



# Role of Zinc–Lysine on Growth and Chromium Uptake in Rice Plants under Cr Stress

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## Abstract

Chromium (Cr) is a very toxic heavy metal present in agricultural soils. Soils contaminated with Cr are the major source of Cr entrance into the food chain. The present experiment was designed to determine the effects of different levels of lysine chelated with zinc (Zn–lys) (0, 10, 20, 30 mg L<sup>-1</sup>) upon photosynthesis, biomass, anti-oxidant enzyme activities, oxidative stress, and the uptake of Cr by rice under various applied levels of Cr (0, 100, 500 mg kg<sup>-1</sup>). Cr stress decreased the physiological and morphological parameters as well as the activities of anti-oxidants enzymes; and caused oxidative stress by enhancing the Cr concentration in plants over that of controls. Zn–lys foliar treatment enhanced photosynthesis, biomass, Zn contents, and enzyme activities. The application of Zn–lys (30 mg L<sup>-1</sup>) under 500 mg kg<sup>-1</sup> of Cr increased plant height by 50% and shoot dry weight by 74% but also the root dry weight by 129% over the control treatment. In addition, Zn–lys reduced oxidative stress and Cr concentration compared to that of Cr treatments alone. The application of Zn–lys (30 mg L<sup>-1</sup>) also reduced Cr contents in roots by 26 and 31% in 100 and 500 mg kg<sup>-1</sup> Cr treatments, respectively. The results of our study revealed that Zn–lys foliar treatment enhanced rice growth and decreased oxidative stress and Cr concentration by stimulating the anti-oxidant defense system as well as by promoting photosynthesis and Zn uptake in rice plants. Overall, the Zn–lys foliar treatment was helpful in increasing plant growth and Zn concentration while reducing the Cr contents in rice. However, further studies at field levels are required to explore the mechanisms of Zn–lys mediated reduction of Cr and possibly other heavy metal toxicity in plants.

**Keywords** Chromium · Zinc-lysine · Biofortification · Photosynthesis · Tolerance

## Introduction

Chromium (Cr) is among the most toxic trace elements present in agricultural soils and is being released through a variety of anthropogenic activities such as electroplating and leather tanning (Gomes et al. 2017; Shahid et al. 2017). Chromium enters plants through roots exposed to Cr-contaminated soils and depends upon many soil factors such as soil pH, electrical conductivity (EC), competition between different metal species present in the soil, as well as the different plant-associated factors such as plant species, growth stages, and root system (Zeng et al. 2012; Ali et al. 2015a; Ertani et al. 2017). In plants, excess Cr is phytotoxic which may cause toxicities at morphological, physiological, biochemical, and molecular levels (Gomes et al. 2017). It has been well-documented that Cr exerts many toxic effects in plants and thus reduces plant shoot parameters such as shoot length, leaf area, and biomass but also reduces plant

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root parameters such as root surface area and number of root hairs (Adrees et al. 2015a; Shahid et al. 2017). Excess Cr also reduces leaf gas exchange attributes and chlorophyll contents (Ma et al. 2016). Previous studies reported Cr-induced reduction in the uptake of micronutrients in plants including zinc (Zn) and iron (Fe) in *Amaranthus viridis* (Liu et al. 2008) and Zn, Fe and copper (Cu) in pea (Rodriguez et al. 2012). Furthermore, there exists a competition between Zn and Cr as well as other nutrient elements during uptake by plants (Dotaniya et al. 2018).

Higher Cr concentrations in plants have also caused ultra-structural alterations in various parts of plants (Gill et al. 2015a; Ma et al. 2016). Moreover, Cr toxicity previously caused oxidative stress in various crop species through the overproduction of the reactive oxygen species (ROS) (Ma et al. 2016). Plants have a well-developed defense system of ROS-scavenging which consists of mainly enzymatic and non-enzymatic antioxidants (Ali et al. 2015a; Gill et al. 2015a). Among the key anti-oxidant enzymes, superoxide dismutase (SOD) constitutes the main defense against enhanced ROS (Gill et al. 2015b) whereas catalase (CAT) and guaiacol peroxidase (POD) eliminate hydrogen peroxide ( $H_2O_2$ ) in plants (Anjum et al. 2016). It has been reported that Cr toxicity altered anti-oxidant enzyme activities depending upon a number of factors both at the soil and plant levels (Ma et al. 2016; Shahid et al. 2017).

Different management strategies have been employed for the reduction of Cr uptake and its toxic effects in plants (Adrees et al. 2015b; Jabeen et al. 2016; Abbas et al. 2017; Shahid et al. 2017). Various fertilizers composed of amino acids such as threonine or lysine (Lys) chelated with different micronutrients such as Zn and Fe can be used to increase the growth and nutritional quality of crop plants (Souri 2016; Rafie et al. 2017). Among these chelated fertilizers, Zn chelated with different amino acids has been reported to enhance plant growth and is considered an eco-friendly plant growth promoter (Ghasemi et al. 2013a, b; Rafie et al. 2017). Micronutrients can be used to grow plants in metal-contaminated soils as these nutrients can enhance plant tolerance to metal stress (Rizwan et al. 2016a, 2017a; Bashir et al. 2018). On the other hand, amino acids have been studied for the alleviation of abiotic stresses in many plant species (Sadak and Abdelhamid 2015). The use of amino acids complexed with micronutrients can ameliorate various types of abiotic stresses in plants but less information is available in the literature in this regard (Souri 2016; Rizwan et al. 2017b). It has been shown that Zn-amino acid complexes effectively reduce salt toxicity in lettuce compared to  $ZnSO_4$  (Mohammadi and Khoshgoftarmansh 2014). Thus, micronutrients and amino acid chelates may be used to decrease the toxic effects of Cr in plants.

Rice (*Oryza sativa* L.) is a grain crop that is widely consumed over the globe (Rizwan et al. 2016b). It has been

reported that Cr caused toxicity in rice and reduced the growth and yield of this important food crop (Chen et al. 2017; Ma et al. 2016; Shraim 2017). On the other hand, 30% of the total agricultural soils all over the globe are Zn deficient where rice suffers Zn deficiency more than any other agricultural crop which negatively affects the rice growth and grain quality (Joy et al. 2017; Zaman et al. 2017). To avoid the hidden hunger, Zn application as a foliar spray is recommended because of its low agronomic efficiency when applied to the soil (Gregory et al. 2017; Rafie et al. 2017).

Due to the importance of Cr stress and Zn deficiency in rice and the use of micronutrient-amino acid complexes as fertilizers for improving plant growth, it was hypothesized that Zn-lys might be effective in reducing the Cr concentration in plants through enhancing plant growth and the defense system under Cr stress. Thus, the current experiment was conducted to determine the effects of foliar-applied Zn-lys on rice growth, biomass, oxidative stress, Cr uptake, and the activities of certain antioxidants under Cr stress.

## Materials and Methods

### Soil Sampling and Analysis

Soil sampling was done by using a stainless-steel spade from a field under agricultural crops located at the University of Agriculture, Faisalabad, Pakistan (31.4303°N, 73.0672°E). Mostly cereal crops were grown in the field where the soil was collected for the current study. Sampling was done at several points in the field at a depth of 0–20 cm after 60 days of wheat harvesting from the field. The samples were thoroughly mixed to homogenize and air-dried under shade. All the debris and other unwanted materials such as previous crop residues were removed and the soil was finally sieved to 2 mm. After this, the soil was analyzed for the determination of initial soil properties (Table 1). The hydrometer method was used for the estimation of size fractions of soil particles (Bouyoucos 1962). Soil electrical conductivity (EC), pH, sodium adsorption ratio (SAR), and other soluble ions were determined by using standard protocols (US Salinity Laboratory Staff 1954; Page et al. 1982). Bioavailable trace elements (AB-DTPA-extractable, ammonium bicarbonate diethylenetriamine pentaacetic acid, pH 7.6) in soil samples were estimated by extracting the samples with the procedure described by Soltanpour (1985).

### Pot Experiment

A pot culture experiment was conducted under ambient conditions during the Kharif season. Rice (cv. Kainat)

**Table 1** Properties of soil used in the pot experiment

Texture	Clay loam
Sand (%)	64.5
Silt (%)	13.3
Clay (%)	22.2
pHs	7.54
ECe (dS m <sup>-1</sup> )	4.85
Cation exchange capacity (CEC) (cmol <sub>c</sub> kg <sup>-1</sup> )	12.9
Soluble CO <sub>3</sub> <sup>2-</sup> (mmol L <sup>-1</sup> )	0.91
Soluble HCO <sub>3</sub> <sup>3-</sup> (mmol L <sup>-1</sup> )	3.96
Soluble Cl <sup>-</sup> (mmol L <sup>-1</sup> )	6.73
Soluble Ca <sup>2+</sup> + Mg <sup>2+</sup> (mmol L <sup>-1</sup> )	15.43
Organic matter (%)	0.78
Available Ni (mg kg <sup>-1</sup> )	0.26
Available Cu (mg kg <sup>-1</sup> )	0.53
Available Zn (mg kg <sup>-1</sup> )	0.82

nursery, 30-days-old, was transferred to the pot having 3.0 kg of air-dried soil. Initially, 5 seedlings were sown in each pot and then thinned to 3 individuals after one week of sowing in the pots. After 2 weeks of transplanting the seedlings, different concentrations of Zn-lys (0, 10, 20, and 30 mg L<sup>-1</sup>) and Cr in the form of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) (0, 100, and 500 mg kg<sup>-1</sup> of soil) were applied to plants at 7-day intervals for the next 4 weeks. Selection of potassium dichromate as the Cr source was based on the fact that it provides Cr in the form of Cr(VI) which is more toxic to plants than the other forms of Cr. Zn-lys was a foliar-applied and, at the same time, a Cr solution was applied in the soil. Total Cr doses were split into four parts to reach the final Cr levels of 100 or 500 mg kg<sup>-1</sup> of soil. For Zn-lys, the total volume of two liters was foliar-applied per treatment during the four applications and the control plants were foliar sprayed with distilled water while the soil was covered during the Zn-lys application to avoid any contamination. All Zn-lys and Cr treatments were applied in split doses to avoid any sudden toxicity to plants and a completely randomized design of the experiment was followed with four replicates of each treatment. Fertilizer levels of 120–50–25 kg ha<sup>-1</sup> were used for urea as a source of nitrogen (N), diammonium phosphate as a source of phosphorus (P), and potassium sulfate as a source of potassium (K). Potassium added from K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was subtracted from the dose and complete doses of PK whereas half of the N was given to the plants after 1 week of transplanting the seedlings and another half was given to the plants after 2 weeks of the 1st fertilizer application. A water level of about 2 cm was kept in the pots during the whole experimental period and pots were rotated on a regular basis to avoid the environmental effects on the plants.

## Plant Harvesting

Rice seedlings were harvested after 10 weeks of growth in pots and separated into shoots and roots. Plant height was measured by the use of a stainless-steel scale. Shoot and roots were washed with distilled water. Some fresh samples were used for photosynthetic pigment and other analyses and the remaining samples were oven-dried at 70 °C until the constant weight and dry biomass of these plant parts were noted.

## Measurement of Photosynthetic Parameters

A given weight of leaf samples was crushed into tubes and 85% acetone (v/v) was added with a pipette. For 24 h, they were placed into the dark for extraction of the pigments, and then centrifuged for 10 min at 4000×g under 4 °C and the supernatant was further analyzed. A spectrophotometer was used for the measurement of different wavelengths (470, 647, and 664.5 nm) and values of chlorophyll contents were calculated as given by Lichtenthaler (1987). Water use efficiency, transpiration rate of leaves and photosynthetic rate, and stomatal conductance were recorded before harvesting the plants. A portable IRGA (Infra-Red Gas Analyzer, Analytical Development Company, Hoddeston, England) was used for measuring these parameters. Gas exchange parameters were recorded during a sunny day (10:00 am to 11:00 am).

## Measurement of Oxidative Stress and Antioxidants

Electrolyte leakage (EL) of the samples was estimated by vertically placing the samples in the tubes having a known volume of distilled water, autoclaving the tubes for 2 h at 32 °C and then noting EC as EC<sub>1</sub> and again the same samples were autoclaved at 121 °C for 20 min and then the EC was noted as EC<sub>2</sub>. The EL was determined by using Eq. 1 (Dionisio-Sese and Tobita 1998).

$$EL = (EC_1/EC_2) \times 100 \quad (1)$$

Malondialdehyde (MDA) contents were determined by using 0.1% thiobarbituric acid according to the method given by Heath and Packer (1968) and a little modification later by Dhindsa et al. (1981) and Zhang and Kirham (1994). For the measurement of H<sub>2</sub>O<sub>2</sub>, each sample (50 mg) was extracted after homogenizing the sample in phosphate buffer solution of 3 mL (50 mM, pH 6.5) and then 1 mL of titanium sulfate (0.1%) and H<sub>2</sub>SO<sub>4</sub> (20% v/v) were used in 3 mL supernatant and centrifugation was done for 15 min at 6000 g. The intensity was measured at 410 nm and H<sub>2</sub>O<sub>2</sub> contents in the supernatant

were estimated by using a  $0.28 \mu\text{mol}^{-1} \text{cm}^{-1}$  extinction coefficient.

Key anti-oxidant enzyme activities in the plant samples were determined after standardizing the samples with 0.05 M phosphate buffer at 7.8 pH. Both POD and SOD activities were estimated according to the method of Zhang (1992) by using a spectrophotometer. The CAT activity was measured according to the standard protocol (Aebi 1984) whereas APX activity was estimated via the method of Nakano and Asada (1981).

### Determination of Plant Cr and Zn Concentrations

For the digestion of samples, 10 mL consisting of  $\text{HNO}_3\text{--HClO}_4$  (3:1, v:v) were used for each plant shoot and root sample of 1.0 g and the mixture was placed overnight, and then the samples were placed on a hot plate and digested after adding 5 mL of  $\text{HNO}_3$  (Ryan et al. 2001). Finally, atomic absorption spectrophotometer was used to measure the Cr and Zn contents in the samples.

### Statistical Analyses

One-way ANOVA was applied to statistically analyze the data at 5.0% of probability by using IBM SPSS software (Version 21.0. Armonk, NY: IBM Corp). Where significant, Tukey's HSD post hoc test was used for the multiple comparisons of means.

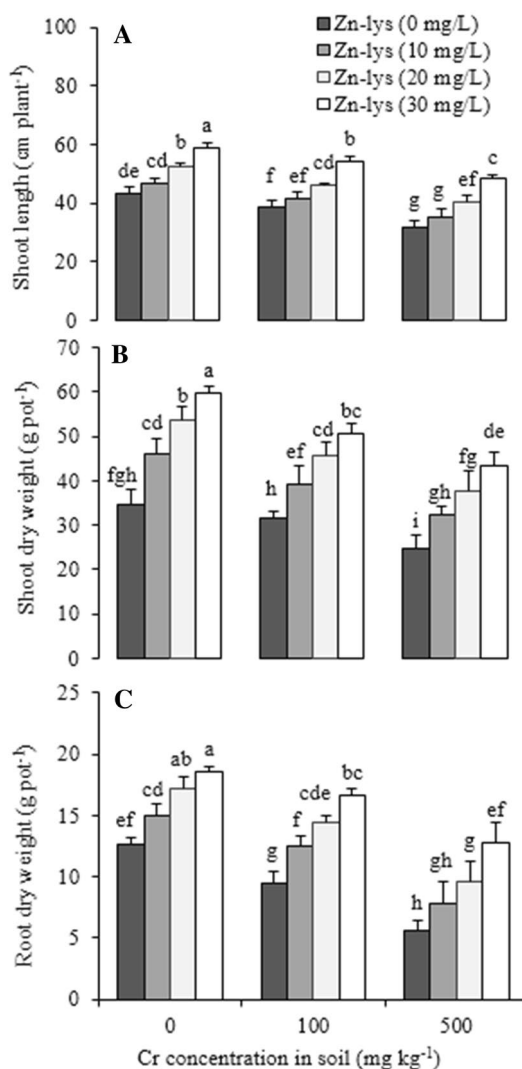
## Results

### Rice Dry Weights

Chromium treatments alone significantly ( $p \leq 0.05$ ) reduced shoot length and dry weights of shoots and roots (Fig. 1). At the highest level of Cr stress alone ( $500 \text{ mg kg}^{-1}$ ), the reduction in shoot length and dry weights of shoots and roots was about 25, 28, and 55% of controls, respectively. Application of Zn-lys increased shoot length as well as dry weights of Cr-stressed plants. The maximum increase in these parameters was with  $30 \text{ mg L}^{-1}$  Zn-lys without Cr. Compared to the control, application of  $30 \text{ mg L}^{-1}$  Zn-lys increased plant height by 37, 40, and 50% and shoot dry weight by 72, 60, and 74%, whereas root dry weight by 46, 74, and 129% in 0, 100, and  $500 \text{ mg kg}^{-1}$  of Cr, respectively.

### Chlorophyll Concentration and Gas Exchange Attributes

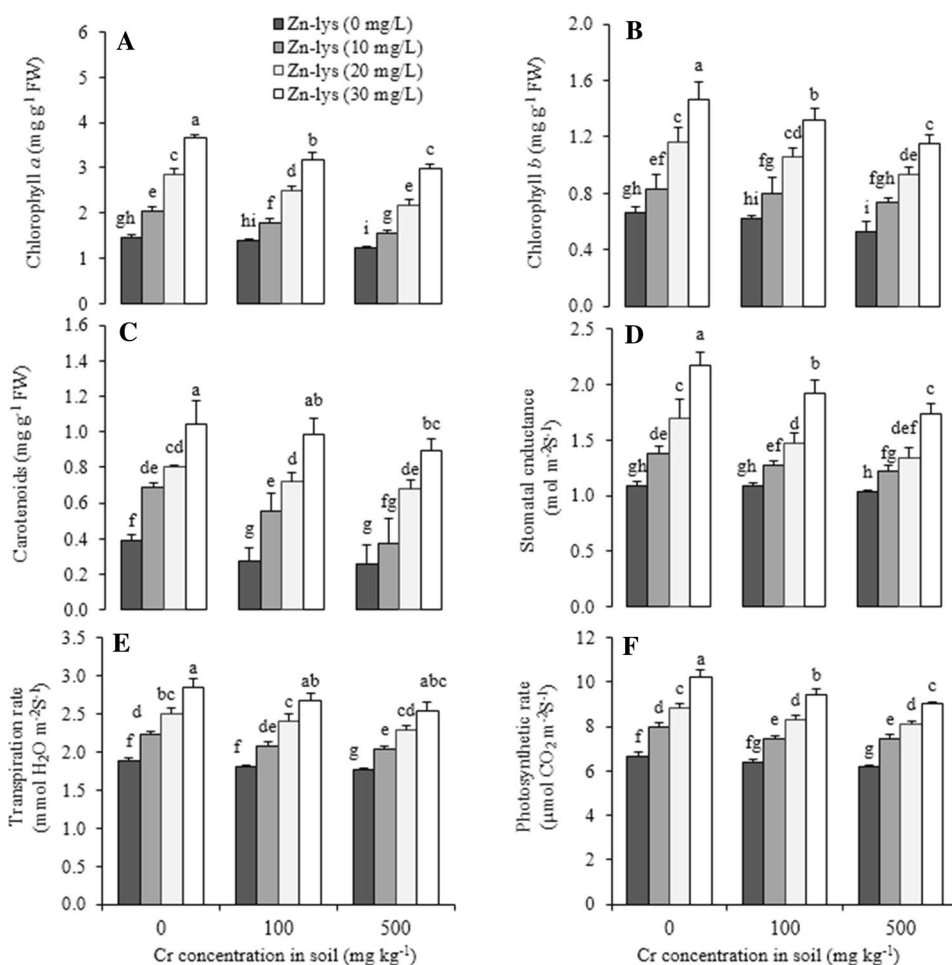
Increasing Cr levels alone in the growth medium reduced the photosynthetic pigments and gas exchange attributes (transpiration rate, stomatal conductance, and photosynthetic



**Fig. 1** Effect of foliar dressing of different concentrations of zinc-lysine (0, 10, 20, 30  $\text{mg L}^{-1}$ ) on plant height and shoot and root dry biomass of rice plants grown in soil with increasing concentrations of Cr (0, 100 and  $500 \text{ mg kg}^{-1}$ ). Bars represent SD of four replicates. Different lower-case letters indicate significant differences among different treatments at  $p \leq 0.05$

rate) in the leaves of rice compared to the control (Fig. 2). The maximum reduction in these photosynthetic parameters was noted in leaves with the highest Cr level applied ( $500 \text{ mg kg}^{-1}$ ) in the soil. Foliar-applied Zn-lys increased these photosynthetic parameters under Cr stress compared to the same Cr treatment without Zn-lys (Fig. 2). As compared to the  $30 \text{ mg kg}^{-1}$  Cr stress alone, the increase in chlorophyll *a* was about 141%, chlorophyll *b* was about 120%, and carotenoids was about 244% in the treatment with  $30 \text{ mg kg}^{-1}$  Cr +  $30 \text{ mg L}^{-1}$  Zn-lys, respectively. Under  $30 \text{ mg L}^{-1}$  of Zn-lys, the stomatal conductance increased by 99, 77, and 68% under 0, 100, and  $500 \text{ mg kg}^{-1}$  Cr treatments compared to the respective Cr treatments without Zn-lys, respectively.

**Fig. 2** Effect of foliar dressing of different concentrations of zinc-lysine (0, 10, 20, 30 mg L<sup>-1</sup>) on chlorophyll and gas exchange parameters of rice plants grown in the soil with increasing concentrations of Cr (0, 100 and 500 mg kg<sup>-1</sup>). Bars represent SD of four replicates. Different lower-case letters indicate significant differences among treatments at  $p \leq 0.05$



Transpiration and photosynthetic rate also significantly increased with Zn-lys application and the response was increased with increasing Zn-lys levels.

### Chromium and Zn Accumulation in Plants

Supply of Cr in the soil enhanced the Cr concentrations in the shoots and roots of rice irrespective of Zn-lys foliar usage (Fig. 3a, b). Foliar application of Zn-lys reduced the Cr concentrations in rice. In the shoots, 30 mg L<sup>-1</sup> Zn-lys decreased the Cr concentrations by 39%, and 41% in 100 and 500 mg kg<sup>-1</sup> Cr treatments, respectively. Similarly, 30 mg L<sup>-1</sup> Zn-lys reduced the Cr contents in roots by 26, and 31% in 100 and 500 mg kg<sup>-1</sup> Cr treatments, respectively. Soil applied Cr significantly ( $p \leq 0.05$ ) decreased the Zn concentrations in rice compared to control (Fig. 3c, d). The highest Zn contents in shoots and roots were found in plants treated with 30 mg L<sup>-1</sup> Zn-lys without Cr. As compared to the control, shoot Zn concentrations were reduced by 3, and 4.5% and root Zn concentrations reduced by 4.8, and 7.1% in 100 and 500 mg kg<sup>-1</sup> Cr treatments, respectively. Under Cr stress, foliar application of Zn-lys significantly enhanced

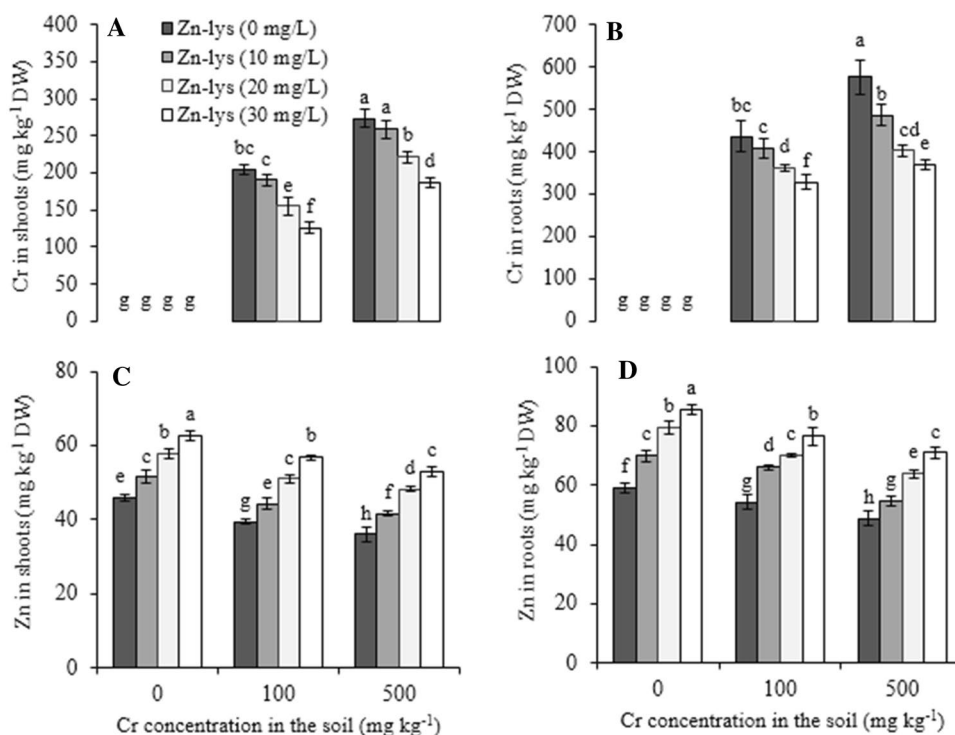
the Zn concentrations in shoots and roots compared to the respective Cr treatments alone. At the highest Zn-lys rate (30 mg L<sup>-1</sup>), the increase in shoot Zn concentrations was about 36, 38.5, and 34% in 0, 100, and 500 mg kg<sup>-1</sup> Cr treatments compared to the respective Cr levels alone, respectively. As compared to the Cr treatments alone, 30 mg L<sup>-1</sup> Zn-lys application increased the root Zn concentrations by 42, 45.4, and 45.7% under 0, 100, and 500 mg kg<sup>-1</sup> Cr treatments, respectively.

### Electrolyte Leakage and Changes in Antioxidants

Chromium toxicity increased EL, MDA, and H<sub>2</sub>O<sub>2</sub> in rice compared to control (Fig. 4). The highest concentrations of EL, MDA, and H<sub>2</sub>O<sub>2</sub> were found in plants treated with 500 mg kg<sup>-1</sup> Cr without Zn-lys application. Exogenous application of Zn-lys decreased EL, MDA, and H<sub>2</sub>O<sub>2</sub> concentrations in rice under Cr stress. The significant reduction in EL, MDA, and H<sub>2</sub>O<sub>2</sub> was found in seedlings treated with the highest level of Zn-lys (30 mg L<sup>-1</sup>) compared to the other levels of Zn-lys. In contrast to the respective Cr treatment alone, 30 mg L<sup>-1</sup> Zn-lys decreased EL in shoots



**Fig. 3** Effect of foliar dressing of different concentrations of zinc-lysine (0, 10, 20, 30 mg L<sup>-1</sup>) on Cr and Zn concentrations in rice plants grown in the soil with increasing concentrations of Cr (0, 100 and 500 mg kg<sup>-1</sup>). Bars represent SD of four replicates. Different letters indicate significant differences among treatments at  $p \leq 0.05$



by 38, 44, 50%, and in roots decreased by 30, 34, and 41%, under 0, 100, and 500 mg kg<sup>-1</sup> Cr treatments, respectively.

The activities of anti-oxidant enzymes (SOD, POD, CAT, and APX) decreased with increasing Cr concentration in the soil (Fig. 5). However, the application of Zn-lys increased the activities of these enzymes. The highest levels of anti-oxidant enzymes were found in 30 mg L<sup>-1</sup> Zn-lys application without Cr stress whereas the lowest levels of these enzymes were found in plants treated with 500 mg kg<sup>-1</sup> Cr treatment alone.

## Discussion

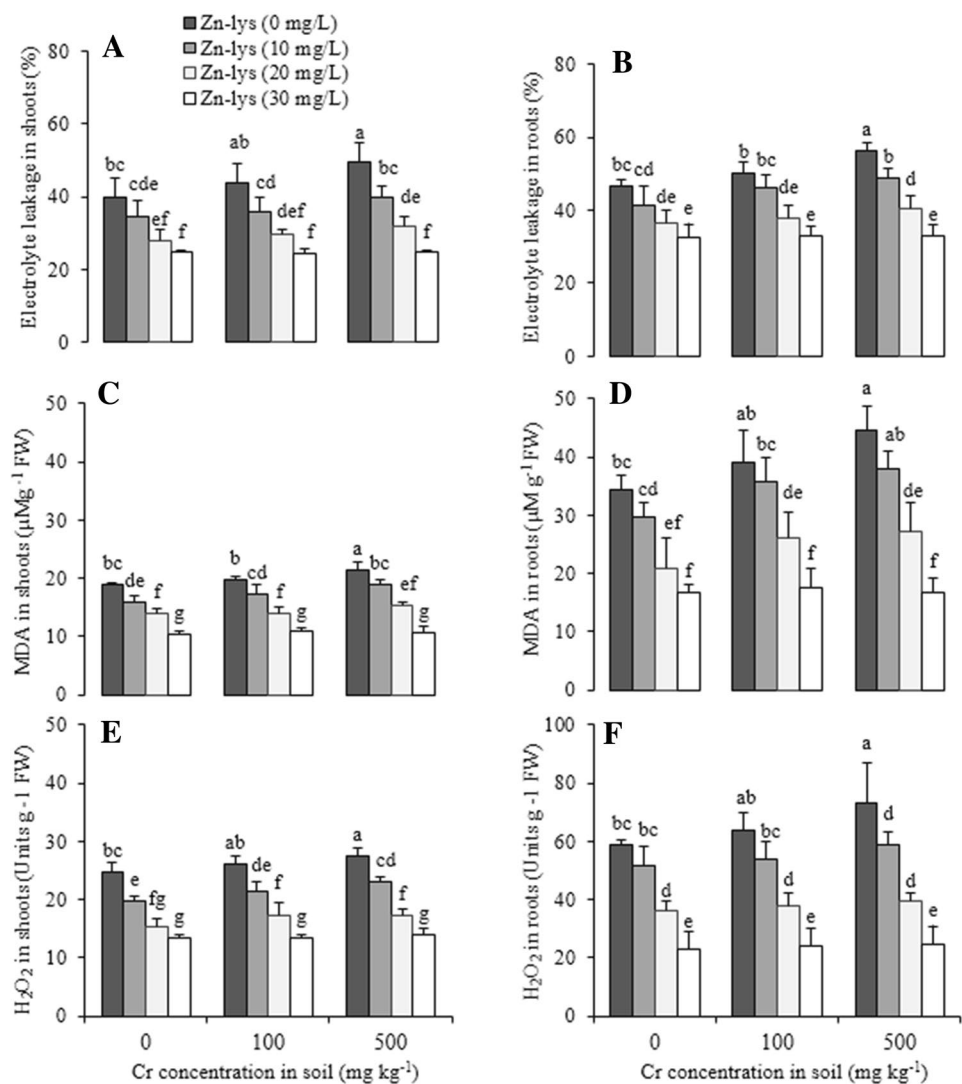
In the present study, dry weights and plant heights of rice decreased by Cr treatment alone (Fig. 1). Chromium-induced reduction in plant biomass and growth has been reported in various crops like rice (Gill et al. 2015a; Shahid et al. 2017), wheat (Adrees et al. 2015a; Ali et al. 2015a), and mung bean (Jabeen et al. 2016). This reduction in dry weights and plant height may be due to the reduction in nutrient uptake of rice plants under Cr stress (Shahid et al. 2017). Also, this reduction may be ascribed to the changes in ultrastructure of mesophyll cells in rice leaves (Gill et al. 2015b). The Zn-lys foliar application significantly alleviated the Cr-induced reduction in production of growth and biomass of rice plants. The valuable role of amino-chelated fertilizers towards morpho-physiological parameters of plants has been reported in many studies under stress (Ghasemi

et al. 2014; Souri 2016; Rafie et al. 2017). Moreover, Zn-amino acid chelates showed better results towards increasing plant growth than that of ZnSO<sub>4</sub> (Rafie et al. 2017). This increase in the plant growth by Zn-lys may be ascribed to the involvement of amino acids in structural components of plants (Nasholm et al. 2009). The Zn-lys was applied as a foliar spray as foliar application of Zn-lys was effective in enhancing plant growth and Zn uptake by plants (Rizwan et al. 2017b).

Chlorophyll contents, carotenoids as well as gas exchange characteristics (GEC) decreased in rice with Cr application alone (Fig. 2). Similar findings have been observed under Cr stress in various crops like mung bean (Jabeen et al. 2016), sunflower (Farid et al. 2017) and wheat (Ali et al. 2015b). The Cr-induced reduction in chlorophyll contents may be due to Cr toxicity towards the photosynthetic machinery of plants (Ali et al. 2015b). However, the Zn-lys foliar application significantly alleviated the Cr-induced reduction in photosynthetic and GEC contents in rice plants. Souri (2016) reported increased plants photosynthesis due to nutrient-amino acid chelates. Increased photosynthesis with Zn-lys under Cr stress may be ascribed to decreased uptake of Cr and increased uptake of Zn in rice plants (Fig. 2).

In our study, we highlighted that with increasing Cr concentrations alone in soil medium, the Cr uptake increased however the Zn contents were decreased both in roots and shoots of rice plants (Fig. 3). A similar increase in Cr concentration has been observed in several crops grown in Cr-contaminated soils (Farid et al. 2017). The

**Fig. 4** Effect of foliar dressing of different concentrations of zinc-lysine (0, 10, 20, 30 mg L<sup>-1</sup>) on electrolyte leakage, MDA, and H<sub>2</sub>O<sub>2</sub> contents in rice plants grown in the soil with increasing concentrations of Cr (0, 100 and 500 mg kg<sup>-1</sup>). Bars represent SD of four replicates. Different letters indicate significant differences among treatments at  $p \leq 0.05$

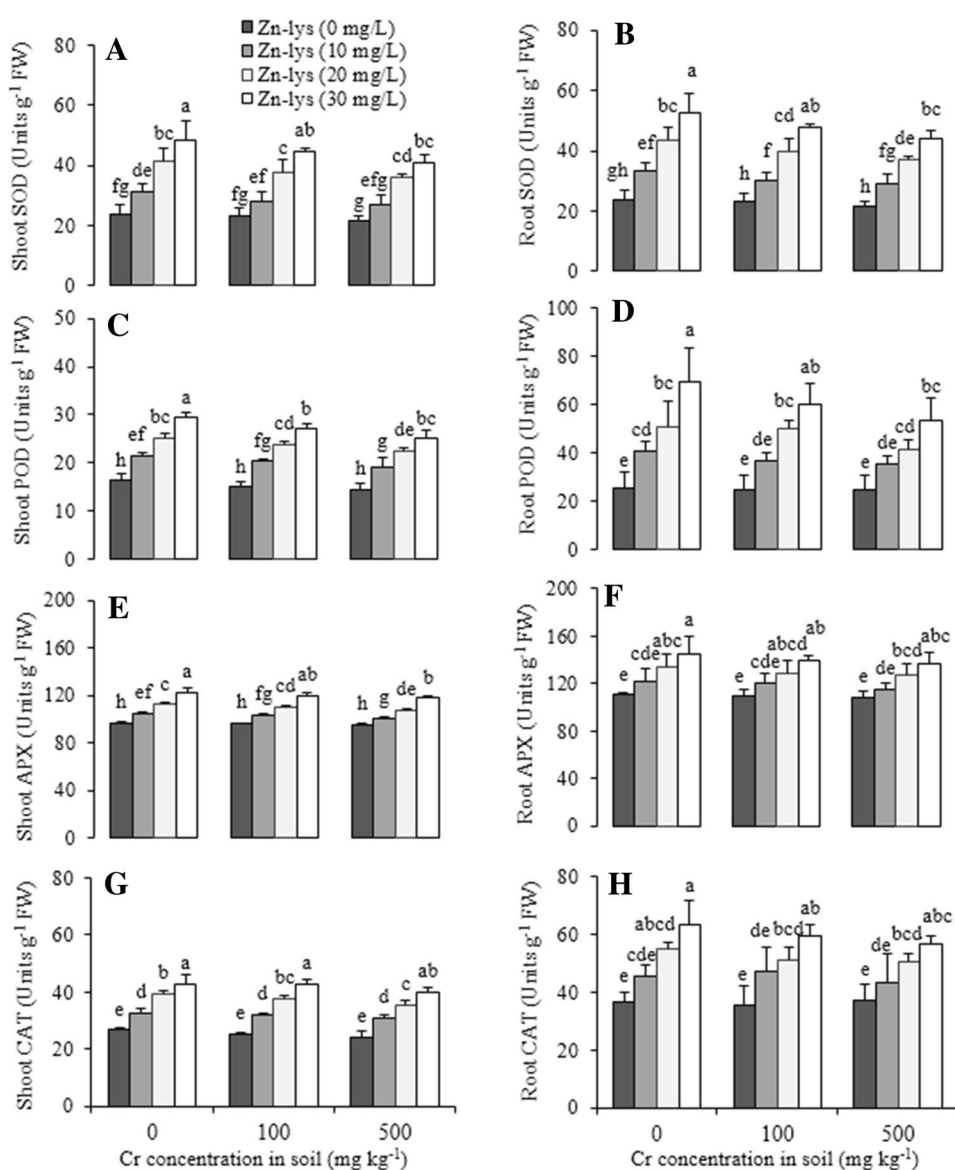


decrease in Zn concentration might be associated with lower nutrient uptake of plants due to Cr toxicity (Tauqeer et al. 2016). However, Zn-lys foliar application significantly reduced Cr uptake and enhanced Zn contents both in roots and shoots of rice (Fig. 3). The decrease in Cr contents may be ascribed to the ability of amino acids to make complexes with metals which reduces their mobility to upper parts of the plants (Sharma et al. 2016). The increased Zn contents with Zn-lys foliar application has been observed in many crops (Rafie et al. 2017; Ghasemi et al. 2014). This increase in Zn contents with Zn-lys foliar supply might be associated with the potential of Zn-lys to counteract and decrease the uptake of Cr in plants. It has been reported that there exists a competition between Zn and Cr as well as other nutrient elements during uptake by plants (Dotaniya et al. 2018). Moreover, Zaman et al. (2017) reported the role of Zn in enhancing chloroplast activity and maintenance of structural integrity of membraned, which increases plant tolerance to Cr

stress. Hence, Zn-lys foliar application is beneficial in Zn biofortification in cereals as in rice in the present study. Zn concentrations increased in shoots and roots of wheat (Fig. 3). This increased uptake of Zn might be effective in Zn biofortification in rice as this crop does not provide enough Zn to humans (Nakandalage et al. 2016).

With increasing levels of Cr alone to the soil media, the contents of EL, MDA, and H<sub>2</sub>O<sub>2</sub> increased both in roots and shoots of rice (Fig. 4). Likewise, results have been observed in various studies under Cr stress (Farid et al. 2017; Habiba et al. 2015). This increase in ROS might be associated with oxidative damage due to Cr (Shahid et al. 2017). However, Zn-lys foliar application reduced Cr-induced overproduction of ROS. Rizwan et al. (2017b) reported that application of Zn reduced ROS overproduction upon heavy metal stress. Moreover, it has also been reported that amino acids have the potential to reduce the overproduction of ROS in plants (Teixeira et al. 2017). The decrease in ROS overproduction due to Zn-lys might be due to the dual role of Zn-lys in

**Fig. 5** Effect of foliar dressing of different concentrations of zinc-lysine (0, 10, 20, 30 mg L<sup>-1</sup>) on SOD, POD, APX, and CAT activities in rice plants grown in the soil with increasing concentrations of Cr (0, 100 and 500 mg kg<sup>-1</sup>). Bars represent SD of four replicates. Different letters indicate significant differences among treatments at  $p \leq 0.05$



protection of rice from toxicity of Cr as well as in scavenging oxidative stress.

Plants develop their self-defense via stimulating anti-oxidants enzymes against heavy metal stress. By increasing Cr concentration alone, the anti-oxidant enzyme activities reduced both in plant roots and shoots (Fig. 5). Similar results have been reported in various plants under Cr stress (Jabeen et al. 2016; Farid et al. 2017). The decrease in anti-oxidant enzyme activities because of Cr stress may be due to the overproduction of ROS through Cr toxicity. However, Zn-lys foliar application minimized the Cr-induced reduction in anti-oxidant enzyme activities (Fig. 5). The amino acid chelated with Fe significantly increased the APX and CAT in leaves of tomato plant (Ghasemi et al. 2012). Also, amino acid foliar application enhanced the anti-oxidant enzyme activities in soybean plants (Teixeira et al. 2017).

The increase in anti-oxidant enzymatic activities by Zn-lys might be due to the reduction in ROS overproduction (Fig. 4) by Zn-lys under Cr stress. So, Zn-lys might be helpful in alleviation of Cr toxicity in rice plants under Cr stress.

## Conclusion

Foliar-applied Zn-lys enhanced the growth and photosynthetic pigments in Cr-stressed rice plants. Foliar-applied Zn-lys also decreased Cr concentrations and increased Zn concentrations in rice plant. Exogenous Zn-lys improved enzymatic anti-oxidant levels but decreased oxidative stress. Finally, foliar Zn-lys applications have shown a significant role in reducing Cr concentrations as well as Zn biofortification in rice thus can be used in soils deficient in Zn and/or



contaminated with Cr in particular and probably with other metals as well.

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**Author Contributions** AH, MR, SA, and MNA conceived the idea and designed research. AH and MR conducted the experiment. AH, SA, and FH did the analysis. MZR, MR, SAA, LW, and AH analyzed the data and developed the first full draft of the manuscript. SAA, MNA, and LW critically reviewed the manuscript. All authors contributed to the subsequent development and approved the final manuscript.

## Compliance with Ethical Standards

**Conflict of interest** No potential conflict of interest was reported by the authors.

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