THE EFFECT OF SILICON FOLIAR AND ROOT APPLICATION ON GROWTH, PHYSIOLOGY, AND ANTIOXIDANT ENZYME ACTIVITY OF WHEAT PLANTS UNDER CADMIUM TOXICITY

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Abstract. Silicon (Si) application can boost plant growth and physiology under heavy metal toxicity. The article aimed to investigate how the foliar and root application of silicon (Si) affects the uptake, translocation, and concentration of cadmium (Cd) for winter wheat (*Triticum aestivum* L.), and in turn the plant's growth, physiology, and antioxidant enzyme activity, using a complete randomized block design (CRBD)? For this purpose, we used twelve treatments consisting of two levels of Si (0 and 3 mM Na_2SiO_3) against four levels of Cd (0, 50, 100, and 200 μM $CdCl_2$) with three replications. Results showed that all levels of Cd significantly enhanced membrane permeability measured as malonyldialdehyde (MDA) content, osmotic stress measured as hydrogen peroxide (H_2O_2) content, and triggered the enzymatic and non-enzymatic antioxidant system, resulting in a severe reduction in the growth of wheat seedlings. Besides, Si application either as root (SiR3) or foliar spray (SiF3) remarkably encountered Cd toxicity by further improving antioxidative enzymes activities, and by hindering Cd uptake, but more significant results were recorded for SiR3. Therefore, it might be established that Si root supplementation inhibited Cd translocation from root to shoot more effectively than foliar application, thereby lowering the potential health risk of Cd pollution.

Keywords: *enzymatic and non-enzymatic antioxidants, membrane permeability, osmoprotectants, photosynthetic pigments*

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Introduction

Wheat (*Triticum aestivum* L.) is the ultimate extensively grown cereal crop which fulfills 20% of the required daily protein for 4.5 billion people in the world (Flister and Galushko, 2016). It is the second most significant food crop in the developing world after rice. The wheat crop has the ability to adapt to various environmental circumstances but, considerable grain yield loss has been reported under heavy metals contamination, predominantly cadmium (Cd) contaminated soils (Rady et al., 2015). Most of the crop production area is under heavy metal stress caused by industrialization, anthropogenic activities, and other agricultural practices, which is currently becoming a worldwide serious threat (Zabin et al., 2015).

Among heavy metals, Cd is a highly lethal element because of its relative mobility, high water solubility, and phytotoxicity (Ali et al., 2011; Wuana and Okieimen, 2011). Due to its dynamic fates, Cd in an ionic form enters into plants through root and being shifted to shoot xylem and phloem through various active transporters and passive transporters (Dong et al., 2019; Takahashi et al., 2012). Higher uptake of Cd reduces photosynthesis by hindering root Fe (III) reductase, affects the activity of the enzyme involved in CO_2 fixation, affects mineral absorption causing a nutritional imbalance (such as N and K^+) and results in stomatal closure (Azevedo et al., 2012). It has been reported that in the intensified oxidative processes occur in cells of Cd-stressed plants which caused the formation of massive amount of reactive oxygen species (ROS) (Balakhnina et al., 2015; Gratão et al., 2005) which caused remarkable reduction of anti-oxidative enzymes activities in plants (Song et al., 2009). Additionally, Cd accumulates in the human body via the food chain, causing kidney, bone, and pulmonary damage (Ali et al., 2014; Fu and Wei, 2013; Li et al., 2014). Thus, developing positive approaches to minimize the potential health risk of heavy metal impurity in food chains is urgent for protecting edible parts of crops being polluted by heavy metals.

To alleviate heavy metals toxicity in plants, various means like soil phytoremediation and soil remediation engineering has been practiced (Rascio and Navari-Izzo, 2011). Although in most cases, these means did not work more effectively, as an alternative and/or accompanied by these means, silicon (Si) as soil or foliar application could be a more effective approach to diminish heavy metals accumulation in plants.

Silicon (Si), as a beneficial element, shows a crucial part in the growth and enlargement of various crops (Keeping, 2017; Zhao et al., 2017). Currently, Si has been conceived by Shi et al. (2014) as a compulsory element for numerous higher plants. However, Si is being proved as useful for plants, and it has the ability to alleviate biotic and abiotic stresses (Dong et al., 2019; Yu et al., 2016; Zhao et al., 2017). Si is the only element that can intake, translocate, and accumulate in considerable quantities in plants without any adverse effects (Ma et al., 2001a). Moreover, Si has been declared as a non-corrosive element (Wang et al., 2016). Consequently, Si, as being high-quality fertilizer may be used for eco-friendly agricultural enhancement.

It has been established in previous findings that Si can improve plant growth by mitigating the adverse influences of heavy metal toxicity (Dong et al., 2019). Numerous scientists have reported that Si treatments detoxify Cd-induced stress in various plants, particularly in graminaceous crops, which are deliberated as Si accumulators (Farooq et al., 2013; Howladar et al., 2018; Keeping, 2017; Zhao et al., 2017). Concerning abiotic stresses, Si counters various stresses, including drought, salinity, and heavy metals toxicity included Cd toxicity in growth medium (Alzahrani et al., 2018). Si, in the form

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of silicates, converts the exchangeable and the soluble fractions of metals into stable chemicals in soil. Such a decline in metal bioavailability is documented to redox reactions, humification, and precipitation (Vieira da Cunha et al., 2008). Besides, Silicates in soil encourages the polymerization of silicate composts and forms heavy-metals complexes (Sommer et al., 2006). However, evidence suggesting that Si addition improves photosynthetic activity modifies nutrient imbalance, minimizes mineral toxicity, and enhances abiotic tolerance (Ma et al., 2001a). Si maintains photosynthetic activity in Cd-stressed plants, reducing Cd uptake from nutrient solution to plant roots and restricts its translocation from root to shoots, which might be the possible mechanism of Cd detoxification (Kabir et al., 2016a).

Reactive oxygen species (ROS) produced in plants by Cd stress, causing a severe distraction of several metabolic processes in plants (Kim et al., 2017). Different responses have been reported against various environmental stresses within the crop, plant species, and/or cultivars according to their antioxidant defense system (Wael et al., 2015). Optimum ingredients of antioxidants in plants either stimulated and/or constitutive induce more tolerance in plants to oxidative burst (Kusvuran et al., 2016). It has been described by Kim et al. (2017) that Si increased plant tolerance to oxidative damage by regulating a substantial amount of different ROS in heavy metal stressed-plants. These findings can be justified based on Si amounts in plants, which involves improving activities of antioxidative enzymes; particularly those antioxidants which participated in the transformation of H_2O_2 in H_2O, and/or minimizing MDA activity to scavenge negative effects of ROS.

It has been widely studied the protective role of foliar as well as soil application of Si to alleviate the heavy metal toxicity in wheat crops. However, to our best knowledge, this is the first kind of study used Si foliar and root application method in wheat crop to evaluate the most effective Si application method to raise the capability of wheat to tolerate Cd toxicity.

Consequently, the primary aim of the present study was to find the constructive impact of Si in two Si application approaches and to identify the optimum way of Si supplementation for plant physiological traits, chlorophyll contents, anti-oxidative enzymes activities, proline, H_2O_2, and MDA content in Cd-stressed wheat plants grown hydroponically.

Materials and methods

Experimental layout

The experiment was conducted at the experimental site of the Farmland Irrigation Research Institute, Chinese Academy of Agricultural Sciences in Xinxiang City, China. Healthy seeds of winter wheat genotype Xin Mai 23 were immersed overnight in deionized water and sown in pasteurized quartz sand trays with the sand layer of 4 inches in width. The sand dishes were placed in a growing chamber of 16 h light/8 h dark with a light intensity of 375 μmole m^{-2} S^{-1}. The temperature of the growth chamber was set at 28 °C to 30 °C with a relative humidity of 85%. After two weeks of sowing, the five uniform seedlings were enfolded with foam at a root-shoot joint and relocated in each hole (15 in. ×17 in. in size) of plastic sheets fluctuating on 10 L capacity plastic basins. These basins occupied with 8 L modified Hoagland's solution (see *Appendix* heading 1). The pH was adjusted to 6.5 throughout the experiment by using acid (HCl) and base (NaOH). The air pumps were applied to the aerated nutrient solution. During

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the whole experiment, the nutrient solution was renewed after three days of intervals. After 65 days of transplantation Cd in the form of cadmium chloride ($CdCl_2$) and 3 mM per litter Si in the form of silicates nanoparticles (made by sodium silicate) (see *Appendix* heading 2) were added to nutrient solutions for 15 days to form twelve treatments with three replications as shown in *Table 1*. Si was applied by two different methods; 1) Si root-application (SiR3), 2) Si foliar-application (SiF3). In Si root application, 3 mM Si as silica gel was applied per liter of nutrient solution after every three days during 15 days of treatments. Alternatively, in Si foliar-application, Si with an amount of 3 mmol/L in the form of silica gel was sprayed five times during 15 days of treatment in 100 days experiment.

Table 1. *Experimental treatments*

Treatments	Cd (µM)	SiR (mM)	SiF (mM)
Ck	0	0	0
T2	0	3	0
T3	0	0	3
T4	50	0	0
T5	50	3	0
T6	50	0	3
T7	100	0	0
T8	100	3	0
T9	100	0	3
T10	200	0	0
T11	200	3	0
T12	200	0	3

Two levels of Si (0 & 3 mM) with two methods of Si applications (SiR3 & SiF3) along with four levels of Cd @ 0, 50, 100, and 200 µM

Determination of plant physiological traits

Growth parameters like roots and shoots fresh and dry weights were measured after 100 days of germination. Two plants from each replication were sampled and stored at -80 °C in the freezer (Thermo Fisher Scientific, USA 702) for enzymatic analysis. Remaining plants were detached into root and shoots and were measured for their fresh weights (kept at 70 °C temperatures in the oven till constant dry weight), which were subsequently measured for Si, Cd, K^+, total N, and total protein contents.

Measurements of photosynthetic pigments

Photosynthetic pigments (carotenoids. Chlorophyll a, b, and total chlorophyll) were measured by an ultraviolet-visible spectrophotometer (TU-1810) using the spectrophotometric method of Metzner et al. (1965).

Biochemical analysis

Anti-oxidative enzymes like superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), of leaves were analyzed with ultraviolet visible spectrophotometer (TU-1810) by using the kits of Beijing Solarbio Science & Technology Co., Ltd

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(http://www.solarbio.com). Briefly, 0.5 g weighted fresh samples of leaves were milled with the help of a motor and pestle and standardized in 0.05 M phosphate buffer with pH 7.8 under chilled condition. The standardized mixture was centrifuged at 12,000 rpm for 10 min at 4 °C after sieving through four layers of muslin cloth.

The activity of CAT was measured by the following formula:

$$CAT\left(\frac{\mu}{mgprot}\right)=\left(OD_{Control}-OD_{Test}\right)\times\frac{271}{60}\times\frac{1}{SQ}\times\frac{1}{Protein\,conc.} \quad \text{(Eq.1)}$$

where: SQ = sample quantity, $OD_{control}$ = absorption of light in control, OD_{test} = absorption of light in test samples.

After mixing all reagents in the standardized mixture, the supernatant was again centrifuged at 3500 rpm for 10 min. The light diameter of 1 cm was adjusted to zero by double streaming water. OD was measured at 420 nm wavelength. The activity of POD was measured by the following equation:

$$POD\left(\frac{\mu}{mgprot}\right)=\left(OD_{Test}-OD_{Control}\right)\times\frac{12}{1\text{ cm}}\times\frac{V_t}{SQ\times RT\times Protein\,conc.}\times 1000 \quad \text{(Eq.2)}$$

where: V_t = total volume of the reaction liquid, SQ = sample quantity, RT = reaction time, $OD_{control}$ = absorption of light in control, OD_{test} = absorption of light in test samples.

After mixing all reagents in a standardized mixture, the supernatant was placed at room temperature for 10 min. OD was measured at 550 nm wavelength. The activity of SOD was measured by the following equation:

$$SOD\left(\frac{\mu}{mgprot}\right)=\left(\frac{OD_{control}-OD_{test}}{OD_{Control}}\right)\times\frac{1}{50}\times\frac{V_t}{SQ\times Protein\,conc.} \quad \text{(Eq.3)}$$

where: V_t = total volume of the reaction liquid, SQ = sample quantity, $OD_{control}$ = absorption of light in control, OD_{test} = absorption of light in test samples.

The level of lipid peroxidation in the leaf tissue was assessed by measuring the contents of malondialdehyde (MDA, a by-product of lipid peroxidation. Briefly, 0.2-0.5 g weighted fresh samples of leaves were milled with the help of a motor and added 2 ml 10% TCA and a small amount of quartz sand, ground to homogenate, add 3 ml TCA, further ground. The homogenized sample was centrifuged at 12000 rpm for 10 min. Took 2 ml supernatant, added 0.67% TBA, mixed and boiled for 15 min in 100 °C water bath. Cooled the sample at room temperature and centrifuged again. Absorption values of samples were measured at 532 nm, 600 nm, and 450 nm, respectively. The activity of MDA was measured by the following formula:

$$CMDA=6.45\left(A532-A600\right)-0.56\times A450 \quad \text{(Eq.4)}$$

$$MDA\left(\frac{\mu mol}{g}\right)=CMDA\times\left(\frac{V_t}{SQ\times 1000}\right) \quad \text{(Eq.5)}$$

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where: V_t = total volume of the reaction liquid, SQ = sample quantity.

Proline was also assessed by using the kit of Beijing Solarbio Science & Technology Co., Ltd. Following formula was used to measure the proline contents:

$$Proline\left(\frac{\mu g}{g}\right)=\left(\frac{OD_{sample}-OD_{blank}}{OD_{st}-OD_{blank}}\right)\times C_{st}\frac{5\mu g}{ml}\times\frac{V_{reagent}}{M_{tissue}}\times COD \quad \text{(Eq.6)}$$

where: CoD = the coefficient of dilution in the pre-treatment process, C_{st} = concentration of standard, OD_{st} = absorption of standard sample.

Hydrogen peroxide levels in leaves of wheat plants were assessed by Sergiev et al. (1997) method (see *Appendix* heading 4).

Translocation and bioaccumulation factors

Cd translocation from shoot to root measured and calculated using the following equation:

$$TF=\left(\frac{C_{shoot}}{C_{root}}\right) \quad \text{(Eq.7)}$$

where C_{shoot} and C_{root} are the concentration of Cd in shoot and root, respectively. TF > 1 showed that metals effectively transported from root to shoot (Zhang et al., 2002).

The bioaccumulation factor (BAF) was calculated using the following equation:

$$BAF=\left(\frac{C_{shoot}}{C_{water}}\right) \quad \text{(Eq.8)}$$

where C_{shoot} and C_{water} presented Cd concentration in shoot and water, respectively. BAF was categorizing further as hyperaccumulators samples, which accumulated metals > 1 mg kg^{-1}, accumulator, and excluder samples, which accumulated metals < 1 mg kg^{-1}(Ma et al., 2001b).

Determination of nutrient elements in plant tissues

The N, K^+, Cd, and Si content in the plants were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS, Agilent, and 7700 X, USA) after being oven-dried by following our previous study method (see *Appendix* heading 3) (Fırat et al., 2017).

Statistical analysis

The data were processed and analyzed using the SPSS 21.0 (SPSS, Chicago, IL), and all the graphs were made using the Sigma plot 12.5 software packages. The means of the three replicates were subjected to analysis of variance (two-way ANOVA), and multiple comparisons were performed using Duncan's multiple range test (DMR) at $P < 0.05$.

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Results

Cadmium attenuates growth parameters

Cadmium (Cd) caused a significant decrease in plant biomass without Si application (*Fig. 1*). Plant biomass in Cd50, Cd100, and Cd200 was decreased by 42%, 49%, and 68%, respectively, than that of the control plants. However, significantly higher biomass (root and shoot fresh weight) was recorded in Si treatments of combined SiR3 and SiF3 (*Fig. 1*). Plant biomass was increased, 71%, 62%, and 119% respectively, in SiR + Cd50, SiR + Cd100, and SiR + Cd200 than that of the Cd50, Cd100 and Cd200. While, in SiF + Cd50, SiF + Cd100, and SiF + Cd200 was increased, 45%, 40%, and 101%, respectively, than that of Cd50, Cd100, and Cd200. The maximum increase in plant biomass recorded in SiR3 while; minimum plant biomass was recorded in Cd200 than control (*Fig. 1*). Results showed that the overall SiR3 application performed well as compared to SiF3. Results showed that plant biomass in SiR3, SiR + Cd50, SiR + Cd100, and SiR + Cd200 was 10%, 18%, 15%, and 9%, respectively than that of SiF3, SiF + Cd50, SiF + Cd100, and SiF + Cd200. Si concentration was found positively correlated with plant growth parameters (*Fig. 2*).

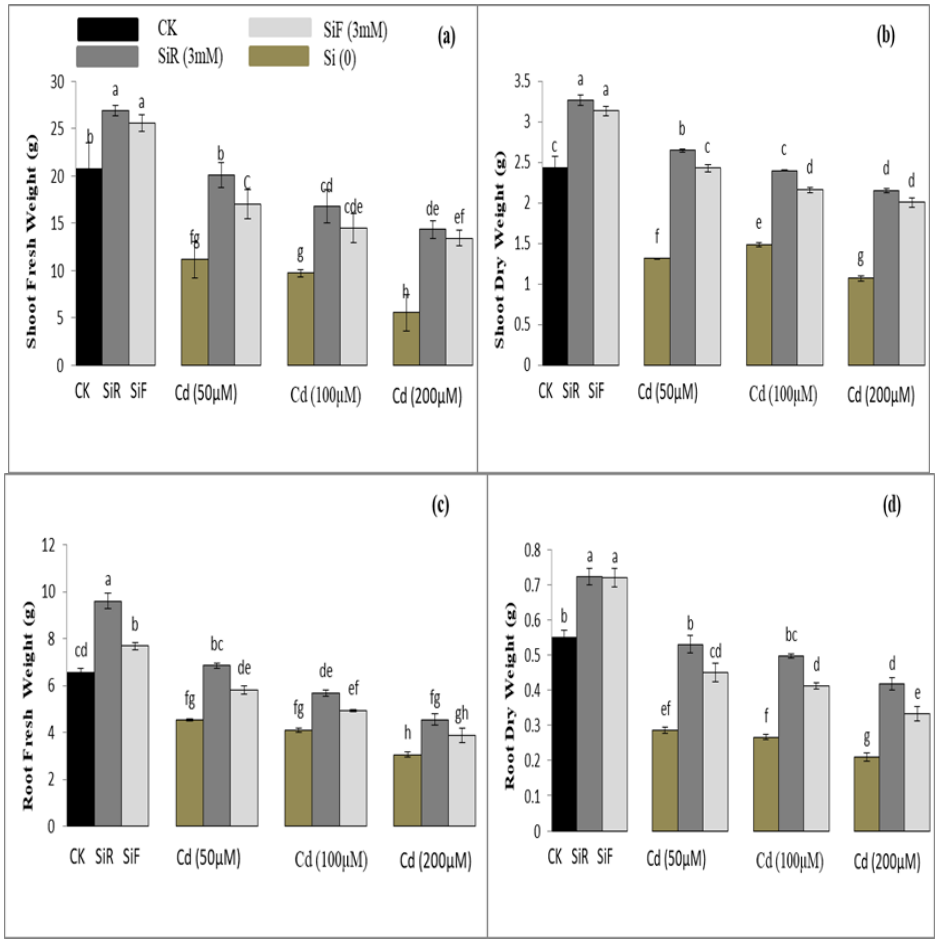

Figure 1. *The effects of silicon application (0, 3 mM) on shoot fresh weight (a), shoot dry weight (b), root fresh weight (c) and root dry weight (d) of the wheat plant grown under 0, 50, 100 and 200 µM Cd containing nutrient solution. Means ± SD (n = 3) with different letters in column indicates significant ($p \leq 0.05$) differences between treatments using Duncan's multiple range test (DMR)*

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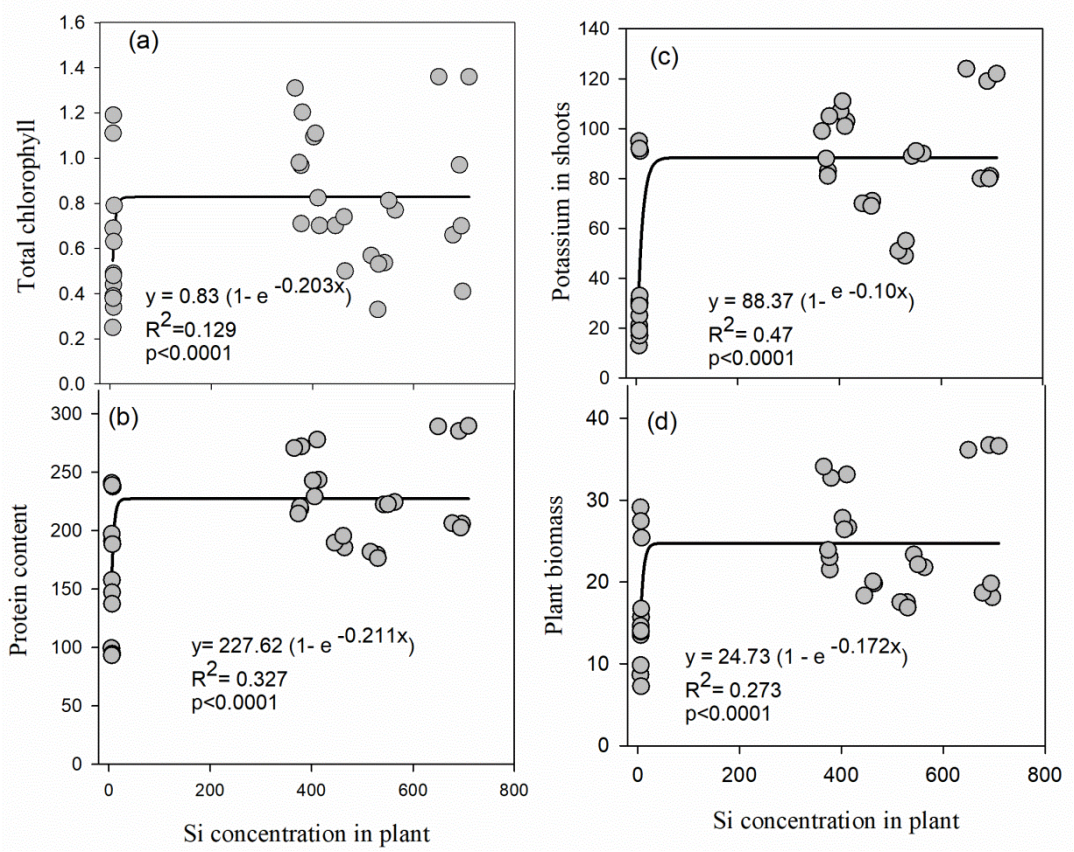

Figure 2. *Relationships among Si concentration in plants with (a) total chlorophyll content (b) protein content (c) potassium in plant and (d) plant biomass*

Cadmium attenuates the photosynthetic pigments

Cadmium (Cd) stress triggered a significant ($p < 0.05$) decline in carotenoids and total chlorophyll contents in wheat plants relative to control (*Table 2*). Total chlorophyll content in Cd50, Cd100, and Cd200 was decreased by 38%, 55%, and 71% respectively, as compared to control. Similarly, carotenoids contents in Cd50 were only 38%, while in Cd100 were 54% and in Cd200 were 71% compared to the control (*Table 2*). In the absence of Cd, Si treatment as SiF3 and SiR3 significantly ($p < 0.05$) elevated carotenoids and total chlorophyll contents. For instance, total chlorophyll contents in SiR + Cd50 were 34% that of Cd50, while, in SiR + Cd100 were 30% that of Cd100 and in SiR + Cd200 were 123% that of the Cd200. Correlation analysis was done between the means of the total concentration of Si in plant and total chlorophyll content, which showed a significantly ($p < 0.05$) positive correlation (*Fig. 2*). Similarly, carotenoids contents in SiR + Cd50 were 67% higher than Cd50, while, in SiR + Cd100 were 69% higher than Cd100 and in SiR + Cd200 were 83% higher than Cd200, respectively. Moreover, total chlorophyll contents in SiF + Cd50 were 43% higher than Cd50, while in SiF + Cd100 were 58% higher than Cd100 and in SiF + Cd200 were 90% higher than Cd200 respectively. Furthermore, the significant increase in total chlorophyll contents observed in SiF3 as compared to SiR3. For instance, total chlorophyll contents in SiF + Cd50 were 6% higher than SiR + Cd50, while in SiF + Cd100 were 18% higher than SiR + Cd100, and in SiF + Cd200 were 17% higher than

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SiR + Cd200 respectively. Contradictory results recorded in carotenoids content like maximum carotenoids contents observed in SiR3 than SiF3. Carotenoids contents in SiR + Cd50 were 29% higher than SiF + Cd50, while in SiR + Cd100 were 146% higher than SiF + Cd100, and in SiR + Cd200 were 66% higher than SiF + Cd200, respectively (*Table 2*).

Table 2. *Effect of different concentration of Cd (0, 50, 100, and 200 µM) and Silica sol. (0, 3 mM) along with different methods of Si application on chlorophyll contents of the wheat crop*

Treatments	Chl a (mg/g)	Chl b (mg/g)	Total Chl (mg/g)	Carotenoids (mg/g)
Ck	0.80 ± 0.005^{bc}	0.34 ± 0.03^{abc}	1.03 ± 0.12^{abc}	0.65 ± 0.02^{ab}
SiR 3mM	0.94 ± 0.017^{a}	0.42 ± 0.02^{a}	1.23 ± 0.13^{a}	0.83 ± 0.05^{a}
SiF 3mM	0.85 ± 0.030^{b}	0.39 ± 0.01^{ab}	1.11 ± 0.15^{ab}	0.71 ± 0.04^{ab}
Cd 50 µM	0.44 ± 0.014^{fg}	0.22 ± 0.01^{de}	0.59 ± 0.08^{ef}	0.34 ± 0.02^{de}
SiR3 + Cd50	0.75 ± 0.02^{cd}	0.32 ± 0.01^{bc}	0.97 ± 0.13^{bc}	0.57 ± 0.01^{bc}
SiF3 + Cd50	0.70 ± 0.00^{5d}	0.26 ± 0.02^{cd}	0.89 ± 0.09^{cd}	0.55 ± 0.21^{bc}
Cd 100 µM	0.36 ± 0.015^{g}	0.16 ± 0.02^{ef}	0.45 ± 0.03^{fg}	0.26 ± 0.01^{de}
SiR3 + Cd100	0.51 ± 0.013^{ef}	0.27 ± 0.02^{cd}	0.71 ± 0.09^{de}	0.44 ± 0.08^{cd}
SiF3 + Cd100	0.50 ± 0.005^{ef}	0.21 ± 0.02^{de}	0.65 ± 0.07^{ef}	0.41 ± 0.02^{cd}
Cd 200 µM	0.27 ± 0.011^{h}	0.08 ± 0.01^{f}	0.32 ± 0.04^{g}	0.18 ± 0.01e
SiR3 + Cd200	0.41 ± 0.014^{e}	0.26 ± 0.01^{cd}	0.73 ± 0.09^{de}	0.33 ± 0.03^{de}
SiF3 + Cd200	0.37 ± 0.023^{g}	0.17 ± 0.02^{e}	0.48 ± 0.07^{fg}	0.28 ± 0.01^{de}

Values show the means of three replications ± SD. Means followed by same small letters are not significantly different at $P \leq 0.05$ by using the Duncan's multiple range test (DMR)

Cadmium accumulation in the wheat plant

Results showed that Cd content significantly enhanced when plants received Cd stress relative to control. Silicon (3 mM) addition as SiR3 and SiF3 significantly reduced Cd concentration in all parts of the plant, and the effect was more significant in SiR3 than SiF3. Moreover, results exhibited that Cd concentration in SiR + Cd50 was 25% less than SiF + Cd50, while in SiR + Cd100 was 25% less than SiF + Cd100, and SiR + Cd200 was 32% less than SiF + Cd200 in shoot (*Table 2*). Whereas, Cd concentration in SiR + Cd50 was 27% higher than SiF + Cd50, while SiR + Cd100 was 10% higher than SiF + Cd100, and SiR + Cd200 was 21% higher than SiF + Cd200 in root (*Table 3*). Both Si treatments minimized Cd accumulation significantly, but SiR3 bound more Cd in root cells and restricted its translocation from root to shoot and detoxify its deleterious effects more efficiently than Si foliar-application (*Table 3*).

Table 3. *Effect of different concentration of silica sol. (0, 3 mM) along with different methods of application and Cd (0, 50, 100 and 200 µM) on Cd and Si accumulation in wheat crop*

Treatments	Cd accumulation (µg/g) in root	Cd accumulation (µg/g) in shoot	Si accumulation (µg/g) in root	Si accumulation (µg/g) in shoot
Ck	5.07 ± 0.1^{j}	0.04 ± 0.02^{i}	2.57 ± 0.2^{g}	4.11 ± 0.5^{d}
SiR 3 mM	0.70 ± 0.1^{j}	0.03 ± 0.01^{i}	423.11 ± 5.9^{d}	139.88 ± 9.2^{c}
SiF 3 mM	0.53 ± 0.1^{j}	0.02 ± 0.005^{i}	85.98 ± 3.6^{ef}	299.55 ± 16.5^{a}

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Cd 50 µM)	1696.84 ± 40.6^{e}	1112.103 ± 7.1^{c}	3.17 ± 0.2^{g}	3.27 ± 0.3^{d}
SiR3 + Cd50	837.89 ± 9.4^{h}	229.1 ± 2.8^{h}	660.05 ± 6.0^{c}	130.98 ± 7.1^{c}
SiF3 + Cd50	659.50 ± 4.63^{i}	309.57 ± 12.3^{g}	93.15 ± 2.9^{ef}	213.48 ± 5.9^{a}
Cd 100 µM	2335.95 ± 18.7^{c}	1313.89 ± 9.9^{b}	3.25 ± 0.1^{g}	3.00 ± 0.2^{d}
SiR3 + Cd100	1347.83 ± 21.9^{f}	453.38 ± 23.3^{f}	797.88 ± 13.2^{b}	125.85 ± 3.7^{c}
SiF3 + Cd100	1216.36 ± 10.1^{g}	605.09 ± 7.9^{e}	101.78 ± 5.2e	210.68 ± 6.1^{d}
Cd 200 µM	3654.55 ± 7.3^{a}	1936.27 ± 26.4^{a}	3.07 ± 0.1^{g}	2.87 ± 0.3^{d}
SiR3 + Cd200	2037.04 ± 42.7^{b}	657.42 ± 23.6b^{d}	997.64 ± 6.9^{a}	117.03 ± 2.5^{c}
SiF3 + Cd200	2474.82 ± 8.3^{b}	978.08 ± 11.4^{c}	63.97 ± 15.8^{f}	281.45 ± 5.9^{b}

Values show the means of three replications ± S.D. Means by the same small letters are not significantly different at $P < 0.05$ by using the Duncan's multiple range test (DMR)

Silicon attenuates translocation factor and bioaccumulation factor of Cd

Results showed that a significant ($p < 0.05$) positive correlation was recorded between Cd concentration and translocation factor (TF) (*Table 4*), which means Cd translocation factor increased with the rise of Cd levels in the nutrient solution. While Si application as SiR3 and SiF3 both minimized Cd translocation from root to shoot, but more significant results observed in case of SiR3. TF of Cd in SiR + Cd50 was 41% less than SiF + Cd50, while in SiR + Cd100 was 32% less than SiF + Cd100, and in SiR + Cd200 was 33% less than SiF + Cd200, respectively. Moreover, in both SiR3 and SiF3, TF < 1 showed Cd incompetently transport from root to shoot in a wheat plant in the presence of Si. In the case of Cd, bioaccumulation factor recorded data showed significantly ($p < 0.05$) negative correlation between BAF of Cd and Cd existence in the nutrient solution (*Table 5*). Silicon both applications SiR3 or SiF3 showed a significantly negative correlation with both Cd bioaccumulation and translocation factors (*Fig. 4*). Moreover, Si treatments as SiR3 and SiF3 along with Cd (50, 100, and 200 µM) further minimized BAF of Cd, but results were more significant in SiR3 as compared to SiF3. For instance, BAF of Cd in SiR + Cd50 was 26% less than SiF + Cd50, while in SiR + Cd100 was 25% less than SiF + Cd100, and in SiR + Cd200 was 33% less than F3 + Cd200 respectively (*Table 4*).

Table 4. *Effect of different application methods as well as levels of Si on Cd translocation and bioaccumulation factors*

Treatments	Translocation factor (%)	Bioaccumulation factor (%)
Ck	0.007 ± 0.003^{f}	0 ± 0^{g}
SiR 3 mM	0.004 ± 0.012^{f}	0 ± 0^{g}
SiF 3 mM	0.05 ± 0.025^{f}	0 ± 0^{g}
Cd 50 µM	0.65 ± 0.011^{a}	0.121 ± 0.0007^{a}
SiR3 + Cd50	0.27 ± 0.003^{e}	0.025 ± 0.0003^{e}
SiF3 + Cd50	0.47 ± 0.001^{c}	0.034 ± 0.001^{d}
Cd 100 µM	0.56 ± 0.008^{b}	0.072 ± 0.0005^{b}
SiR3 + Cd100	0.34 ± 0.021^{de}	0.025 ± 0.001^{e}
SiF3 + Cd100	0.49 ± 0.01^{bc}	0.033 ± 0.0004^{d}
Cd 200 µM	0.55 ± 0.006^{bc}	0.053 ± 0.0007^{c}
SiR3 + Cd200	0.32 ± 0.017^{e}	0.018 ± 0.0006^{f}
SiF3 + Cd200	0.39 ± 0.005^{d}	0.027 ± 0.0003^{e}

Values show the means of three replications ± SD. Means followed by same small letters are not significantly different at $P \leq 0.05$ by using the Duncan's multiple range test (DMR)

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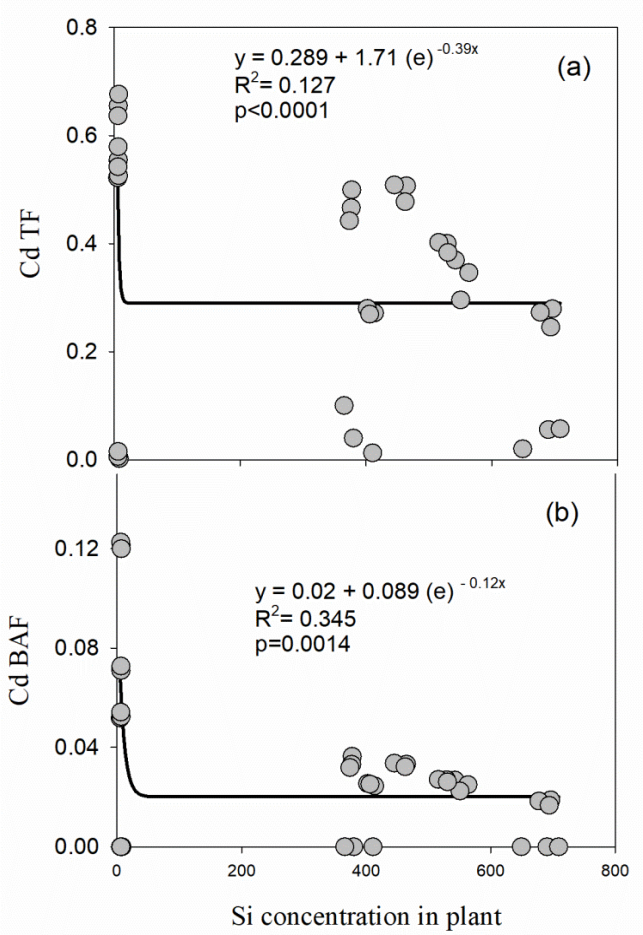

Figure 4. *The relationship among Si concentration in the plant, cadmium translocation factor (Cd TF), and cadmium bioaccumulation factor (Cd BAF)*

Silicon accumulation under cadmium toxicity

Silicon concentration was significantly ($p < 0.05$) increased when plants exposed to Si treatments as SiF3 and SiR3 relative to the control. Si concentration in SiF3 application was higher in shoots as compared to SiR3 application. For instance, Si concentration in SiF + Cd50 was 62% higher than in the SiR + Cd50, while, in SiF + Cd100 was 33% higher than in the SiR + Cd100, and in SiF + Cd200 was 31% higher than in the SiR + Cd200 respectively. While in SiR3 application, Si concentration in SiR3 was higher in root than shoot as compared to SiF3 application. For instance, our results showed that, Si concentration in SiR + Cd50 was 70% higher than in the SiF + Cd50, while, in SiR + Cd100 was 129% higher than in the SiF + Cd100, and in SiR + Cd200 was 189% higher than in the SiF + Cd200 respectively in root. The recorded data showed that Si concentration increased with the increase in Cd levels (*Table 3*). Si accumulation in root was more significant in SiR3 as relatively SiF3 while, Si accumulation in the shoot was more significant in SiF3 as relatively SiR3 (*Table 3*).

Cadmium enhances reactive oxygen species (ROS)

A significantly higher concentration of H_2O_2 and MDA in the shoot was recorded in Cd-treated plants than control (*Fig. 3b*).

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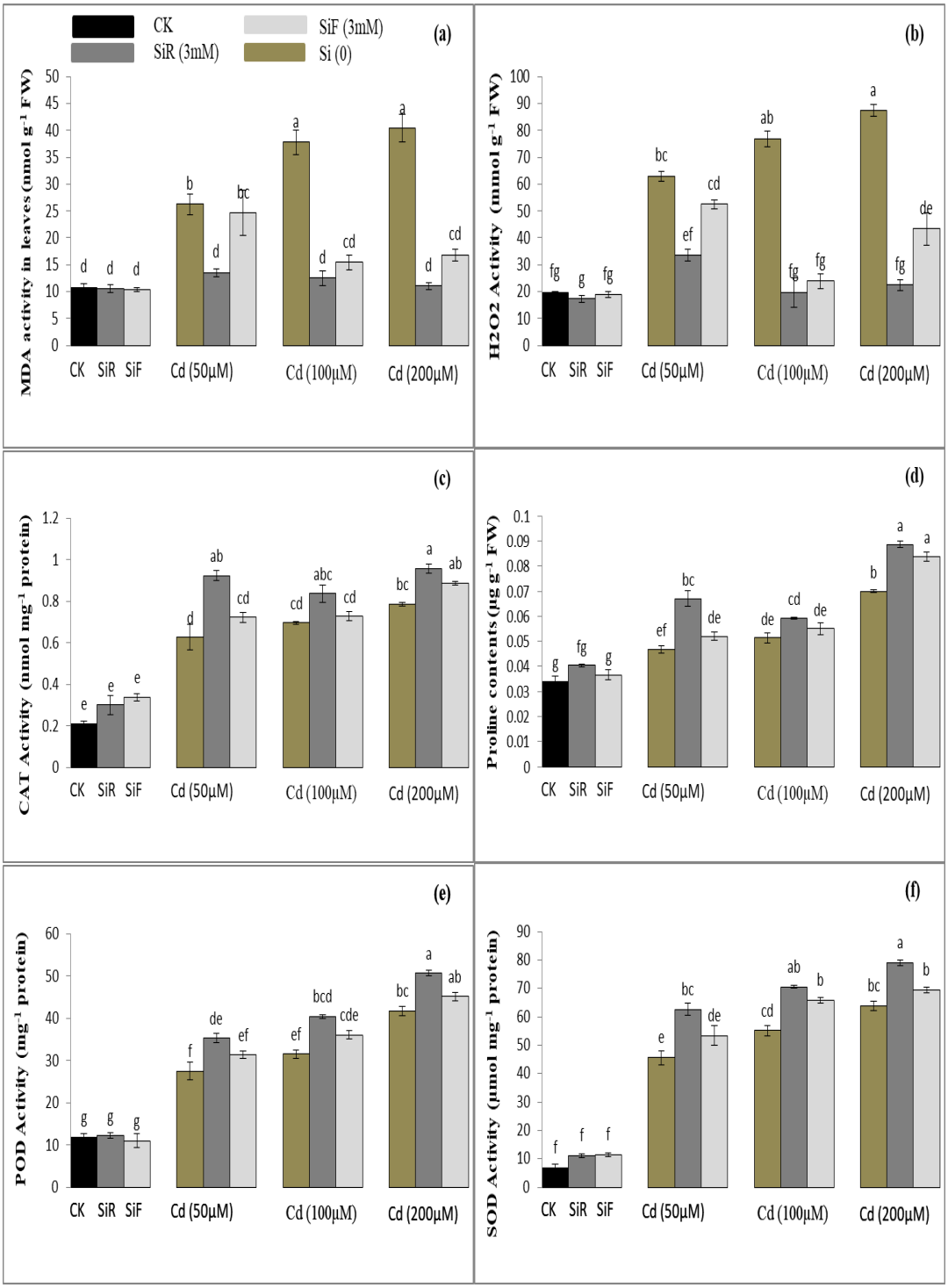

Figure 3. *The effects of silicon application (0, 3 mM) on reactive oxygen species (ROS) activity i.e. MDA (a), H_2O_2 (b), and antioxidative enzymes activity i.e. CAT (c), proline (d), POD (e) and SOD (f) of the wheat plant grown under 0, 50, 100 and 200 µM Cd containing nutrient solution. Bars show S.E. of three replications, the different letter above column within CAT, SOD, POD, MDA, H_2O_2, and proline represents a significant difference at $P < 0.05$ by using Duncan's multiple range test (DMR)*

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For instance, H_2O_2 concentration was 219% higher in Cd50, was 290% higher in Cd100, and was 343% higher in Cd200 than in control, respectively. Si application as SiR3 and SiF3 significantly ($P < 0.05$) decreased the concentration of H_2O_2 in shoots (*Fig. 3b*), but SiR3 proved more effective than SiF3. For instance, H_2O_2 concentration in SiR3 + Cd50 was 36% lower than in the SiF3 + Cd50, while in SiR + Cd100 was 17% lower than in SiF + Cd100, and in the SiR + Cd200 was 48% lower than in the SiF + Cd200 respectively (*Fig. 3*b). The same trend recorded in MDA contents. MDA concentration was significantly high in Cd-treated shoots as being compared to control. For instance, MDA concentration was, respectively, 144%, 251%, and 276% in Cd50, Cd100, and Cd200 that of the control (*Fig. 3a*). Added Si as SiR3 and SiF3 significantly lower MDA concentration in shoots of wheat plants at all Cd supply levels (*Fig. 3a*), but the maximum decreased in MDA concentration was noted in SiR3-treated plants along with or without Cd50, Cd100, and Cd200 as compared to all other treatments. For instance, MDA concentration was 45%, 18%, and 34% lower in SiR + Cd50, SiR + Cd100, and SiR + Cd200 that of the SiF + Cd50, SiF + Cd100, and SiF + Cd200 respectively (*Fig. 3a*).

Cadmium meliorates enzymatic and non-enzymatic antioxidants in wheat seedlings

A significant elevation was recorded in enzymatic (superoxide dismutase; SOD, peroxidase; POD and catalase; CAT) and non-enzymatic (proline) antioxidants concentration in Cd-treated plants as being compared to control. For instance, SOD activity was, 568%, 708%, 835% higher respectively, POD activity was 133%, 166%, 253% higher respectively, CAT activity was 198%, 231%, 275% higher respectively, and proline concentration was 37%, 51%, 106% higher in Cd50, Cd100, and Cd200 that of the control (*Fig. 3c, d, e, f*). Added Si as SiF3 and SiR3 further elevated SOD, POD, CAT, and proline activities significantly with or without Cd50, Cd100, and Cd200. Overall, SiR3 alone, as well as with Cd50, Cd100, and Cd200 performed well to boost up SOD, POD, CAT, and proline activities as compared to SiF3, but the difference was not significant. For instance, POD activity in SiR3 along with Cd (50, 100 and 200 μmol/L) was 31%, 36%, 45% respectively, SOD activity was 17%, 7%, and 14% higher, CAT activity was 27%, 15%, and 8% higher, and proline concentration was 28%, 8%, and 6% higher than that of higher than of SiF3 along with same levels of Cd (*Fig. 3*).

Cadmium attenuate essential nutrients in wheat

A significant decreasing trend was recorded in K^+ and N concentration in Cd-stressed plants than that of control (*Table 5*). For instance, K^+ and N concentration was, 66% and 73%, 82% and 19%, 38%, 59% respectively, in Cd50, Cd100, and Cd200 that of the control. Added Si as SiF3 and SiR3 alone, as well as with Cd50, Cd100, and Cd200, significantly increased K^+ and N concentration in the shoot of the wheat plant (*Table 5*). The maximum increase in results recorded in case of SiR3 compared to SiF3. For instance, K^+ and N concentration in shoot was, respectively, 27%, 28%, 55% and 24%, 51%, 113% higher in SiR + Cd50, SiR + Cd100, and SiR + Cd200 that of the SiF + Cd50, SiF + Cd100, and SiF + Cd200. Total Si concentration in plant showed a strong affirmative association with K^+ concentration in shoots of wheat plants (*Fig. 2*). With the increase of Si concentration from 0 to 3 mM significantly increased K^+ concentration from 16 mg $g^{-}1$ to 107 mg g^{-1} (*Table 5*).

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Table 5. *Effect of different concentrations of Cd (0, 50, 100, and 200 µM), and Silica sol. (0 and 3 mM) along with different ways of Si application on K^+, total N and total Protein concentration in shoots of the wheat plant*

Treatments	K^+ concentration in shoot (mg/g)	Total N in shoot (mg/g)	Total protein in shoot (mg/g)
Ck	92.67 ± 1.20^{c}	38.25 ± 0.15^{c}	239.06 ± 0.93^{c}
SiR 3 mM	121.67 ± 1.45^{a}	46.07 ± 0.22^{a}	287.96 ± 1.36^{a}
SiF 3 mM	101.67 ± 1.76^{b}	43.75 ± 0.35^{b}	273.44 ± 2.21^{b}
Cd 50 µM	31.33 ± 0.88^{h}	30.73 ± 0.43^{fg}	192.08 ± 2.68^{fg}
SiR3 + Cd50	107.00 ± 2.31^{b}	38.15 ± 0.74^{c}	238.41 ± 4.64^{c}
SiF3 + Cd50	84.00 ± 2.08^{de}	34.86 ± 0.27^{de}	217.84 ± 1.70^{de}
Cd 100 µM	25.00 ± 2.31^{h}	23.57 ± 0.94^{h}	147.32 ± 5.88^{h}
SiR3 + Cd100	90.00 ± 0.58^{cd}	35.68 ± 0.10^{d}	223.02 ± 0.64^{d}
SiF3 + Cd100	70.00 ± 0.58^{f}	30.42 ± 0.46^{g}	190.14 ± 2.87^{g}
Cd 200 µM	16.33 ± 1.76^{i}	15.32 ± 0.30^{i}	95.72 ± 1.90^{i}
SiR3 + Cd200	80.33 ± 0.33^{e}	32.77 ± 0.18^{ef}	204.81 ± 1.16^{ef}
SiF3 + Cd200	51.67 ± 1.76^{g}	28.67 ± 0.25^{g}	179.21 ± 1.59^{g}

Values show the means of three replications ± SD. Means followed by same small letters are not significantly different at $P \leq 0.05$ by using Duncan's multiple range test (DMR)

Discussion

Plants exposed to several biotic and abiotic stressors in different agriculture systems. Abiotic stressors like heavy metals stress, restrict plant biomass, and limit its yield. In present findings, Cd existence in nutrient solution at the concentration of 50 µM, 100 µM, and 200 µM severely decreased wheat plant growth (*Fig. 1*), silicon (Si) contents (*Table 2*), chlorophyll contents (*Table 2*), potassium (K^+) and nitrogen (N) contents (*Table 5*), while it significantly increased Cd content (*Table 3*), Cd translocation and bioaccumulation factors (*Table 4*), lipid peroxidation measured as malondialdehyde (MDA) content (*Fig. 3*), enzymatic and non-enzymatic antioxidative contents (*Fig. 3*). However, Si application either as root or foliar method at a level of 3 mM encountered the negativity of cadmium toxicity in wheat plants by further elevating of enzymatic and non-enzymatic antioxidative contents, reducing Cd, MDA, and H_2O_2 contents, resulting in high plant growth. These findings are consistent with Farooq et al. (2013), Howladar et al. (2018), Silva et al. (2017), Alzahrani et al. (2018), Wang et al. (2016), and Rady et al. (2015). The dominant and diverse thing in the current findings that two separate methods of Si supplementation (e.g., foliar spray, root treatment) were tested and the optimum results were attained with Si root application.

Results of the present study demonstrated that Cd stress interrupted several biological, chemical, and metabolic processes in various plant species (Kim et al., 2014; Shi et al., 2010; Zhang et al., 2014; Zhao et al., 2017). Foyer and Noctor (2005) has been identified Cd as a disruptor of antioxidant defense system by overproducing oxidative burst and reactive oxygen species (ROS), which enhances lipid peroxidation by MDA contents (Howladar et al., 2018). Cd accumulation gradually increased in plant various parts with preference of root, with the rise of Cd levels in growth medium. Our data showed that higher Cd accumulation in root than shoot of wheat plants. Plants take up lower amount of metal solution in upper ground part in any event with highest tolerance (Yang et al., 2004). In this study, Cd stress generated high levels of ROS,

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which indicated by severing reduction of photosynthetic gas exchange parameters in leaf, and significant elevations in MDA and H_2O_2 contents. Therefore, to meet these adverse conditions, plants are grown under Cd-induced oxidative damage to develop complex antioxidant systems (Dixit et al., 2001; Farooq et al., 2013; Howladar et al., 2018).

Silicon as a high-quality non-corrosive fertilizer enhanced plant existence against Cd toxicity by significantly interrupted uptake and/or accumulation of Cd in Cd-toxic plants (Farooq et al., 2013; Song et al., 2009; Wang et al., 2016; Zhao et al., 2017). The constructive effects of Si on plant physio-biochemical traits under various environmental stresses have been well documented (Hashemi et al., 2010). Previous researchers with different findings (Ali et al., 2013; Kabir et al., 2016b; Shi et al., 2005; Song et al., 2009; Zhao et al., 2017) have suggested optimum methods by which Si can enhance plant tolerance against abiotic stresses including Cd that considered the most critical toxic elements, causing limited crop yield in both arid and semiarid regions. The researchers have been conducting various studies to establish Si beneficial effects on the development and yield of wheat cultivars under Cd toxicity. In our present study, Si found effective fertilizer in demolishing Cd toxicity in the wheat plant. The growth traits were significantly elevated in stressed-plants to normal with Si supplementation. Si causes nutritional balance and increases dry matter production of plants while applied in suitable dose (Silva et al., 2017). According to the results of the present study, nutrient medium receiving Si or plants received Si as foliar spray increased growth of stress wheat plants. This upgrading in wheat growth may be recognized to that Si improved photosynthetic rate that is correlated to activity of ribulose biphosphate carboxylase, leaf ultrastructure, and leaf chlorophyll contents (Hamayun et al., 2010).

Oxidative damage generally caused in plants due to ROS, e.g., superoxide radical, H_2O_2, and hydroxyl radical (OH^{-1}) (Shahid et al., 2014; Tamás et al., 2017). Along with the overproduction of ROS, membrane permeability with lipid peroxidation is also the main mechanism in higher plants due to Cd exposure (Farooq et al., 2013). MDA, as a derivative of lipid peroxidation, destabilized cell membrane integrity, and higher the risk of its permeability in various crops (Moussa, 2006; Soylemezoglu et al., 2009). In our findings, Si supplementation significantly lowers the malondialdehyde (MDA) contents in Cd-stressed wheat plants, resulting in sustain cell membranes integrity and reduce their permeability in Cd stress. Plants have various enzymatic (CAT, SOD, and POD) and non-enzymatic (proline) antioxidant defense systems to control different productions of ROS. Among these protective defense systems, the fusion of osmolytes/osmoprotectants is one of the integral protective mechanisms of plants grown in stress conditions (Rios et al., 2017). Proline as osmoprotectants protects plant cells by neutralizing osmotic strength of external environment and the cytosol and vacuole osmotic strengths under salt stress (Gadallah, 1999).

Furthermore, proline, as an impotent osmolyte accumulated in response to osmotic stress, contributes to osmotic adjustments in plant cells (Gadallah, 1999). Proline contents scavenge free radicals of oxidative stress molecules, stabilizes protein and sub-cellular structures under Cd stress (Sharma and Dubey, 2005). As an optimum Si application method, Si root-application improved proline accumulation in wheat plants to increase plant chances to withstand under oxidative stress induced by Cd (*Fig. 3*).

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Enzymatic antioxidants included catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), etc. is another defense system in various stresses including Cd (Şen, 2012). In the current study, enzymatic antioxidant activities were higher with Cd-induced oxidative stress and were further improved with Si supplementation with the preference of Si root-application. Cd-induced oxidative stress resulted superoxide radicals that were transformed into H_2O_2 by SOD. H_2O_2 is a powerful oxidant that is accumulated in plant tissues from SOD canalization reaction and is prohibited by the cycle of ascorbate-glutathione. Except H_2O_2, another lethal oxide is the OH^{-1}, which can combine with all macro-molecules. By assimilating their actions, both CAT and SOD can stop OH^{-1} formation in plant tissues (Kusvuran et al., 2016). It has established in previous findings that Si application elevated CAT and SOD (Howladar et al., 2018). Kabir et al. (2016b) found that addition of Si under stress considerably improved CAT and SOD activities in alfalfa plants. Peroxidases (PODs) due to their role in consuming and scavenging H_2O_2 can modify ROS levels in plants. Compared to SOD and CAT, PODs have high attraction to H_2O_2, though PODs can also produce H_2O_2 by the oxidation of NAD(P)H (Ranieri et al., 2005). Several scientists have reported in their findings that Si application elevated POD activity in plants grown under oxidative stress. In general, Si treatment reduced ROS generation and led to raising enzymatic and non-enzymatic anti-oxidants used to scavenge ROS (Rios et al., 2017). Therefore, Si uses ROS scavenging metabolic pathways more effectively, which makes it able to alleviate Cd-induced oxidative stress at cellular level, which may recover the integrity of cell membranes.

Cd accumulation in shoots and roots of wheat plants followed the same trend as shoots, and roots levels reduced with increasing Si dose as a foliar application. The same results found by previous reports (Treder and Cieslinski, 2005), but our results were conflicted with the finding of several studies that examined decreased shoots Cd concentration with increasing Si levels (Rizwan et al., 2012). Interestingly, contradictory results were obtained in case of Si root application as Cd concentration did not follow the same trend as Cd reduced in all three parts of plants than that of Cd alone treatments. But our consequences were conflicted with the finding of several studies which examined decreased Cd shoots concentration with increasing Si levels (Rizwan et al., 2012). Interestingly, contradictory results were obtained in case of Si root application as Cd concentration did not follow the same trend, i.e., Cd shoot concentrations declined while root concentrations elevated with increasing Si dose. Similar results were also found in durum wheat developed in a soil with aged impurity (Rizwan et al., 2012), rice is grown hydroponically (Shi et al., 2005; Zhang et al., 2008), in peanut and cucumber (Shi et al., 2010) and *Brassica Chinensis* (Song et al., 2009), but our results in case of SiR3 disagreed with authors who found Cd increased in both root and shoot with the increase in Si doses (Vaculík et al., 2009), or a decrease in Cd concentration in both roots and leaves with increase in Si levels (Nwugo and Huerta, 2008). In Si root application, increased Cd concentration in roots could be due to the deposition of Cd in roots and formed metal complexes with silicates as reported by numerous studies. i.e., Zhang et al. (2008) established that Cd usually deposited in the section of roots endodermis and epidermis in rice plants, while, Shi et al. (2010) found that Si often stored in the cell walls of the endodermis and formed metal complexes with silicates. However, in SiF3 application Cd concentration in both roots and shoots was decreased with increasing Si levels, it could be due to Si inhibitory behavior, Si deposited in leaves cells and translocated from leaves to shoot and then roots and

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inhibited Cd translocation through xylem and phloem (Treder and Cieslinski, 2005). In both methods of Si, application plant withstand against Cd toxicity may be attributed due to the improvement of plant tolerance and/or due to uptake and transport of Cd which was also reported by Wang et al. (2016). In our study Cd concentration was high in Cd alone treatments as compared to Cd + Si (*Table 2*) that were conflicted with the findings of various authors who found that the Cd concentration in both roots and shoots was higher in Cd + Si treated plants than alone Cd-treated plants (Prabagar et al., 2011; Ye et al., 2012). Our results showed TF < 1 suggesting that Cd could ineffectively be translocated from root to shoots in case of both SiF3 and SiR3, but more significant effects observed in the case of SiR3 (*Table 5*) which is in line of previous findings (Howladar et al., 2018). In our study Si concentration under Cd stress was significantly increased with Si supplementation (Silva et al., 2017), which clarified that in Si both application methods, Si absorbed by roots should have translocation into shoots, where it deposits in the leaf apoplast as a polymer, forming a crucial barrier to protect plants from various environmental stresses counting Cd stress. Furthermore, Si concentration in plant showed a negative correlation with TF, BAF, and BCF of Cd (*Fig. 4*), which suggests that with the increase of Si concentration in plants, Cd translocation and bioaccumulation will decrease simultaneously. Our results showed that BAF of Cd decreased with the rise of Cd concentration in nutrient solution (*Table 5*) which was in line of previous findings (Zhao et al., 2003) but was contrary with some scientists who found increase in BAF with increase of heavy metal accumulation in soil (Rezvani and Zaefarian, 2011).

Micro and macronutrients play an optimum role in the maintenance of biochemical processes, plant growth, and high plant yield if they are up taken in a controlled way, transported to a long distance, and then correctly utilize. Si application against chromium (Cr) and Cd stress improved the utilization of macro (Ca^{++}, Mg^{++}, and K^{+}) and microelements (Fe and Zn) (Tripathi et al., 2012). Our results showed a strong positive correlation between Si and K^{+} concentration, which means with the increase of Si concentration in solution K^{+} would also be increased simultaneously (*Fig. 4*). Previous studies (Jayakannan et al., 2013) showed that Si application increased K^{+} concentration plants under abiotic stresses. According to our hypothesis, Si might improve K^{+} concentration in stressed-plants by mitigating nutrient imbalance created by Cd-stress. Our study showed that Si and K^{+} have positive effects on the production of ROS in plants under stress conditions (*Table 4*).

Conclusion

In conclusion, the addition of Si has been proved high-quality non-corrosive fertilizer under the hostile conditions of Cd-stressed wheat seedlings as a study herein. Our data demonstrated that Si supplementation, with the preference of root application, significantly improved growth traits by successfully encountered toxic belongings of Cd stress. Si root-application enhanced all recorded parameters such as photosynthetic pigments, enzymatic (CAT, SOD, POD), and non-enzymatic (proline) antioxidants and demolished MDA, H_2O_2, and Cd contents. Si showed the antagonistic effect with Cd concentration in plants and showed synergetic effect with essential nutrients like K and N. After analyzing the recorded data, we can be strongly emphasized that Si supplementation with the preference of root-application must be used as an optimum approach to reduce Cd toxicity in wheat plants.

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APPENDIX

1. Recipe of Hoagland's solution

The Hoagland's solution had composition (mg L^{-1}): (NH4)2SO4 48.2, MgSO4 65.9, K2SO4 15.9, KNO3 18.5, Ca (NO3)2 59.9, KH2PO4 24.8, Fe citrate 6.8, MnCl2.4H2O 0.9, ZnSO4.7H2O 0.11, CuSO4.5H2O 0.04, H3BO3 2.9, H2MoO4 0.01.

2. Recipe of Si treatment

Silicon (Si) as a silica nanoparticle prepared from sodium silicate. A measured amount of sodium silicate put into the double amount of boiling deionized distilled water in a petri dish for 10 min. For increasing its solubility, we added a small amount of KOH and further heated for 10 min. Continue stirring with a long-handled spoon and at the end added 2 to 3 drops of H_2O_2. The solution removed from heat stove and allowed it to cool at room temperature. Cooled solution transferred into a plastic bottle and sealed it up. From the prepared stock solution, we made subsolution of 3 mM/L and applied as SiF3 and SiR3. Both treatments Si and Cd were applied after 65 days of transplantation. The twelve treatments were arranged factorially in a randomized complete block design with three replications per treatments. A total number of pots were 36, and each pot contained 15 plants.

3. Recipe to determine nutrient elements in plant tissues

Cd and Si were measured in plant root and shoot dry masses by atomic absorption spectrometry method with some necessary modifications (Fırat et al., 2017). After 35 days of treatments, wheat plants were harvested and washed thoroughly with tap water, distilled water, and then with double distilled water. Plant samples were separated into roots and shoots and dried at 70 °C in the oven for 48 h, and ground into powder. Weighed 0.2 g of dried plant sample was placed into a microwave digestion tube. Added 10 ml of nitric acid and covered every sample with a lid. Later, it was put into the microwave digestion instrument and started to dissolve after selection of an appropriate program. After completion of digestion, tubes were removed and put the digestion solution into the PTFE digestion cup with a small number of repeated flushing. Digestion cups were placed on a hot plate at 220 °C temperature until the removal of 2-3 ml liquid. In the end, the digestion fluid was transferred into the 50 ml volumetric flask and made its volume up to 50 ml with double distilled water. Digestion solution used to measure Cd and Si concentrations in root and shoots by using an atomic absorption spectrophotometer (AAS) model AA-6300 SHIMADZU. The total contents of Cd and Si per plant were calculated from Cd and Si concentration (μg g^{-1}) and meant

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the dry weight of below- and above- ground parts. Total nitrogen was measured in plants by the method of (Brookes et al., 1985) with some modifications. Weighted 0.3 g of plant dry sample in digestion tubes with 4 ml of sulphuric acid was put on the curved stem small funnel into the digestion furnace and initiated to dissolve. The temperature was set to 220 °C for 2 h and boiled till brown, yellow endpoint. First, samples were heated at 380 °C and removed these digestion tubes from digestion furnace. Added 20 drops of hydrogen peroxide (H_2O_2) and samples were placed on hot plate and continue to heat again. This cycle was repeated 2-5 times to clear the digestion solution. At every cycle, the amount of H_2O_2 was decreased. The amount of H_2O_2 added to the blank was consistent with the maximum number of samples. After that, a few amounts of multiple flushing methods were transferred to the 100 ml volumetric flask. In the end, total nitrogen was determined in a solution using flow analyzer-3 of brand BRAN + LUEBBE, and potassium was determined in a solution using flame photometer FP6410.

4. Determination of biochemical parameters

For MDA measurement, 0.25 g leaf sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10,000 ×g for 10 min. to 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in the ice bath. After centrifugation at 10,000 ×g for 10 min, the absorbance of the supernatant at 532 nm was read and the value of the nonspecific absorption at 600 nm was subtract. The MDA content was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}. For the measurement of H2O2, we weighted 0.5 g leaf tissues were homogenized in an ice bath with 5 ml 0.1% (W/V) trichloroacetic acid (TCA). The homogenate was centrifuged at 12000 × g for 15 min and 0.5 ml of the supernatant was added to 0.05 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M KI. The absorbency of supernatant was assessed at 390 nm.

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