

THE EFFECTS OF ARSENIC AND SILICON ON THE OXIDATIVE AND NON-OXIDATIVE ENZYMES IN THE SEEDLINGS OF THREE DIFFERENT RICE (*ORYZA SATIVA* L.) VARIETIES IN DIFFERENT GROWTH PERIODS

BOORBOORI, M. R. – LIN, W. X.* – FANG, C. X.*

Fujian Provincial Key Laboratory of Agroecological Processing and Safety Monitoring, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, P. R. China

*Corresponding authors

e-mail: wenxiong181@163.com, changfangxingyx@163.com

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Abstract. Silicon plays an important role in reducing the damage done to plants by environmental stress. Arsenic is an important stress factor, and silicon can reduce arsenic-induced stress on plants. In this study, we examined the affection of silicon on different enzymatic, and non-enzymatic antioxidants in the roots and shoots of different rice that exposed to arsenic at different time periods. The rice varieties studied during this experiment included LE-WT, LE-OE, and LE-R. This study showed addition of silicon increased APX, CAT, GSH, and GST in the first and second weeks after treatment, while it also significantly decreased MDA, POD, and SOD in roots and shoots of three varieties of rice. Addition of arsenic increased MDA content in all rice, but also decreased the rest of enzymes compared to control. Treatment of seedlings with As + Si significantly decreased APX, CAT, GSH, GST, POD, and SOD in rice shoots and roots compared to control in the first and second weeks after treatment. The results indicated the effect on enzymes activity in rice roots and shoots, which are attributable to arsenic, and silicon levels in the culture medium.

Keywords: *rice (Oryza sativa L.), wild type, transgenic, silicon, arsenic (III), antioxidants*

Introduction

Arsenic (As) is a carcinogenic metalloid (Dey et al., 2014), which accumulates in soil and water is a significant problem in the environment, which negatively affects human health. In addition, contaminated soil causes a negative result on economic growth and development because of its deep effects on growth of crops (Farquhar et al., 2002; Huq et al., 2006; Celik et al., 2008). Contamination of water by As needs specific attention due to high toxicity of it, even at low concentrations, due to its rapid transfer to the environment. High concentrations of arsenic reported in France, Bangladesh, Brazil, Vietnam, China, Nicaragua, United States, and many other countries (McClintock et al., 2012), making As contamination an important public health issue around the world. Therefore, special attention should be paid to the arsenic accumulation in indirectly consumed crops, such as rice. Indeed, arsenic accumulates in rice, and as rice is a staple diet in many countries, this increases the risk of arsenic-related diseases in humans living in those areas (Tchounwou and Centeno, 2004; Mondal, 2008; Su et al., 2010; Fontcuberta et al., 2011; Awasthi et al., 2017). Moreover, extreme accumulation of As in soil and irrigation water negatively affects both the growth and quality of rice which presses the importance of this issue further.

Silicon (Si) is a numerous metalloid in the soil, and that is useful for rice. Si is absorbed in the form of silicic acid (H_4SiO_4) in high quantities via plants. Si content in rice reaches up to 10% of its dry weight. Si fertilizers are commonly used to increase rice growth and

production capacity. Si also makes plants resistant to biological and non-biological stresses (Ma et al., 2001, 2006; Feng et al., 2011; Singh et al., 2011; Guntzer et al., 2012; Pontigo et al., 2017). Some studies showed the application of Si can reduce the biological toxicity of As, as well as As accumulation decreased in rice, which grows in pots or hydroponics (Seyfferth and Fendorf, 2012; Tripathi et al., 2013; Fleck et al., 2013).

A number of different methods have been used to reduce soil As contamination, but the application of fertilizers is one of the simplest for this purpose. Si widely uses to make a better soil condition for plant growth. Besides its suitability for basic manure, the high potential of sodium silicate to reduce As content in rice has also been reported. Effects of Si on As-induced oxidative stress is a way to increase As tolerance in the early stages of growth. In present study, we investigate effect of Si on oxidative stress behaviour on three different rice seedlings exposed to As. To recognize possible mechanisms responsible for stress responses in rice, the impact of anti-oxidative enzymes in short (one week after As and Si treatment) and long-term (two weeks after As and Si treatment) were analyzed. This experiment evaluated the time-dependent responses in rice plants after adding Si and As in both periods. We hypothesized that the addition of Si in the early stages of growth would help the rice to compensate for the toxic effects of As via anti-oxidative enzyme protective mechanisms. The present study particularly addresses the questions: (1) if does the addition of Si in the early stages of rice increases the tolerance to As toxicity, and (2) whether does the combination of As and Si in different rice varieties affects the amount of anti-oxidative enzymes.

Material and method

Planting conditions

This study was done in the greenhouse of Fujin agriculture and forestry university in Fuzhou city of China. Rice seeds selected from two different varieties of rice, Lemont wild type (Le-WT), *Lsi1*-overexpression transgenic Lemont (LE-OE), and *Lsi1*-RNAi transgenic Lemont rice (LE-R). Before planting, the seeds sterilized by 1% H₂O₂ for 15 minutes, rinsed in deionized water for 48 hours, and then kept in a Petri dish in an incubator for 4 days, at 28°C. After germination, suitable seedlings selected and transferred to the greenhouse for cultivation under hydroponic conditions. The pots (2.5 L) filled with a modified version of the nutrient solution described by Yoshida et al. (1971). The nutrient solution included: (NH₄)₂SO₄ (48.2 mg L⁻¹), Ca(NO₃)₂·4H₂O (86.43 mg L⁻¹), K₂SO₄ (14.9 mg L⁻¹), Na₂SiO₄·9H₂O (200 mg L⁻¹), KNO₃ (18.5 mg L⁻¹), FeSO₄·7H₂O (45.7 mg L⁻¹), H₃BO₃ (1.43 mg L⁻¹), EDTA (48.44 mg L⁻¹), CuSO₄·5H₂O (0.04 mg L⁻¹), KH₂PO₄ (24.8 mg L⁻¹), MnCl₂·4H₂O (0.905 mg L⁻¹), Na₂MoO₄·2H₂O (0.045 mg L⁻¹), ZnSO₄·7H₂O (0.11 mg L⁻¹), and MgSO₄·7H₂O (135.06 mg L⁻¹). The nutrient solution pH set to 5.8 by using HCl or NaOH rice seedlings planted, when they had three leaves, and the nutrient solution changed once a week. In each pot, we planted 4 seedlings. The plants exposed to two levels of Si (Na₂SiO₄·9H₂O) (0 and 0.70 mM), and two levels of As (III) (NaAsO₂) (0 and 30 μM), thus, four different treatments formed, control (CK, I), 0.70 mM Si + no As (II), 30 μM As + no Si (III), 30 μM As + 0.70 mM Si (IV). For each treatment we had three pots.

Random plant samples were selected one and two weeks after treatment. For both times of sampling, we harvested two seedlings from each pots. The roots and shoots of plants washed separately by distilled water, and remaining residue in the root zone washed by 0.5 mM CaCl₂ solution for 30 minutes before being washed with distilled

water again. All samples were instantly transferred to a freezer at -10°C , and stored until the enzyme activity determined.

The amount of sodium added to the solution compensated for adding $\text{Na}_2\text{SiO}_4 \cdot 9\text{H}_2\text{O}$, the equivalent of NaCl added to the solution.

Superoxide dismutase (SOD) activity measurement

SOD activity specified with some modification of the nitro blue tetrazolium method (NBT) (Dias et al., 2011; Esposito et al., 2015). Approximately, 0.5 g of samples homogenized by potassium phosphate buffer (50 mM, pH 7.8) including 1% polyvinylpolypyrrolidone, and centrifuged for 20 minutes, at $15000 \times g$ speed, and 4°C . The supernatants used to find SOD activity (Sajedi et al., 2011). Every 3 ml of the reaction mixture included 0.2 ml of NBT (750 μM), 130 mM Met (0.2 ml), 2.2 ml of PBS (50 mM pH 7.8), 20 μM EDTA- Na_2 (0.1 ml), 100 μM riboflavin (0.2 ml), and 0.1 ml of supernatant. The enzyme activity expressed as the amount of extract requirement to control the decrease of NBT by 50%.

Catalase (CAT) activity measurement

Azevedo et al. (2007) reported, CAT activity specified by the absorbance amount of 240 nm because of H_2O_2 consumption. Reaction mixture included H_2O_2 (0.3 ml, 0.1 M), distilled water (1 ml), 50 mM PBS (1.5 ml, pH 7.8) and sample (0.2 ml).

Ascorbate peroxidase (APX) activity measurement

The supernatant was used to measure APX activity. It made by using 1 g sample homogeneous solution, 0.1 M sodium phosphate buffer (5 ml, pH 7) including 10% PVP. Then it centrifuged for 20 minutes at $12000 \times g$ speed, and 4°C (Nakano and Asada, 1981). Reaction mixture included 0.1 mM EDTA (0.3 ml), 0.1 M phosphate buffer (0.7 ml, pH 7), 0.5 mM ascorbic acid (0.3 ml), 0.1 mM H_2O_2 (0.3 ml), and the supernatant (0.4 ml). The reaction mixture absorbance variation determined at 290 nm after 5 minutes. APX activity expressed by consuming extinction coefficient $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$, then showed decomposed ascorbic acid protein $\text{mg}^{-1} \text{ min}^{-1}$.

Peroxidase (POD) activity measurement

POD activity characterized by some changes according to Bai et al. (2006) method. Almost 0.5 g of samples of the stored shoots and roots homogenized. We used potassium phosphate buffer (50 mM, pH 7.8) for homogenized. That included 1% polyvinylpolypyrrolidone, and centrifuged for 20 minutes, at $15000 \times g$ speed, and 4°C . The supernatants used to find POD activity. Reaction mixture included 0.3% H_2O_2 (1.0 ml), 0.9 ml Guaiaco 10.2%, 0.05 M PBS (1 ml, pH 7.8), and supernatants (0.1 ml), and absorbance determined at 470 nm in a minute period.

Malondialdehyde (MDA) content measurement

The MDA content estimated from a reaction with Thiobarbituric Acid (Heath and Packer, 1968). We subjected sample (0.5 g) with potassium phosphate buffer (1.5 mM, PBS, pH 7.8) contains 1% polyvinylpolypyrrolidone. We centrifuged it for 20 minutes, at $15,000 \times g$ speed, and 4°C (Sajedi et al., 2011). The supernatant (2 ml) mixed by 0.5% TBA (2 ml), and 20% TCA (2 ml). The mixture made hot with a temperature around 95°C for about 30 minutes, after that rapidly made it cold. Non-specific adsorption measured

at 600 nm, and reaction mixture minus absorbance from sample solution at 532 nm. To find MDA content, contraction coefficient ($155 \text{ mM}^{-1} \text{ cm}^{-1}$) used.

Glutathione reduced (GSH) content measurement

GSH content measured by Sedlak and Lindsay (1968) method. The plant sample (1 g) was prepared in a homogeneous solution; 5% (w / v) SSA (5 ml) including EDTA (10 mM), then it centrifuged for 20 minutes, at $10,000 \times g$ speed, and 4°C . The supernatant (10 μl) added to reaction mixture (140 μl). The reaction mixture included potassium phosphate buffer (100 mM, pH 7.5), 6 ml^{-1} GR, EDTA (1 mM), 5% SSA (10 μl), and 10 mM DTNB, and then put in Incubator for 3 hours at a dark room. The reaction started via increasing 2 mM NADPH (50 μl). Absorbance occurred at 412 nm with a spacing of 1 minute and 5 minutes. We found GSH content by a standard curve, then demonstrated as nmol.g^{-1} (FW).

Glutathione-S-transferase (GST) activity measurement

The roots and shoots (1 g) homogenized by 100 mM Tris-HCl (5 ml, pH 7.5) including 14 mM β -mercaptoethanol, 7.5% PVP (w/v), and EDTA (2 mM) (Ando et al., 1988). The collected supernatants centrifuged for 15 minutes, and $15,000 \times g$ speed, and 4°C . Measurement did in 100 mM potassium phosphate buffer (2 ml, pH 6.5), which included 1 mM CDNB (250 ml), 5 mM GSH, and extracted enzyme (0.5 ml). The absorbance changes determined at 340 nm with a spacing of 1 minute and 5 minutes. The GST activity computed by consuming an extinction coefficient ($9.6 \text{ mM}^{-1} \text{ cm}^{-1}$), then showed as $\text{U.min}^{-1}.\text{mg}^{-1}$ protein.

Statistical analyses

This experiment arranged in the completely randomized factorial design, and three replications. All the findings analyzed by one-way ANOVAs pursued by LSD test ($p \leq 0.05$), and SPSS software (19.0).

Results and Discussion

Effects of Si and As on SOD activity of rice roots and shoots

In our study, we tried to understand the result of Si in reducing As stress by measuring physiological responses of rice to different treatments, at different periods. Our results show that SOD activity in roots and shoots of different rice decreased As exposure compared to CK ($p \leq 0.05$). The highest reduction observed in LE-R line root in the first week, but the highest decrease observed in the roots of LE-WT after two weeks (*Fig. 1*). In addition, Si treatment showed a significant difference in SOD activity of rice roots and shoots compared to CK ($p \leq 0.05$). The greatest decrease in the first and second weeks after exposure to Si treatment observed in the roots of LE-R. In the present study, reduction in SOD activity observed in the seedlings, which treated by As + Si in shoots and roots in both exposure periods. The highest decrease was in the roots of LE-R in the first and second weeks.

Abiotic stress same as metalloids and heavy metals increases the amount of reactive oxygen species (ROS) in plants, and stimulates oxidative stress. ROS, by containing O_2^- , OH and H_2O_2 attack important metabolic functions via destroying vital biomolecules

same as proteins, lipids, and nucleic acids. Plants have several defense mechanisms against ROS. One of those defense pathways in plants is through different enzymes same as SOD, POD, and CAT. These enzymes detoxify ROS, and destroy free radicals, that are responsible for cellular signaling. The effects of SOD, POD, and CAT highly majored to produce one of them usually turns into another enzyme-substrate, resulting in the production of non-toxic substances. First defensive line of organisms versus the toxicity of ROS is SOD, and it catalyzes the disproportionate reaction of O_2^- to hydrogen peroxide, oxygen, and works as a significant defense mechanism versus ROS. Thus, SOD considered a suitable index for assessing toxicity in nature.

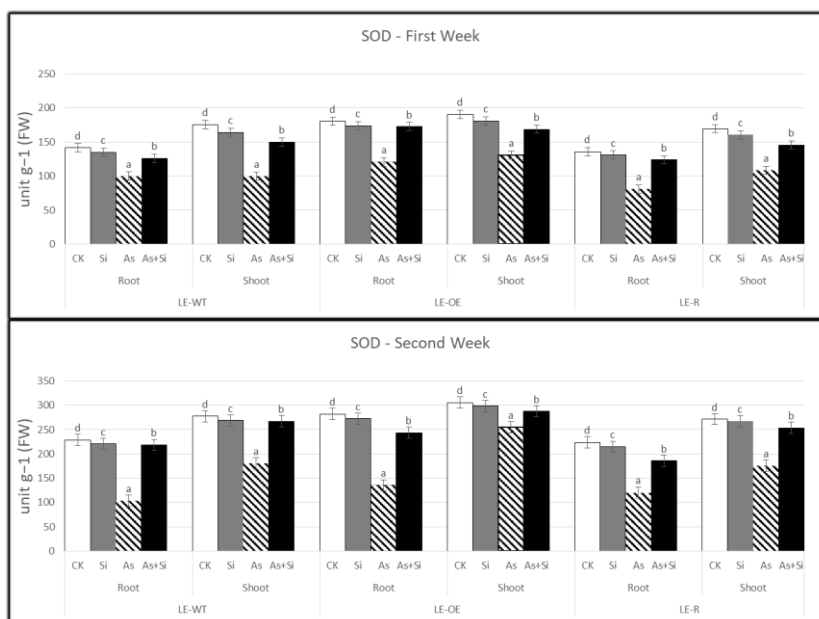


Figure 1. SOD activity in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

In theory, as ROS increases, plants increase SOD activities to remove ROS. In this study, SOD activity was significantly reduced with increasing ROS, which indicates rice seedlings tolerated oxidative stress caused by As toxicity. Including giving less area for As accumulation in rice, or oxidative stress that damages SOD, thereby diminishing SOD activity, or stresses that cause intense loss to rice cells, leading to restraint of more SOD amount (Shen et al., 2010; Farooq et al., 2013; Wu et al., 2017). In our study, As + Si treatment significantly grew SOD activity compared to the single As treatment, which indicates Si can activate SOD to eliminate additional free radicals, and can effectively increase defense capacity versus oxidative stress caused via As toxicity in rice. Some studies showed SOD activities will also increase under other stress in rice (Song et al., 2009; Tripathi et al., 2013; Ju et al., 2017). Moreover, we also observed the reduction in SOD activity in roots under As stress was greater than in the shoots, indicating that the reduction in SOD activity related to rice tissue characteristics, too (Zeng et al., 2011).

Effects of Si and As on CAT activity of rice roots and shoots

Treatment containing As decreased CAT activity compared to CK ($p \leq 0.05$). The highest reduction observed both in the first week and second weeks in the roots of LE-WT

(63.98% and 44.79% decrease compared to CK, respectively) (Fig. 2). The present study showed Si treatment in different rice in the first week and second weeks increased CAT activity. The highest increase in CAT activity observed in LE-R shoots compared to CK ($p \leq 0.05$). The increase in CAT activity in the first week and second weeks was respectively 13.84% and 12.3% compared to CK. By adding As + Si treatment, CAT activity significantly reduced in roots and shoots of different rice compared to CK ($p \leq 0.05$). The highest reduction saw in LE-WT roots, which was 54.62% lower in the first week than in the control, and in the second week decreased by 38.62% compared to control.

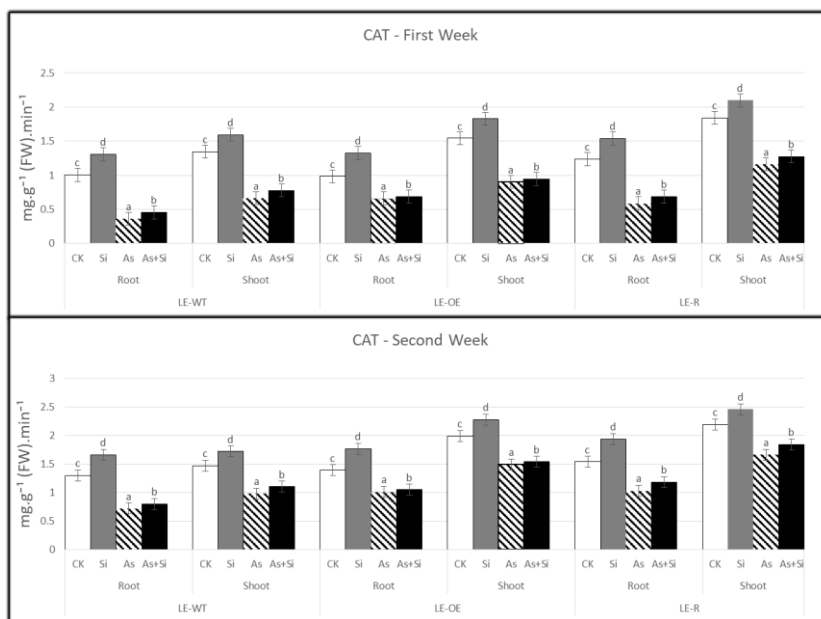


Figure 2. CAT activity in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

There are some non-enzymatic and enzymatic ROS elimination systems in plants. These systems act as a vital role in the membrane defense systems structure and function, and in maintaining the redox state of cells (Chen et al., 2018). SOD naturally converts O_2^- to harmful H_2O_2 and O_2 , and although H_2O_2 is still harmful, CAT can instantly transform that to water (Liu et al., 2015). CAT is likely to be an important enzyme in facilitating ROS modification in rice, making that essential section of ROS detoxification system, and a key in defense mechanism versus ROS. Thus, CAT activity is ever subject to increased oxidative stress (Wang et al., 2015). As treatments significantly decreased CAT activity, which can be because of 1) ROS levels are higher than those CAT is able to control 2) CAT may have a less role in detoxifying As in rice. In the latter case this maybe because As inhibits rice growth, or stronger oxidative stress damages CAT. Similar results related in rice and other plants (Shi et al., 2005; Song et al., 2009; Zeng et al., 2011; Farooq et al., 2013; Tripathi et al., 2015; Rahman et al., 2017). Treatments with both As and Si increased CAT activity compared to As alone treatment, probably due to the CAT synthesis increasing effect of Si. Higher CAT activities increased via Si addition may further eliminate ROS as well as H_2O_2 , and defense rice organs against As membrane oxidative damage. Other studies have shown similar results in different plants under

different stress, and this indicates different effects on different varieties, plant growth stages, and even plant tissue types (Shi et al., 2005, 2010, 2014; Song et al., 2009, 2011; Zeng et al., 2011; Farooq et al., 2013; Habibi and Hajiboland, 2013; Zhang et al., 2013; Vaculíková et al., 2014; Tripathi et al., 2015; Kang et al., 2016; Ju et al., 2017; Rahman et al., 2017; Wu et al., 2017; Shekari et al., 2017).

Effects of Si and As on APX activity of rice roots and shoots

Compared to CK, APX activity significantly ($p \leq 0.05$) decreased in both roots and shoots of all three rice varieties, when they were exposed to As, with the highest decrease observed for both the first and second weeks in the roots of LE-WT (Fig. 3). In addition, Si treatment showed a significant ($p \leq 0.05$) grow in APX activity both in the shoots and roots of rice plants compared to control. The highest increase was observed in the LE-OE line shoots in the first week and second weeks after treatment with Si, which respectively represents 8.85% and 14.40% growth compared to CK. APX activity was decreased in plants treated with As + Si compared to CK ($p \leq 0.05$), and the largest reduction saw in the roots of LE-WT. One week after treatment this decrease was 28.02%, and was 22.80% two weeks after treatment. When we compared APX activity in shoots, the highest reduction in APX activity observed in LE-WT shoots. APX activity decreased 2.94%, one week after treatment, and 5.57%, two weeks after treatment, indicating different effects of the same treatment on different tissues. APX is important in the ascorbate-glutathione cycle, and can break down H_2O_2 (Mittler et al., 2004; Liang et al., 2005; Mhamdi et al., 2010; Zeng et al., 2011; Foyer and Noctor, 2011). Since Si has a positive effect on APX activity in rice, which are under stress, treating plants with Si, can effectively increase the defense capacity versus oxidative stress, either caused by arsenic or other stressors. The function of APX to eliminate H_2O_2 partially overlaps with that of CAT in non-photosynthetic tissues, and research has shown, that APX's role in eliminating H_2O_2 in rice can be replaced or offset by CAT (Zhao et al., 2018).

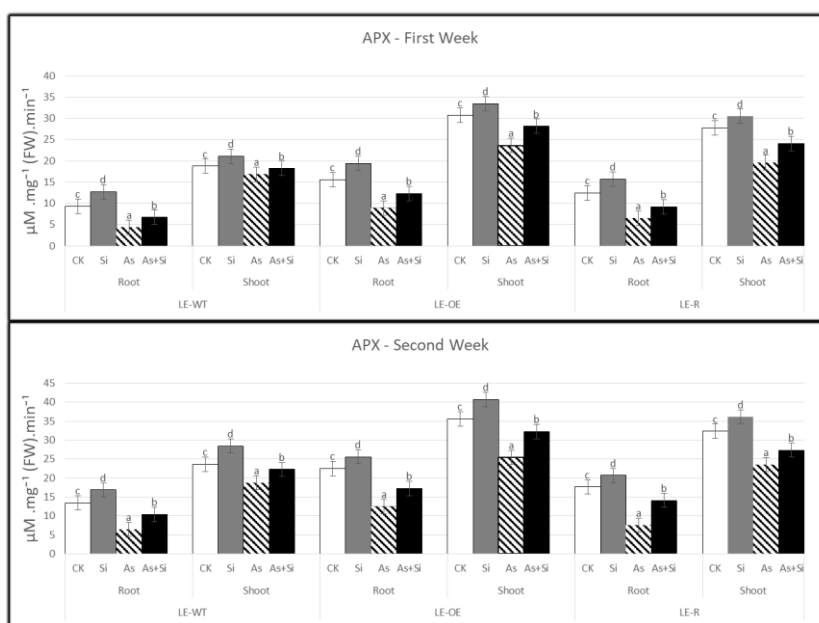


Figure 3. APX activity in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

Effects of Si and As on POD activity of rice roots and shoots

Treatment of seedlings by Si significantly ($p \leq 0.05$) decreased POD activity in both the first and second weeks after treatment with the lowest POD activity being observed in LE-WT shoots (Fig. 4). POD activity decreased by 9.99% in the first week after treatment but only reduced 5.95% in the second week. One and two weeks after As treatment, POD activity was significantly different in the roots and shoots of rice, when it was compared to CK ($p \leq 0.05$). The lowest POD activity observed in the shoots of LE-WT in the first week (47.51% decreased compared to control), and in the roots of LE-WT in the second week (39.16% reduced compared to control). However, POD activity in rice treated with As + Si was higher than in rice treated only with As. This indicates that the addition of Si positively affects POD activities and effectively enhances the defense capacity versus oxidative stress-induced via As exposure in rice. Same results found in rice treated by heavy metals, acidic rain, and other stresses, and also was seen in different plants like barley ‘Brassica Chinensis L. ‘cotton, xerophyte *Zygophyllum xanthoxylum* ‘peas ‘peanut, pakchoi ‘banana, dill ‘pistachio (Song et al., 2009; Shi et al., 2010; Zeng et al., 2011; Li et al., 2012; Zhang et al., 2013; Bharwana et al., 2013; Farooq et al., 2013; Habibi and Hajiboland, 2013; Kang et al., 2016; Rahman et al., 2017; Shekari et al., 2017; Ju et al., 2017). Furthermore, the efficacy of Si addition can depends on plant varieties and tissues (Song et al., 2009; Shi et al., 2010; Zeng et al., 2011; Zhang et al., 2013; Kang et al., 2016). At extreme As doses CAT activity may not be sufficient in protecting the plants against the large amounts of released H_2O_2 and, the POD enzyme can be involved in the detoxification process. POD can take part in lignin biosynthesis to create physiological obstacles versus heavy metal stress, and it also can detoxify H_2O_2 using different substrates (same as phenols) as an electron donor. Thus, POD activity changes considered as the most trustworthy oxidative stress indicators, the decrease in POD may be because of severe oxidative stress damage (Farooq et al., 2013).

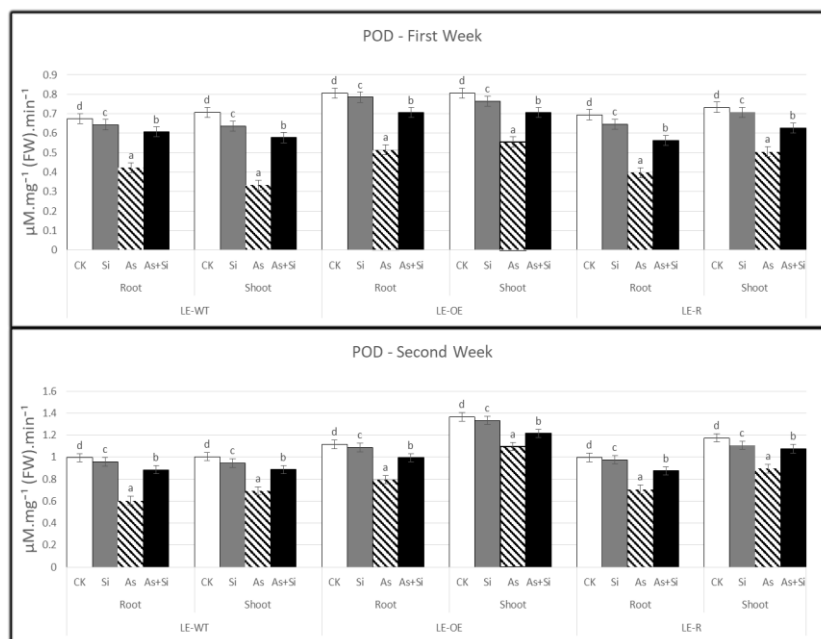


Figure 4. POD activity in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

Effects of Si and As on MDA content of rice roots and shoots

MDA levels increased after adding As to the culture solution compared to CK ($p \leq 0.05$); the highest MDA content in the first week was observed in shoots of LE-R line (Fig. 5). However, the highest MDA content in the second week was observed in the LE-WT shoots. This increase in MDA content compared to CK respectively was 186% and 98%. In most of the plants, free radicals inevitably made in non-infected plants and plants, which evolved elimination systems (non-enzymatic antioxidants, and antioxidant enzymes) to control ROS levels and prevent excessive accumulation of ROS. This balance can be broken by environmental stresses. The excessive presence of heavy metals in plants causes accumulation of free radicals. Accumulation of As stimulates the increase of ROS, the effect on the activity of chlorophyll synthesis and biomass, membrane permeability, enzymes, and photosynthetic reactions. ROS due to As directly attack to the hydrogen atom on the methylene group alongside an unsaturated carbon atom, thus, peroxidation, as the chain of degenerate fatty acids, polyunsaturated fatty acids, induce membranes, and eventually produce lipid peroxides. MDA is an important product of the degradation of unsaturated fatty acids by hydroperoxides, is generally regarded as an indirect index of oxidative stress. In present experiment, MDA content grew in whole rice treated with As compared to CK, and it shows As can cause superoxide radicals. Increasing the MDA content proves that rice was under oxidative stress conditions. In addition, the increase in MDA in a rice cultivar indicates that the cultivar is weak against As stress. This can be related to rice tissue, rice genotype, stages and conditions of plant growth, and the concentration and duration of As exposure. Studies showed similar results for MDA content in different plants under different stresses (Liu et al., 2009; Song et al., 2011; Vaculíková et al., 2014; Tripathi et al., 2015; Begum et al., 2016; Wu et al., 2017).

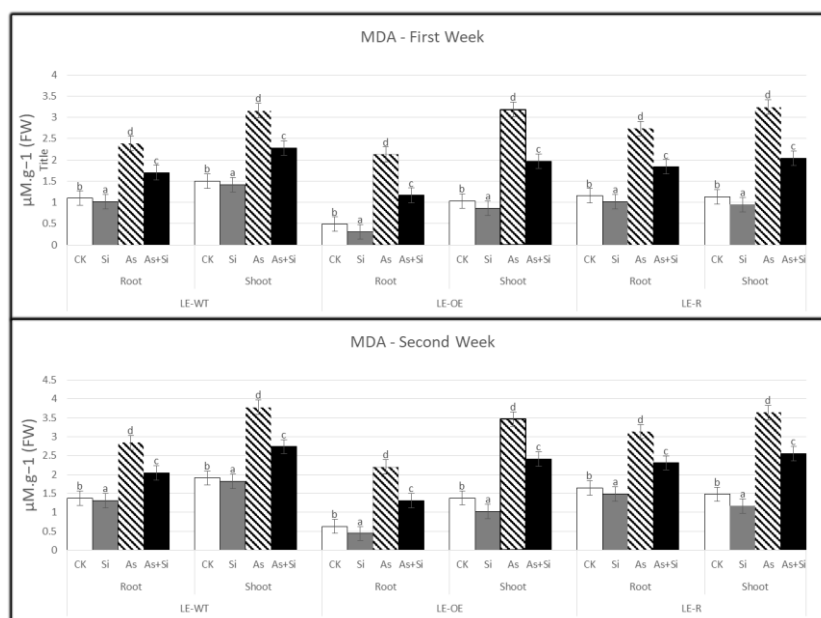


Figure 5. MDA content in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

Treated with Si rice seedlings significantly reduced MDA content, with the lowest MDA content, observed in the first and second weeks being in roots of Lsi1-OE line

(37.61% and 30.11% decrease, compared to CK ($p \leq 0.05$), respectively). MDA content in rice treated with As + Si grew compared to CK ($p \leq 0.05$), and the highest MDA content, both in the first and second weeks, was observed in LE-WT shoots (52.11% and 43.39% increase, respectively). In the present study, MDA contents in roots and shoots of different rice varieties treated by As + Si decreased compared to As exposure, indicating Si, indeed, is important in ROS metabolism of rice subjected to stress, and mitigates effect of As toxicity. The reason maybe that 1) Si reduces superoxide accumulation, and H_2O_2 radicals, as a result reducing LPO in As-stressed rice, and improving the antioxidant defense system, or 2) Si minimizes the penetrate plasma cell membranes by increasing the lipid strength and preventing harm to the structures and function of rice cell membranes during stress (Shi et al., 2005, 2014; Liu et al., 2009; Song et al., 2009, 2011; Li et al., 2012; Bharwana et al., 2013; Farooq et al., 2013; Tripathi et al., 2013, 2015; Cao et al., 2015; Vaculíková et al., 2014; Wu et al., 2017).

Effects of Si and As on GSH content of rice roots and shoots

Si treatment significantly ($p \leq 0.05$) increased GSH content in roots and shoots of different rice compared to control (*Fig. 6*). The highest GSH content observed in the LE-WT shoots both in the first and second weeks (12.81% and 8.68% increase compared to CK, respectively). GSH content significantly decreased in all rice cultivars, which treated with As. When compared to CK, the highest decrease of GSH content was in LE-WT roots, both in the first and second weeks (80.78% and 66.16% decrease, respectively). At the time of application of As + Si treatment, GSH content increased compared to the application of As alone, but it decreased in all rice cultivars in both the first and second weeks. The highest decrease observed in the roots of LE-WT in both first and second weeks, with respectively, 68.67% and 52.84% decreases compared to CK ($p \leq 0.05$).

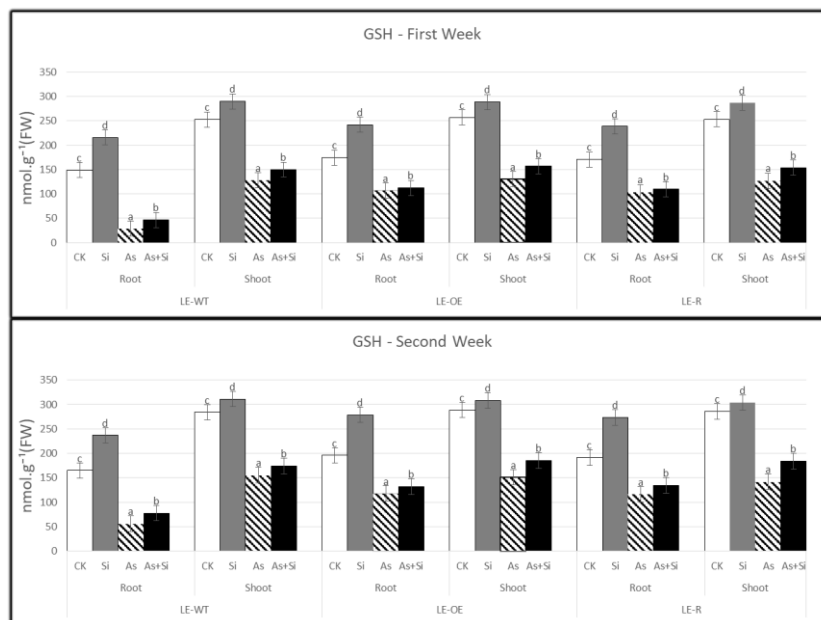


Figure 6. GSH content in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

GSH is an important non-protein triols sources in plant cells. High levels of GSH effect on reducing the toxicity of heavy metals via direct cleaning of ROS or making a complex with the toxic metals by GST activity. Meanwhile, GSH is involved in the biological transmission of As. GSH is used as an enzymatic or non-enzymatic depressant to decrease As (V) to As (III), therefore, GSH is a good indicator for assessing the toxicity of As in the environment. Binding of inorganic GSH to As is a good mechanism by which may control the toxicity of As. GSH uses GST to synthesize toxic molecules. GST is a present enzyme, which stimulates with toxic metals, in detoxification by combining GSH to toxic molecules. GSH nucleophilic attack on the electrophilic center of toxic compounds, and targets them for ATP-dependent transport into the vacuole, and it protects plant cells against their detrimental effects. In this experiment, the presence of As clearly decreased GSH content in three rice. This decrease in GSH levels may be due to 1) shortening of NAD(P)H as a substrate in possible reaction, through the reduction of GSSG to GSH by GR; 2) its reduction phytoclutins via acute or chronic exposure to stress; 3) Interference with the reproduction of AsA from its oxidized form in AsA-GSH cycle (Shi et al., 2005; Ellis et al., 2006; Hasanuzzaman et al., 2017). In our study adding Si increased GSH content in different rice roots and shoots, indicating an increase in GSH activity by Si application. According to reports, Si is important in the activity enzymes of ROS inhibitors in chloroplasts, in ASA-GSH pathway. When rice is in a stressful environment, Si will be involved in ROS metabolism. Similar results observed in rice under heavy metals stress, and other stress. According to similar studies, Si has a different effect on GSH content in different plant tissues as well as different rice (Shi et al., 2005; Song et al., 2009; Liu et al., 2009; Li et al., 2012; Wang et al., 2015; Cao et al., 2015; Rahman et al., 2017).

Effects of Si and As on GST activity of rice roots and shoots

In our study, GST activity levels significantly decreased by As treatments compared to CK ($p \leq 0.05$). The highest reduction in GST activity compared to control observed in LE-WT shoots in both first and second weeks (32.26% and 31.07% decline, compared to CK, respectively) (*Fig. 7*). When As + Si treatment added, GST activity increased compared to the As application alone. However, the GST activity under As + Si treatment showed a significant reduction compared to CK ($p \leq 0.05$). The highest reduction observed in LE-WT shoots, with 6.39% and 4.20%, respectively decreased compared to CK, in the first and second weeks. Treating rice seedlings with Si ($p \leq 0.05$) significantly increased GST activity compared to control in both the first and second weeks after treatment. The highest GST activity observed in LE-OE line roots in the first week, and the highest GST activity in the second weeks observed in LE-OE line shoots, showing a 23.22% and 20.31% increase compared to control, respectively.

GSTs are concentrated in apoplast, cytosol, chloroplast, mitochondria, and nucleus (Gechev et al., 2006). GST activity has important function in the ascorbate-glutathione cycle relate to ascorbate, and its regulatory enzymes. GSTs do detoxification in plants via combining toxic molecules with GSH (Ellis et al., 2006). Increased GST activity in rice seedlings treated by As + Si compared to treated with As alone indicates Si prevents oxidative damage induced by As, probably by reducing GSSG to GSH (Tripathi et al., 2013). Same results reported for the effects of GST activity against various stresses on the tissues of rice, pea, maize, wheat and Phragmites (Mauch and Dudler, 1993; Marrs and Walbot, 1997; Dixit et al., 2001; Iannelli et al., 2002; Adamis et al., 2004; Zhang et al., 2013).

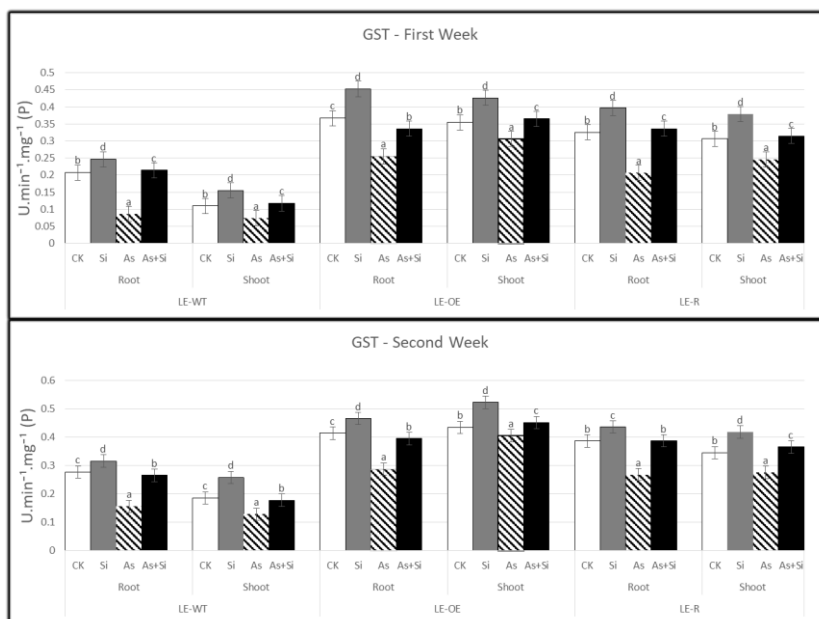


Figure 7. GST content in shoots and roots of LE-WT, LE-OE line and LE-R first and second weeks after adding different treatments

Conclusions

By decreasing the antioxidant capacity, exposure to As in rice plants can be harmful to the plant. The use of Si with As-toxicated plants partially reduces the harmful effects of As alone by modulating the antioxidant activities. Si application can also reduce stress induced by As by reducing MDA content in rice seedlings. Findings of the present experiment partly indicate Si is capable to enhance significantly the antioxidant defense capacity in rice seedlings, thus, it increases the resistance of different plant organs to As stress over time.

We also found that different varieties of rice have different resistance to As stress, and the addition of Si in different varieties has a different effect on the plant's resistance to As stress. Therefore, choosing As tolerant varieties, along with the use of Si in fertilizers can substantially reduce the damage caused in rice, and compensate for the economic loss. Also, suggest doing RNA-seq and Transgenic experiments, to know more details about the mechanisms and the genes relate to As and Si in these three rice varieties.

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