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Silicon alleviates arsenic-induced toxicity in wheat through vacuolar sequestration and ROS scavenging

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Abstract

Arsenic (As) is a phytotoxic element causing health hazards. This work investigates whether and how silicon (Si) alleviates As toxicity in wheat. The addition of Si under As-stress significantly improved morpho-physiological characteristics, total protein and membrane stability compared to As-stressed plants, suggesting that Si does have critical roles in As detoxification in wheat. Analysis of arsenate reductase activity and phytosiderophore release reveals their no involvement in the Si-mediated alleviation of As in wheat. Furthermore, Si supplementation in As-stressed plants showed a significant increase of As in roots but not in shoots compared with the plants grown under As stress. Further, gene expression analysis of two chelating molecules, *TaPCS1* (phytochelatin synthase) and *TaMT1* (metallothionein synthase) showed significant induction due to Si application under As stress compared with As-stressed plants. It is consistent with the physiological observations and suggests that alleviation of As toxicity in rice might be associated with As sequestration in roots leading to reduced As translocation in shoots. Furthermore, increased CAT, POD, and GR activities in roots imply the active involvement of ROS scavenging for protecting wheat plants from As-induced oxidative injury. The study provides mechanistic evidence on the beneficial effect of Si on As toxicity in wheat plants.

Keywords: As stress; wheat; vacuolar sequestration.

Introduction

Arsenic (As) is a toxic chemical element. Millions of people affected by As-contaminated water and food materials in South and Southeast Asia (Nordstrom 2002). Among the different As derivatives, arsenate is the dominant form of As in aerobic soils and is an analog of phosphate. Concentrations of As have increased substantially in soils as a result of the industrial revolution (Smith et al. 1998), and from the use of growth promoters for poultry and pigs (Christen 2001). High soil As is phytotoxic to plants causing decreases in plant growth and yields; discolored and stunted roots; reductions in protein contents, and photosynthetic capacity (Marin et al. 1993). Excessive uptake of As by crop plants may present a food safety problem. Soil contamination with As is now a serious concern, particularly in agricultural land (Mishra et al. 2014). It leads higher accumulation of As in crop grain that may pose a health risk to people (Jaishankar et al. 2014).

Silicon (Si) consists about 28% of the earth's crust (Sommer et al., 2006). Si has been proved to mitigate the adverse effect of few heavy metal and abiotic stresses in plants (Kabir et al. 2016; Greger et al. 2016). Importantly, mechanisms by which Si facilitate alleviation of heavy metals vary from plants to plants and the metals involved. These include modifying metal uptake and translocation and metal binding to the cell (Rogalla and Romheld 2002; Greger et al. 2016; Kabir et al. 2016). Ma et al (2008) reported that arsenite transport in rice roots shares the same pathway as Si. Further, Si converts soluble and exchangeable fractions of metals in the soil into stable chemical forms through various reactions (Chen et al. 2000; Liang et al. 2005). In plants, Si may alter translocation and distribution of metals in different plant parts to get rid of metal toxicity (Shi et al. 2005, Zhang et al. 2008). Recent studies revealed that the application of high Si (1mM) is useful for alleviating Cd toxicity in wheat (Greger et al. 2016) and alfalfa (Kabir et al. 2016). Therefore, the application of Si to reduce heavy metal toxicity in plants becomes an emerging practice to decrease environmental contamination and agricultural loss.

The adaptive mechanisms of plants to withstand heavy metal are linked with a correlation of physiological and molecular mechanisms. One important mechanism is the complexation of

heavy metal with various substances, such as organic acids, phytochelatins (PC), metallothioneins (MT) and amino acids (Sanita di Toppi et al. 2002; Cho et al. 2003) to regulate the translocation to the shoot. PCs and MTs are different classes of cysteine-rich, heavy metal-binding protein molecules (Cobbett and Goldsbrough 2002). Advancement in understanding the regulation of PC and MT gene expression has explored the knowledge of heavy metal detoxification in plants. In addition to vacuolar storage, plants have evolved antioxidant enzymes and antioxidant non-enzyme molecules to counteract the ROS (reactive oxygen species) induced by heavy metal stress (Kabir et al. 2016; Romero-Puertas et al. 2007). These antioxidant enzymes include catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), etc. HMs, in particular, Cd activates antioxidant enzymes such as SOD and GR (Romero-Puertas et al. 2007).

Wheat (*Triticum aestivum* L.) is the most produced cereal crop in Europe and the second most produced in the world. A continuous supply of As in the soil from As-contaminated irrigation water reduces wheat yields and poses a serious threat on human and livestock health. This study, therefore, was designed to investigate (i) whether Si does have beneficial roles in improving morphological characteristics (ii) restoring cellular activities and (iii) how Si facilitates this alleviation strategy in cellular and molecular levels in wheat plants under As stress.

Materials and Methods

Plant material and growth conditions

Seeds of wheat (var. Vinjett) were disinfected by superficial treatment with 95% (v/v) ethanol for 10 min before washing thoroughly in deionized water. Afterward, the seeds were placed in Petri dishes in wet tissue paper for three days in the dark at room temperature for germination. Uniform seedlings were transplanted to solution culture (Hoagland and Arnon 1950) containing the following nutrients (μM): KNO_3 (4000), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (4000), $\text{NH}_4\text{H}_2\text{PO}_4$ (1300), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2000), KCl (50), H_3BO_3 (25), Fe-EDTA (25), ZnSO_4 (2), $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (2), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5). The 3-day old wheat seedlings were grown in a plastic container (2 L) in a growth chamber (25 °C temperature and 70% humidity) under 16 h light and 8 h dark (550–560 $\mu\text{mol s}^{-1}$ per μA). The pH was adjusted to 6.0. The medium was treated with 50 μM As(V) (Na_2HAsO_4) and 0 or 1.0 mM $\text{H}_4\text{O}_4\text{Si}$. The concentration of As used in study was

optimized after several trials, which was consistent with the previous report (Ghosh et al. 2016). The solution was replaced every 3 days. Plants were grown concurrently for 5-day after treatment was imposed and harvested at the same time.

Measurement of morphological characters and chlorophyll concentrations

Morphological features such as shoot height, root length, shoot dry weight, and root dry weight was measured on 5-d old plants. Each root was washed in distilled water to remove nutrient and then quickly blotted in tissue paper. Shoot and root tissues were then dried in an oven at 70°C for 3 days before taking the dry weight.

The chlorophyll concentration (a and b) of leaves was estimated as previously described (Lichtenthaler and Wellburn 1985). Firstly, leaves were weighed and placed in 95% acetone in a 5 mL falcon tube. The leaf sample was then ground using mortar and pestle and was filtered through Whatman filter. The samples were then centrifuged at 2500 rpm for 10 min. The supernatant was separated, and the absorbance was recorded at 662 nm (chlorophyll a) and 646 nm (chlorophyll b) by spectrophotometer (UV-1650PC, Shimadzu). The concentration of chlorophyll a and b was calculated according to the formula (Lichtenthaler and Wellburn 1985).

As, Fe and P analysis by AAS (atomic absorption spectroscopy)

Roots and leaves were harvested from 5-day old plants and were digested in 3 mL HNO₃. The tissue samples were then heated in microwave oven at 75°C for 10 min, followed by 109°C for 15 min. The samples were then cooled for 10 mins before adding 1 mL of perchloric acid and heated at 109°C for 15 min. Further, the samples were analyzed by Flame Atomic Absorption Spectroscopy (AAS) outfitted (AA-6800, Shimadzu). The standard solutions (As, Fe and P) were separately prepared from their respective concentration from which further diluted for the standard calibration curve (Begum et al. 2016).

Determination of phyto siderophore (PS) release in roots

The PS release in roots was determined by analyzing the PS content in root washings. Briefly, plants were harvested 2 h after the onset of the light period and washed with deionized water for 1 min. Afterward, harvested roots were submerged in 500 ml deionized water aerated by the air

pump for 3 h. Later, collected exudates were passed through micropur (Roth, Germany) to prevent microbial degradation of PS (Valentinuzzi et al. 2015). Further, exudates were filtered through filter paper and concentrated to 20 ml at 50 °C under vacuum. Finally, PS release per gram tissue was calculated (Reichman and Parker 2006).

Determination of Si concentrations in root and shoot

Once harvested, root and shoot were separated from plants before washed with CaSO₄ (1mM) and deionized water. The tissue was then ground in mortar and pestle in deionized water. The samples were then centrifuged at 12,000 g for 5 min and the clear supernatant was transferred to 10% ammonium molybdate. Afterwards, 10% oxalic acid was added to each sample for forming silico-molybdate complex. Further, 0.5% ascorbic acid was added to each sample mixture and kept at room temperature for 20 mins until blue color is formed. Finally, the absorbance of the reaction mixture was measured at 660 nm in a UV spectrophotometer (Kabir et al. 2016).

Measurement of electrolyte leakage and total soluble protein

The electrolyte leakage (EL) was measured in roots and shoots using an electrical conductivity as previously described (Lutts et al. 1996), with some modifications. Initially, seedlings were washed with deionized water to remove surface contamination. Afterward, samples were weighed and placed in individual vials containing 20 mL of deionized water. These samples were incubated in a shaker at 25°C for 2h. The electrical conductivity of the solution was then recorded by conductivity meter.

Total soluble proteins in both roots and shoots were measured spectrophotometrically using different concentrations of bovine serum albumin (BSA) in calibration curve (Guy et al. 1992). Briefly, tissues were washed with deionized water and were ground with a chilled mortar and pestle in buffer supplemented with ice-cold 50 mM Tris-HCl, pH 7.5; 2 mM EDTA and 0.04% (v/v) 2- mercaptoethanol. The homogenate was centrifuged at 12,000g for 10 min at room temperature. Later, the supernatant (100 µl) was transferred to glass cuvette containing 1 mL Coomassie Brilliant Blue. Finally, absorbance was read at 595 nm in a spectrophotometer, and the concentration of total soluble proteins was calculated using the calibration curve of BSA.

Determination of arsenate reductase activity

Arsenate reductase activity was assayed in roots using the coupled enzymatic reaction as previously described (Shi et al. 1999) with some modifications. The assay solution contained 50 mM MOPS, 50 mM MES, pH 6.5, 1.5 mM NADPH, 1 unit yeast (*Saccharomyces cerevisiae*), GR (Roth, Germany), 1 mM GSH and 10 mM sodium arsenate. Firstly, 100 mL of root extract were pre-incubated for 5 min in buffer containing GR and GSH at 30°C. Further, NADPH and arsenate were mixed thoroughly in small volumes. Afterward, GR-specific NADPH oxidation was monitored by recording the decrease in A₃₄₀ in a spectrophotometer (Model No. AA-6800, Shimadzu) and the amount of NADPH oxidized was calculated using a molar extinction coefficient of 6200 m⁻¹ cm⁻¹ for NADPH at 340 nm.

RNA isolation and quantitative real-time PCR

Expression analysis of *Actin* (AB181991.1), *TaPCS1* (AF093752.1) and *TaMT1* (AF470355.1) was studied by quantitative qRT-PCR (reverse transcription PCR) in roots of 5d old plants. Briefly, tissues (50-80 mg) were ground to a fine powder with a mortar and pestle in liquid nitrogen. The total RNA was isolated as instructed by SV Total RNA Isolation System (cat. no. Z3100), Promega Corporation, United States. The integrity of RNA was further checked by denaturing agarose gel electrophoresis and quantified by NanoDrop 2000 UV-Vis Spectrophotometer.

The first-strand cDNA from RNA was synthesized by using GoScript™ Reverse Transcription System (Cat no. A5001), Promega Corporation, United States. Prior to real-time analysis, the cDNA samples were treated with RNAase for removing RNA contamination. Real-time PCR was performed in triplicate using the Eco™ real-time PCR system (Illumina, United States) using GoTaq® qPCR Master Mix (Promega United States) and gene-specific primers (*Actin*: F- GTCGGTGAAGGGGACTTACA, R- TTCATACAGCAGGCAAGCAC; *TaPCS1*: F- GGGTATCATGCCGAGAAAGA, R- GCCGCAACTCACTGTGTAGA; *TaMT1*: F- GATCATCACCAACCGACCAT, R- CACTTGCATCCTCCGTTCTC). Expression data was normalized with Actin as an internal control (Eco Software v4.0.7.0). The real-time PCR program was as follows: 3 min at 95°C, 40 cycles of 30 s at 94°C, 15 s at 58°C and 30 s at 72°C.

Enzymatic assay

CAT, POD, SOD and GR enzymes were extracted in roots of plants as previously described with slight modifications (Goud and Kachole 2012). Briefly, roots were ground in 5 mL of 100 mM phosphate buffer (pH 7.0) and the homogenate was centrifuged for 10 min (8000 rpm) before separating the supernatant in Eppendorf tubes. CAT was analyzed in a reaction mixture containing 100 mM potassium phosphate buffer (pH 7.0), 6% H₂O₂ and 100 µl root extract. Once root extract is added, the changes in absorbance were monitored at 240 nm (extinction coefficient of 0.036 mM⁻¹ cm⁻¹) in a UV spectrophotometer at 30s intervals up to 1 min. The activity of CAT is expressed as µmol of H₂O₂ oxidized min⁻¹ (mg protein)⁻¹. For POD analysis, root extract was mixed with 100 mM potassium phosphate buffer (pH 6.5), 1 0.05 M pyrogallol solution, 200 mM H₂O₂. The change of absorbance was then monitored at 430 nm (extinction coefficient 12 mM⁻¹cm⁻¹) from 30 sec up to 1.5 min in a spectrophotometer. The specific activity of the enzyme is expressed as µmol pyrogallol oxidized min⁻¹(mg protein)⁻¹. In the case of SOD, assay mixture was mixed with 50 mM sodium carbonate/bicarbonate buffer (pH 9.8), 0.1 mM EDTA and 0.6 mM epinephrine (Sun and Zigman 1978). The adrenochrome formation for 4 min was then read at 475 nm in a UV-Vis spectrophotometer. For GR activity, 100 µl of root extract was mixed with 0.2 M phosphate buffer (pH 7.0), 1 mM EDTA, 20 mM oxidized glutathione (GSSG) and 0.2 mM NADPH. Oxidation of NADPH by GR was subsequently monitored at 340 nm. The GR activity was calculated using the extinction coefficient of 6.12 mM⁻¹ cm⁻¹ (Halliwell and Foyer 1978).

Statistical analysis

All experiments were set in a completely randomized block design (RBD) having at least three independent biological replications for each biological sample. Statistical significance for each group of data was set at $P \leq 0.05$ by ANOVA one-way followed by Duncan's Multiple Range Test (DMRT) in SPSS Statistics 20 Software. Further, the graphical presentation was prepared using GraphPad Prism 6.

Results

Morpho-physiological features

The root length, shoot height, root dry weight and shoot dry weight significantly ($P < 0.05$) decreased due to As stress compared with non-treated controls (Table 1, Fig. 1). When Si was added in combination with As, plant showed no significant increase in root dry length and root dry weight compared with As-stressed plants. However, shoot parameters (shoot height and shoot dry weight) significantly increased under Si supplementation in combination with As treatment compared with As-stressed plants (Table 1). Si supplementation in non-stressed plants showed similar morphological characteristics to that of control plants (As-). Leaf chlorophyll concentration (*a* and *b*) showed no significant changes in any of the treatments used in this study (Fig. 2).

Fe, P and As contents in roots and shoots

Fe and P concentration in root and shoot showed no significant changes in either control and As-stressed conditions cultivated in the presence or absence of Si (Fig. 2 and 3). Plants grown without As in the absence or presence of Si showed no As concentration (trace amount) in either roots or shoots (Fig. 3). However, As concentration significantly increased in both roots and shoots under As stress compared to control, showing higher concentration of As in roots than in shoots (Fig. 3). Supplementation of Si along with As significantly increased the accumulation of As in roots but decreased in shoots compared with the plants grown under As stress alone (Fig. 3).

Effect of Si in planta

Addition of Si either in control of As-treated hydroponic plants showed significant increase of Si in both root and shoot compared to the plants grown without Si (As- and As+) supplementation (Table 2).

Effect of Si on electrolyte leakage and total soluble protein

The electrolyte leakage significantly increased in both roots and shoots under As stress compared with control plants (As-). However, the electrolyte leakage significantly decreased in both tissues due to Si supplementation under As stress compared with As-stressed wheat plants (Fig. 4). Si

applied solely on non-treated plants showed similar electrolyte leakage to that of plants treated without As (Fig. 4). Further, As stress showed a significant decrease in total soluble protein content in both roots and shoots compared with control plants. However, application of Si under As supplementation significantly increased the protein content in both tissues compared with As-stressed wheat plants. When treated solely with Si without As, plants showed protein content similar to that of control plants (Fig. 4).

Effect of Si on arsenate reductase activity and PS release

Arsenate reductase activity showed a significant increase due to As stress compared with control plants in both roots and shoots (Fig. 4). Further, application of Si along with As did not show any significant changes in arsenate reductase activity in either roots and shoots. Plants treated with Si in control plants showed similar arsenate reductase activity in roots and shoots to that of controls plants (Fig. 4). In addition, PS release in roots showed no significant changes in either control and As-stressed conditions cultivated in the presence or absence of Si (Fig. 4).

Expression analysis of candidate genes in roots

Gene expression analysis showed no significant changes of two metal chelators (*TaPCS1* and *TaMT1*) in roots of wheat plants compared to the plants grown without As (Fig. 5). However, expression of these two genes significantly increased in roots due to Si supplementation under As stress compared with As-stressed plants. Si applied on non-treated plants showed similar expression pattern for these genes to that of control plants (Fig. 5).

Changes in antioxidant enzyme activities

Enzymatic activity of CAT, POD, GR and SOD showed no significant changes in roots under As stress compared with control plants (Fig. 6). However, application of Si along with As caused a significant increase in CAT, POD and GR activities compared with As-stressed plants. Si applied solely to non-treated plants showed similar activities of CAT, POD, GR and SOD to that of control plants (Fig. 6). In shoots, As stress showed a significant increase in CAT activity under As stress compared with controls. However, application of Si along with As showed no significant changes in any of the enzymes other than CAT in shoot (Fig. 6). Si applied to plants

grown without As showed similar CAT, POD, GR and SOD activities to that of control plants (Fig. 6).

Discussion

Silicon (Si) is highly abundant in soil and proved to be efficient for heavy metal (HM) detoxification in plants (Kabir et al. 2016). In the present study, As stress in hydroponic conditions showed significant damage in the shoot and root growth in wheat. However, Si supplementation during cultivation with As showed marked improvement in growth parameters in wheat. Vijayarengan (2012) reported that high concentration of As decreased the shoot dry weight in rice. Also, phosphate and higher concentrations of Si showed alleviation of As toxicity by reducing As uptake in wheat roots (Luo et al. 2015). In another report, Fujii et al. (1999) reported that rice seedlings that were treated with silica gel exhibited high shoot dry weight. In this study, application of Si in As-treated nutrient media significantly restored the total protein content in both roots and leaves compared with As-stressed Wheat plants. Previous reports reveal that Si acts on increasing total soluble protein in wheat leaves under the oxidative stress of drought (Gong et al. 2005) by reduction of free radicals and ROS regeneration. Si restores total soluble protein in both roots and shoot. Furthermore, application of Si in As-stressed plants diminished the Electrolyte leakage in both roots and shoots. It suggests that exogenous Si might provide membrane stability in As-stressed Wheat plants. In similar studies, Cadmium (Cd) and lead (Pb) stress induced a significant increase in the membrane injury but Si application noticeably eliminated Electrolyte leakage in Alfalfa cotton plants (Kabir et al. 2016; Bharwana et al. 2013).

AAS data showed the significant increase of As concentrations in both root and shoot tissue under As stressed. Although the dual application of Si and As showed a significant increase in root As concentration, this combined treatments significantly inhibited the translocation of As in shoots, suggesting that Si inhibits As translocation from roots to shoots in Wheat plants. This evidence is consistent with previous reports on rice (Zhang et al. 2008). However, Si supplementation reduced the As uptake by roots and accumulation in the shoot of rice plant (Guo et al. 2005, 2007; Li et al. 2009). Results also suggest that translocation of As into the rice grain from the shoots is mainly achieved by P rather than Si transport pathway (Norton et al. 2010). We observed Fe and P concentration in root and shoot showed no significant changes in either

control or As-stressed conditions cultivated in the presence or absence of Si. These findings support that inhibition of As translocation in shoots of wheat plants is not regulated with Fe and P transport under Si supplementation. When plants were treated with AsV (arsenate), more than 90% As in roots and shoots was found as AsIII (arsenite) (Pickering et al. 2000; Dhankher et al. 2006; Xu et al. 2007). AsV is reduced to AsIII by plants, which is considered as the first strategy for As detoxification in plants (Pickering et al. 2000; Schmöger et al. 2000). This study further showed that Si does have in planta effect to increase its concentration in both roots and shoot, which is eventually used by wheat plants to overcome As toxicity. Mitigation of As toxicity through Si application was previously reported in rice (Fleck et al. 2013; Zhao et al. 2012) and tomato (Marmioli et al. 2014). In the present study, application of Si along or with As did not show any significant changes in arsenate reductase activity in either roots and shoots. It indicates that arsenate reductase activity was not induced by exogenous Si in wheat plants.

Based on morpho-physiological observations, the mechanistic basis for As toxicity tolerance through Si supplementation was further investigated in roots of Wheat plants. We performed PC analysis and two candidate genes (*TaPCS1*, *TaMT1*) responsible for vacuolar sequestration in roots. Sequestration of As and other heavy metal ions into vacuoles is a well-characterized mechanism of detoxification (Rea et al. 1998). Findings of this study indicate that As detoxification in Wheat plants following Si application may be involved in the change of metal sequestrations in roots. These findings are consistent with AAS data revealing significant As accumulation in roots due to Si application. Our expression analysis revealed that under As stress condition, the expression of two metal chelators (*TaPCS1* and *TaMT2*) in roots of wheat plants showed no significant changes compared to the plants grown without As. However, expression of these two genes *strongly* upregulated due to *Si application under As stress* compared with As-stressed plants. Further, Si applied to non-treated plants showed similar expression pattern for these genes to that of control plants. Alleviation of As uptake in rice at the whole plant as well as the cellular level was reported to be involved with vacuolar sequestration of the phloem companion cells (Song et al. 2014). In the present study, upregulation of *TaPCS1* and PC accumulation suggest that PC does play critical role to withstand As stress due to Si supplementation being consistent with the previous findings (Huda et al. 2016). This molecular evidence is consistent with our AAS data confirming the hypothesis that though increased As is entered through root intake in Wheat plants under As stress; it gets stored in root vacuole leading

to decreased translocation of As in shoots. Both inhibition of transporters and As-PC formation confers Si-mediated mitigation of As toxicity in wheat. MT also plays a major role in HM detoxification and homeostasis of intracellular metal ions in the plants. As like *TaPCS1*, *TaMT1* gene significantly upregulated due to Si supplementation under As stress. This data suggest that upregulation of *TaMT1* gene might form complex with As and increase their accumulation in root tissues in Wheat plants. Furthermore, the protection by *TaMT1* against oxidative stress damage is apparent in Wheat plants which are supported by the hypothesis of an MT based mechanism acting against radical attack in poplar plants (Balestrazzi et al. 2009). There is substantial evidence that complexation of arsenite by PCs is an important mechanism of As detoxification in few plants (Kabir et al. 2016).

We also analyzed antioxidant enzymes involved in antioxidant defense in plants under abiotic stress. Among these enzymes, CAT, POD and GR activities significantly increased in roots under Si supplementation in As-stressed Wheat plants. In shoots, Application of Si Showed a significant ($P < 0.05$) increase in CAT activity compared with As stress. ROS production is a common feature in plant under As exposure (Srivastava et al. 2005; Singh et al. 2006). A number of enzymes are involved in ROS defense strategies. Among them, CAT is involved in the primary defense mechanism against ROS playing critical roles in decreasing H_2O_2 level in plant cells (Malar et al. 2014). In the present work, Wheat plants have a tendency to increase CAT activity under As stress in shoots, suggesting better efficiency in converting O_2 to H_2O_2 following Si treatment. The higher CAT activities due to Si on As-stressed wheat plants may be partially correlated with the O_2 decreased H_2O_2 in roots. CAT mainly occurs in peroxisomes requiring no reductant for catalyzing a dismutation reaction (Sofo et al. 2015). Kabir et al. (2016) also reported that the Si-mediated increases in antioxidant enzymes might be an adaptive response to counteract heavy metal stress in Alfalfa. In addition to vacuolar storage, plants have evolved antioxidant enzymes and antioxidant non-enzyme molecules to prevent the ROS (reactive oxygen species) induced by heavy metal stress (Kabir et al. 2016; Romero-Puertas et al. 2007). ROS is involved in damaging amino acids, proteins and nucleic acids and may cause peroxidation of membrane lipids (Møller et al. 2007). Lipid peroxidation not only inhibits cellular function but also increase the production of lipid-derived radicals (Van Breusegem and Dat 2006; Møller et al. 2007). POD is responsible for degradation of lipid peroxides. POD significantly increased in roots under Si supplementation in As-stressed Wheat plants. This result

suggests that Si may induce POD activity in root and thus, contribute a part of the antioxidative defense under As stress in Wheat. Another important antioxidant enzyme, GR was only increased in roots under the dual application Si and As. This result suggests that Si may induce GR activity in roots and thus, contribute a part of the antioxidative defense under As stress in Wheat. The activity of GR, which catalyzes the NADPH-dependent reduction of oxidized glutathione into glutathione (Rendon et al. 1995), is induced under metal stressed in plants (Laspina et al. 2005; Dixit et al. 2001). It also maintains the optimum level of GSH for scavenging of ROS by other enzymes in plant cells. This fact also suggests that Si facilitates As alleviation by activating not only the main antioxidant enzyme activities but also the ASC–GSH cycle, thus regenerating antioxidant metabolites (ASC, GSH), which are supported the higher glutathione concentration in shoots. Silicon may also reduce the cellular toxicity of As within plant cells, by increasing the antioxidant activities which alleviate the adverse effects of the reactive oxygen species (ROS) generated in plants exposed to As (Liu et al. 2009). In the light of our findings, it is evident that active involvement of ROS scavenging is also associated with the Si-mediated alleviation of As stress in Wheat.

Conclusion

The present study reveals the critical role of Si alleviating As toxicity in wheat. Biochemical and molecular evidence support that alleviation of As toxicity in wheat due to Si application is associated with vacuolar sequestration of As in roots along with antioxidant defense. These findings will be useful for further researches on phytoremediation of As and development of As-tolerant wheat.

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