 mL; sulphuric acid, 150 mL; perchloric acid 60 per cent, 300 mL). The digest was cooled and made up to 50 mL and filtered through acid washed Whatmann No.1 filter paper. One mL of digest was mixed with 2 mL of 2N nitric acid and diluted to 8 mL. One mL of molybdovanadate reagent (25 g of ammonium molybdate in 500 mL water, 1.25 g ammonium vanadate in 500 mL of 1 N nitric acid; both were mixed in equal volumes) was added, make up to 10 mL, shacked and the absorbance was measured at 420 nm in a spectrophotometer.

Statistical Analysis

Design of the experiment was completely randomized factorial with three replications per treatment. Analysis of variance of data for all parameter was carried out to find out the significance of variance sources and Duncan's New Multiple Range test ($P \leq 0.05$) was used to find differences among the treatments by using a computer software COSTAT (Cohort software Berkeley, California).

Results

Growth Parameters

The data for different growth parameters indicated significant ($P<0.05$) differences amongst the barley varieties as well as amongst different treatments. The growth data indicated that addition of Cd to the nutrient medium caused a visually noticeable reduction in growth parameters. At low concentration of Cd (100 µM), minor effect was noticed, while at high concentration of cadmium 700 µM very strong inhibitory effects were observed in among genotypes. The extent of reduction was more pronounced in root length as compared to shoot length (Fig. 1). Cd-induced growth

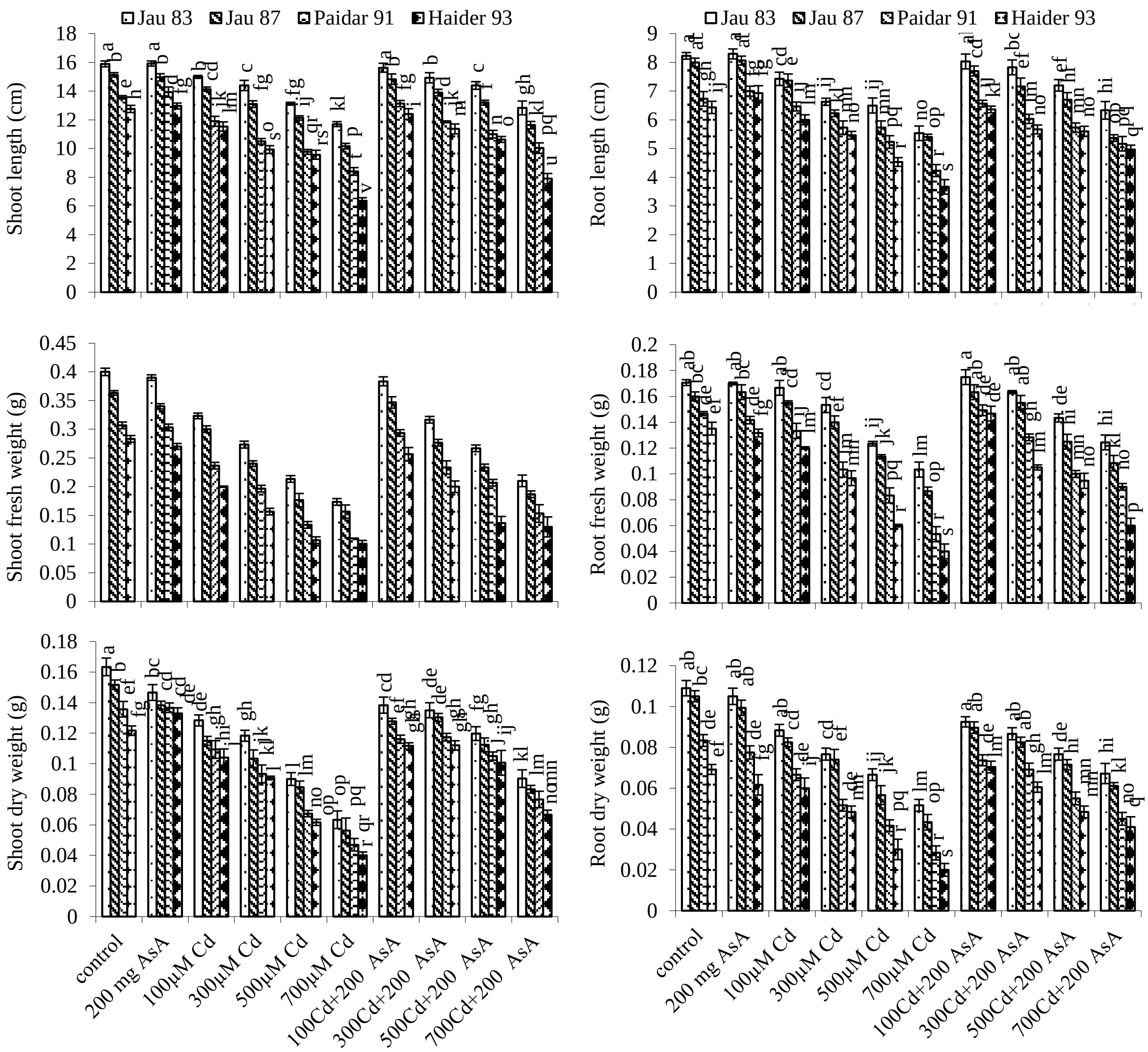

Fig. 1: Main effects of cadmium (Cd), ascorbic acid (AsA) and their interactions on shoot and root growth of four barley genotypes

diminishing tendency was in the order: root length, shoot length, and shoot fresh and dry weight (Fig. 1). Among the genotypes Jau-83 indicated better tolerance to cadmium toxicity and more improvement was recorded with the medium supplementation of AsA in the order: Jau-83 < Jau-87 < Paidar 91 < and Haider 93.

Mineral Nutrients

Changes observed with increased levels Cd on the shoot and root K^+, Ca^{2+}, P and Mg^{2+} contents. The data indicated significant ($P<0.01$) differences in Cd and AsA treatments and genotypes for the accumulation of the mineral ions. Medium applied Cd levels declined the tissue concentrations of all the nutrients measured in four barley genotypes, compared to control.

In the present study, Cd addition decreased shoots and root K^+ contents of all the four barley genotypes (Fig. 2). The decrease in K^+ contents was higher at Cd level 700 µM in comparison to 100 µM, 300 µM and 500 µM Cd levels. The rate of decrease in K^+ contents was higher in Haider 93 followed by Paidar 91, Jau-87 and a least decline was observed in Jau-83 (Fig. 2). Ca^{2+} content of Cd treated barley seedlings decreased as Cd level increased in the growth medium. A minor decrease in Ca^{2+} contents was seen at Cd level 100 µM, while this reduction increased with the increase in Cd level. At 700 µM Cd level, maximum reduction was observed in all barley genotypes. Jau-83 was generally higher in Ca^{2+} contents of shoot and root than in Jau-87, Paider 91 and Haider 93 under both control and Cd stress conditions (Fig. 2).

Cadmium application in present study greatly affected

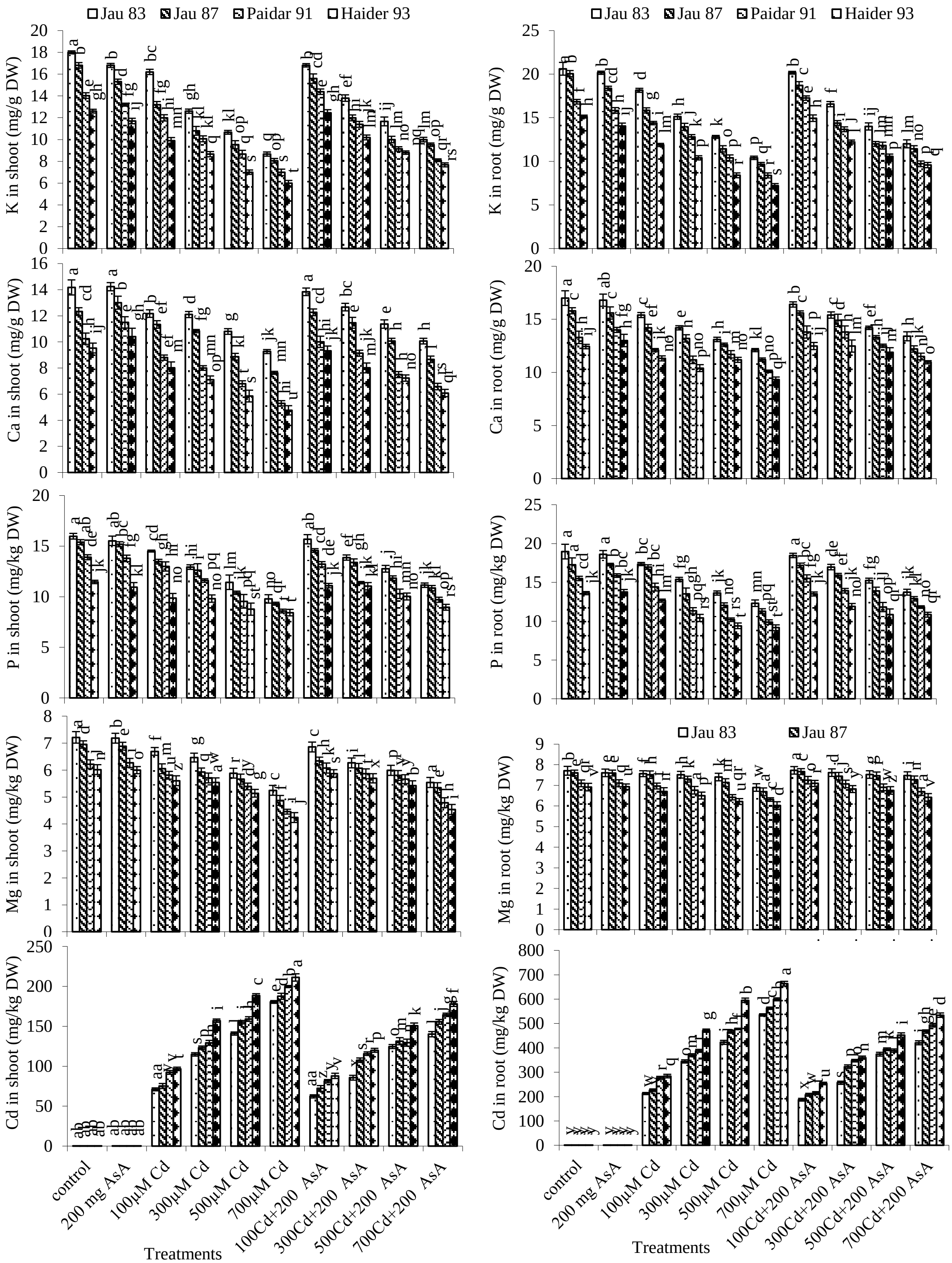

Fig. 1: Main effects of cadmium (Cd), ascorbic acid (AsA) and their interactions on shoot and root nutrinet and Cd contents in barley gentypes

P contents in both shoot and root of all the barley genotypes compared to control. This reduction was in a Cd-concentration dependent manner. At 100 µM Cd, a slight reduction in P contents was noticed, while this reduction was more pronounced as the Cd level increased and at 700 µM P content was affected the most. Decrease in tissue P order of genotypes was; Jau-83<Jau-87<Paidar-91<Haider-93 (Fig. 2).

Increasing Cd levels also led to the continuous decrease in Mg^{2+} contents both shoot and root in all the four barley genotypes. Mg^{2+} contents exhibited the corresponding reduction at lower level 100 µM of Cd stress compared to control, while this reduction increased at 300 and 500 µM Cd level but was the highest at 700 µM. The order of genotypes for changes in Mg^{2+} accumulation was: Jau-83 < Jau-87 < Paidar 91 <Haider 93. On the other hand AsA used in the current exploration substantially enhancing Mg^{2+} in all the barley genotypes (Fig. 2).

Cadmium Contents

All the treatments and genotypes differed significantly (P<0.01) for the accumulation of Cd in shoot and root. As predicted, Cd contents were consistently higher in roots compared to shoots with increased in cadmium concentration. Similarly, Cd contents increased in all the genotypes with the increasing Cd concentration, maximum accumulation was detected at 700 µM Cd level showed in (Fig. 2). Among the genotypes, Haider 93 accumulated the highest Cd followed by Paidar 91, Jau-87 but it was the least in Jau-83 (Fig. 2). Addition of AsA in the growth medium reduced the accumulation of Cd contents in all genotypes. Jau-83 showed more tolerance against Cd toxicity with or without ascorbic acid application and accumulated less Cd contents.

Discussion

Adverse effects produced by Cd toxicity diminished the growth of the seedlings, as evident from different growth parameters interpreted above. The effect of increased Cd concentrations on these growth parameters revealed that low levels of Cd had small effect on growth parameters, while higher levels were strongly damaging. Among Cd concentrations, the most damaging was 700 µM (Fig. 1). Amongst different growth parameters, the most deteriorating effects due to excessive Cd were noticed on the root elongation, which was expected because the roots are in direct contact with Cd from the soil solution. A decline in growth might be due to impeded normal physiological functions and replacement of essential mineral nutrients by the Cd. In the current study, external supply of AsA appreciably enhanced growth parameters (shoot, root lengths, shoot, and root fresh and dry weights). Being a growth promoter, AsA positively influences the mineral uptake and diminishes the adverse effect of abiotic stresses (Wu and Zhang, 2002; Sheteawi, 2007; Athar *et al*., 2008).

Studies show that, among other effects, Cd induces deficiency and imbalance of mineral nutrients in different plant species (Eun *et al*., 2000; Wahid *et al*., 2008; Mane *et al*., 2010), while the toxicity of Cd can be diminished with the use of growth promoters (Raza *et al*., 2013; Fatima *et al*., 2014; Perveen *et al*., 2015). Ideal plant development can be attained with the optimal physiological levels of essential nutrients. Deficiency of even a single nutrient may lead to death of plant (Taiz and Zeiger, 2015). In the present case, Cd stress considerably reduced the mineral nutrients (Ca, K, P and Mg) contents in shoots and roots in all the barley genotypes, this reduction was more pronounced in Haider 93 as compared to other genotypes, and root tissue was more adversely affected (Fig. 2). It has been argued that Cd impinges negative impact on the uptake of beneficial nutrient since it competes with them at plasma lemma level (Wahid *et al*., 2009; Asgher *et al*., 2015).

It is important to mention that AsA application enhanced the uptake of mineral nutrients in this study by reducing the tissue contents of Cd and thereby reducing its toxicity more on the shoot than on the root of all barley genotypes. In such instances, the prolific root system and retention of Cd by AsA may defend the shoots as a strategy of evasion from Cd toxicity (Chaoui *et al*., 1997). Ascorbic acid application in combination with elevating Cd stress caused decline in root Cd accumulations, and thus encountered the Cd toxicity (Wu and Zhang, 2002). Hussein *et al*. (2011) reported that AsA being antioxidant and potential growth regulator usually stimulates the mineral uptake in many plant species and diminished the harmful effect of abiotic stresses. Different genotypes differed greatly in their behavior toward Cd uptake and tolerance, which provided room for future research in the field of Cd tolerance using exogenous application of AsA.

Conclusion

Although with large varietal difference, Cd toxicity adversely affected the growth and nutrients uptake whilst the AsA application, due to its antioxidant properties, partially reduced the Cd toxicity on barley. The genotypes Jau-83 emerged as most tolerant Cd because growth and mineral nutrients were less affected as compared to other genotypes. Further studies are needed to find the possible mechanism(s) of the growth improvement in barley and possibly other crop species.

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