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THE ROLE OF SILICON TO INCREASE ARSENIC TOLERANCE IN RICE (*Oryza sativa* L.) SEEDLINGS BY REINFORCING ANTI-OXIDATIVE DEFENSE

Mohammad Reza Boorboori[1], Wenxiong Lin[1], Wenshan Zhan[1] and Changxun Fang[1]

ABSTRACT

Arsenic is a toxic metalloid which can cause severe problems to plants. On the other hand, silicon is a beneficial element, which supports plants to build resistance under stressed conditions. The objective of the present study was to assess the effect of silicon and arsenic on the various enzymatic, and non-enzymatic antioxidants, in shoots and roots of two rice seedlings (Du-WT and DU-OE), for one and two weeks. Seedlings were exposed to four different culture media: a) Control; b) 0.70 mM Si+no As; c) 30 μM As+no Si; d) 30 μM As+0.70 mM Si. Culture media and rice genotypes were arranged in a 8-treatment factorial with three replications. Results showed that response to silicon, arsenic and or combination of them in unstressed rice plants followed similar patterns, and varied depending upon the antioxidant. The addition of As always decreased the values, but together with silicon there was a partial recovery of them. The pattern of plant response was similar regardless the plant tissue or time of exposure to As. Transgenic Dullar rice, under As stress conditions, activated the highest level of antioxidants, especially when seedlings were treated with silicon.
Additional keywords: Antioxidants, enzymes, plant resistance, toxic element

RESUMEN

Rol del silicio en el incremento de la tolerancia al arsénico en plántulas de arroz mediante el refuerzo de la defensa antioxidativa
El arsénico es un metaloide tóxico que puede causar graves problemas a las plantas. Por su parte, el silicio es un elemento beneficioso, que ayuda a desarrollar resistencia en condiciones de estrés. El objetivo del presente estudio fue evaluar el efecto del silicio y el arsénico sobre diversos antioxidantes enzimáticos y no enzimáticos, en brotes y raíces de plántulas de dos genotipos de arroz (Du-WT y DU-OE), durante 1 y 2 semanas. Las plántulas fueron expuestas a cuatro medios de cultivo diferentes: a) Control; b) 0,70 mM Si+no As; c) 30 μM As+no Si; d) 30 μM As+0.70 mM Si. Los medios de cultivo y los genotipos de arroz se organizaron en un factorial de 8 tratamientos con three repeticiones. La respuesta al silicio, el arsénico, y la combinación de ellos en plantas sin estrés siguió patrones similares y varió según el antioxidante. La adición de As siempre disminuyó los valores, pero junto con el silicio produjo una recuperación parcial de los mismos. El patrón de respuesta de la planta fue similar, independientemente del tejido o el tiempo de exposición al As. El arroz Dullar transgénico, bajo condiciones de estrés, activó el nivel más alto de antioxidantes, especialmente cuando las plántulas fueron tratadas con silicio.
Palabras clave adicionales: Antioxidantes, elemento tóxico, enzimas, resistencia de las plantas

INTRODUCTION

Soil contamination by heavy metals and metalloids became serious problems in the recent decades because they negatively affect the growth and productivity of crops all around the world. Arsenic is a toxic heavy metalloid, which easily pollutes the soil, because of its high water solubility (Rodríguez et al., 2013). Soil contaminated with As is the main source of entrance of the element in the food chain (Pan et al., 2014).

On the other hand, silicon is a beneficial element for crop growth, especially in rice, which can accumulate large amounts of the element, in the range of 230-470 kg·ha^{-1}. Silica strengthens the rice plant and can protect it against biotic and abiotic stresses (Rao et al., 2017).

Abiotic stresses such as heavy metalloids and metals stimulate production of reactive oxygen

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[1] Fujian Key Laboratory of Agroecological Processing and Safety Monitoring, College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, P.R.China. e-mail: m.boorboori@yahoo.com; wenxiong181@163.com; 1170525027@fafu.edu.com; changfangxingyx@163.com (corresponding author)

species (ROS) and oxidative stress in plants. ROS attacks main metabolic functions via destroying vital biomolecules such as nucleic acids, proteins, and lipids. One of the mechanisms that rice plants utilizes to reduce the impact of abiotic stresses and ROS production can be associated to the action of a complex of various enzymatic, and non-enzymatic antioxidants.

The anti-oxidative complex includes substances that can increase the plant tolerance to stresses caused by various types of environmental factors and provides defense against damages caused by ROS (Cobbett and Goldsbrough, 2002). Some of the most important correspond to antioxidant substances such as superoxide dismutase (SOD) (Abreu and Cabli, 2010), catalase (CAT) (He et al., 2011), peroxidase (POD) (Ju et al., 2017), Ascorbate peroxidase (APX) (Gill and Tuteja, 2010), glutathione (GSH) (Xiang et al., 2001; Shri et al., 2009), and glutathione-S-transferase (GST) (Ding et al., 2017; Cummins et al., 2011). On the other hand, Malondialdehyde (MDA), an organic compound resulting from lipid peroxidation of polyunsaturated fatty acids, can be used as a convenient biomarker to determine damages caused by ROS (Kong et al., 2016).

In this study, we tested the effect of Si on oxidative stress behaviors of different rice genotypes, which were exposed to As. To identify possible mechanisms for the stress response in rice plants, anti-oxidative enzymes were analyzed. We hypothesized that the addition of Si in the early stages of rice growth would help the seedlings to mitigate the toxic effects of As via anti-oxidative enzymatic protective mechanisms. Thus, the objective of this study was to gain insight into the effect of additional Si on the activities of anti-oxidative enzymes at the early stages growth of two As affected genotypes of rice.

MATERIALS AND METHODS

Planting conditions. Healthy looking rice seeds were selected from two different rice varieties, Dullar wild type (DU-WT), and *Lsi1*-over expression transgenic Dullar (DU-OE). Before planting, the seeds sterilized in 1 % H_2O_2 (15 min), rinsed with deionized water and placed in Petri dishes in an incubator for 4 days at 28 °C. After germination, healthy seedlings were selected and transferred to the greenhouse for cultivation under hydroponic's conditions. Pots (2.5 L) filled with a nutrient solution modified from Cock et al. (1976). The base nutrient solution (culture medium) included the following substances (in $mg \cdot L^{-1}$): $(NH_4)_2SO_4$ (48.2), $Ca(NO_3)_2 \cdot 4H_2O$ (86.43), K_2SO_4 (14.9), $Na_2SiO_4 \cdot 9H_2O$ (200), KNO_3 (18.5), $FeSO_4 \cdot 7H_2O$ (45.7), H_3BO_3 (1.43), EDTA (48.44), $CuSO_4$ $5H_2O$ (0.04), KH_2PO_4 (24.8), $MnCl_2 \cdot 4H_2O$ (0.905), $Na_2MoO_4 \cdot 2H_2O$ (0.045), $ZnSO_4 \cdot 7H_2O$ (0.11), and $MgSO_4 \cdot 7H_2O$ (135.06). The pH was set to 5.8 by adding HCl or NaOH, and it was refilled once a week. Rice seedlings were transferred to the culture medium when they had three leaves, and were exposed to two additional levels of Si ($Na_2SiO_4 \cdot 9H_2O$) (0 and 0.70 mM) and two levels of As ($NaAsO_2$) (0 and 30 µM). Hence, there were four treatments: a) control (base medium only); b) 0.70 mM Si; c) 30 µM As; and d) 30 µM As + 0.70 mM Si.

The experiment was conducted in a completely randomized design with three replications, and factorial arrangement of the culture medium and rice varieties.

Rice samples were collected at one and two weeks after treatment. Shoots and roots were washed separately in distilled water, and the remaining residues in the root zone was washed with $CaCl_2$ solution (0.5 mM) for 30 minutes before being washed with distilled water. All samples were quickly transferred to a freezer (-10 °C) and stored until the enzyme activity was specified.

The amount of sodium added to the treated culture medium by $Na_2SiO_4 \cdot 9H_2O$ was compensated by adding the equivalent of NaCl to the base culture medium.

SOD activity. It was measured using the nitro blue tetrazolium method (NBT) with some modification (Esposito et al., 2015). Samples (0.5 g) were homogenized with 50 mM potassium phosphate buffer (PBS, pH 7.8) containing 1 % polyvinylpolypyrrolidone. Then they were centrifuged at 15,000*g* at 4 °C (20 min). Supernatants were used to measure SOD activity (Sajedi et al., 2011). Reaction mixture (3 mL) contained 750 µM NBT (0.2 mL), 130 mM Met (0.2 mL), 50 mM PBS (2.2 mL, pH 7.8), 20 µM EDTA-Na_2 (0.1 mL), 100 µM riboflavin (0.2 mL), and supernatant (0.1 mL). SOD activity expressed as the amount of enzyme required to cause 50 % inhibition.

CAT activity. It was specified via the absorbance amount at 240 nm due to H_2O_2 consumption, as reported by Azevedo et al. (2007). Reaction mixture contained 0.1 M H_2O_2 (0.3 mL), distilled water (1 mL), 50 mM PBS (1.5 mL, pH 7.8), and the sample (0.2 mL).

POD activity. It was characterized according to Li-Ping et al. (2006) with some modifications. Samples (0.5 g) homogenized in 50 mM potassium phosphate buffer (PBS, pH 7.8) containing 1 % polyvinylpolypyrrolidone, then centrifuged at 15,000*g* and 4 °C (20 min), and supernatants used to measure POD activity. Reaction mixture contained 0.05 M PBS (1.0 mL, pH 7.8), 0.2 % Guaiacol (0.9 mL), 0.3 % H_2O_2 (1.0 mL) and supernatants (0.1 mL). An increase in absorbance was determined at 470 nm in periods of 1 min period.

APX activity. One gram of sample was homogenized in 0.1 M sodium phosphate buffer (5 mL, pH 7.0) including 10 % PVP. Then it centrifuged at 12,000*g* at 4 °C (20 min) (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), 0.1 mM H_2O_2 (0.3 mL), 0.5 mM ascorbic acid (0.3 mL), and supernatant (0.4 mL). The change in the reaction mixture absorbance recorded at 290 nm after 5 min. The enzyme activity was expressed as the decomposed ascorbic acid protein in mg^{-1} min^{-1} by using an extinction coefficient of 2.8 $mM^{-1}{\cdot}cm^{-1}$.

MDA content. It was determined via the thiobarbituric acid reaction (Heath and Packer 1968). Using 0.5 g of plant sample with 1.5 mM potassium phosphate buffer (PBS, pH 7.8) including 1 % polyvinylpolypyrrolidone. Sample was centrifuged at a rate of 15,000*g* and 4 °C (20 min) (Sajedi et al., 2011). Supernatant mixed (2 mL) with 0.5 % TBA (2 mL) in 20 % TCA (2 mL). The mixture was heated to 95 °C (30 min), and then immediately cooled. Non-specific adsorption of the reaction solution was measured at 600 nm minus absorbance at 532 nm from the solution. To estimate the MDA content, an extinction coefficient (155 mM^{-1} cm^{-1}) was used.

GSH content. It was measured by Sedlak and Lindsay (1968) method. One gram of plant sample was homogeneous in a solution of 5 % (w/v) SSA (5 mL) included 10 mM EDTA, Then it centrifuged at 10,000*g* at 4 °C (20 min), then supernatant collected. The supernatant (10 µl), 140 µl working mix (100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, and 5 % SSA (10 µl), 10 mM DTNB and 6 U mL^{-1} GR and Incubated for 3 hours in a dark room) added. The reaction started via adding 2 mM NADPH (50 µl), and absorbance occurred at 412 nm after 1 min in 5 min intervals. GSH content calculated via a standard curve, and finally expressed as $nmol{\cdot}\ g^{-1}$ FW.

GST activity. One gram of each sample was homogenized in 100 mM Tris-HCl (5 mL, pH 7.5) solution, containing 14 mM β-mercaptoethanol, 2 mM EDTA, and 7.5 % PVP (w/v) (Ando et al., 1988). The supernatants collected after centrifugation at 15,000*g* at 4 °C (15 min). Measurement was done in 100 mM potassium phosphate buffer (2 mL, pH 6.5) containing 1 mM CDNB (250 mL), 5 mM GSH, and enzyme extract (0.5 mL). The change in absorbance done at 340 nm with a spacing of 1 min and 5 min. GST activity computed via using an extinction coefficient of 9.6 mM^{-1} cm^{-1} and expressed as µmole-conjugate formed in $mg^{-1}{\cdot}protein{\cdot}min^{-1}$.

Statistical analysis. Data from culture media and rice varieties were analyzed in factorial arrangement by Anova and Tukey tests (SPSS 26.0 software). Results are presented separately for shoots and roots, for two different weeks. Since at the second week after adding As treatment to the culture medium, all seedlings of DU-OE variety dried out, and thus all enzymatic and non-enzymatic antioxidant levels became zero, these values were excluded from the analysis.

RESULTS

The addition of As to the culture medium caused increase of this toxic element in the plant tissue whose levels reached values of 9.68 $mg{\cdot}kg^{-1}$ in shoots and 226.6 $mg{\cdot}kg^{-1}$ (dry weight) in roots. The response of the anti-oxidant substances was as follows:

SOD activity. Treatment with Si alone caused a slight but significant decrease ($P \leq 0.05$) of SOD activity as compared to control, although a much larger decreased occurred under the As treatment alone (Table 1). As+Si treatment significantly increased SOD activity compared to As treatment showing an important recovery of the enzyme. The most notorious response was seen in DU-WT roots in the second week where the activity of

SOD decreased from 221.9 to 103.6 unit·g^{-1} when adding As, but it recovered to 210.3 unit·g^{-1} when combined with Si. This behavior was seen in the roots and shoots of rice at both exposure times (one and two weeks). With this enzyme, the rice DU-OE showed the highest activity except for the shoot in the second week where DU-WT presented the highest values.

Table 1. SOD activity (unit·g^{-1} FW) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture medium	Control	170.85 a	158.48 a	291.42 a	230.42 a
	As	106.81 d	106.37 d	182.75 d	103.61 d
	Si	163.39 b	154.89 b	287.28 b	227.47 b
	As+Si	144.49 c	144.96 c	274.63 c	203.80 c
Rice genotype	DU-WT	141.16 b	126.86 b	254.16 a	188.40 b
	DU-OE	151.61 a	155.50 a	218.19 b	224.47 a

Means from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

CAT activity. Silicon treatment caused a significant increase of CAT activity compared to the control non stressed rice seedlings ($P \leq 0.05$) (Table 2). When exposed to As, a large reduction was observed in roots and shoots of both rice lines. Seedlings treated with As+Si increased CAT activity compared to As treatment alone, but this recovery was small and the reached values were still much lower than that of the control plants. One of the best responses occurred in DU-WT shoots in the second week where the activity of CAT decreased from 1.288 to 0.794 mg·g^{-1}·min^{-1} when adding As, and the recovery was only to 0.841 mg·g^{-1}·min^{-1} when combined with Si. Regarding this enzyme, the rice line with highest values was DU-WT most of the times, except DU-OE in the shoots of the second week.

Table 2. CAT activity (mg·g^{-1} FW·min^{-1}) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture médium	Control	1.3074 b	0.8228 b	1.2629 b	0.9174 b
	As	0.6671 d	0.3300 d	0.7941 d	0.5821 c
	Si	1.5730 a	1.1254 a	1.5141 a	1.2844 a
	As+Si	0.7461 c	0.3896 c	0.8605 c	0.4969 d
Rice genotype	DU-WT	1.1460 a	0.7573 a	1.1169 b	0.8734 a
	DU-OE	1.0008 b	0.5766 b	1.2006 a	0.8286 b

Mean values from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

POD activity. Si alone caused a slight but significant decrease ($P \leq 0.05$) of POD activity respect to control and an even larger decrease was seen in the As treatment alone (Table 3). As+Si treatment significantly increased POD activity compared to As treatment but still lower

than the control plants. A good response was observed in DU-WT shoots in the second week where the activity of POD decreased from 0.888 to 0.610 $\mu M \cdot mg^{-1} \cdot min^{-1}$ when adding As, and recovered to 0.801 $\mu M \cdot mg^{-1} \cdot min^{-1}$ when combined with Si. This behavior was observed in the roots and shoots of rice at both exposure times. With this enzyme, the rice line with highest values was DU-OE most of the times, except for DU-WT in the roots of the first week.

Table 3. POD activity ($\mu mol \cdot mg^{-1}$ FW$\cdot min^{-1}$) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture médium	Control	0.5906 a	0.6439 a	0.9199 a	1.0451 a
	As	0.3706 d	0.4101 d	0.6102 c	0.6718 d
	Si	0.5521 b	0.6112 b	0.8763 b	1.0173 b
	As+Si	0.5050 c	0.5575 c	0.8374 b	0.8951 c
Rice genotype	DU-WT	0.4564 b	0.5663 a	0.7787 b	0.8861 b
	DU-OE	0.5527 a	0.5451 b	0.9209 a	1.0141 a

Means from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

APX activity. Si treatment caused a significant increase of APX activity compared to control ($P \leq 0.05$) (Table 4). When exposed to As, a reduction was observed. Seedlings treated with As+Si increased APX activity as compared to As treatment alone, even though the reached values were still lower than those of the control plants. A good response was seen in DU-OE shoots in the first week when there was a large decrease of the activity of APX from 25.830 to 19.036 $\mu M \cdot mg^{-1} \cdot min^{-1}$ when adding As, and recovered to 24.219 $\mu M \cdot mg^{-1} \cdot min^{-1}$ when it was combined with Si. The observed trend of treatments was similar for the roots and shoots of rice at both exposure times. Regarding activity of this enzyme, the rice DU-OE was always superior to DU-WT considering both plant tissues and both periods of time (weeks).

Table 4. APX activity ($\mu mol \cdot mg^{-1}$ FW$\cdot min^{-1}$) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture médium	Control	22.326 b	11.473 a	27.302 b	14.089 b
	As	17.709 d	5.159 c	17.724 d	6.558 d
	Si	24.890 a	13.073 a	29.804 a	17.043 a
	As+Si	21.020 c	7.662 b	22.811 c	11.586 c
Rice genotype	DU-WT	18.480 b	7.832 b	22.259 b	10.769 b
	DU-OE	24.493 a	10.852 a	29.506 a	16.305 a

Means from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

MDA content. Si treatment caused a significant decrease of MDA content compared to control ($P \leq 0.05$) (Table 5). When exposed to As alone, a large increase was detected in roots and shoots of both rice lines. The most notorious response of MDA was its large increase when the rice seedling was exposed to As, and the content became double or even triple compared with the control non stressed plant, as seen in the MDA content in shoots in the first week which increased from 1.747 to 3.419 $\mu M \cdot g^{-1}$ in DU-WT and from 1.139 to 3.302 $\mu M \cdot g^{-1}$ in DU-OE after exposure in the culture medium. Plants treated with As+Si decreased MDA content compared to As treatment, although this content was still higher than that of control plants. Similar trend was found in roots and shoots of rice at both exposure time. In relation to MDA, DU-WT rice seedling was always superior to DU-OE regardless the plant tissue and times of exposure.

Table 5. MDA content ($\mu mol \cdot g^{-1}$ FW) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture médium	Control	1.4434 c	1.1279 c	2.1814 c	1.2284 c
	As	3.3606 a	2.6027 a	3.9527 a	3.0745 a
	Si	1.3118 d	1.0266 d	1.9555 d	1.1217 d
	As+Si	2.3123 b	1.7963 b	3.1517 b	1.9052 b
Rice genotype	DU-WT	2.3433 a	1.9146 a	2.7504 a	2.1469 a
	DU-OE	1.8707 b	1.3621 b	2.5095 b	0.9992 b

Means from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

GSH content. Si treatment caused a significant increase of GSH content compared to the control plants ($P \leq 0.05$) (Table 6). When exposed to As, a reduction was observed. Seedlings treated with As+Si showed significant but low increased in GSH content respect to As treatment alone, and the reached values were still much lower than those of the control plants. For instance, in DU-WT roots of the second week the content of GSH showed a large drop from 191.46 to 66.262 $nmol \cdot g^{-1}$ and when As was applied together with Si the recovery was only to 83.295 $nmol \cdot g^{-1}$.

Table 6. GSH content ($nmol \cdot g^{-1}$ FW) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture médium	Control	272.68 b	169.63 b	287.79 b	193.96 b
	As	123.41 d	65.99 d	158.82 d	66.262 d
	Si	281.28 a	225.09 a	302.82 a	256.89 a
	As+Si	144.21 c	78.58 c	177.10 c	101.92 c
Rice genotype	DU-WT	212.41 a	115.85 b	234.68 b	146.83 b
	DU-OE	198.38 b	153.80 a	251.84 a	194.82 a

Means from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

The observed trend of treatments was similar for the roots and shoots of rice at both exposure times. With GSH, the rice DU-OE showed the highest content except for the shoot in the first week where DU-WT presented the highest values.

GST activity. Si treatment caused a significant increase of GST activity compared to control ($P \leq 0.05$) (Table 7). When exposed to As, a reduction was observed. Seedlings treated with As+Si increased GST activity compared to As treatment alone, and the reached values were lower but somewhat close to those of the control plants. We can see that in DU-WT shoots of the first week the activity of GST showed a decrease from 0.208 to 0.172 $U \cdot min^{-1} \cdot mg^{-1}$, and when the As was applied together with Si the activity reached a value close to the original control (0.2046 $U \cdot min^{-1} \cdot mg^{-1}$) The observed trend of treatments was similar for the roots and shoots of rice at both exposure times. Regarding the activity of this enzyme, rice DU-OE was always superior to DU-WT considering both plant tissues in both periods of time.

Table 7. GST activity ($U \cdot mg^{-1}$ $P \cdot min^{-1}$) in roots and shoots of DU-WT and DU-OE rice genotypes at the first and second week after exposed to different treatments with arsenic (As) and/or silicon (Si)

		First week		Second week	
		Shoot	Root	Shoot	Root
Culture médium	Control	0.2227 ab	0.2698 b	0.2686 b	0.2868 ab
	As	0.1727 c	0.1670 c	0.2117 c	0.2090 c
	Si	0.2545 a	0.3127 a	0.3298 a	0.3155 a
	As+Si	0.2172 b	0.2450 b	0.2550 b	0.2663 b
Rice genotype	DU-WT	0.2070 b	0.2569 a	0.2616 b	0.2838 a
	DU-OE	0.2265 a	0.2404 a	0.2907 a	0.2703 a

Means from culture medium or rice genotype followed by different letters are significantly different according to Tukey test ($P \leq 0.05$)

DISCUSSION

The action of the culture medium was independent of the rice varieties, and no significant interactions were detected among 28 analyses (7 compounds x 2 rice varieties x 2 weeks). Thus, only the simple or main effect of treatments was considered for data interpretation.

First of all, it was found that addition of Si to the control non stressed plants could effectively increase the activities or content of CAT, APX, GSH and GST, while the activity of SOD and POD decreased. These results were very consistent, regardless if measurements were made in either variety, in shoot or root, and in first or second week, meaning that they were independent of rice genotype, plant tissue or time of measurement.

On the other hand, when Si was added to As stressed plants the activity or content of the antioxidants increased, showing the capacity of Si as a factor that can help to decrease As toxicity in rice (Kang et al., 2016; Rahman et al., 2017; Khan and Gupta, 2018). Furthermore, Si is also involved in ROS metabolism, when rice is under stress (Raza et al., 2016; Geng et al., 2018).

The addition of As to the culture medium without the addition of Si, resulted in a consistent decrease of activity or content of the antioxidants. This is attributed to levels of ROS higher than enzyme's ability to eliminate them or detoxify arsenic in rice.

In contrast to the enzymes and antioxidants, the addition of As alone to the culture medium increased dramatically MDA content in rice tissues suggesting some membrane damage in rice seedlings. Since MDA is an important product of degradation of unsaturated fatty acids and due to cell membrane is built of phospholipids (fats), the increase of MDA and the concomitant degradation of fatty acids will conduct to membrane damage by lipid peroxidation (Begum et al., 2016). Other

studies have also shown elevated MDA contents in rice under arsenic compounds stresses (Geng et al., 2018; Khan and Gupta, 2018).

When Si was added to As stressed plants, MDA content decrease significantly as compared to plants treated with As alone. This supports the positive effect of Si for protection of plants under stress. As mentioned before, we found that the addition of Si increased activity or content of the antioxidants.

One of the main defense pathways in plants against abiotic stresses that may cause oxidative stress by ROS are the various enzymes such SOD and CAT. ROS contains O_2-, OH- and H_2O_2, which attack main metabolic functions of the plant and are responsible for destroying cellular signaling; however, the antioxidant effect of those enzymes can catalyze the disproportionate O_2- reaction to hydrogen peroxide and oxygen and operates as a defense mechanism.

Metalloenzymes, such as SOD, are the first defensive line against toxicity of ROS, by detoxifying toxic O_2- to H_2O_2; and as the peroxide is still harmful, CAT can immediately catalyze it to harmless water and molecular oxygen (Liu et al., 2015).

Treatment with As+Si significantly increased SOD and CAT activity compared to As alone, which indicates that Si can trigger SOD under As stress, which facilitates elimination of excess of free radicals, and effectively increase defense capacity. SOD activity in rice treated with Si under other stresses have also increased (Ju et al., 2017). Geng et al. (2018) found that silicon improves growth and alleviates oxidative stress in rice seedlings by strengthening antioxidant defense and enhancing protein metabolism under exposure to an organoarsenic compound.

Seedlings treated with As+Si increased CAT activity compared to As treatment alone showing that Si increased CAT synthesis. CAT is an important enzyme in rice and a key defense mechanism against ROS toxicity (Wang et al., 2015). The high CAT activities induced by adding Si has strengthen enzyme activities or provided protection against heavy metal toxicities in different plant species (Kang et al., 2016; Rahman et al., 2017).

The rest of antioxidants showed similar response when plants were treated with As+Si, and showed increments respect to plants receiving As alone. This response supports the beneficial effect of silicon due to the fact that the studied enzymes and non enzymatic compounds have proven to be involved in defense against ROS. For instance, the increase in POD activity suggests that the addition of Si was efficient in increasing the defense capacity of the plant because the enzyme can interfere in lignin biosynthesis to create physiological obstacles against heavy metals stresses (Ju et al., 2017) and crystallize H_2O_2 when CAT activity does not adequately protect against the high amounts of the peroxide (Xiang et al., 2001). APX has an important role in eliminating reactive oxygen (Gill and Tuteja, 2010), and H_2O_2 (Khan and Gupta, 2018; Ju et al., 2017) for protection of cells against toxic effects of ROS. GST can reduce toxic oxygen levels and plays an important role in detoxification reactions from different stresses, such as those from heavy metals (Cummins et al., 2011; Ding et al., 2017; Das et al., 2018). Additionally, the increase observed in GSH when Si was added indicates that this non enzymatic compound can act in defense against ROS induced by the As toxicity (Shri et al., 2009).

Regarding the presence of the antioxidants in different plant tissues or rice lines, we found that, when adding Si, the response of antioxidants was similar for both shoot and root, and independent of rice variety; however, Rahman et al. (2017) found that the role of Si in increasing GSH content can depends on plant tissues and genotype of plants.

Finally, it has to be highlighted that transgenic Dullar rice (DU-OE) showed in most situations (except with CAT) higher activities or contents of enzymes and non enzymatic compounds with antioxidant capacities, which suggests that this line of rice has better possibility to minimize high level of toxic substances such as arsenic, especially when the plant is treated with silicon.

CONCLUSIONS

Response of antioxidants to silicon, arsenic and or combination of them in rice plants follows similar patterns. The enzymes always decrease when exposing plants to As. The content of GSH and activity of CAT, APX and GST increased, and SOD and POD in rice seedlings decreased, after exposure of unstressed plants to silicon. Addition

of arsenic alone always decreased the values. Addition of silicon to arsenic stressed plants increased activity or content of enzymatic and non enzymatic compounds meaning partial recovery of the original values of the control non stressed plants. The effects of the analyzed plant tissue and time of exposure to As were consistently similar throughout the study. MDA shows to be highly responsive to plant stress with high accumulation under arsenic exposure. Transgenic Dullar rice (DU-OE) showed better possibility to minimize high level of toxic substances such as arsenic. The use of silicon fertilizers can help to reduce food contamination during the early growth period of rice seedlings.

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