



# Silicon deposition in roots minimizes the cadmium accumulation and oxidative stress in leaves of cowpea plants

Talitha Soares Pereira<sup>1</sup> · Thaís Soares Pereira<sup>1</sup> · Carla Leticia Figueredo de Carvalho Souza<sup>1</sup> · Emilly Juliane Alvino Lima<sup>1</sup> · Bruno Lemos Batista<sup>2</sup> · Allan Klynger da Silva Lobato<sup>1</sup>

Received: 1 June 2017/Revised: 15 November 2017/Accepted: 7 December 2017/Published online: 19 December 2017  
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**Abstract** Silicon (Si) frequently accumulates in plants tissues, mainly in roots of dicotyledons, such as cowpea. By contrast, Cadmium (Cd) is a metal that is extremely toxic to plant metabolism. This research aims to investigate if the deposition of Si in root can reduce Cd contents and minimize its negative effects on leaves, measuring gas exchange, chlorophyll fluorescence, antioxidant metabolism, photosynthetic pigments and growth, which may explain the possible role of Si in the attenuation of Cd toxicity in cowpea. This study had a factorial design, with all factors completely randomized and two Cd concentrations (0 and 500  $\mu\text{M}$  Cd, termed as  $-$  Cd and  $+$  Cd, respectively) and three Si concentrations (0, 1.25 and 2.50 mM Si). Si reduced Cd contents in the roots and in other plant organs, such as stems and leaves. The Si contents were highest in roots, followed by stems and leaves, which was explained by the passive absorption of Si. The application of Si promoted increase in both the macro- and micronutrient contents in all tissues, suggesting that Si mitigates the effect of Cd on nutrient uptake. Si attenuated Cd-mediated effects on light absorption of photosystem II (PSII), increasing the effective quantum yield of PSII photochemistry and the electron transport rate. Additionally, toxic effects induced by Cd on gas exchange were mitigated by the action of Si. Plants treated with Cd + Si showed increase in the activities of antioxidant enzymes and reductions in oxidant compounds; these modifications

were promoted by Si via detoxification mechanisms. Increases in the photosynthetic pigments and growth of plants treated with Si and exposed to Cd stress were detected and were due to the reduced deterioration of cell membranes and maintenance of chloroplasts, which had positive repercussions on growth and development. This study validated the hypothesis that the accumulation of Si in roots induces benefits on metabolism and alleviates the toxic effects caused by Cd in leaves of cowpea.

**Keywords** Antioxidant system · Beneficial element · Chlorophyll fluorescence · Growth · Heavy metal · *Vigna unguiculata*

## Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important high protein food legume that is used in human and livestock diets (Agbicodo et al. 2009). This crop is moderately tolerant to drought and largely cultivated in semi-arid tropical regions covering Asia, Africa, Central and South America. Cowpea also has a high capacity to fix atmospheric nitrogen and shows adequate growth, even on soils with low fertility (Ehlers and Hall 1997; Singh et al. 2003).

Cadmium (Cd) is a highly toxic heavy metal that can reach high concentrations in agricultural soils, and it is easily assimilated (Krantev et al. 2008). Cd can be found in high amounts in mining areas or in areas that have received large amounts of herbicides and/or fertilizers, mainly phosphate fertilizers (Tran and Popova 2013). Organic matter, temperature, pH, the concentration of other minerals in the soil and the redox potential are factors that can favour Cd absorption by plants (Benavides et al. 2005).

✉ Allan Klynger da Silva Lobato  
allanlobato@yahoo.com.br

<sup>1</sup> Núcleo de Pesquisa Vegetal Básica e Aplicada, Universidade Federal Rural da Amazônia, Rodovia PA 256, Paragominas, Pará, Brazil

<sup>2</sup> Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, São Paulo, Brazil

When in excess, Cd causes phytotoxicity and interferes negatively with diverse metabolic processes, such as the decreased absorption of essential nutrients, leading to nutritional deficiency (Irfan et al. 2013), damage to the photosynthetic apparatus (Chen et al. 2011; Dias et al. 2013), reduction of the growth rate (Gill et al. 2012), increase of lipid peroxidation (Hassan et al. 2005), and induction of oxidative stress due to the production of reactive oxygen species (Cho and Seo 2005; Gill and Tuteja 2010). The accumulation of Cd in plant tissues may also represent a risk to the health of humans and animals feeding on these plants (Vecchia et al. 2005).

Silicon (Si) is the second most abundant element in soil (Vaculíková et al. 2016). Plants can assimilate Si using their roots in the form of silicic acid [Si(OH)<sub>4</sub>], in both passive and active forms. Si is translocated to shoot via the xylem (Ma and Yamaji 2006). Si is not recognized as an essential element, but its beneficial effects on biotic and abiotic stress have been found in several species (Côté-Beaulieu et al. 2009; Farooq et al. 2013).

The action of Si on the attenuation of the negative effects caused by Cd has been described in the literature, including the improvement of antioxidant responses in *Arachis hypogaea* (Shi et al. 2010), maintenance of membrane integrity in *Echium amoenum* (Amiri et al. 2012), increase in biomass of *Zea mays* (Dresler et al. 2015), maximization of growth by *Vicia faba* (Abu-Muriefah 2015), *Cucumis sativus* and *Solanum lycopersicum* (Wu et al. 2015), higher photosynthetic performance of *Cucumis sativus* (Harizanova et al. 2014), and reduction of Cd content in *Triticum turgidum* (Rizwan et al. 2012). However, there are no studies on *Vigna unguiculata*.

There are different tolerance mechanisms to metal induced by Si in higher plants, being described as compartmentation (Jesus et al. 2017), coprecipitation (Neumann and Nieden 2001) and/or chelation of heavy metals in different plant parts, mainly root and stem (Wu et al. 2013; Adrees et al. 2015). In relation to soil, studies conducted by Treder and Cieslinski (2005) and da Cunha et al. (2008) showed that Si immobilizes Cd and Zn. Specifically to cowpea plants, the probable strategy used to avoid the Cd toxicity is currently unknown.

Si frequently accumulates in plants tissues, mainly in the roots of dicotyledons, such as cowpea. By contrast, Cd is a metal that is extremely toxic to plant metabolism. This study, therefore, aimed to investigate whether Si deposition in roots could reduce Cd contents and minimize its negative effects on leaves, evaluating gas exchange, chlorophyll fluorescence, antioxidant metabolism, photosynthetic pigments and growth, demonstrating the possible roles of Si related to the attenuation of Cd toxicity in cowpea plants.

## Materials and methods

### Location and growth conditions

The experiment was performed at the Campus of Paragominas of the Universidade Federal Rural da Amazônia, Paragominas, Brazil (2°55'S, 47°34'W). The study was conducted in a greenhouse with controlled temperature and humidity. The minimum, maximum, and median temperatures were 23, 32 and 26.5 °C, respectively. The relative humidity during the experimental period varied between 60 and 80%.

### Plants, containers and acclimation

*Vigna unguiculata* L. cv. BR3-Tracuateua seeds were germinated and grown in 1.2-L pots (0.15 m in height and 0.10 m in diameter) filled with a mixed substrate of sand and vermiculite at a ratio of 3:1. Plants were cultivated under semi-hydroponic conditions, and the pots had one hole in the bottom covered with mesh to maintain the substrate and aerate the roots. Solution absorption was by capillarity, with these pots placed into other containers (0.15 m in height and 0.15 m in diameter) containing 500 mL of distilled water for five days. A modified Hoagland and Arnon (1950) solution was used for nutrients, and the ionic strength began at 50% and was modified to 100% after one day. After one day, the nutritive solution remained at total ionic strength.

### Experimental design

The experiment had factorial design with the two factors completely randomized, being two Cd concentrations (0 and 500 μM Cd, described as – Cd and + Cd, respectively) and three Si concentrations (0, 1.25 and 2.50 mM Si). With five replicates for each of the six treatments, a total of 30 experimental units were used in the experiment, with one plant in each unit.

### Plant growth and treatments with Si and Al

Six-day-old plants received the following macro- and micronutrients from the nutritive solution: 8.75 mM KNO<sub>3</sub>, 7.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 3.25 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 1.5 mM MgSO<sub>4</sub>·7 H<sub>2</sub>O, 62.50 μM KCl, 31.25 μM H<sub>3</sub>BO<sub>3</sub>, 2.50 μM MnSO<sub>4</sub>·H<sub>2</sub>O, 2.50 μM ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.63 μM CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.63 μM NaMoO<sub>4</sub>·5H<sub>2</sub>O, and 250.0 μM NaEDTAFe·3H<sub>2</sub>O. One plant per pot was used during plant conduction. For Si treatment, Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O was used at concentrations of 0, 1.25 and 2.50 mM Si and applied for 18 days (days 7–25 after the initiation of the experiment).

To simulate Cd exposure, CdCl<sub>2</sub> was used at concentrations of 0 and 500 µM Cd, which was applied over 10 days (days 15–25 after the initiation of the experiment). During the study, the solutions were changed at 07:00 h at 3-day intervals, with the pH adjusted to 5.0 using HCl or NaOH. All reagents used in this study were obtained from Sigma-Aldrich™. On day 25 of the experiment, the physiological and morphological parameters were measured for all plants, and leaf tissues were harvested for nutritional and biochemical analyses.

### Measurement of chlorophyll fluorescence

The effective quantum yield of PSII photochemistry ( $\Phi_{PSII}$ ), photochemical quenching coefficient ( $q_p$ ), non-photochemical quenching (NPQ), electron transport rate (ETR), relative energy excess at the PSII level (EXC) and ratio between the electron transport rate and net photosynthetic rate ( $ETR/P_N$ ) were determined using a modulated chlorophyll fluorometer (model OS5p; Opti-Sciences). The chlorophyll fluorescence was measured in fully expanded leaves under light. Preliminary tests determined the location of the leaf, part of the leaf and time required to obtain the greatest  $F_v/F_m$  ratio. Therefore, the acropetal third of leaves that was in the middle third of the plant and adapted to the dark for 30 min was used in the evaluation. The intensity and duration of the saturation light pulse were  $7500 \mu\text{mol m}^{-2} \text{s}^{-1}$  and 0.7 s, respectively.

### Evaluation of gas exchange

The net photosynthetic rate ( $P_N$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), and intercellular CO<sub>2</sub> concentration ( $C_i$ ) were evaluated using an infrared gas analyser (model LCPro<sup>+</sup>; ADC BioScientific). These parameters were measured at the adaxial surface of fully expanded leaves that were collected from the middle region of the plant. The water-use efficiency (WUE) was estimated according to Ma et al. (2004a), and the instantaneous carboxylation efficiency ( $P_N/C_i$ ) was calculated using the formula described by Aragão et al. (2012). Gas exchange was evaluated in all plants under a constant CO<sub>2</sub> concentration, photosynthetically active radiation, air-flow rate and temperature conditions in the chamber of  $360 \mu\text{mol mol}^{-1} \text{CO}_2$ ,  $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ,  $300 \mu\text{mol s}^{-1}$  and 28 °C, respectively, between 10:00 and 12:00 h.

### Extraction of antioxidant enzymes, superoxide and soluble proteins

Antioxidant enzymes (SOD, CAT, APX and POX), superoxide and soluble proteins were extracted from leaf tissues according to the method of Badawi et al. (2004). The extraction mixture was prepared by homogenizing 500 mg of fresh plant material in 5 ml of extraction buffer, which consisted of 50 mM phosphate buffer (pH 7.6), 1.0 mM ascorbate and 1.0 mM EDTA. Samples were centrifuged at  $14,000 \times g$  for 4 min at 3 °C, and the supernatant was collected. Quantification of the total soluble proteins was performed using the method described by Bradford (1976). Absorbance was measured at 595 nm, using bovine albumin as a standard.

### Superoxide dismutase assay

For the SOD assay (EC 1.15.1.1), 2.8 ml of a reaction mixture containing 50 mM phosphate buffer (pH 7.6), 0.1 mM EDTA, 13 mM methionine (pH 7.6), 75 µM NBT, and 4 µM riboflavin was mixed with 0.2 ml of supernatant. The absorbance was then measured at 560 nm (Giannopolitis and Ries 1977). One SOD unit was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction. The SOD activity was expressed in unit  $\text{mg}^{-1}$  protein.

### Catalase assay

For the CAT assay (EC 1.11.1.6), 0.2 ml of supernatant and 1.8 ml of a reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 12.5 mM hydrogen peroxide were mixed, and the absorbance was measured at 240 nm (Havir and McHale 1987). The CAT activity was expressed in  $\mu\text{mol H}_2\text{O}_2 \text{mg}^{-1} \text{protein min}^{-1}$ .

### Ascorbate peroxidase assay

For the APX assay (EC 1.11.1.11), 1.8 ml of a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 1.0 mM hydrogen peroxide was mixed with 0.2 ml of supernatant, and the absorbance was measured at 290 nm (Nakano and Asada 1981). The APX activity was expressed in  $\mu\text{mol AsA mg}^{-1} \text{protein min}^{-1}$ .

### Peroxidase assay

For the POX assay (EC 1.11.1.7), 1.78 ml of a reaction mixture containing 50 mM phosphate buffer (pH 7.0) and 0.05% guaiacol was mixed with 0.2 ml of supernatant, followed by addition of 20 µL of 10 mM hydrogen

peroxide. The absorbance was then measured at 470 nm (Cakmak and Marschner 1992). The POX activity was expressed in  $\mu\text{mol tetraguaiacol mg}^{-1} \text{ protein min}^{-1}$ .

#### Determination of superoxide concentration

For the determination of  $\text{O}_2^-$ , 1 ml of extract was incubated with 30 mM phosphate buffer [pH 7.6] and 0.51 mM hydroxylamine hydrochloride for 20 min at 25 °C. Then, 17 mM sulphanilamide and 7 mM  $\alpha$ -naphthylamine were added to the incubation mixture for 20 min at 25 °C. After the reaction, ethyl ether was added in the identical volume and centrifuged at  $3000 \times g$  for 5 min. The absorbance was measured at 530 nm (Elstner and Heupel 1976).

#### Extraction of nonenzymatic compounds

Nonenzymatic compounds ( $\text{H}_2\text{O}_2$  and MDA) were extracted as described by Wu et al. (2006). Briefly, a mixture designed to extract  $\text{H}_2\text{O}_2$  and MDA was prepared by homogenizing 500 mg of fresh leaf material in 5 mL of 5% (w/v) trichloroacetic acid. Then, samples were centrifuged at  $15,000 \times g$  for 15 min at 3 °C to collect the supernatant.

#### Determination of hydrogen peroxide concentration

To measure  $\text{H}_2\text{O}_2$ , 200  $\mu\text{L}$  of supernatant and 1800  $\mu\text{L}$  of reaction mixture (2.5 mM potassium phosphate buffer [pH 7.0] and 500 mM potassium iodide) were mixed, and the absorbance was measured at 390 nm (Velikova et al. 2000).

#### Quantification of malondialdehyde concentration

MDA was determined by mixing 500  $\mu\text{L}$  of supernatant with 1 mL of the reaction mixture, which contained 0.5% (w/v) thiobarbituric acid in 20% trichloroacetic acid. The mixture was incubated in boiling water at 95 °C for 20 min, with the reaction terminated by placing the reaction container in an ice bath. The samples were centrifuged at  $10,000 \times g$  for 10 min, and the absorbance was measured at 532 nm. The nonspecific absorption at 600 nm was subtracted from the absorbance data. The amount of MDA–TBA complex (red pigment) was calculated based on the method of Cakmak and Horst (1991), with minor modifications and using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### Determination of electrolyte leakage

Electrolyte leakage was measured according to the method of Gong et al. (1998) with minor modifications. Fresh tissue (200 mg) was cut into pieces 1 cm in length and placed

in containers with 8 mL of distilled deionized water. The containers were incubated in a water bath at 40 °C for 30 min, and the initial electrical conductivity of the medium ( $\text{EC}_1$ ) was measured. Then, the samples were boiled at 95 °C for 20 min to release the electrolytes. After cooling, the final electrical conductivity ( $\text{EC}_2$ ) was measured. The percentage of electrolyte leakage was calculated using the formula  $\text{EL} (\%) = (\text{EC}_1/\text{EC}_2) \times 100$ .

#### Determination of photosynthetic pigments

Determinations of the chlorophyll and carotenoid levels were performed with 40 mg of leaf tissue. The samples were homogenized in the dark with 8 mL of 90% methanol. The homogenate was centrifuged at  $6000 \times g$  for 10 min at 5 °C. The supernatant was removed, and the chlorophyll *a* (Chl *a*) and *b* (Chl *b*), carotenoid (Car) and total chlorophyll (total Chl) levels were quantified using a spectrophotometer (model UV-M51; Bel Photonics) according to the methodology of (Lichtenthaler and Buschmann 2001).

#### Measurements of morphological parameters

The growth of roots, stems and leaves was measured based on constant dry weights (g) after drying in a forced-air ventilation oven at 65 °C.

#### Extraction and Si determination

Samples containing 100 mg of dry matter were placed in a muffle furnace and maintained for 3 h at 500 °C. The material was removed and mixed in 10 mL of 1% NaOH. For the determination of Si, 200  $\mu\text{L}$  of supernatant and 1720  $\mu\text{L}$  of the reaction mixture (0.078 N HCl, 3.45 mM  $\text{NH}_4\text{Mo}_7\text{O}_{24}$ , 54 mM tartaric acid) were mixed with 80  $\mu\text{L}$  of a reducing agent. The reducing agent was prepared with 40 mM  $\text{Na}_2\text{SO}_3$ , 10.5 mM 1-amino-2-naphthol-4-sulfonic acid, and 1.45 mM  $\text{NaHSO}_3$ . The absorbance was measured at 600 nm (Ma et al. 2004b).

#### Determination of Cd and nutrients

Samples with 100 mg of milled samples were weighed in 50 ml conical tubes (Falcon<sup>®</sup>, Corning, Mexico) and pre-digested (48 h) with 2 ml of sub boiled  $\text{HNO}_3$  (DST 1000, Savillex, USA). Thereafter, 8 ml of a solution containing 4 ml of  $\text{H}_2\text{O}_2$  (30% v/v, Synth, Brasil) and 4 ml of ultra-pure water (Milli-Q System, Millipore, USA) were added and the mixture was transferred to a Teflon digestion vessel, closed and heated in a block digester (EasyDigest<sup>®</sup>, Analab, France) according to the following program: (1) 100 °C for 30 min; (2) 150 °C for 30 min; (3) 130 °C for

10 min; (4) 100 °C for 30 min and, (5) left to cool. The volume was made up to 50 mL with ultra-pure water and iridium was used as internal standard at 10 µg l<sup>-1</sup>, in agreement with Batista et al. (2014). The determinations of Cd, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn, and Mo were carried out by using an inductively coupled plasma mass spectrometer (ICP-MS 7900, Agilent, USA). Certified reference materials (NIST 1570a and NIST 1577c) were run in each batch for quality control purposes. All found values were in agreement with certified values.

**Bioconcentration factor**

The bioconcentration factor (BCF) was calculated by the equation  $BCF = E_{pt}/E_{ns}$  as described by Yoon et al. (2006), where  $E_{pt}$  represents the element concentration in plant tissue and  $E_{ns}$  represents the element concentration in the nutritive solution.

**Data analysis**

The data were subjected to two-way ANOVA, and significant differences between the means were determined by the Scott–Knott test at a probability level of 5% (Steel et al. 2006). Standard deviations were calculated for each treatment. The statistical analyses were performed with Assistat software.

**Results**

**Si reduces Cd contents**

The application of 2.50 mM Si to *Vigna unguiculata* plants exposed to Cd, contributed to significant reductions in the contents of this metal. We observed 23, 37 and 44% reductions in roots, stems and leaves (Table 1),

respectively, compared to the 500 µM Cd + 0 mM Si treatment. The action of Si also induced significant reductions in Cd BCF in plants subjected to treatment with 500 µM Cd + 2.50 mM Si compared to control plants (0 mM Si) and plants treated with Cd.

**Si accumulation in tissues**

Plants treated with Si had increased ( $P < 0.05$ ) Si contents for all tissues studied in both conditions (with or without Cd), and the contents in the root were highest, followed by the stem and leaf (Table 2). In relation to Si BCF, plants without Cd showed higher values at a 1.25 mM Si concentration, whereas plants subjected to metal toxicity suffered reductions in Si BCF in the root and stem compared to plants treated under the same Si concentration without Cd.

**Si mitigates the effects of Cd on macro- and micronutrients**

Cd reduced the contents of macronutrients (P, K, Ca, Mg and S) (Table 3). However, the 500 µM Cd + 2.50 mM Si treatment resulted in significant increases in P, K, Ca, Mg and S of 46, 17, 51, 22 and 20% (root); 25, 36, 70, 14 and 44% (stem); and 14, 27, 22, 23 and 22% (leaf), respectively, compared to the treatment with 500 µM Cd + 0 mM Si. The micronutrient (Zn, Cu, Fe, Mn and Mo) contents were reduced when plants were treated with Cd (Table 4). However, treatment with 500 µM Cd + 2.50 mM Si promoted increases in Zn, Cu, Fe, Mn and Mo of 23, 29, 30, 22 and 10% (root); 51, 55, 37, 151 and 20% (stem); and 25, 31, 36, 33 and 35% (leaf), respectively, compared to the treatment with 500 µM Cd + 0 mM Si.

**Table 1** Cd contents and bioconcentration factor in cowpea plants treated with Si and exposed to Cd toxicity

Cd (µM)	Si (mM)	Cd content (µg g <sup>-1</sup> )			Cd BCF		
		Root	Stem	Leaf	Root	Stem	Leaf
0	0	0.38 ± 0.03Ab	0.02 ± 0.01Ab	0.01 ± 0.01Ab	–	–	–
0	1.25	0.08 ± 0.01Ab	0.02 ± 0.01Ab	0.01 ± 0.01Ab	–	–	–
0	2.50	0.07 ± 0.01Ab	0.03 ± 0.01Ab	0.01 ± 0.01Ab	–	–	–
500	0	1036.74 ± 61.08Aa	42.76 ± 3.21Aa	100.40 ± 9.60Aa	18.45 ± 1.09Aa	0.76 ± 0.06Aa	1.79 ± 0.17Aa
500	1.25	855.74 ± 67.34Ba	29.41 ± 1.89Ba	58.56 ± 2.85Ba	15.23 ± 1.20Ba	0.52 ± 0.03Ba	1.04 ± 0.05Ba
500	2.50	794.80 ± 70.56Ba	27.00 ± 2.11Ca	56.31 ± 3.18Ba	14.14 ± 1.26Ba	0.48 ± 0.04Ca	1.00 ± 0.06Ba

*Cd BCF* Bioconcentration factor of Cd. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500 µM Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

**Table 2** Si contents and bioconcentration factor in cowpea plants treated with Si and exposed to Cd toxicity

Cd ( $\mu\text{M}$ )	Si (mM)	Si content ( $\mu\text{g g}^{-1}$ )			Si BCF		
		Root	Stem	Leaf	Root	Stem	Leaf
0	0	56.7 $\pm$ 5.4Ca	32.4 $\pm$ 2.3Ca	15.3 $\pm$ 1.2Ca	–	–	–
0	1.25	2423.0 $\pm$ 151.0Ba	614.4 $\pm$ 26.5Ba	518.3 $\pm$ 26.3Aa	69.0 $\pm$ 4.3Aa	17.5 $\pm$ 0.3Aa	14.8 $\pm$ 0.7Aa
0	2.50	3613.8 $\pm$ 208.8Aa	767.8 $\pm$ 55.5Aa	365.1 $\pm$ 27.6Bb	51.4 $\pm$ 3.4Ba	10.9 $\pm$ 0.5Ba	5.2 $\pm$ 0.4Bb
500	0	44.1 $\pm$ 9.9Ca	23.4 $\pm$ 1.4Cb	14.4 $\pm$ 1.3Ca	–	–	–
500	1.25	2294.9 $\pm$ 228.3Ba	565.9 $\pm$ 48.0Ba	190.7 $\pm$ 11.2Bb	65.3 $\pm$ 5.5Aa	16.1 $\pm$ 0.4Ab	5.4 $\pm$ 0.3Bb
500	2.50	2958.4 $\pm$ 304.3Ab	720.6 $\pm$ 50.1Aa	432.5 $\pm$ 17.4Aa	42.1 $\pm$ 4.0Bb	10.3 $\pm$ 0.3Ba	6.2 $\pm$ 0.2Aa

*Si BCF* Bioconcentration factor of Si. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu\text{M}$  Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

**Table 3** Macronutrient contents in cowpea plants treated with Si and exposed to Cd toxicity

Cd ( $\mu\text{M}$ )	Si (mM)	P (mg g $\text{DM}^{-1}$ )	K (mg g $\text{DM}^{-1}$ )	Ca (mg g $\text{DM}^{-1}$ )	Mg (mg g $\text{DM}^{-1}$ )	S (mg g $\text{DM}^{-1}$ )
<i>Contents in root</i>						
0	0	4.48 $\pm$ 0.27Aa	5.90 $\pm$ 0.17Aa	0.50 $\pm$ 0.04Ba	12.74 $\pm$ 0.97Aa	42.74 $\pm$ 1.82Aa
0	1.25	4.61 $\pm$ 0.39Aa	6.06 $\pm$ 0.16Aa	0.80 $\pm$ 0.13Aa	12.26 $\pm$ 0.70Aa	44.21 $\pm$ 1.69Aa
0	2.50	4.52 $\pm$ 0.33Aa	6.06 $\pm$ 0.19Aa	0.85 $\pm$ 0.09Aa	12.00 $\pm$ 1.01Aa	43.06 $\pm$ 1.31Aa
500	0	2.54 $\pm$ 0.18Cb	3.12 $\pm$ 0.11Bb	0.40 $\pm$ 0.03Cb	5.00 $\pm$ 0.37Cb	31.93 $\pm$ 0.56Bb
500	1.25	3.20 $\pm$ 0.11Bb	3.32 $\pm$ 0.13Bb	0.50 $\pm$ 0.04Bb	7.81 $\pm$ 0.70Ab	33.03 $\pm$ 1.53Bb
500	2.50	3.71 $\pm$ 0.16Ab	3.66 $\pm$ 0.15Ab	0.60 $\pm$ 0.03Ab	6.09 $\pm$ 0.26Bb	38.51 $\pm$ 1.09Ab
<i>Contents in stem</i>						
0	0	5.19 $\pm$ 0.24Aa	4.06 $\pm$ 0.17Aa	0.45 $\pm$ 0.04Ba	3.00 $\pm$ 0.04Ba	17.52 $\pm$ 0.62Aa
0	1.25	5.29 $\pm$ 0.23Aa	4.24 $\pm$ 0.18Aa	0.45 $\pm$ 0.02Ba	3.01 $\pm$ 0.08Ba	18.67 $\pm$ 0.71Aa
0	2.50	5.21 $\pm$ 0.30Aa	4.12 $\pm$ 0.20Aa	0.60 $\pm$ 0.05Aa	3.40 $\pm$ 0.24Aa	17.97 $\pm$ 0.85Aa
500	0	3.21 $\pm$ 0.29Bb	2.30 $\pm$ 0.09Bb	0.30 $\pm$ 0.03Cb	2.19 $\pm$ 0.13Bb	10.09 $\pm$ 0.47Cb
500	1.25	3.73 $\pm$ 0.30Ab	3.16 $\pm$ 0.05Ab	0.40 $\pm$ 0.02Bb	2.39 $\pm$ 0.12Ab	13.13 $\pm$ 1.14Bb
500	2.50	4.01 $\pm$ 0.33Ab	3.14 $\pm$ 0.14Ab	0.50 $\pm$ 0.03Ab	2.51 $\pm$ 0.21Ab	14.57 $\pm$ 1.14Ab
<i>Contents in leaf</i>						
0	0	8.61 $\pm$ 0.42Aa	4.20 $\pm$ 0.15Aa	1.60 $\pm$ 0.01Ca	3.14 $\pm$ 0.18Ba	22.93 $\pm$ 1.42Aa
0	1.25	8.44 $\pm$ 0.38Aa	4.32 $\pm$ 0.14Aa	1.70 $\pm$ 0.02Ba	3.59 $\pm$ 0.19Aa	23.33 $\pm$ 1.77Aa
0	2.50	8.39 $\pm$ 0.81Aa	4.22 $\pm$ 0.10Aa	2.01 $\pm$ 0.03Aa	3.22 $\pm$ 0.17Ba	23.08 $\pm$ 1.30Aa
500	0	6.84 $\pm$ 0.18Bb	2.82 $\pm$ 0.09Bb	1.15 $\pm$ 0.02Cb	2.35 $\pm$ 0.21Bb	17.00 $\pm$ 1.06Bb
500	1.25	7.20 $\pm$ 0.10Bb	3.26 $\pm$ 0.12Ab	1.30 $\pm$ 0.01Bb	2.47 $\pm$ 0.11Bb	19.75 $\pm$ 0.80Ab
500	2.50	7.77 $\pm$ 0.35Ab	3.58 $\pm$ 0.29Ab	1.40 $\pm$ 0.01Ab	2.89 $\pm$ 0.16Ab	20.77 $\pm$ 0.95Ab

*P* Phosphorus, *K* Potassium, *Ca* Calcium, *Mg* Magnesium, *S* Sulfur. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu\text{M}$  Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

### Si improves the chlorophyll fluorescence

Although Cd induced reductions in  $\Phi_{\text{PSII}}$ ,  $q_{\text{P}}$  and ETR, the concentration of 2.50 mM Si attenuated the negative effects by inducing increases of 37, 61 and 55%, respectively, compared with the treatment with 500  $\mu\text{M}$  Cd + Si

0 mM (Table 5). In the absence of Cd,  $\Phi_{\text{PSII}}$ ,  $q_{\text{P}}$  and ETR showed the highest values at 1.25 mM Si. For NPQ, EXC and  $\text{ETR}/P_{\text{N}}$ , Cd resulted in increases, whereas the treatment with 2.50 mM Si significantly reduced these values by 27, 8 and 18%, respectively, compared to the treatment with 500  $\mu\text{M}$  Cd + 0 mM Si (Table 5).

**Table 4** Micronutrient contents in cowpea plants treated with Si and exposed to Cd toxicity

Cd (μM)	Si (mM)	Zn (μg g DM <sup>-1</sup> )	Cu (μg g DM <sup>-1</sup> )	Fe (μg g DM <sup>-1</sup> )	Mn (μg g DM <sup>-1</sup> )	Mo (μg g DM <sup>-1</sup> )
<i>Contents in root</i>						
0	0	49.92 ± 2.57Aa	10.70 ± 0.71Ba	2465.45 ± 123.34Aa	408.15 ± 25.35Aa	21.08 ± 1.35Ba
0	1.25	51.03 ± 2.96Aa	12.03 ± 0.93Aa	2680.28 ± 221.92Aa	411.04 ± 20.00Aa	23.26 ± 2.08Aa
0	2.50	50.52 ± 3.46Aa	11.73 ± 0.98Aa	2585.81 ± 242.36Aa	410.38 ± 15.08Aa	23.02 ± 1.00Aa
500	0	35.09 ± 0.34Bb	7.42 ± 0.70Bb	1760.94 ± 166.22Bb	296.71 ± 5.58Bb	12.81 ± 1.27Ab
500	1.25	41.35 ± 3.69Ab	9.37 ± 0.71Ab	2116.48 ± 116.60Ab	338.84 ± 32.86Ab	13.72 ± 0.27Ab
500	2.50	43.35 ± 3.04Ab	9.60 ± 0.51Ab	2285.84 ± 70.07Ab	362.35 ± 15.62Ab	14.05 ± 0.23Ab
<i>Contents in stem</i>						
0	0	14.92 ± 0.16Ba	1.75 ± 0.06Aa	8.85 ± 0.86Ba	130.03 ± 2.71Ba	9.74 ± 0.87Aa
0	1.25	17.35 ± 1.51Aa	1.85 ± 0.13Aa	9.69 ± 0.57Ba	132.01 ± 5.08Ba	9.78 ± 0.63Aa
0	2.50	17.15 ± 1.41Aa	1.78 ± 0.07Aa	11.71 ± 0.93Aa	140.56 ± 11.75Aa	9.89 ± 0.75Aa
500	0	7.75 ± 0.27Bb	0.96 ± 0.05Cb	7.03 ± 0.68Cb	30.40 ± 2.76Bb	7.56 ± 0.46Bb
500	1.25	11.22 ± 1.12Ab	1.35 ± 0.08Bb	8.37 ± 0.72Bb	76.28 ± 6.86Ab	8.89 ± 0.67Ab
500	2.50	11.70 ± 0.51Ab	1.49 ± 0.11Ab	9.63 ± 0.91Ab	76.39 ± 4.43Ab	9.08 ± 0.30Aa
<i>Contents in leaf</i>						
0	0	33.37 ± 1.55Aa	2.45 ± 0.09Aa	24.70 ± 1.48Aa	242.32 ± 10.73Aa	3.37 ± 0.24Aa
0	1.25	35.23 ± 2.52Aa	2.46 ± 0.12Aa	25.66 ± 2.16Aa	249.24 ± 11.67Aa	3.37 ± 0.26Aa
0	2.50	34.79 ± 2.53Aa	2.35 ± 0.06Aa	24.78 ± 0.05Aa	247.88 ± 9.96Aa	3.15 ± 0.19Aa
500	0	23.16 ± 2.07Bb	1.43 ± 0.07Bb	13.26 ± 1.05Cb	131.57 ± 12.89Cb	1.10 ± 0.07Bb
500	1.25	25.66 ± 1.82Bb	1.83 ± 0.11Ab	15.82 ± 1.39Bb	151.20 ± 12.73Bb	1.34 ± 0.12Ab
500	2.50	28.99 ± 1.25Ab	1.87 ± 0.17Ab	18.09 ± 0.62Ab	174.88 ± 12.60Ab	1.49 ± 0.09Ab

Zn Zinc, Cu Copper, Fe Iron, Mn Manganese, Mo Molybdenum. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500 μM Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

**Table 5** Chlorophyll fluorescence in cowpea plants treated with Si and exposed to Cd toxicity

Cd (μM)	Si (mM)	$\Phi_{PSII}$	$q_p$	NPQ	ETR (μmol m <sup>-2</sup> s <sup>-1</sup> )	EXC (μmol m <sup>-2</sup> s <sup>-1</sup> )	ETR/ $P_N$
0	0	0.78 ± 0.01Ca	0.61 ± 0.08Ba	0.36 ± 0.02Ab	62.32 ± 2.0Ba	0.46 ± 0.03Ab	3.81 ± 0.27Ab
0	1.25	0.84 ± 0.01Aa	0.88 ± 0.09Aa	0.30 ± 0.01Bb	67.62 ± 1.0Aa	0.45 ± 0.01Ab	3.66 ± 0.15Ab
0	2.50	0.81 ± 0.01Ba	0.64 ± 0.05Ba	0.33 ± 0.01Ab	64.38 ± 1.4Ba	0.45 ± 0.02Ab	3.79 ± 0.25Ab
500	0	0.56 ± 0.02Cb	0.34 ± 0.03Cb	0.60 ± 0.04Aa	34.39 ± 1.7Bb	0.58 ± 0.02Aa	6.97 ± 0.31Aa
500	1.25	0.74 ± 0.01Bb	0.44 ± 0.04Bb	0.51 ± 0.04Ba	49.39 ± 4.9Ab	0.55 ± 0.04Ba	6.15 ± 0.45Ba
500	2.50	0.77 ± 0.01Ab	0.55 ± 0.01Ab	0.44 ± 0.02Ca	53.21 ± 3.9Ab	0.53 ± 0.03Ba	5.68 ± 0.49Ba

$\Phi_{PSII}$  Effective quantum yield of PSII photochemistry,  $q_p$  Photochemical quenching coefficient, NPQ Nonphotochemical quenching, ETR Electron transport rate, EXC Relative energy excess at the PSII level, ETR/ $P_N$  Ratio between the electron transport rate and net photosynthetic rate. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500 μM Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

**Si improves gas exchange in plants affected by Cd**

Plants exposed to Cd suffered reductions ( $P < 0.05$ ) in  $P_N$ ,  $E$ ,  $g_s$ , WUE and  $P_N/C_i$ . However application of Si at a concentration of 2.50 mM resulted in a 90, 31, 214, 112 and 147% increase, respectively, compared to the treatment

with 500 μM Cd + Si 0 mM (Table 6). Cd promoted an increase in  $C_i$ , whereas applying 2.50 mM Si led to a reduction of 20% compared to the treatment with 500 μM Cd + 0 mM Si. In treatments without the addition of Cd, applying 1.25 mM Si induced a significant increase in  $P_N$ , WUE and  $P_N/C_i$  (Table 6).

**Table 6** Gas exchange in cowpea plants treated with Si and exposed to Cd toxicity

Cd ( $\mu\text{M}$ )	Si (mM)	$P_N$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	$g_s$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	$C_i$ ( $\mu\text{mol mol}^{-1}$ )	WUE ( $\mu\text{mol mmol}^{-1}$ )	$P_N/C_i$ ( $\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$ )
0	0	16.38 $\pm$ 0.5Ba	3.24 $\pm$ 0.07Aa	0.44 $\pm$ 0.01Aa	242 $\pm$ 18Ab	5.04 $\pm$ 0.19Ba	0.067 $\pm$ 0.005Ba
0	1.25	18.45 $\pm$ 0.4Aa	3.14 $\pm$ 0.08Aa	0.40 $\pm$ 0.02Ba	230 $\pm$ 14Ab	5.88 $\pm$ 0.12Aa	0.080 $\pm$ 0.003Aa
0	2.50	17.02 $\pm$ 0.6Ba	3.14 $\pm$ 0.09Aa	0.39 $\pm$ 0.03Ba	242 $\pm$ 15Aa	5.42 $\pm$ 0.23Ba	0.070 $\pm$ 0.005Ba
500	0	4.95 $\pm$ 0.2Cb	1.34 $\pm$ 0.03Cb	0.07 $\pm$ 0.01Cb	315 $\pm$ 13Aa	2.51 $\pm$ 0.09Cb	0.015 $\pm$ 0.001Cb
500	1.25	8.06 $\pm$ 0.4Bb	1.48 $\pm$ 0.09Bb	0.10 $\pm$ 0.01Bb	270 $\pm$ 23Ba	4.78 $\pm$ 0.20Bb	0.029 $\pm$ 0.003Bb
500	2.50	9.39 $\pm$ 0.3Ab	1.76 $\pm$ 0.05Ab	0.22 $\pm$ 0.02Ab	251 $\pm$ 15Ba	5.33 $\pm$ 0.46Aa	0.037 $\pm$ 0.003Ab

$P_N$  Net photosynthetic rate,  $E$  Transpiration rate,  $g_s$  Stomatal conductance,  $C_i$  Intercellular  $\text{CO}_2$  concentration,  $WUE$  Water-use efficiency,  $P_N/C_i$  Carboxylation instantaneous efficiency. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu\text{M}$  Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

### Si increases the enzymatic activity of plants suffering Cd toxicity

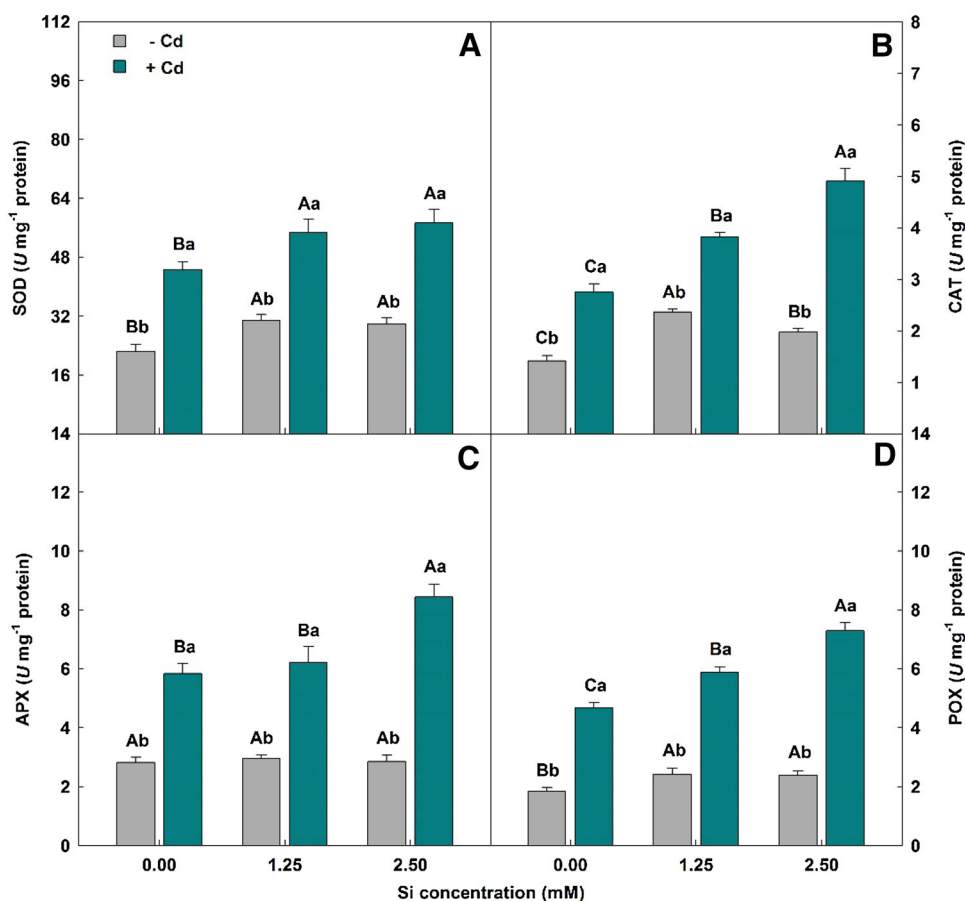
The stress caused by Cd increased the activity of SOD, CAT, APX and POX. We also detected increases in the enzymatic activity as the Si concentrations increased (Fig. 1). Compared to treatment with the 500  $\mu\text{M}$  Cd + 0 mM Si, the treatment with 500  $\mu\text{M}$

Cd + 2.50 mM Si caused increases of 28, 79, 44 and 56% in the SOD, CAT, APX and POX activities, respectively.

### Si contributes to the detoxification of ROS

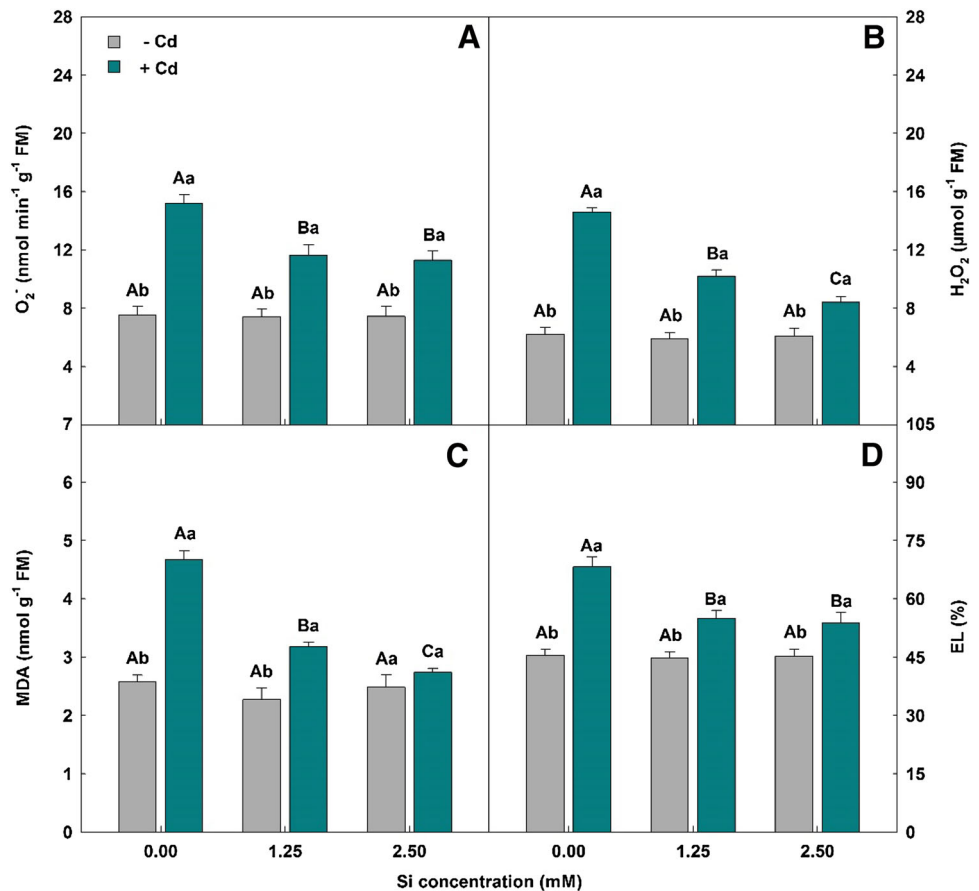
Significant increases were observed in  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ , MDA and EL in plants subjected to Cd (Fig. 2). However, the addition of 2.50 mM Si caused significant reductions of

**Fig. 1** Activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) in cowpea plants treated with Si and exposed to Cd toxicity. Different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu\text{M}$  Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Columns described corresponding to means from five repetitions and SD





**Fig. 2** Superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), malondialdehyde (MDA) and electrolyte leakage (EL) in cowpea plants treated with Si and exposed to Cd toxicity. Different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu$ M Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Columns described corresponding to means from five repetitions and SD



ROS (26, 39, 41 and 21%, respectively) compared to the control (500  $\mu$ M Cd + 0 mM Si). In treatments without Cd, the addition of Si did not cause significant changes in these variables.

**Si maximizes the photosynthetic pigments in plants exposed to Cd stress**

Plants administered Cd showed significant decreases in the levels of Chl *a*, Chl *b*, Chl Total, and CAR as well as the Chl *a*/Chl *b* ratio, whereas treatment with 500  $\mu$ M Cd + 2.50 mM Si resulted in increases of 93, 59, 90 and 54%, respectively, compared with the 500  $\mu$ M Cd + 0 mM Si treatment (Fig. 3). Additionally, Cd caused an increase in the Chl total/Car ratio ( $P < 0.05$ ). In plants without Cd, we found that 1.25 mM Si promoted the highest values for Chl *a*, Chl *b*, Chl Total and Car.

**Si attenuates the toxic effects on growth promoted by Cd**

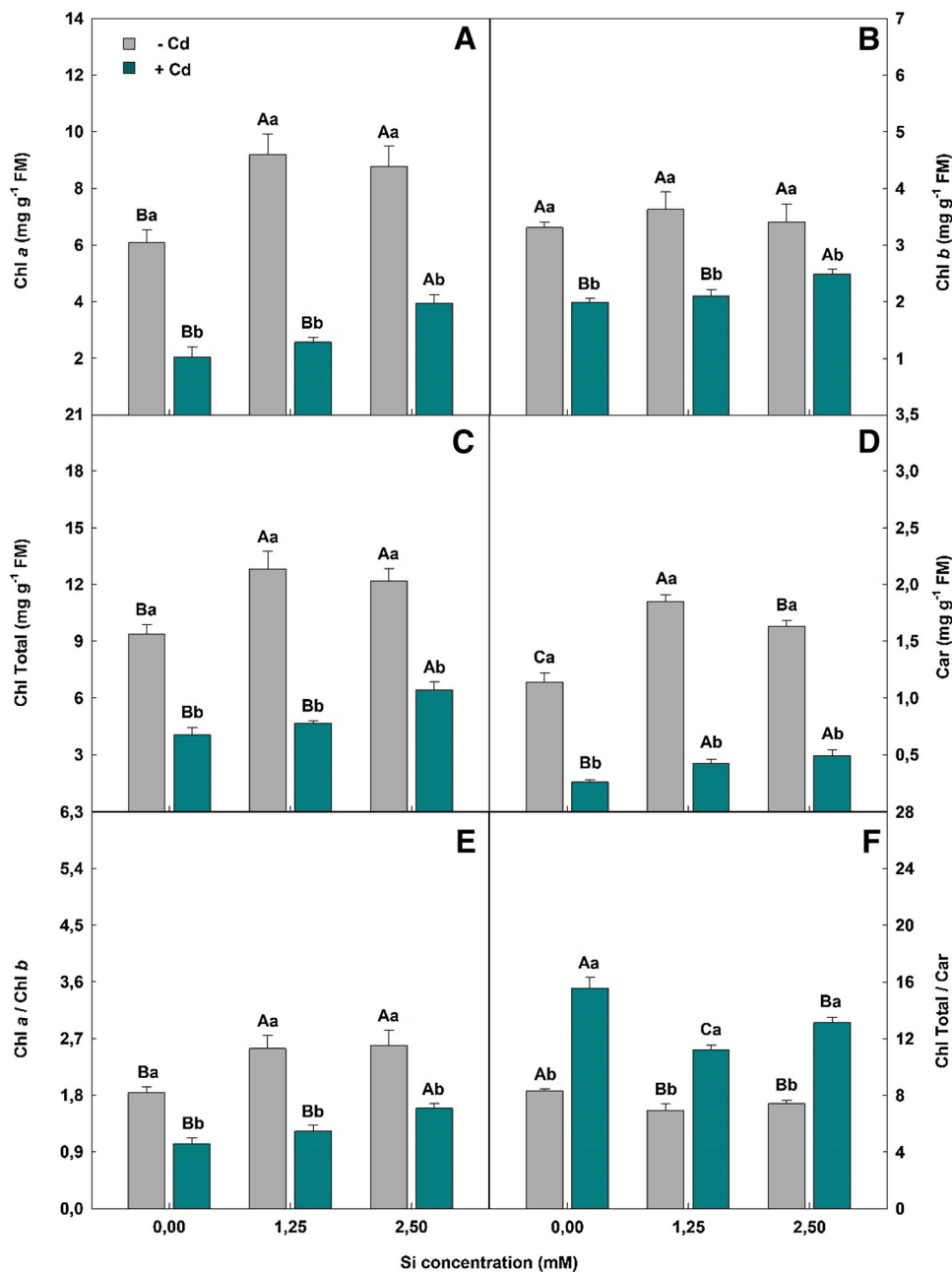
The treatment with 0  $\mu$ M Cd + 1.25 mM Si promoted increases of 7, 21, 4 and 29% in the values of Leaf dry matter (LDM), Stem dry matter (SDM), Total dry matter

(TDM) and the Shoot dry matter/Root dry matter (ShDM/RDM) Ratio, respectively, compared to the control treatment (Table 7). In plants treated with 500  $\mu$ M Cd + 2.50 mM Si, the LDM, SDM, RDM and TDM variables showed increases of 12, 9, 48 and 20%, respectively, compared to those planted with 500  $\mu$ M Cd + 0 mM Si (Table 7).

**Discussion**

Cd contents were reduced in all tissues after the application of Si, suggesting that Si interfered with Cd assimilation in cowpea. These decreases in Cd contents can be explained by the reduction in Cd BCF obtained in this study and by the deposition of silicon in the root endoderm (Ma and Guo 2014), which inhibits the transport of this metal to stems and leaves due to a physical blockade of the passage of Cd by the root system apoplast, preventing its translocation to other plant organs (da Cunha and Nascimento 2009). The results found in this study corroborate findings by Liang et al. (2005b) and Shi et al. (2005b), who investigated the effect of Si on *Zea mays* and *Oryza sativa*, respectively. Both studies found that in soil contaminated with Cd, Cd

**Fig. 3** Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (total Chl), carotenoids (Car) Chl *a*/Chl *b* ratio and Chl Total/Car ratio in cowpea plants treated with Si and exposed to Cd toxicity. Different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu$ M Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Columns described corresponding to means from five repetitions and SD



transport from the root system to the shoot was inhibited due to the complexation of metal in the root apoplast.

The Si contents were highest in the root, followed by the stem and leaf tissues, which can be explained by Si passive absorption. Cowpea is a species that absorbs Si in a passive way through mass flow, which has direct influence of the *E* rate (Liang et al. 2005a; Imtiaz et al. 2016). There are species of the *Poaceae* family, such as *Zea mays* (Mitani et al. 2009), *Triticum aestivum* (Rains et al. 2006), and *Oryza sativa* (Köster et al. 2009), that perform Si uptake and transport by an active process, and they accumulate larger amounts of Si in the shoot (Epstein 1999; Fuhrs et al. 2012). Mitani and Ma (2005) compared Si uptake and

transport in *Cucumis sativus*, *Lycopersicon esculentum* and *Oryza sativa*. In their study, it was observed that *Cucumis sativus* and *Lycopersicon esculentum* were unable to accumulate Si in the shoot, whereas *Oryza sativa* showed greater accumulation of Si in the leaf and stem. Wu et al. (2015) analysed the physiological responses of *Cucumis sativus* and *Solanum lycopersicum* under Cd stress and treated them with Si, leading to the observation that there was no statistically significant difference in the Si content when the Si and Si + Cd treatments were compared.

Cd caused reductions in the macro- and micronutrient contents; these results are related to the interference in the assimilation of the elements. Cd is a bivalent cation that

**Table 7** Growth in cowpea plants treated with Si and exposed to Cd toxicity

Cd ( $\mu\text{M}$ )	Si (mM)	LDM(g)	SDM(g)	RDM (g)	TDM (g)	Ratio ShDM/RDM
0	0	3.28 $\pm$ 0.05Ba	2.30 $\pm$ 0.16Ca	2.23 $\pm$ 0.11Aa	7.80 $\pm$ 0.08Ca	2.51 $\pm$ 0.17Bb
0	1.25	3.51 $\pm$ 0.09Aa	2.78 $\pm$ 0.07Aa	1.94 $\pm$ 0.04Ba	8.23 $\pm$ 0.13Aa	3.24 $\pm$ 0.07Aa
0	2.50	3.50 $\pm$ 0.13Aa	2.61 $\pm$ 0.05Ba	1.90 $\pm$ 0.07Ba	8.02 $\pm$ 0.05Ba	3.21 $\pm$ 0.15Aa
500	0	1.16 $\pm$ 0.05Bb	1.00 $\pm$ 0.01Ab	0.75 $\pm$ 0.05Bb	2.91 $\pm$ 0.07Bb	2.87 $\pm$ 0.08Aa
500	1.25	1.21 $\pm$ 0.04Bb	1.00 $\pm$ 0.05Ab	1.10 $\pm$ 0.06Ab	3.31 $\pm$ 0.15Ab	2.00 $\pm$ 0.14Bb
500	2.50	1.30 $\pm$ 0.07Ab	1.09 $\pm$ 0.03Ab	1.11 $\pm$ 0.08Ab	3.50 $\pm$ 0.11Ab	2.17 $\pm$ 0.17Bb

LDM Leaf dry matter, SDM Stem dry matter, RDM Root dry matter, TDM Total dry matter, ShDM/RDM Ratio Shoot dry matter/Root dry matter. Columns with different uppercase letters between Si treatments (0, 1.25 and 2.50 mM Si under equal Cd concentration) and lowercase letters between Cd treatments (0 and 500  $\mu\text{M}$  Cd under equal Si concentration) indicate significant differences from the Scott-Knott test ( $P < 0.05$ ). Values described corresponding to means from five repetitions and SD

frequently competes with Ca, Fe, Mg, Mn and Zn during transport across membranes (Llamas et al. 2000; Sarwar et al. 2010; Nocito et al. 2011) because the metal transporters are non-selective and can absorb nutrients that are toxic metals, such as Cd (Clemens et al. 1998; Maria et al. 2011). However, inhibition of Cd transport caused by the application of Si promoted increases in the macro- and micronutrient contents in leaf, stem, and root tissues. These results suggest that Si mitigates the Cd effect on nutrient uptake and transport mechanisms, providing better development for plants.

Plants require P, K Ca, Mg, and S in large quantities ( $> 0.1\%$  of dry matter), and each one of these macronutrients is essential for the plant to complete its life cycle (Maathuis 2009). K availability is important for stomatal regulation and osmotic adjustment by controlling the turgor pressure of guard cells (Oosterhuis et al. 2013). Ca is essential for several structural roles, improving the integrity of the cell wall and membranes (Mali and Aery 2008; Maathuis 2009). Mg is largely related to protein synthesis and photosynthesis since this element is the central atom of the chlorophyll molecule (Shaul 2002; Karley and White 2009).

Micronutrients (Zn, Cu, Fe, Mn and Mo), although required in only small amounts, are essential components of enzymes and play important roles, such as in photosynthesis, respiration and antioxidant defence (Ducic and Polle 2005; Kaiser et al. 2005; Broadley et al. 2007; Hänsch and Mendel 2009). Fe, for example, plays fundamental roles in electron transport, maintenance of PSI levels, and photosystem II (PSII) quantum yield (Yadavalli et al. 2012). Mn is a constituent of the enzyme that breaks water molecules in PSII via manganese superoxide dismutase (MnSOD) (Allen et al. 2007), whereas Mo is used by nitrate and nitrogenase reductase (Mendel and Hänsch 2002).

Nano-silicon application in *Oryza sativa* seedlings alleviated the toxicity caused by Cd, in which the Mg, Fe and Zn contents increased in plants treated with Si (Wang et al. 2015). Tripathi et al. (2012) evaluated the impact of Si in *Oryza sativa* exposed to Cr stress and observed that the macro—(Mg, Ca and K) and micronutrients (Zn and Fe) contents in leaves and roots decreased in plants treated with Cr, whereas the Si + Cr treatment prevented the reduction of these contents. These results corroborate the affirmation that Si mitigates the oxidative effect of metals.

Si utilization increased  $\Phi_{\text{PSII}}$ , qP and ETR values, these responses being related to Si absorption and essential nutrients to the proper functioning of the photosynthetic apparatus, such as Mn and Fe (Hänsch and Mendel 2009). In relation to EXC and ETR/ $P_{\text{N}}$ , plants subjected to Si presented reductions and were associated with a Si-mediated photoprotective mechanism, with the aim of allowing the photosystem II electron acceptors to work because there was a reduction in damage caused by Cd in chloroplasts, as confirmed by the reduction in NPQ. Silveira et al. (2015) working with *Elephantopus mollis* subjected to 10, 50 and 100  $\mu\text{M}$  Cd and found that there was interference in the photochemical phase of photosynthesis, leading to significant reductions in the ETR of plants at concentrations of 50 and 100  $\mu\text{M}$  Cd. López-Millán et al. (2009) studied *Lycopersicon esculentum* under hydroponic cultivation and subjected them to 10 and 100  $\mu\text{M}$  Cd, which led to a significant increase in NPQ under 100  $\mu\text{M}$  Cd toxicity. Nwugo and Huerta (2008) evaluated the relationship among concentrations of 0.0, 0.2, or 0.6 mM Si and 0.0 or 2.5  $\mu\text{M}$  Cd and reported that the addition of 0.2 mM Si induced a significant increase in qP in *Oryza sativa* plants under Cd stress.

Si indirectly attenuated the toxic Cd effects on  $P_{\text{N}}$ , because this element caused increases in ETR and  $\Phi_{\text{PSII}}$ , detected in this research. The increase in energy absorption in photosystem II and consequent higher electron flow

carried out by ferredoxins resulted in an increase in  $P_N$  (Baker and Rosenqvist 2004; Bodek and Blum 2013). Ali et al. (2013) investigated the influence of three Si concentrations (0, 1 and 2 mM) on *Hordeum vulgare* stressed by 100  $\mu\text{M}$  Cr and observed that Si alleviated Cr toxicity, which was reflected by a significant increase in  $P_N$ , with better results for 2 mM Si. In addition, Ali et al. (2016) studied *Gossypium hirsutum* under stress by three different Cu concentrations (0, 25 and 50  $\mu\text{M}$ ) and found that the application (1 mM) of Si alleviated the toxic effect of this metal, optimizing the  $P_N$  performance, reaffirming that Si can mitigate the effects of heavy metal toxicity.

In plants subjected to Cd + Si, the variables  $E$  and  $g$  increased, whereas  $C_i$  was reduced. This decrease in  $C_i$  may be related to the higher performance of the RUBISCO enzyme during  $\text{CO}_2$  fixation, an effect that is possibly due to the addition of Si in plants exposed to metal toxicity (Nwugo and Huerta 2011). In studies conducted by Feng et al. (2010) with *Cucumis sativus*, it was found that 1.5 mM Si alleviated the negative effects caused by the toxicity of Cd on gas exchange; a significant increase of  $E$  and  $g_s$ , as well as a considerable reduction of  $C_i$  were observed after 10 and 15 days of treatment.

Si provided increases in WUE and  $P_N/C_i$  in plants exposed to Cd + Si. The higher performance of WUE is explained by the benefits promoted by Si over  $P_N$ , whereas the increase observed in  $P_N/C_i$  occurred due to the decrease obtained in  $C_i$ . Li et al. (2015) studied the Cd effect on *Elsholtzia argyi* in hydroponic cultivation over 21 days and observed a reduction of 47.1% in WUE in plants subjected to 100  $\mu\text{mol}$  Cd compared to the control treatment (0  $\mu\text{mol}$  Cd). Farooq et al. (2013) found reductions in the gas exchange parameters in *Gossypium hirsutum* subjected to 0.1 and 5  $\mu\text{M}$  Cd. However, the application of Si prevented the stress conditions, improving WUE.

Plants treated with Cd + Si showed increases in SOD, CAT, APX and POX enzyme activities. These detected increases are related to ROS detoxification, produced during the intoxication process by Cd (Mohamed et al. 2012; Irfan et al. 2014). Si also caused a reduction in EXC and increases in ETR and  $\Delta F/F_m$ , by attenuating oxidation on photosystems and membranes. Farooq et al. (2013) observed increases in the activities of the antioxidant enzymes SOD, POX, APX, and CAT in *Gossypium hirsutum* under Cd toxicity, indicating that the addition of Si can significantly increase its defensive capability against oxidative damage. Song et al. (2009) also observed increases in the SOD, CAT and APX activities in *Brassica chinensis* treated with Si and subjected to stress by Cd. Tang et al. (2015) found an increase of 42.4% in SOD activity in *Boehmeria nivea* treated with Si + Cd, compared to treatment with Cd alone, corroborating the results found in our study. Lukačová et al. (2013) studied the

action of Si in *Zea mays* and observed increases in POX activity, suggesting the mitigation of Cd toxicity.

Si significantly decreased the  $\text{O}_2^-$  and  $\text{H}_2\text{O}_2$  concentrations in plants under stress by Cd, and this result showed that Si interfered positively in the antioxidant metabolism (Song et al. 2009) and was supported by the increase in the activity of SOD, CAT and APX in plants treated with Si. SOD is the enzyme responsible for converting  $\text{O}_2^-$  radicals into  $\text{H}_2\text{O}_2$ . CAT and APX are responsible for the dismutation of  $\text{H}_2\text{O}_2$  to the formation of  $\text{H}_2\text{O}$  and  $\text{O}_2$  (Zhang et al. 2009). Lin et al. (2012) studied *Oryza sativa* plants grown under hydroponic conditions and found that Cd utilization for the period of 5–15 days also promoted increases (60–71%) in  $\text{O}_2^-$  values. Wu et al. (2015) in a study with *Cucumis sativus*, obtained a reduction of  $\text{H}_2\text{O}_2$  with the addition of Si in plants under Cd toxicity, a behaviour that these authors described was based on increased enzymatic activities. Shi et al. (2005a) also identified the increase of  $\text{H}_2\text{O}_2$  in *Cucumis sativus* under a high Mn concentration and a decrease in the concentration of this compound when Si was applied. Tripathi et al. (2013) evaluated the defence mechanisms after Si application in two cultivars of *Oryza sativa* and found reductions of 16 and 11% in  $\text{O}_2^-$  concentrations of As tolerant and As sensitive cultivars, respectively.

The decreases in MDA and EL in the Cd + Si treatment occurred due to lower lipid peroxidation and increased antioxidant enzyme activities, indicating that Si contributes to the improvement of the cell membrane structure and integrity (Shahnaz et al. 2011; Lukačová et al. 2013). Shen et al. (2014) worked with *Arachis hypogaea* under stress by Al and found increases of 37 and 34% in the MDA and EL levels, but Si exposition caused decreases of 26 and 15%, respectively, confirming the results obtained in this research. Shi et al. (2010) evaluated the effect of Si on Cd toxicity in leaves of two cultivars of *Arachis hypogaea* and observed that Cd significantly increased (40 and 11%) the MDA levels. However treatment with Si reduced MDA in the cultivar tolerant to Cd. Liu et al. 2013 studied *Solanum nigrum* seedlings and detected that EL and  $\text{H}_2\text{O}_2$  were significantly reduced in the Cd + Si treatment compared to the Cd treatment.

The increase in Chl  $a$ , Chl  $b$ , total Chl and Car levels in plants treated with Si and exposed to Cd stress can be attributed to increases in the activities of antioxidant enzymes (CAT, APX and POX), combined with the greater accumulation of Mn (Benavides et al. 2005). In agreement with Gunes et al. (2008), increases in antioxidant enzymes promote higher tolerance to abiotic stresses. The negative effects were attenuated by Si, a fact that Hossain et al. (2007) stated was related to the preservation of the cell membrane, increased plant extensibility and resistance to damage because accumulation of Mn facilitates the transfer

of electrons to photosystem II. López-Millán et al. (2009) observed a reduction of 40 and 35% for Chl *a* and 46 and 23% for Chl *b* in *Lycopersicon esculentum* in a hydroponic culture subjected to concentrations of 10 and 100  $\mu\text{M}$  Cd, respectively. Vaculík et al. (2015) investigated the effect of Si on the photosynthetic process in *Zea mays* exposed to two Cd concentrations (5  $\mu\text{M}$  and 50  $\mu\text{M}$ ) and detected reductions in the levels of total Chl, and Car as well as the Chl *a*/Chl *b* ratio in plants under Cd stress. However this negative effect was mitigated by Si, supporting the results described in our study. In addition, Feng et al. (2010) described that the exposure of Cd for 10 and 15 days in *Cucumis sativus* caused significant reductions in Chl *a*, Chl *b*, total Chl and Car. However, when Si was added, the pigment values increased.

The addition of Si attenuated the negative effects linked to Cd on LDM, SDM, RDM and TDM, as these results are related to lower lipid peroxidation and reduced ROS accumulation. These compounds are responsible for causing damage to cell membranes, reducing cell expansion, and consequently reducing vegetative growth (Shah et al. 2001; Nwugo and Huerta 2008). Additionally, Si promotes the greater accumulation of dry matter (Amiri et al. 2012) because it inhibits the deterioration of cell membranes and improves rates of growth and development. Farooq et al. (2016), studied how Si can reverse the effects caused by Cd toxicity in *Oryza sativa* plants and reported an increase in the dry biomass of shoots and roots after treatment with Si. Vaculík et al. (2009) also found similar results when studying the influence of Si on the growth of *Zea mays* cultivated under hydroponic conditions in the presence of Cd, noting an increase of approximately 11% in root dry matter and approximately 20% for shoot. Our results corroborate these studies related to the benefits of Si on the vegetative growth of plants under Cd stress.

Si reduced Cd contents in roots and other plant organs, such as stems and leaves. The Si contents in roots were highest, followed by stems and leaves, which can be explained by the passive absorption of Si. The application of Si promoted increases in the macro- and micronutrient contents in all tissues, suggesting that Si mitigates the Cd effect on nutrient uptake. Si attenuates the Cd effects on light absorption of photosystem II (PSII), increasing the effective quantum yield of PSII photochemistry and the electron transport rate. Toxic effects induced by Cd on gas exchange were mitigated by the action of Si. Plants treated with Cd + Si showed increases in the activities of antioxidant enzymes and reductions in oxidant compounds, with these modifications promoted by Si via a detoxification mechanism. Increases in photosynthetic pigments and the growth of plants treated with Si and exposed to Cd stress were observed and were explained by the reduced deterioration of cell membranes and maintenance of

chloroplast, which led to positive effects on growth and development. This study validated the hypothesis that Si accumulation in roots induces benefits on metabolism and alleviates the toxic effects caused by Cd in leaves of cowpea plants.

**Acknowledgements** This research had financial supports from Fundação Amazônia Paraense de Amparo à Pesquisa (FAPESPA/Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) and Universidade Federal Rural da Amazônia (UFRA/Brazil) to Lobato A.K.S. Pereira T.S. and Souza C.L.F.C. were supported by undergraduate scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil).

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