RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Effects of Nanoscale Silica Sol Foliar Application on Arsenic Uptake, Distribution and Oxidative Damage Defense in Rice (*Oryza sativa* L.) under Arsenic Stress

Chuanping Liu [a], Lan Wei[b], Shirong Zhang [a], Xianghua Xu [a], Fangbai Li [a*]

[a] *Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, China*

[b] *Institute of Agricultural Resources and Environment, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China*

***Corresponding author**

Dr. Fangbai Li

Professor in Environmental Soil Chemistry

Guangdong Institute of Eco-Environmental and Soil Sciences

Guangzhou 510650, P. R. China.

Phone: +86-20-37021396; Fax: +86-20-87024123

E-mail: cefbli@soil.gd.cn

Abstract:

It has been widely reported that silicon (Si) can enhance rice resistance and/or tolerance to arsenic (As) toxicity; however, there was very little evidence suggesting that nanoscale silica foliar applications can decrease As accumulation in rice grains grown in As-contaminated environments. This study aimed at investigating the mechanism of nanoscale silica sol foliar application on alleviation As toxicity and reduction its accumulation in rice. Pot, field and hydroponics experiments were conducted to study effects of nanoscale silica sol foliar application on biomass and As accumulation in rice plants. The electrolyte leakage (EL), malondialdehyde (MDA) contents, and activity of antioxidant enzymes were also examined in rice grown in 50 or 100 mM As contained nutrition with or without 5 mM nanoscale silica sol foliar pretreatment. Results showed that nanoscale silica sol foliar application significantly increased grain yields, while decreased As concentration of brown rice in pot and field experiments, and induced more As combined on cell walls of shoots, decreased EL and MDA contents in root and increased activities of antioxidant enzymes including superoxide dismutase (SOD), preoxidase (POD), catalase (CAT), and asorbate peroxidase (APX) in root of rice seedlings grown in As contained nutrition. These results indicated that nanoscale silica sol foliar application was effective in alleviation As toxicity and accumulation in rice. These were mainly attributed to enhanced antioxidant defense capacity by Si treatments. The mechanism of nanoscale silica foliar application to alleviate the toxicity and accumulation of As in grains of rice may be also related to the probable As sequestration in the shoot cell walls.

Keywords: Arsenic contamination; Antioxidant defense; Foliar application; Rice; Nanoscale silica sol

1. Introduction

Arsenic (As) is a non-threshold carcinogenic metalloid that occurs ubiquitously in the environment, with both natural and anthropogenic sources[1, 2]. Generally, mining and smelting are the main anthropogenic sources of As contamination in water and soil[1, 3-5]. The oral intake of food and beverages is the most significant pathway of exposure to As for healthy humans while that of water may be the major source in areas where drinking water contains >50 μg[6]. Recent studies indicated that rice may be the primary source of inorganic arsenic for human exposure in Southeast Asia where the rice is the dominant source of diet for populations[2]. Hence, it becomes a growing public concern on amelioration the As contamination in soils and reduction the As transfer to rice, especially in mining area of Southeast Asia.

Rice is easy to accumulate more As in grains than other cereal grains when grown on As contaminated soil or irrigated with As tainted water[7, 8]. It was reported that the concentration of As even reached to 7.5 mg kg^{-1} in grain of rice grown in some As contaminated soils[3]. In paddy soils, which are flooded during much of the rice growing season, arsenite (the more mobile As species) becomes the predominant chemical species of As[7] and arsenite is taken up by the highly efficient Si pathway[9], which explains why rice is efficient in As accumulation. According to the report of Ma et al. (2008)[10], that As(III) is uptaken through Si influx transporter Lis1, and transported to the xylem and accumulation in shoots and grain of rice through Si efflux transporter Lsi2. Therefore, exogenous Si would inhibit As uptake and accumulation in rice due to the competition with As(III) for the same transporter, and the Si application as a measure of reducing As accumulation in rice has received increasing attention in recent years.

Si is the second most abundant element in soils. Although it is not an essential element for most plants, it has been shown to be beneficial for the healthy growth and development of many plant species, particularly graminaceous plants, such as rice[11]. Rice is a typical Si accumulator, and it accumulates Si to levels of up to 10.0% of the shoot dry weight[12]. It has

been reported that Si can enhance the resistance and/or tolerance of plants to toxicity from heavy metals, such as Pb[13], Cd[14], Zn[15], Cu[16] and Mn[17]. Recently, studies showed that Si supply to root of rice alleviated As toxicity and reduced As accumulation in rice[18, 19]. It has been shown that As concentrations in brown rice and polished rice were reduced by 23% and 22% with the application of Si, respectively[19], and Li et al. (2009) also reported that Si fertilization decreased the total As concentration in straw and grain by 78% and 16%, respectively in pot experiments[18]. However, the Si used for the applications in these studies was primarily sourced from furnace slag, steel sludge, and Si chemicals, and it was directly applied to the soil. The availability of Si fertilizer when applied to the soil is very low because silicate slag is difficult to dissolve, or it is immobilized by minerals in the soils[20].

Despite evidence of a role for Si radical application in ameliorating As toxicity in rice, with Si reducing the uptake and accumulation of As in rice, there is very little evidence to suggest whether foliar nanoscale silica applications to rice could decrease As accumulation in rice grains. Nanoscale silica sols have been widely used in industry for several decades. In recent years, it has been reported that nanoscale silica sol could be applied in agricultural application such as pesticide carrier. A mixture of hydrosol SiO_2 (nano-SiO_2) and TiO_2 (nano-TiO_2) could increase the nitrate reductase in soybeans (*Glycine max*), enhance its abilities to absorb and use water and fertilizer, stimulate its antioxidant system, and hasten its germination and growth[21]. Furthermore, it has been established that silica sol has a role in protecting rice from diseases such as blast[22]. Our previous studies have shown nanoscale silica sol foliar application could reduce cadmium accumulation in rice and the use of silica sol for heavy metal control in rice was both economically viable and environmentally friendly[14].

In this study, a nanoscale silica sol was prepared by hydrothermal method and applied to rice as a Si foliage fertilizer. We hypothesize that foliar supplementations of nanoscale silica sol could ameliorate As toxicity in paddy rice and thereby reduce As accumulation in rice.

Consequently, the objectives of this study were as follows: 1) to investigate the effects of nanoscale silica sol applications on rice growth and As uptake and distribution in field and pot experiments; and (2) the possible mechanisms of Si to alleviate the As toxicity and reduce its accumulation in plants.

2. Materials and methods

2.1 Preparation of nano-sized silica sols

The nanometer silica sols were prepared from tetraethyl orthosilicate as our previous research[14]. 10 mL ethanol was blended with 5 mL 25% (v:v) ammonia for about 0.5 h. About 5 mmol tetraethyl orthosilicate was added to the mixed solution, heated in a water bath at 25 ºC-30 ºC for about 14 h. The solution was distillated to vaporize the ethanol and ammonia at 40 ºC and then dialyzed with dialysis bag (MWCO=8000-12000) at room temperature to eliminate the organic impurities and the inorganic ions. The obtained silica sols were diluted to 1000 mL with deionized water. The silica sols were semitransparent solutions with a light-blue color and a particle size distribution (PSD) of 10-30 nm. The prepared silica sol was slightly acidic with pH of 6.5.

2.2 Field and pots experiments: As accumulation in plants and grains

The field near Lianhuashan tungsten mine contaminated with As was selected for the field experiments. The physical and chemical properties of the soils are as follows: pH: 6.12, CEC: 8.75 Cmol kg^{-1}, organic matter: 2.73%, total nitrogen: 0.15%, available nitrogen: 153 mg kg^{-1}, total phosphorus: 0.10%, available phosphorus: 142 mg kg^{-1}, total potassium: 1.13%, available potassium: 97.4 mg kg^{-1} and total As: 93.6 mg kg^{-1}. The experiment was performed from April 5th to July 14th, 2008. During the tillering stage, the rice leaves were sprayed with 3000 L ha^{-1} silica sol (5 mM), and with the same quantity of deionized water spraying was set as control. The treatments in a completely-randomized design with four replicates per

treatment were set up in experimental plots of 20 m^2 (4×5 m) each, with 0.5 m distance between plots. Periodic irrigation was applied during the growing season and agricultural measures such as insecticides and herbicides were applied when necessary. The plants were harvested at maturity, and the available tillering number and thousand-grain weights were detected; the yields were tested by the dried weight of grains; the As concentration in grain of rice was measured after removed the chaff and ground in a carnelian mortar.

For the pot experiments, soil was collected from the same site as the field experiments. Air-dried soils were passed through a 2 mm-diameter sieve. The composition of basal fertilizers applied to the soils was 100 mg N kg^{-1} dry soil as urea, 80 mg P kg^{-1} and 100 mg K kg^{-1} as KH_2PO_4[18]. After undergoing three cycles (10 d per cycle) of saturation with deionized water and air-drying, the soils were placed in plastic pots at 10 kg of soil per pot.

Rice seeds (*Oryza sativa* L. cv Youyou 128) were purchased from the Rice Research Institute of Guangdong Agricultural Academy. They were surface-sterilized with 0.5% NaClO, rinsed thoroughly with deionized water, and then germinated for 3 d. The germinated seeds were grown in uncontaminated soils. After 20 days, the seedlings with two tillers were transplanted into pots (three plants per pot). The pot soil was maintained under flooded conditions (with 2-3 cm of water above the soil surface) during the entire 120 d growth period.

Nanoscale silica sols (600 ml) were sprayed individually onto the leaves of rice seedlings grown in the As contaminated soil three times at the tillering stage (60-70 d). The concentration of silica sol in the treatment was 5 mM. The control plants were sprayed with the same quantity of deionized water. Each treatment was replicated three times. The rice shoots and grains were harvested after maturity and dried at 80 °C for 72 h. The dry weights of the shoots and grains were measured. The chaff of the grains was removed and the parts were then ground in a carnelian mortar for further chemical analysis.

2.3 Hydroponics experiment: Antioxidant system under As stress

The rice seeds (*Oryza sativa* L. cv Youyou 128) were surface sterilized as described for the pot experiments and then germinated for 3 d. After germination, the seedlings were transferred to 2 dm^3 pots containing Kimura B nutrient solution. Twenty plants were placed in each pot. After growing for 20 d, the leaves were pre-treated with 200 ml of nanoscale silica sol per pot. The plants were sprayed with the same quantity of deionized water as the control. Three days after the silica sol application, Na_2HAsO_4 was added to the solution at concentrations of 0 μM, 50 μM and 100 μM. Each treatment was replicated three times. The experiment was performed in a greenhouse with day/night temperatures of 28/20 °C under natural light. The pH of the nutrient solutions was adjusted to 5.5 with 1 mM HCl or 1 mM NaOH. Nutrient solutions were renewed every 3 d. The rice shoots and roots were harvested 7 d after Na_2HAsO_4 treatments. The roots were washed twice with distilled water (acidified to pH 4.0 with HCl) and then washed with deionized water. The shoots were washed twice with deionized water.

2.4. Chemical Analyses

2.4.1 Determination of As

As concentrations in soil or plant samples were detected after digested with a mixture of HNO_3-H_2O_2 and then analyzed for total arsenic concentration with a hydrogen generation-atomic fluorescence spectrometer (*AFS-820, Beijing Titan Instruments Co., Beijing, China*). To ensure accuracy and precision in the analysis, certified standard reference materials, reagent blanks and analytical duplicates were used as described in our pervious reports[5].

As bound on the root or shoot cell wall was measured with the method of Hart et al. (1992)[23]. Washed roots or shoots were dipped in a mixture of methanol: chloroform (1:1, v/v) solution for 3 d, and then washed with deionized water. The materials were dried at 80 °C,

and then acid-digested with a mixture of with HNO_3-H_2O_2 to analyze the arsenic contents (AFS).

2.4.2 Determination of MDA Contents in root of rice

Fresh tissues (0.5 g) were homogenized in 5 ml of 5% TCA (trichloroacetic acid) solution. The homogenate was centrifuged at 3000 r·min^{-1} for 10 min, and 4 ml of the supernatant was mixed with 4 ml of 0.67% TBA (thiobarbituric acid) in a water bath at 100 °C for 30 min and centrifuged again after the solution was cold. The absorbance of the supernatant was tested at 450 nm, 532 nm, and 600 nm[24].

24.3 Determination of root Electrolyte Leakage

The procedure used was based on the method of Lutts et al. (1996)[25]. About 0.2 g fresh roots were rinsed thoroughly with de-ionized water to remove surface contamination, then were cut into 1 cm segments and placed in individual vials containing 10 ml of distilled water. These samples were vacuumized at room temperature (25 ºC) for 3 h. Electrical conductivity of bathing solution (EC1) was measured after vacuumization by electrical conductivity meter (*SY-2*, *Nanjing, China*). Samples were then placed in thermostatic water bath at 100 ºC for 15 min and the second reading (EC2) was determined after the solutions were cooled down to room temperature. EL was calculated as EL = $\frac{EC1}{EC2} \times 100$ and expressed as percent.

2.4.5 Assays of enzymatic antioxidants in rice roots

Fresh root samples (0.5 g) were ground in an ice bath with 10 ml of homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8) and then centrifuged at 10, 000 g at 4 °C for 30 min. The supernatants were used for assay the activity of enzymes. The SOD activity was measured by using the photochemical method

described by Giannopolitis and Ries (1977)[26] in terms of the ability to inhibit the reduction of NBT (r-nitroblue tetrazolium chloride). One unit of SOD activity was defined as the amount of enzyme required for a 50% inhibition in the rate of NBT reduction as measured at 560 nm. The POD activity was assayed by following the method of Cakmak et al. (1993)[27]. In brief, the assay mixture contained 2.9 ml of 100 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 7.8), 1.0 ml 2% H_2O_2, 1.0 mL of 0.05 mM guaiacol and 0.1 mL of enzyme extract in a total volume of 5.0 mL. Changes in the absorbance of brown guaiacol at 470 nm in the presence of H_2O_2 were recorded to calculate the POD activity. The CAT activity was assayed using the method described by Cakmak and Marschner (1992)[28]. The assay mixture contained 2.5 mL of NaH_2PO_4-Na_2HPO_4 buffer (pH 7.8), 0.3 mL of 0.1 mM H_2O_2 and 0.2 mL of enzyme extract in each total volume of 3.0 mL. The activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. The APX activity was assayed using the method described by Nakano and Asada (1981)[29]. The reaction was started with 0.2 mL enzyme extract and 2 mL of NaH_2PO_4-Na_2HPO_4 buffer (pH 7.0), 0.3 ml of 1 mM EDTA-Na_2 and 0.3 mL of 5 mM Vc, and 0.3 mL of 1 mM H_2O_2. The activity was assayed by monitoring the decrease in absorbance at 290 nm. Enzyme activities were expressed as unit per milligram protein. The protein content was determined according to the method of Bradford (1976)[30] using bovine serum albumin (BSA, Sigma) as standard.

2.4.6 Assays of ASA and GSH concentrations in rice roots

The concentration of ascorbic acid (AsA) was measured according to Cakmak and Marschner (1992)[27]. Fresh tissues (0.5 g) were homogenized in 5 ml of NaH_2PO_4-Na_2HPO_4 buffer (pH 7.4) and centrifuged at 12000 r min^{-1} at 4 °C for 15 min. 100 μL of supernatant was collected with 300 μL of NaH_2PO_4-Na_2HPO_4 buffer (pH 7.4) and 100 μL of ultrapure water. The assay mixture (1 mL) contained 0.1 mL of 10% TCA, 0.1 mL of 44% H_3PO_4, 0.2 mL of 4% 2,2'-bipyridine-ethanol, 0.1 mL of 3% $FeCl_3$-ethanol and 0.5 mL of supernatant. The assay

was placed in a 40 °C water bath for 1 h and its absorbance was monitored at 525 nm. Glutathione (GSH) was fluorimetrically quantified according to Ellman (1959)[31]. Fresh roots (0.5 g) were ground in a mixture with 4 mL of 15% H_3PO_3 at 0 °C. The homogenate was centrifuged at 10,000 g for 30 min at 4 °C, and the supernatant was diluted five-fold in sodium phosphate EDTA buffer (pH 8.0). The final assay mixture (2.0 mL) contained 100 μL of the diluted supernatant, 1.8 mL of phosphate EDTA buffer and 100 μL of *O*-phthalaldehyde (1 mg mL^{-1}). After being mixed thoroughly and incubated at room temperature for 15 min, the solution was transferred to a quartz cuvette and the fluorescence was measured at 420 nm after excitation at 350 nm.

2.5 Statistical analysis

All data in the tables were subject to an analysis of variance and were expressed as the means with standard errors for four replicates. The statistical significance of the means was compared by Duncan's New Multiple Range Test at a 5% probability level using SPSS software.

3. Results

3.1 Effects of nanoscale silica sol foliar application on rice yield and As uptake in field and pot exprimentss

In comparison with the control, the nanoscale silica sol foliar application at the tillering stage led to a significant increase in the rice yield by 22% in the field experiments. The elevated yield by silica foliar application was partly explained by a significant increase in the available tillering number and the thousand-grain weight of rice by the treatment of nanoscale silica sol foliar application. At the same time, the grain As concentration was significantly decreased by nanoscale silica sol foliar application. The As concentration in the brown rice treated with nanoscale silica sol foliar application was about 59.8% of the

control (Table 1).

In the pot experiment, nanoscale silica sol foliar applications could significantly mitigate the arsenic toxicity to rice grown on the contaminated soil (in which the As concentration was 93.6 mg kg^{-1}). Comparing with the control, that nanoscale silica sol foliar application at the tillering stage significantly increased the dry weight of the grains and stalks by 29.6% and 36.9%, respectively. And the As concentration in straws and grains of rice was reduced significantly with nanoscale silica sol foliar application. Comparing with the control, that nanoscale silica sol foliar application lead to a decrease of As concentrations in grains and stalks by 28.0% and 54.5%, respectively (Table 2).

3.2 Effects of nanoscale silica sol foliar application on plant growth, arsenic uptake and accumulation in hydroponics experiments

As shown in Fig. 1, that nanoscale silica sol foliar application also mitigated the growth inhibition in rice seedlings caused by As stress in hydroponics experiments. The root dry weight was significantly increased by nanoscale silica sol foliar pretreatment under 50 and 100 μM Na_2HAsO_4 stresses, which was 1.46 and 1.66 times of the treatments without silica sol application (Fig. 1). The shoot biomass was also increased by nanoscale silica sol foliar pretreatment under 50 and 100 μM Na_2HAsO_4 stresses, but only when rice seedlings were grown in 100 μM Na_2HAsO_4 contained solution, the difference was significantly. Under the 100 μM Na_2HAsO_4 treatment, the shoot biomass of rice with the nanoscale silica sol foliar pretreatment was about 1.18 times of the samples without silica sol application (Fig. 1).

Nanoscale silica sol foliar pretreatment reduced the As concentration in both of the shoots and roots of rice seedlings grown in As contained nutrition. When compared with the 50 and 100 μM Na_2HAsO_4 treatment, the shoot As concentrations in the nanoscale silica sol foliar pretreatments decreased by 53.2% and 41.3%; and the root As concentration in the nanoscale silica sol foliar pretreatment decreased by 12.2% and 22.3%, respectively (Fig. 2A).

Nanoscale silica sol foliar pretreatment also significantly reduced As accumulation in rice shoots. When compared with the 50 and 100 μM Na_2HAsO_4 treatment, the As contents in shoots pretreated with the nanoscale silica sol foliar application decreased by 48.2% and 30.0%, respectively. Meanwhile, the nanoscale silica sol foliar application significantly increased the root As contents by 30.0% under the 100 μM Na_2HAsO_4 treatment, but there was no significant difference for the root As contents with or without nanoscale silica sol foliar application under 50 μM Na_2HAsO_4 stress (Fig. 2B). The proportions of As in shoot of rice exposed to 50 or 100 μM As were significantly affected by nanoscale silica sol foliar application. With nanoscale silica sol foliar application, that the As percentages in shoot of rice seedlings exposed to 50 or 100 μM As(V) were decreased from 24.9% or 20.6% to 20.0% or 12.2%, respectively (Fig. 2C).

3.3 Effects of nanoscale silica sol foliar application on As distribution in rice seedlings

The 23-d-old rice seedlings grown in the Kimura B nutrient solution were foliar applied with nanoscale silica sol or deioned water, and then transplanted to the 50 or 100 μM As contained Kimura B nutrient solution. 7 d after the As treatments, the As concentration combined on the cell walls of the shoots and roots was detected. The results showed that nanoscale silica sol applications significantly increased the percentage of As combined on the shoot cell walls (Fig. 3). When compared with the solitary 50 and 100 μM Na_2HAsO_4 treatments, the percentage of As in the cell walls of shoots treated with nanoscale silica sol foliar application increased by 125% and 75%, respectively. However, there was no significant difference in the percentage of As in cell wall of roots with or without Si-sol application (Fig. 3).

3.4 Effects of nanoscale silica sol foliar application on plant oxidative damage and enzymatic or non-enzymatic antioxidants defense systems under As stress

Table 3 shows the effects of nanoscale silica sol foliar application on oxidative damage,

enzymatic and non-enzymatic antioxidants defense systems in root of rice seedlings exposed to As stress. The MDA levels in 50 and 100 μM As-stressed roots were significantly higher than that of the control by 50% and 86%, respectively (Table 3). Nanoscale silica sol pretreatment significantly reduced the MDA concentrations in root of rice seedlings exposed to 50 and 100 μM As-stressed roots by 16% and 23%, respectively (Table 3). The EL of rice roots under As stress were increased significantly with the increasing As concentration. When compared with 0 μM Na_2HAsO_4 treatments, the EL levels under 50 and 100 μM Na_2HAsO_4 treatments were increased by 101.5% and 130.2%, respectively (Table 3). At the same time, the nanoscale silica sol pretreatment lead to a significant decrease in the EL levels of rice roots grown in the 50 or 100μM As contained Kimura B nutrient solution by 34.8% or 33.8% (P<0.01), respectively (Table 3). These results demonstrated that the nanoscale silica sol application reduced oxidative damage in rice roots and increased the integrity of the cell membrane.

The activities of SOD, POD and CAT in rice roots were decreased significantly (P<0.05) by 50 or 100 μM Na_2HAsO_4 treatments (Table 3), while the activity of APX were increased with the 50 μM Na_2HAsO_4 treatment. And no significant differences in APX activity were found between 100 μM Na_2HAsO_4 and 0μM Na_2HAsO_4 treatments (Table 3). When compared with the 0 μM Na_2HAsO_4 treatment, the SOD activity of the 50 and 100 μM Na_2HAsO_4 treatments decreased by 19.3% and 38.5%; the POD activities decreased by 33.0% and 49.0%; and the CAT activities decreased by 23.5% and 34.2%, respectively.

Nanoscale silica sol foliar pretreatments alleviated the activity inhabitation of antioxidative enzymes induced by As stress. In comparison with 50 and100 μM As stress alone, nanoscale silica sol foliar applications led to an increase in the SOD activities by 25.2% and 54.0%; an increase in POD activities by 38.7% and 41.9%; and an increase in APX activities by 16.5% and 16.8%, respectively. However, there was no significant difference in the CAT activity in root of rice seedlings grown in same leave As contained nutrition with or without nanoscale

silica sol foliar pretreatment (Table 3).

As shown in Table 3, both non-enzymatic antioxidants AsA and GSH contents in rice roots were significantly increased under As stress. When compared with the 0 μM Na_2HAsO_4 treatment, the ASA contents in roots of rice seedlings exposed to 50 and 100 μM As stress increased by 27.8% and 32.7%, at the same time, the GSH contents increased by 19.1% and 26.0%, respectively. Nanoscale silica sol foliar application further increased ASA and GSH contents in root of rice seedlings under As stress. In comparison with 50 and100 μM As stress alone, nanoscale silica sol foliar applications led to an increase in the AsA contents by 7.0% and 11.9%; an increase in GSH contents by 13.9% and 2.7% However, there was no significant difference in the ASA and GSH in root of rice seedlings grown in same leave As contained nutrition with or without silica sol foliar pretreatment (Table 3).

4. Discussion

Arsenic is a non-essential and generally toxic element to plants. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield and fruit production[32]. Si can improve plant growth under biotical or abiotic stress conditions[12, 14, 33]. In the present research, the foliar applications of nanoscale silica sol significantly increased the yields of grains by 22.1% and 29.6% for pot and field experiments, respectively (Table 1 and 2). In the 50 or 100 μM As contained Kimura B nutrient solutions, the dry weight of the shoots and the roots were also increased significantly by the pre-treatment with nanoscale silica sol on the rice leaves (Fig. 1). These results suggested that nanoscale silica sol foliar application could alleviate the As toxicity on rice. The enhancement of rice growth might be due to the lower As-induced oxidative stress by nanoscale silica sol foliar application[32].

The main toxicity of arsenic to rice is the accumulation of free radicals and reactive

oxygen species (ROS) in the plant, which results in the injury of lipid peroxidation and the loss of membrane integrity[34]. In present research, the electrolyte leakage and MDA contents, the indicates of reactive oxygen species (ROS), in roots of rice were enhanced significantly by silica sol foliar application under 50 or 100μM As stress in the hydroponics experiments. The results of hydroponics experiments also showed that anti-oxidant enzymes such as SOD, POD, and CAT decreased significantly under 50 or 100μM As stress，while APX increased significantly under 50 μM As stress. SOD is the major superoxide ($O_2^{\cdot-}$) scavenger and can convert highly reactive $O_2^{\cdot-}$ to H_2O_2. POD is responsible for degradation of lipid peroxides. CAT is a H_2O_2 decomposing enzyme; it has a capacity to rapidly degrade H_2O_2 into H_2O and O_2 without consuming cellular reducing equivalents. The reduction in these antioxidant enzymes at high As concentration might be due to the sever stress of oxidative damage to antioxidant enzymes[34]. However, nanoscale silica sol foliar application increased activities of these antioxidant enzymes and consequently decreased EL level and MDA concentration in root of rice under 50 or 100μM As stress. The activities of APX, the contents of AsA and GSH increased in root of rice seedlings exposed to 50 or 100 μM As and further increased by nanoscale silica sol foliar application, suggesting the important role of APX in the detoxification of H_2O_2 through the ascorbate-glutathione circle cycle and Si could increase plant tolerance to oxidative stress under As toxicity by increasing the efficiency of ascorbate-glutathione cycle. Similarly, Li et al. (2012) found that Si supply could increase plant tolerant to oxidative stress under Mn toxicity by increasing AsA and GSH concentration in cucumber under high Mn stress[18]. Therefore, our results indicated that nanoscale silica sol foliar application enhanced the activity of ROS scavengers might be one mechanism of nanoscale silica sol foliar application to alleviate As toxicity on rice.

It was well documented that exogenous Si supplication could enhance antioxidant defense system in plants exposed to heavy metal stress. Hu et al. (2013) reported that addition of Si extensively improved rice plant's tolerance to As through improving the membrane

permeability and enhancing the plant's antioxidant capacity[32]. Similarly, it was found by Song et al. (2009) that addition of Si increased antioxidant enzyme activities such as SOD, CAT and APX in leaves of *Brassci chinensis* L. consequently alleviated the lipid peroxidation as indicated by the decrease H_2O_2 leval and MDA concentrations[35]. Similar role of Si in mediation antioxidative regulation has been confirmed in the excess Mn-stressed cucumber[11] and rice[18], excess Zn-stressed rice[15], excess Cu-stressed *Arabidopsis thaliana* L.[16], Al-stressed maize[36], Pb-stress banana[13]. So, Si-enhanced antioxidant defense capacity is probably the main mechanism for Si-enhanced tolerance to heavy metal stress in plants.

In present research, we also found that nanoscale silica sol foliar application was able to reduce As uptake and accumulation in rice. The As concentration in rice grain was decreased by 28% and 40% for pot and field experiments, respectively (Table 1 and 2), and nanoscale silica sol foliar application also significantly decreased As concentration in root and shoot of rice grown in 50 and 100 μM As contained Kimura B nutrient solution (Fig. 2A). At the same time, the nanoscale Si foliar application changed the distribution of As in the shoots (Fig. 3). The percentages of As combined on the shoot cell walls were significantly increased by the Si foliar application in the present study. This was similar to our previous finds that the foliar application of Si could alleviate Cd toxicity and reduce Cd accumulation in rice grains by sequestrating Cd in the shoot cell walls[14]. Our current results were in agreement with the report of Ma and Yamaji (2006) who considered that Si deposited lignin in cell walls and induced heavy metal ions bound to cell walls, thus reduced translocation of metal from roots to shoots[37]. The present results suggested that As and Si co-localization in shoot cell walls may play an important role in alleviating As toxicity and restricting As translocation from the shoots to the grains in rice. Silicon is a structural component of plants' cell walls[38]. Most Si in the cell walls was bound to aliphatic polyols (simple sugar-like molecules) via the formation of Si-O-C bonds in the suspension cells[39]. Yan et al (2012) reported that cell wall played key roles in the accumulation and detoxification of As in *P. notoginseng* in

accordance with more than half of total subcellular As located in the cell walls of plants. The relative reason was amylase, protein or polysaccharide molecules in the cell wall contain large amounts of metal ion coordination groups[40]. The nanoscale silica sol foliar application increased As bond to shoot cell walls might be due to that Si and As co-bound to aliphatic polyols in cell wall, but this needs further investigation.

In present study, nanoscale silica sol foliar application also significantly decreased the As contents and As percentage in shoot of rice seedlings exposed to As(V) (in the form of Na_2HAsO_4) stress in the hydroponics experiments (Fig. 2B, and Fig. 2C), indicating that nanoscale silica sol foliar application inhibited As translocation from root to shoot. Silicic acid competing with As(III) for the same Si transporters during uptake and xylem unloading is the main mechanism of external Si-mediated reduction in As uptake and accumulation in rice. As(V), an analogue of phosphate, enters the root cells via phosphate transporters, and then gets reduced to As(III) inside cells for subsequent extrusion to the xylem and accumulation in shoots and grain of rice through Si efflux transporter Lsi2. The gene expression of *Lis1* and *Lis2* can be inhabited by external Si supply[9, 10, 37]. Silica sol foliar application may inhibit the expression of *Lsi1* and *Lsi2* in the roots, and then reduce As uptake and translocation from root to shoot and grain. But the expression of *Lsi1* and *Lsi2* in root of rice supply with silica sol on leaves in present experiment needs further investigation.

Although previous reports suggest that root-application of Si could reduce heavy metal uptake or accumulation in the seedlings of rice11 and maize[36], only few studies investigated the effects of Si on the accumulation of As in the grains of rice. Alexander et al. reported that with 10 g Si per kg soil application, the grain yield of rice grown on three As contaminated soils increased on average by 17% and concentration of As in brown rice decreased by 23%[19]. Li et al. (2009) reported that Si at the dosage of 20 g kg^{-1} significantly decreased grain As contents by 16%, and increased the grain yield by 13%[33]. In the present study, Si foliar applications led to an increase in rice production by 29.6% or 22%, and a decrease in grain

As content by 28.0% or 22% in the pot or field experiments respectively. In addition, the Si foliar application was more economical than root-application. The dosage of silica at 18 mg kg^{-1} in the present study was less than 2% of that used by Fleck et al. (2013)[19] and Li et al. (2009)[33], respectively. The proposed nanoscale silica sol foliar application method may provide an effective option to reduce Cd accumulation in rice grains.

5. Conclusions

The foliar application of nanoscale silica sol provided an effective alternative to reduce As accumulation in rice grains grown in As-contaminated soils. With the foliar application of nanoscale silica sol, the percentage of As combined on cell walls in shoot of rice grown in 50 and 100 μM As contained Kimura B nutrient solutions increased significantly. It was probably attribute to the foliar application of nanoscale silica sols to alleviate the toxicity and accumulation of As in grains of rice. In addition, the nanoscale silica sol foliar application significantly increased the enzymatic and non-enzymatic antioxidants and consequently alleviated the lipid peroxidation caused by As stress to rice plants. Therefore, silica sol foliar application enhanced Antioxidant defense capacity was also involved in reduction of As toxicity to rice. Future work should be concentrated on the speciation of arsenic in the shoots and grains of rice, and on gene expression related to arsenic uptake and transformation in response to foliar applications of silica sol.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Nos. 31270546, 41301243), the China Postdoctoral Science Foundation (No. 2012M520074), the “863” program (No. 2013AA06A209) and the Natural Science Foundation of Guangdong Province, China (No. S2012010010132).

References

1. A.A. Duker, E.J.M. Carranza and M. Hale, *Environ. Int.,* 2005, **31**, 631-641.

2. Y.G. Zhu, P.N. Williams and A.A. Meharg, *Environ. Poll.,* 2008, **154**,169-171.

3. X.Y. Liao, T. B. Chen, H. Xie and Y.R. Liu, *Environ. Int.*, 2005, **31**, 91–798.

4. R.Q. Huang, S.F. Gao, W.L.Wang, S. Staunton and G. Wang, *Sci. Total Environ.*, 2006, **368,** 531-541.

5. C.P. Liu, C.L. Luo, Y. Gao, F.B. Li, L.W. Lin, C.A. Wu and X.D. Li, *Environ. Poll.*, 2010, **158**, 820-826.

6. WHO, Arsenic and Arsenic Compounds. In: Environmental Health Criteria 224. 2nd ed. World Health Organization, Geneva. 2001.

7. Y. Takahashi, R. Minamikawa, K.H. Hattori, K. Kurishima, N. Kihou and K. Yuita, *Environ. Sci. Technol.*, 2004, **38**, 1038-1044.

8. F.J. Zhao, S.P. McGrath and A.A. Meharg, *Annu. Rev. Plant Biol.*, 2010, **61**, 535-559.

9. J.F. Ma, N. Yamaji, N. Mitani, X.Y. Xu, Y.H. Su, SP. McGrath and F.J. Zhao, *Proc. Nat. Acad. Sci. USA.*, 2008, **105**, 9931-9935. 10. J.F. Ma and N. Yamaji, *Cell Mol. Life Sci.*, 2008, **6**, 3049-3057.

11. X.H. Shi, C.H. Zhang, H. Wang and F.S. Zhang, *Plant Soil*, 2005, **272**, 53-60.

12. N.K. Savant, G.H. Snyder and L.E. Datnoff, *Adv. Agron.,* 1997, **58**, 151-199.

13. L.B. Li, C. Zheng, Y.Q. Fu, D.M. Wu, X.J. Yang and H. Shen, *Biol. Trace Elem. Res.*, 2012, **145**, 101-108.

14. C.P. Liu, F.B. Li, C.L. Luo, X.M. Liu, S.H. Wang, T.X. Liu and X.D. Li, *J. Hazard Materi.,* 2009, **161**, 1466-1472.

15. A.L. Song, Li P., Z.J. Li, F.L. Fan, M. Nikolic and Y.C. Liang, *Plant Soil*, 2011, **344**, 319-333.

16. S. Khandekar and S. Leisner, *J. Plant. Physiol.,* 2011, **168**, 699-705.

17. K. Iwasaki, P. Maier, M. Fecht and W.J. Horst, *Plant Soil,* 2002, **238**, 281-288.

18. P. Li, A.L. Song, Z.J. Li, F.L. Fan and Y.C. Liang, *Plant Soil,* 2012, **354**, 407-419.

19. A.T. Fleck, J. Mattusch and M.K. Schenk, *J. Plant Nutr. Soil Sci.,* 2013, **176**, 785-794.

20. L.R. Drees, L.P. Wilding, N.E. Smeck and A.L Sankayi. In: Minerals in soil environments, ed. J.B. Dixon and S.B. Weed, Soil Science Society of America, 1989, pp. 913-974.

21. C.M. Lu, C.Y. Zhang, J.Q. Wen, G.R. Wu and M.X. Tao, *Soybean Sci.,* 2002, **21**, 168-172.

22. F.A. Rodrigues, D.J. McNally, L.E. Datnoff, J.B.J. Ones, C. Labbé, N.Benhamou, J.G.Menzies and R.R. Bélanger, *Phytopatho*., 2004. **94**, 177-183.

23. J.J. Hart, J.M. DiTomaso, D.L. Linscott and L.V. Kochian, *Pestic. Biochem. Physiol.* 1992, **43**, 212-222.

24. R.S. Dhindsa and W. Matowe, *J. Exp. Bot.,* 1981, **32**, 79-91.

25. S. Lutts, J.M. Kinet and J. Bouharmont, *Ann. Bot.*, *1996*, **78**, 389-398.

26. C.N. Giannopolitis and S.K. Ries, *Plant physiol.*, 1977, **59**, 309-314.

27. I. Cakmak, D. Strboe and H. Marschner, *J. Exp. Bot.,* 1993, **44,** 127-132.

28. I. Cakmak and H. Marschner, *Plant Physiol.,* 1992, **98**, 1222-1227.

29. Y. Nakano, K. Asada, *Plant Cell Physiol.,* 1981, **22**, 867-880.

30. M.M. Bradford, *Anal. Biochem.,* 1976, **72**, 248 – 254.

31. G.L. Ellman, *Arch. Biochem. Biophys.,* 1959, **82**, 70-77.

32. H.C. Hu, J.T. Zhang, H. Wang, R.C. Li, F.S. Pan, J. Wu, Y. Feng, Y.Q. Ying and Q.P. Liu, *Environ. Sci. Pollut. Res.*, 2013, **20**, 8579-8589.

33. R.Y. Li, J.L. Stroud, J.F. Ma, S.P. Mcgrath and F.J. Zhao, *Environ. Sci. Technol.*, 2009, **43**, 3778-3783.

34. M. Shri, S. Kumar, D. Chakrabarty, P.K. Trivedi, S. Mallick, P. Misra, D. Shukla, S. Mishra, S. Srivastava, R.D. Tripathi and R. Tuli, *Ecotoxicol. Environ. Saf.*, 2009, **72**, 1102-10.

35. A.L. Song, Z.J. Li, J. Zhang, G. Xue, F.L. Fan and Y.C. Liang, *J. Hazard Mater.*, 2009, **172**, 74-83.

36. P.S. Kidd, M. Lluugan, C. Poschhenrieder, B. Gunsĕ and J. Barceló, *J. Exp. Bot.,* 2001, **52**, 1339-1352.

37. J.F. Ma and N. Yamaji, *Trends Plant Sci.*, 2006, **11**, 392–397.

38. J. Liu, J. Ma, C.W. He, X.L. Li, W.J. Zhang, F.S. Xu, Y.J. Lin and L.J. Wang, *New Phytol.*, 2013, **200**, 691-699.

39. C.W. He, L.J. Wang, J. Liu, X. Liu, X.L. Li, J. Ma, Y.J. Lin and F.S. Xu, *New Phytol.*, 2013, **200**, 700-709.

40. X.L. Yan, L.Y. Lin, X.Y. Liao and W.B. Zhang, *Chemosphere,* 2012, **87**, 31–36.

Tables:

Table 1 The effects of silica sol foliar applications on characteristics of rice production in the field experiments

Treatment	Available tillering number	Thousand-grain weight (g)	Yield (Kg ha^{-1})	As content in Grains (mg Kg^{-1})
Control	12.6 ± 3.0 b	22.3 ± 0.1 b	5178.2 ± 709.5 b	0.76 ± 0.02 a
Silica sol	15.0 ± 2.3 a	24.1 ± 0.2 a	6319.5 ± 769.5 a	0.55 ± 0.04 b

Data represent as means ± S.D. (n=4). Different letters within the same column are significantly different at $P< 0.05$ according to Duncan's New Multiple Range Test.

Table 2 The effects of silica sol foliar application on the yield (g pot^{-1}) and As content (mg kg^{-1}) in grains and shoots of rice grown in contaminated soil in the pot experiments

Treatment	Yield (g pot^{-1})		As content (mg kg^{-1})	
	Grains	Stalks	Grains	Stalks
Control	25.3 ± 1.2 b	43.6 ± 1.6 b	0.78 ± 0.02 a	41.8 ± 1.73 a
Silica sol	32.8 ± 0.9 a	59.7 ± 1.7 a	0.47 ± 0.04 b	19.0 ± 1.69 b

Data represent as means ± S.D. (n = 3). Different letters within the same column are significantly different at $P < 0.05$ according to Duncan's New Multiple Range Test.

Table 3 The effects of silica sol foliar application on the alleviation of oxidative damage, the activity of antioxidant enzymes, ASA and GSH contents in rice roots as induced by As toxicity in hydroponics experiments

Treatment	Oxidative damage		Enzymatic antioxidants				Non-enzymatic metabolites	
	MDA (nmol g^{-1} FW)	ELR (%)	SOD (U mg^{-1} protein)	POD (A470 min^{-1} mg^{-1} protein)	CAT (A240 $min^{-1}mg^{-1}$ protein)	APX (A290 min^{-1} mg^{-1} protein)	ASA (μmol g^{-1} FW)	GSH (μmol g^{-1} FW)
0As-Si	10.46 ± 1.93 c	14.80 ± 1.84 d	85.15 ± 0.83 ab	92.96 ± 10.25 ab	6.31 ± 0.33 a	2.61 ± 0.15 c	0.45 ± 0.06 b	7.01 ± 0.72 b
0As+Si	10.27 ± 2.11 c	11.63 ± 1.53 d	87.55 ± 4.00 a	104.96 ± 14.77 a	6.38 ± 0.71 a	2.75 ± 0.15 bc	0.60 ± 0.04 a	8.03 ± 0.66 ab
50As-Si	15.65 ± 1.10 b	29.82 ± 2.47 ab	68.70 ± 1.78 c	62.25 ± 4.17 cd	4.83 ± 0.05 b	2.97 ± 0.16 b	0.57 ± 0.03 a	8.41 ± 0.23 ab
50As+Si	13.14 ± 0.62 bc	19.44 ± 6.66 cd	86.00 ± 5.51 ab	86.63 ± 6.60 b	4.93 ± 0.37 b	3.46 ± 0.09 a	0.61 ± 0.05 a	9.58 ± 0.72 a
100As-Si	19.49 ± 2.24 a	36.58 ± 5.95 a	52.37 ± 1.40 d	47.45 ± 5.14 d	4.15 ± 0.61 b	2.79 ± 0.16 bc	0.59 ± 0.02 a	8.82 ± 0.60 a
100As+Si	15.04 ± 1.89 b	24.21 ± 0.56 bc	80.63 ± 3.91 b	67.31 ± 3.99 c	4.90 ± 0.17 b	3.26 ± 0.03 a	0.66 ± 0.02 a	9.06 ± 1.54 ab

Data represent as means ± S.D. (n = 3). Different letters within the same column are significantly different at $P < 0.05$ according to Duncan's New Multiple Range Test.

Figure captions:

Fig. 1 The effects of silica sol foliar applications on the biomass of rice seedlings grown in 50 or 100 μM Na_2HAsO_4 contained solution in the hydroponics experiments. Bars represent S.D. of three replicates, the different letter above column indicates a significant difference at $P<0.05$ according to Duncan's New Multiple Range Test.

Fig. 2 The effects of silica sol foliar applications on the As concentrations (A), As contents (B) in the shoots and roots, and the As percentages in shoot (C) of rice seedlings grown in 50 or 100 μM Na_2HAsO_4 contained solution in the hydroponics experiment. Bars represent S.D. of three replicates, the different letter above column indicates a significant difference at $P<0.05$ according to Duncan's New Multiple Range Test.

Fig. 3 The effects of silica sol foliar applications on the percentage of As combined on the cell walls of rice seedlings grown in 50 or 100 μM Na_2HAsO_4 contained solution in the hydroponics experiment. Bars represent S.D. of three replicates, the different letter above column indicates a significant difference at $P<0.05$ according to Duncan's New Multiple Range Test.

Fig. 1

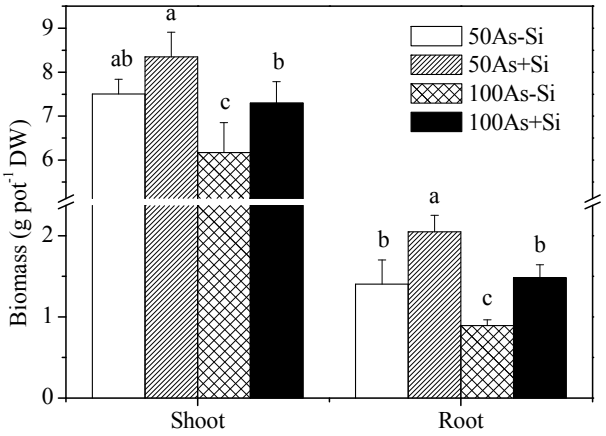
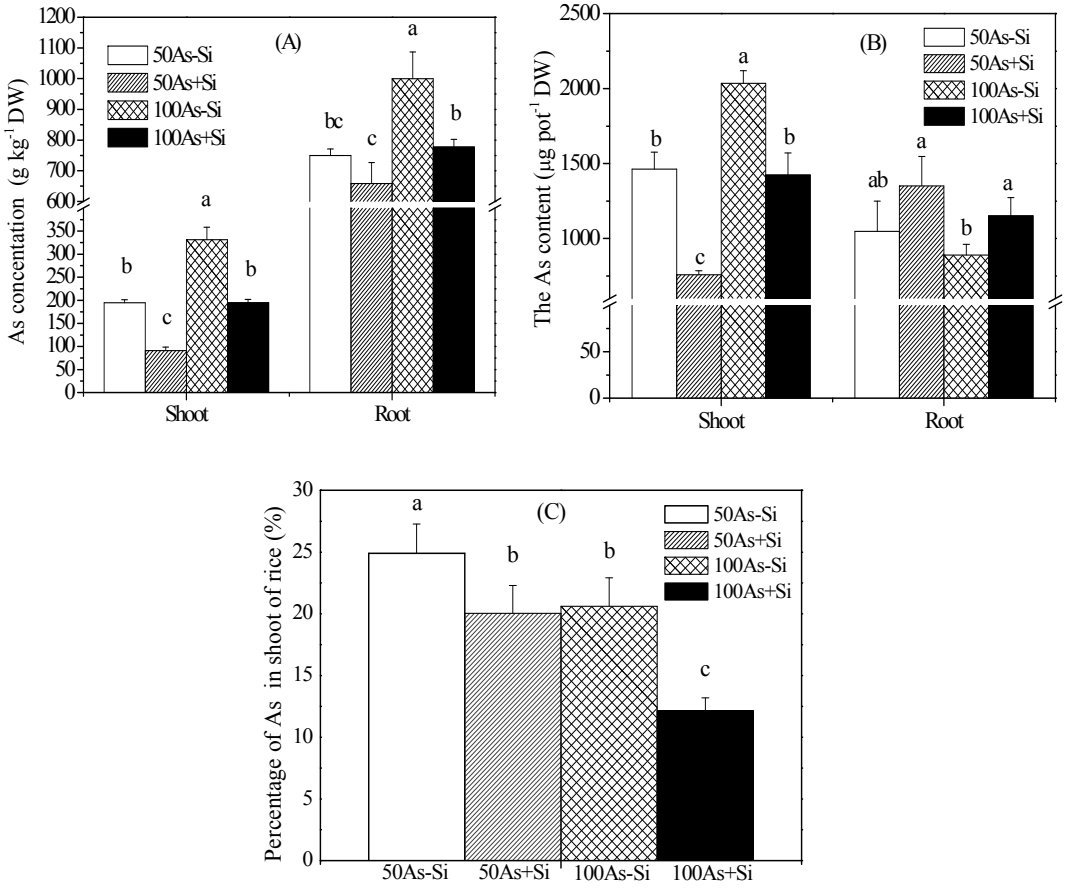
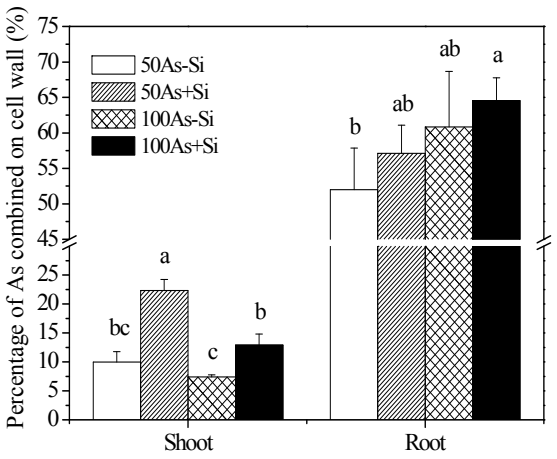


Fig. 2



1 Fig. 3

2



3