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RESEARCH ARTICLE

Silicon (Si) alleviates cotton (*Gossypium hirsutum* L.) from zinc (Zn) toxicity stress by limiting Zn uptake and oxidative damage

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Abstract Silicon (Si) is as an important fertilizer element, which has been found effective in enhancing plant tolerance to variety of biotic and a-biotic stresses. This study investigates the Si potential to alleviate zinc (Zn) toxicity stress in cotton (*Gossypium hirsutum* L.). Cotton plants were grown in hydroponics and exposed to different Zn concentration, 0, 25, and 50 μM, alone and/or in combination with 1 mM Si. Incremental Zn concentration in growth media instigated the cellular oxidative damage that was evident from elevated levels of hydrogen peroxide (H_2O_2), electrolyte leakage, and malondialdehyde (MDA) and consequently inhibited cotton growth, biomass, chlorophyll pigments, and photosynthetic process. Application of Si significantly suppressed Zn accumulation in various plant parts, i.e., roots, stems, and leaves and thus promoted biomass, photosynthetic, growth parameters, and antioxidant enzymes activity of Zn-stressed as well unstressed plants. In addition, Si reduced the MDA and H_2O_2 production and electrolyte leakage suggesting its role in protecting cotton plants from Zn toxicity-induced oxidative damage. Thus, the study indicated that exogenous Si application could improve growth and development of cotton crop experiencing Zn toxicity stress by limiting Zn bioavailability and oxidative damage.

Keywords Antioxidant enzymes · Biomass · Zinc · Silicon · Growth

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Introduction

The term heavy metal refers to the elements with an atomic density greater than 6 g cm^{-3} (Adriano 2001). These elements include copper (Cu), manganese (Mn), iron (Fe), cadmium (Cd), mercury (Hg), nickel (Ni), and lead (Pb). Some heavy metals such as Cu, Mn, Fe, and zinc (Zn) are essential for proper plant growth as micronutrients. Despite of their role in plant growth and multiple functions in daily human life, elevated levels of these elements pose serious negative environmental concerns (Ali et al. 2013a; Han et al. 2002). These heavy metals are naturally present in soils in low concentration but unrestrained geological and anthropogenic activities have increased the concentrations of some of these elements to unsafe levels for living organisms (Basta et al. 2001; Dembitsky and Rezanka 2003; Farid et al. 2013a). Higher concentrations of these heavy metals exert strong toxicological effects to all forms of life including microorganisms, plants, animals, and human, although degree of the toxicity varies for different organisms.

Zinc is widely used in different industrial processes for the production of variety of useful products. However, excessive release of Zn from industrial processes such as mine tailings, smelting, and waste disposal or leakage of wastes and use of

these industrial effluents for agriculture purpose has tremendously increased the Zn concentration in agricultural soils (Luo et al. 2000; Zhao et al. 2003).

Zinc deficiency is one of the most widespread micronutrient deficiencies in plants and causes severe reductions in crop production. There are a number of physiological impairments in Zn-deficient cells causing inhibition of the growth, changes in metabolism stunted growth and chlorosis, and differentiation and development of plants (Falkengren-Grerup et al. 1987; Cakmak 2000). Above a certain optimum concentrations (300 mg kg^{-1}), Zn becomes toxic to plant growth in soil (Ehsan et al. 2013; Marschner 1995). Elevated Zn concentrations in soil usually lead to physiological, morphological, and biochemical disturbances that eventually limits plant yield (Cuypers et al. 1999). Major symptoms of Zn toxicity include yield and growth inhibition, and leaf chlorosis (Broadley et al. 2007; Tewari et al. 2008). Elevated Zn levels in soil interfere with the uptake, translocation, and regularization of essential ions by changing their ionic homeostatic system. Nutrient deficiency, in turn, impairs various metabolic processes vital for plant growth such as photosynthesis, transpiration, and enzymatic activities (Ali et al. 2013b; Abbas et al. 2009; Broadley et al. 2007).

Silicon is the second most abundant element and fills up 28 % of the total earth crust (Epstein 1994; Gong et al. 2006; Ming et al. 2012). Its concentration in the soil solution ranges from 0.01 to 1.99 mM and is controlled by silicate minerals. Generally, silicon (Si) is not considered an essential micronutrient for growth of higher plants (Epstein 1999). However, it has been applied as an important fertilizer component which enhances plant tolerance to variety of biotic and a-biotic stresses (Hattori et al. 2005; Liang et al. 2005).

Silicon has been found effective to ameliorate heavy metal toxicity stress in many plant species (Rogalla and Romheld 2002; Shi et al. 2005) mainly by increasing solution pH and limiting metal phyto-availability (Cocker et al. 1998); therefore, Si could be the potential candidate for the alleviation of Zn toxicity and uptake.

The present study was planned to explore the potential of Si in ameliorating negative effects of Zn stress on growth, biomass, antioxidant enzymes activities, photosynthetic pigments, electrolyte leakage, and gas exchange attributes of cotton (*Gossypium hirsutum* L.) seedlings.

Materials and methods

Experimental site

The complete set of experiments for study was conducted at the wire house of Ayub Agricultural Research Institute (AARI), Pakistan, and all biochemical analyses were performed in labs of Government College University, Faisalabad, Pakistan.

Plant material and growth conditions

Cotton leaf curl virus (CLCuV) is one of the major biotic constraints of cotton production (Akhtar et al. 2005). Healthy seeds of high-yielding and CLCuV-resistant cotton genotype (MNH 886) were immersed in the concentrated sulfuric acid solution for 15 min to remove the short fiber from the seed surface. The seeds were then thoroughly rinsed with distilled water and sown in 2-in. layers of sterilized quartz sand trays in a growth chamber under a photoperiod of 16-/8-h light/dark and light intensity of 400±25 $\mu molm^{-2}$ s^{-1}. The day/night temperature was set at 30/25 °C with relative humidity at 85 %. After 2 weeks of germination, the uniform seedlings were wrapped with foam at a root shoot junction and transplanted in thermo pore sheets having evenly spaced holes floating on 40-L capacity tubs, lined with polyethylene sheet containing modified Hoagland's solution (Ehsan et al 2014). The basic nutrient medium had composition $(Ca(NO_3)_2$ 2.5 mM, $MgSO_4$ 1 mM, KCL 0.5 mM, KH_2PO_4 0.5 mM, $FeCl_3$ 0.1 μM, $CuSO_4$ 0.2 μM, $ZnSO_4$ 1 μM, H_3BO_3 20 μM, H_2MoO_4 0.005 μM, $MnSO_4$ 2 μM). Continuous aeration to nutrient solution was supplied by air pump. The solution was changed every week. Plants were grown in a complete randomized design (CRD) with three replicates. Two weeks after transplanting, Zn levels (0, 25, and 50 μM) were developed by $ZnCl_2$ application and Si was as sodium silicate (Na_2SiO_3) (0 and 1 mM). The pH of solution was maintained to 6.0±0.1 by adding 1 M H_2SO_4 or NaOH.

Measurement of plant growth and biomass

After the 60 days of Zn and Si treatment, plants were harvested and various growth parameters such as plant height, root length, and number of leaves per plant were measured. Fresh biomass measurements were recorded immediately after harvesting, and same samples were used for biochemical analysis. For dry biomass measurements, the plant parts (leaf, stem, and root) were stored in oven with 70–80 °C for 72 h and then dry biomass was recorded and same samples were used for measurement of Zn content. Leaf area of individual plants was measured by leaf area meter (LI-2000, LI-COR, USA).

Soil-plant analysis development value

For the analysis of leaf greenness/soil-plant analysis development (SPAD) value, SPAD-502 (Zheijang Top Instruments Co., Ltd., China) meter was used.

Gas exchange parameters

At the end of experiment (60 days after treatment), various gas exchange parameters such as photosynthetic rate (A), stomatal conductance (Gs), transpiration rate (E), and water use efficiency (A/E) of second fully expanded leaves were determined using Infra-Red Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England).

Assessment of electrolyte leakage

At the end of experiment (after 6 weeks), the topmost fully expanded leaves were cut into 5-mm-long fragments and positioned in test tubes filled with 8-mL distilled water. The tubes were incubated in a water bath at 32 °C for 2 h, and initial electrical conductivity (EC) of the medium EC_1 was noted. The samples were autoclaved at 121 °C for 20 min to discharge all electrolytes, and then cooled at 25 °C, and final EC_2 was measured (Dionisio-Sese and Tobita, 1998). Electrolyte leakage (EL) was recorded by using pH/conductivity meter (model 720, INCO-LAB Company, Kuwait) and calculated by the following formula:

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

Measurement of chlorophyll contents and total carotenoids

Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids of the topmost fully expanded cotton leaves were determined by spectrophotometer after the 60 days of treatment. Fresh weight (0.2 g) of leaves were extracted in 85 % (*v*/*v*) aqueous acetone for an overnight and the absorbance at of extract was measured at 452.5, 644, and 663 nm using a spectrophotometer (AA6300, Shimadzu, Kyoto, Japan). Chl a, b, total chlorophyll, and carotenoids contents were calculated using the following equations:

$$\text{Chlorophyll a } (\mu g/mL) = 10.3 * E_{663} - 0.98 * E_{644}$$
$$\text{Chlorophyll b } (\mu g/mL) = 19.7 * E_{644} - 3,87 * E_{663}$$
$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b}$$
$$\text{Totalcarotenoids}(\mu g/mL) = 4.2 * E_{452.5} - \{(0.0264 * \text{chla}) + (0,426 * \text{chlb})\}$$

Finally, the pigment fractions were calculated as milligrams per gram leaf fresh biomass.

Measurements of anti-oxidant enzymes activities

For evaluation of antioxidant enzymes, 0.5-g fresh leaf and root samples were extracted in a cold buffer solution. Different buffers solutions were employed for each enzyme. The extracted samples were centrifuged at 15,000 rpm for 20 min under 4 °C. The supernatant was collected in micro centrifuge tubes and used for determining the activities of antioxidant enzymes.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was analyzed by nitroblue tetrazolium (NBT) method (Beauchamp and Fridovich 1971) by calculating the NBT photoreduction. The reaction mixture (3 mL) used for measuring absorbance at 560 nm was consisted of 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 10 μM EDTA, 2 mM riboflavin, and enzyme extract (100 μL). Reaction in mixture was initiated by placing tubes under two 15-W fluorescent lamps for 10 min.

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed according to method of Putter and Becker (1974) with some modification. Reaction mixture (3 mL) consisted of 100 μL enzyme extract, 100 μL guaiacol (1.5 %, v/v), 100 μL hydrogen peroxide (H_2O_2) (300 mM), and 2.7 mL (25 mM) potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring the change absorbance due to oxidation of guaiacol at 470 nm (ε=26.6 mM^{-1} cm^{-1}).

Catalase (CAT, EC 1.11.1.6*)* activity was determined by Aebi (1984) method. The assay mixture (3.0 mL) was comprised of 100 μL enzyme extract, 100 μL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was assayed by monitoring reduction in absorbance at 240 nm as a consequence of H_2O_2 disappearance (ε=39.4 mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to Nakano and Asada (1981) method. The reaction mixture containing 100 μL enzyme extract, 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation of ascorbate was observed by monitoring change in absorbance at 290 nm (ε=2.8 mM^{-1} cm^{-1}).

Hydrogen peroxide contents

H_2O_2 was extracted by homogenizing 50 mg leaf or root tissues with 3 mL of phosphate buffer (50 mM, pH 6.5). Then, the homogenate was centrifuged at 6000×*g* for 25 min. To measure H_2O_2 content, 3 mL extracted solution was mixed with 1 mL 0.1 % titanium sulfate in 20 % (*v*/*v*) H_2SO_4, and mixture was centrifuged at 6,000×*g* for 15 min. The intensity of yellow color of the supernatant was measured at 410 nm. H_2O_2 content was computed using the extinction coefficient of 0.28 μmol^{-1} cm^{-1}.

Malondialdehyde content

Lipid peroxidation of leaf and root tissues was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) contents by thiobarbituric acid (TBA) reaction method

(Heath and Packer 1968), with minor modifications as described by Dhindsa et al. (1981) and Zhang and Kirham (1994).

Soluble protein content

Estimation of soluble protein contents was carried out according to Bradford (1976) method, using Coomassie Brilliant Blue G-250 as a dye and albumin as a standard.

Estimation of zinc concentration and accumulation in cotton

After 60 days treatments, cotton plants from each treatment were harvested and thoroughly washed with tap water, distilled water, and deionized water three times, respectively. Plants were then separated into roots, shoots, and leaves and dried at 80 °C in an oven for 48 h, and then ground into powder. Of each sample, 0.5 g were dry-ashed, extracted with and dissolved in 10 mL of concentrated H_2SO_4, and then centrifuged it at 400 rpm for 15 min. Zn concentrations in root, shoot, and leaf tissues were determined by flame atomic absorption spectrometry (Nov Aa 400 Analytik Jena, Germany).

The concentration of Zn in plant root, stem and leaf was measured by the following formula:

$$\text{Zn concentration}\left(\text{mg kg}^{-1}\right) = \text{reading} \times \text{dilution factor}/\text{dry wt. of plant part}$$

The accumulation of Zn in plant shoot and root was estimated by the following formula:

$$\text{Zn accumulation}\left(\mu\text{g plant}^{-1}\right) = \text{conc. of Zn} \times \text{dry wt. of plant organ}$$

Statistical analysis

All values presented in this experiment are mean of three replicates. A statistical package, SPSS version 21.0 (SPSS, Chicago, IL), was used for analyzing the data. A three-way variance analysis (ANOVA) was carried out, followed by the Duncan's multiple range tests to define the substantial difference among means of treatments.

Results

Increasing Zn concentrations in the growth media significantly inhibited cotton growth. Although, effects of lower Zn concentration were not significantly different in comparison to Zn 50 μM. Induction of Si enhanced ability of these plants to cope the Zn toxicity stress and significantly increased the plant growth in term of plant height, root length, number of leaves per plant, and leaf area in each respective treatment. The effect of exogenous Si on biomass of different plant parts under Zn stress is shown in Table 1.

In addition, Si-treated cotton plants contained significantly higher biomass compared with control treatment; illustrating the multidimensional ability of Si to mitigate environmental stresses. Higher Zn concentration (50 μM) significantly reduced fresh and dry weight of leaf, stem, and root compared with control. Exogenous Si application significantly recovered the biomass production in Zn (25 and 50 μM) toxicity-stressed cotton plants.

Toxic Zn concentrations in the growth media significantly inhibited various leaf gas exchange parameters such as net photosynthetic rate (E), transpiration rate (A), stomatal conductance (Gs), and water use efficiency (A/E) of cotton plants (Fig. 1a–c). Application of higher Zn concentration (Zn 50 μM) alone caused maximum reduction in all the gas exchange parameters compared with any other treatment. Silicon significantly recovered the gas exchange parameters of Zn toxicity-stressed plants; it provided assistance to Zn_{50}+Si and Zn_{25}+Si, up to 32 and 25 %, respectively. In addition, Si treatment to non-stressed caused 20 % increase in all gas exchange.

Effect of Zn toxicity on chlorophyll a, b, total chlorophyll, and total carotenoids contents of cotton plants with supplementation of Si is illustrated in the in Fig. 2a–d. Chlorophyll contents (a, b, and total) significantly decreased with the increasing Zn concentrations in growth media Chlorophyll contents of cotton leaves showed a decreasing trend under various treatment, i.e., Zn_{50}>Zn_{50}+Si>Zn_{25}>Zn_{25}+Si>Si> control (from maximum to minimum reduction). Zn_{50} treatment caused 66 and 60 % reduction in chlorophyll a and chlorophyll b contents, respectively, compared with control, while 45 % reduction was recorded in each of chlorophyll a and b contents under Zn_{25}. Adding Si to growth media caused significant increase in the chlorophyll a, 23.2 % for Zn_{25}, 26.2 % for Zn_{50} than the respective Zn treatments alone. In addition, it caused 10 % increase in chlorophyll a content in comparison to control, while addition of Si created accommodating condition for growth and alleviated the effect of Zn more than 21 and 31 % for Zn_{50} and Zn_{25}, respectively. Similar trend was followed by the protein content and SPAD value in cotton plant (Fig. 2c, d). The protein content was higher in leaves than roots while the maximum protein content was measured in treatment having Si alone while the minimum was measured observed in Zn_{50}.

Zn-induced solute leakage in cotton leaves and roots is presented in Fig. 2c. A significant increase in the electrolyte leakage was recorded under Zn stress (25 and 50 μM). Silicon

Table 1 Effect of different concentrations of zinc and silicon on growth and biomass characteristics of cotton

Treatments	Root length (cm)	Plant height (cm)	Leaf area (cm^2)	No. of leaves per plant	Root fresh weight (g)	Root dry weight (g)	Stem fresh weight (g)	Stem dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)
Control	32.33±1.18b	39.40±1.04a	65.23±2.14a	28.33±0.98b	4.13±0.20b	1.33±0.07b	9.30±0.40a	2.15±0.04b	13.20±0.78b	2.50±0.07b
Si 1 mM	36.61±1.21a	42.70±3.10a	68.74±1.43a	32.67±1.12a	5.10±0.12a	1.57±0.08a	10.36±1.26a	2.30±0.13a	14.70±1.21a	2.84±0.09a
Zn25	18.73±1.49d	21.50±1.61c	36.19±2.49c	17.67±1.76d	2.90±0.17d	0.75±0.04d	5.93±0.73c	1.24±0.08d	7.89±0.76d	1.34±0.06d
Zn25+Si	23.63±2.21c	31.47±2.71b	48.48±3.68b	20.33±0.78c	3.63±0.05c	0.91±0.05c	7.93±0.54b	1.56±0.03c	10.16±0.56c	1.86±0.05c
Zn50	9.10±0.81f	14.47±0.22e	26.31±1.62e	7.67±0.54f	1.46±0.09f	0.32±0.04f	3.36±0.27e	0.45±0.02f	5.30±0.12f	0.47±0.01f
Zn50+Si	14.46±1.15e	18.13±1.69d	31.27±2.58d	10.67±1.01e	2.10±0.10e	0.52±0.03e	4.80±0.15d	0.95±0.05e	6.33±0.21e	0.83±0.03e

Values are the means of replicates±S.D. Means followed by similar small letters are not significantly different at $P \leq 0.05$

application to Zn-stressed or Zn-unstressed cotton plants significantly lowered the solute leakages in leaf and root tissues.

Modification in antioxidant enzymes such as SOD, POD, CAT, and APX of cotton leaves and roots under various treatments of Zn and Si as alone or in combination is described in Fig. 3a, b. Control cotton plants showed the higher values of antioxidant enzymes. Under Zn toxicity stress at 25 μM, all four tested antioxidant enzymes showed different behavior as compared to control. While, as the concentration of Zn increased, the activities of enzymes decreased but exogenous application of silicon to the zinc-treated plants had synergetic effect on the anti-oxidant enzyme activities on both Zn (25 and 50 μM).

Increasing Zn concentration significantly elevated the MDA and H_2O_2 levels in both cotton root and leaf tissues. (Fig. 3c, d). Si application significantly inhibited the Zn toxicity-induced oxidative damage to cotton plants by lowering MDA and H_2O_2 levels.

Increasing levels of Zn in growth media significantly increased the leaf Zn contents, i.e., Zn accumulation was 352 mg kg^{-1} DW under Zn_{25} that almost doubled under Zn_{50} treatments (653 mg kg^{-1} DW) (Fig. 4). Silicon treatments helped plant to limit Zn uptake to under no Zn toxic conditions. Thirty-four and 19 % reduction in Zn uptake was observed under Zn_{50}+Si and Zn_{25}+Si, respectively, compared with Zn only treatments. Zn uptake increased in order of control <Si<Zn_{25}+Si<Zn_{25}<Zn_{50}+Si<Zn_{50}.

Zinc accumulation in cotton stem tissues was to 1055 mg kg^{-1} DW under Zn_{50}, while Si treatment caused up to 23 % reduction in Zn uptake.

Cotton roots accumulated maximum Zn concentration compared with leaves and stem. Under Zn_{50} treatment, Zn concentrations in root tissues reached as high as 3600 mg kg^{-1} DW that was approximately 20 times higher compared with control. Zn uptake under Zn_{25} concentration was 2177 mg kg^{-1} DW that was significantly higher compared with control plants and significantly lowers than the Zn_{50} treatment. Addition of Si significantly decreased metal accumulation in cotton root tissues under various Zn levels, i.e., 30 and 48 % reduction in Zn_{50} and Zn_{25} treatments, respectively.

Discussion

This study investigated the Zn toxicity effects on morphological, physiological, and biochemical properties of cotton plants and role of Si in alleviating Zn-induced damage. Zn toxicity negatively influenced the various plant growth parameters such as fresh and dry biomass and length of root, stem, and leaves suggesting that toxic levels of Zn in the environments could potentially limit the cotton yield. Previous studies suggested growth and yield reduction of various crop species under Zn toxicity (Morina et al. 2010; Kaya et al.

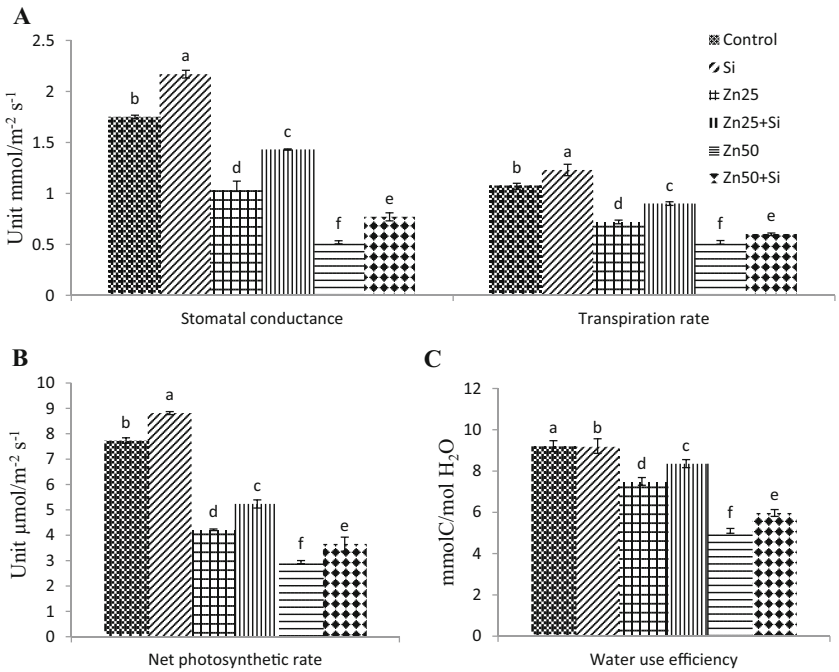

Fig. 1 Effect of Zn and Si on transpiration rate, stomatal conductance (**a**), net photosynthetic rate (**b**), and water use efficiency (**c**) of cotton seedlings grown in solution culture with different Zn treatments (0, 25, and 50 μM) treated or not with 1 mM Si. Values are mean±SD (n=3). *Different letters* indicate that values are significantly different at $P<0.05$

2009). Cotton growth reduction observed in the present study could be the result of Zn-induced oxidative damage to membranes which are caused by upregulation of H_2O_2 and MDA levels under Zn toxicity. Zinc toxicity-induced lipid membrane peroxidation impaired plant biomass producing machinery (chlorophyll pigments) and photosynthetic process leading towards growth inhibition. Reduction in leaf chlorophyll contents could be associated with peroxidation of chloroplast membranes and inhibited chlorophyll biosynthesis and protochlorophyllide reductase activity through Zn-induced activation of chlorophyll degrading species chlorophyllase (Drazkiewice 1994; Rao et al. 2007).

Increasing Zn concentrations in growth media significantly increased Zn concentrations in all plant tissues (root, stem, and leaf) which results in decreasing of plant biomass, chlorophyll content, and gas exchange characteristics along with antioxidant enzymes activities. In addition, elevated Zn concentration in growth media competed with the uptake of micro and macro nutrients and the damage was exacerbated by root growth inhibition. This idea was supported by Cuypers et al. (2001), who observed Zn toxicity evoked visible damage and physiological disorders in common bean (*Phaseolus vulgaris* L.) by disturbing cell division.

In response to elevated levels of tissue Zn concentrations and oxidative stress, cotton plants exhibited modification in anti-oxidant enzyme activity. Environmental adverse effects such as metal stresses usually leads to unbalanced hyper generation of ROS while antioxidant enzymes are known to have important roles in plant stress tolerance (Mittler et al. 2004). Upregulation of SOD and POD activity under lower Zn concentration, i.e., Zn_{25} and reduction under Zn_{50} suggested limited capacity of cotton to defy Zn toxicity-induced oxidative burst.

Modifications in activities of antioxidative enzymes are related to Zn toxicity that causes redox imbalances (Morin et al. 2010). Superoxide dismutase principally plays an important role in ROS detoxification by catalyzing of free O_2^- to O_2 and H_2O_2 in (Bonnet et al. 2000). Increased SOD activities in both root and leaf tissues were recorded in (plant name) under Zn toxicity (Morin et al. 2010), while increased POD activity was discussed by Luo et al. (2010) in jatropha. The enzymes act as scavengers of H_2O_2 and other ROS and are actively involved in the biosynthesis of lignin (Gonzalez et al. 1999). Degenhardt and Gimmler (2000) and Küpper et al. (2000) also discovered that Zn prefers to accumulate at root parts; in cell walls, of the xylem parenchyma and in endodermal layer more than any other part of plant. Morina et al. (2010) referred Zn toxicity-induced oxidative stress is due to high affinity of Zn to root cell walls in *Verbascum thapsus* L. plants.

Significantly higher Zn concentrations in roots compared with leaf tissues suggested cotton roots restrict Zn mobility, it was also evident from little or no change in Zn translocation from roots to leaves by raising Zn concentrations from 25 to 50 μM in the growth media which leads to release of electrolytes in root and leaf tissues. However, increase in Zn contents in cotton root tissues to toxic level (20-fold) under higher Zn levels inhibited plant growth. Although Zn is an essential micronutrient for plant growth and physiological processes, its concentration above a certain limit could impair physiological function.

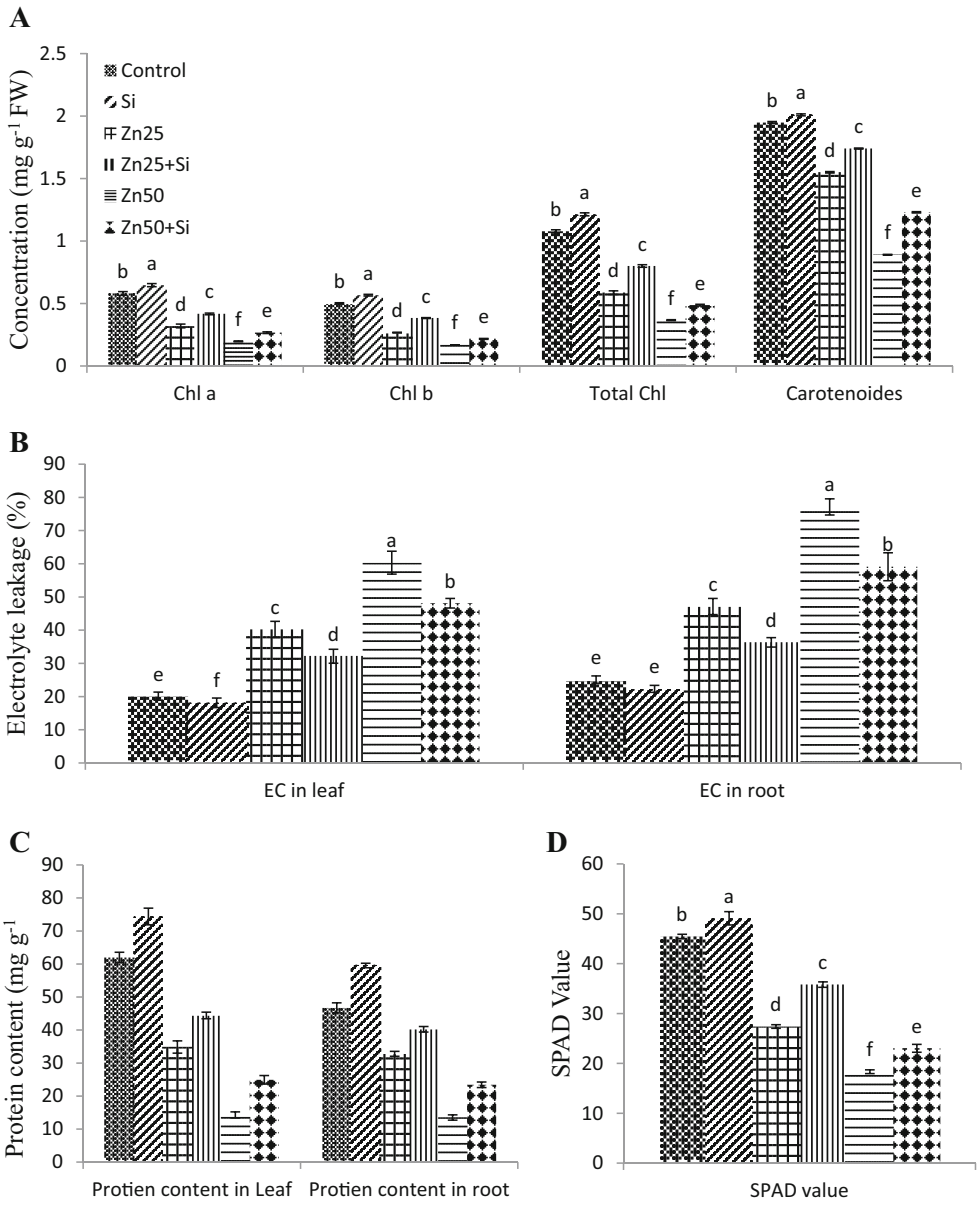

Fig. 2 Effect of Zn and Si on chlorophyll a and b, total chlorophyll, and total carotenoids (**a**), electrolyte leakage (**b**), protein content (**c**), and SPAD value (**d**) in leaf and roots of cotton seedlings grown in solution culture with different Zn treatments (0, 25, and 50 μM) treated or not with 1 mM Si. Values are mean±SD (n=3). *Different letters* indicate that values are significantly different at $P<0.05$

Zinc-induced significant increase in leaf chlorophyll and gas exchange parameters of Zn-stressed plants improved biomass accumulation process. Previous studies suggested effectiveness of Si in plant against biotic and a-biotic stresses (Gurmani et al. 2013; Currie and Perry 2007; Liang et al. 2007). It is likely that the high plant biomass in Si-treated soil provoked a dilution effect on Zn concentration, whereby minimizing Zn-induced damages to plants. Positive effect of Si on Zn-stressed maize and tobacco plants were result of increased Fe concentration in leaf tissues stimulating chlorophyll synthesis (Szlek et al. 1990; Miller et al. 1995; Pei et al. 2010).

In the present study, Si greatly reduced Zn accumulation in cotton suggesting its role in reducing Zn bioavailability by changing the solution pH (Chen et al. 2000; Liang et al. 2005, 2007). Siliconmediated detrimental effects of Zn toxicity on cotton by limiting Zn uptake and translocation and thus the cellular oxidative damage. Under lower Zn levels, Si was relatively more effective in controlling Zn uptake possibly through reducing Zn bioavailability and exclusion from root tissues, as was evident from 48 % reduction in root Zn concentration. However, Si induced a parallel reduction in root and leaf Zn under Zn_{50} suggested Si was effective to suppress Zn bioavailability up to certain levels, and higher Zn influx into root tissues could be expected under increased Zn concentration. Inside the plant cells, Si improves tolerance to Zn toxicity by forming Zn-silicate in cytoplasm (Neumann and Nieden 2001; Gong et al. 2005). Silicon-induced inhibited

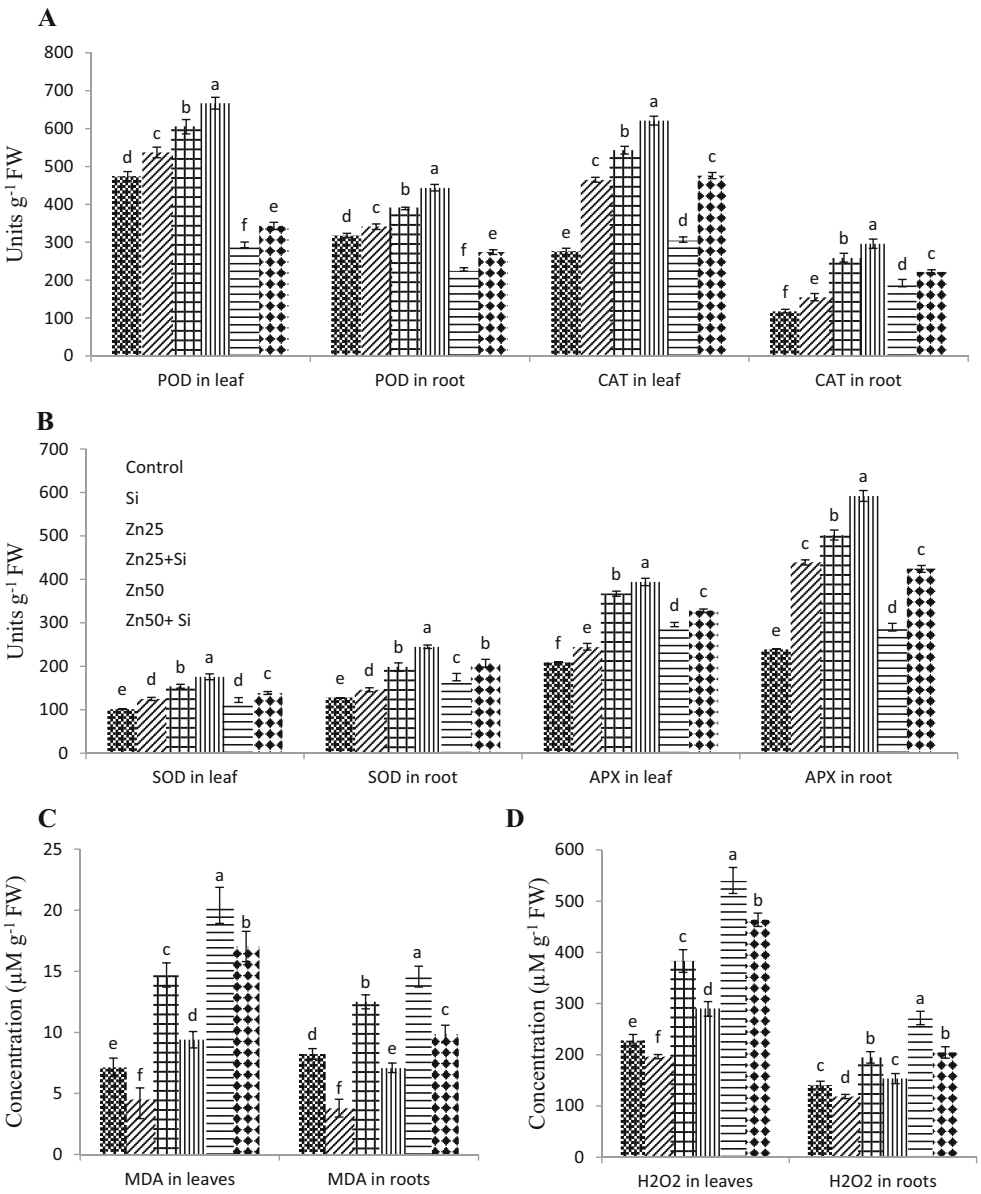

Fig. 3 Ant-oxidative enzyme activities POD and CAT (**a**), SOD and APX (**b**), MDA (**c**), and H_2O_2 (**d**) in leaves and roots of cotton seedlings grown in solution culture with different Zn treatments (0, 25, and 50 μM) treated or not with 1 mM Si. Values are mean±SD (n=3). *Different letters* indicate that values are significantly different at $P<0.05$

Zn translocation to cotton shoots was result of Si deposition on cell wall forming a barrier to apoplastic route (Shi et al. 2005; Gong et al. 2008; Farid et al. 2013b). In addition to limiting Zn uptake and translocation, Si upregulated the activities of anti-oxidant enzymes (SOD and POD) assisting plant to defy Zn-induced oxidative damage.

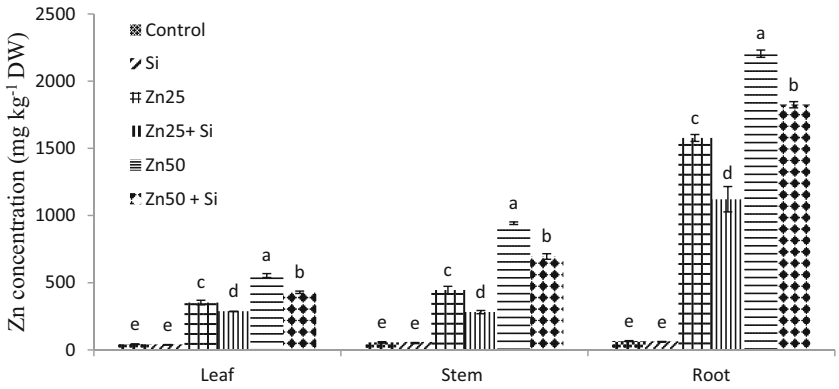

Fig. 4 Zinc concentration in leaf, stem, and roots of cotton seedlings grown in solution culture with different Zn treatments (0, 25, and 50 μM) treated or not with 1 mM Si. Values are mean±SD (n=3). *Different letters* indicate that values are significantly different at $P<0.05$

Conclusion

In present study, Si application significantly reduced Zn uptake and toxicity to cotton and improved plant growth, biomass, chlorophyll content, photosynthetic parameters, and gas exchange attributes. Since higher translocation of metals to the aboveground plant parts invokes the risk of metal entry into food chain. Si-induced reduced metal bioavailability and uptake, and its translocation suggests its potential use for plants, especially vegetable crops growing under metal-polluted soils or irrigated with contaminated waters.

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