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Effect of selenium on uptake and translocation of arsenic in rice seedlings (*Oryza sativa* L.)

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ABSTRACT

Arsenic (As) disrupts the biological functions of plants by inhibiting their developmental process. Selenium (Se) is a beneficial plant micronutrient when it is administered at the optimal doses. The present study investigated the possible mediatory role of selenite (Se(IV)) or selenate (Se(VI)) in arsenite (As(III)) or arsenate (As(V)) uptake by rice seedlings (*Oryza sativa* L.). Simultaneous exposure of rice seedlings to As(III) (5 μM) and Se(IV) (5 μM) increased the root As content after ≥30 h. The maximum increase in root As level (42.2%) was measured after 150 h. At the same time, the shoot As level decreased by 46.4% as the root-to-shoot As translocation rate declined. In contrast, Se(VI) supplementation caused the As content to decrease by 48.8% and 16.1% after 150 h in the roots and shoots, respectively. Nevertheless, when the As exposure duration was < 48 h, no significant differences in the shoot As levels were detected between treatments. The addition of Se(IV) to the As(V) solution applied to the rice seedlings did not significantly affect root As content but strongly decreased shoot As levels. Se (VI) supplementation had the opposite effect. Lower Se(IV) concentrations slightly increased root As content in seedlings treated with As(III) but strongly decreased shoot As content and the root-to-shoot translocation rate. At lower concentrations, Se(VI) addition significantly decreased both root and shoot As content after 7 d. On the other hand, the root-to-shoot translocation rate did not significantly decrease relative to that observed with the As(III) treatment alone. Our results indicate that Se(IV) could effectively mitigate As translocation from roots to shoots in rice.

1. Introduction

Arsenic (As) is an environmental contaminant and is highly toxic to both animals and plants. Over the last decade, As pollution has become a serious problem in West Bengal as a result of extensive groundwater consumption for irrigation purposes. The As level in the groundwater there has exceeded the maximum permissible limit of 10 μg L^{-1} (Ghosh et al., 2013). Arsenic exists in different forms like arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA). The major forms, however, are the inorganic species (Tripathi et al., 2012; Zheng et al., 2013). The main sources of environmental As are mining (such as colliery spoil, coal waste, tailings, etc.), coal ash, dust, offgas, pesticides, and sewage sludge (Meunier et al., 2011). The accumulation of As in the soil impedes plant growth and development and poses human health risks when it enters the food chain. Terrestrial plants can accumulate high concentrations of inorganic As from soils and transfer it to the aerial shoots (Zhang et al., 2002). The most important factors controlling soil As mobility are pH, sorption, competition or synergy of uptake among mineral elements, soil temperature, the presence and concentrations of organic- and inorganic ligands, root exudates, and nutrient levels (Wang et al., 2002). Studies have shown that the presence of selenium (Se) hinders plant As absorption (Yang et al., 1997).

Selenium (Se) is widely distributed in the Earth's crust. Most of it is associated with sulfide minerals. There is no evidence that plants require Se, but this element is essential for humans and animals (Fordyce, 2013). Se enhances antioxidant capacity, scavenges free radicals and reactive oxygen species (ROS), inhibits lipid peroxidation, delays senescence, and promotes plant growth (Lin et al., 2012; Pandey and Gupta, 2015). It is believed that Se also detoxifies As (Malik et al., 2012) and cadmium (Cd) in plants (Saidi et al., 2014). Han et al. (2013) found that low doses of Se (≤4.4 mg kg^{-1}) enhanced the growth of flue-cured tobacco.

Rice is a major food crop consumed by about three billion people worldwide, especially in Asian countries. In many regions, however, rice is also a major dietary source of As (Li et al., 2011). Rice more

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efficiently absorbs and translocate As than either wheat or barley (Williams et al., 2007; Su et al., 2010).

As and Se usually pair in biogeochemical cycles (Couture et al., 2012). The optimal Se dosage in plants has not yet been established. It is believed that Se is primarily beneficial to plants capable of accumulating it in high concentrations (Terry et al., 2000). At low doses, Se stimulates plant growth by increasing dry matter yield, especially in the absence of sulfur (Hartikainen, 2005). Lower levels of Se also counteract the adverse effects of many types of environmental stress including heavy metals. At excessively high doses, however, Se causes oxidative injury to plant tissues (Pezzarossa et al., 2012).

Se and As are situated close to each other in the periodic table and play similar roles in many metabolic functions of prokaryotes plants (Ghosh et al., 2013). In paddy soil, As and Se occur mainly in inorganic form. Trace amounts of organic forms exist there as well due to microbial biotransformation (Goh and Lim, 2004). The application of selenate reverses the toxic effects of arsenate and supports normal growth in wheat seedlings (Ghosh et al., 2013). As(III) usually predominates in anaerobic environments such as flooded paddy soil whereas As(V) is the main species in aerobic soil (Abedin and Meharg, 2002). Competition between As(III) and Se(IV) uptake may be mediated by silicon transporters (Zhao et al., 2010) whereas As(V) and phosphate share the same uptake system as that of Se(IV). As(V) may inhibit xylem Se(VI) transport (Li et al., 2008). Se(IV) uptake is energy-dependent and mediated by the symport of H^+ and Se(IV) (Zhang et al., 2013). To date, however, there are very few reports of systematic investigations into the interactions between inorganic As and Se as they affect plant uptake and translocation (Hu et al., 2014). These interactions could influence plant As accumulation. In rice, As binds to the thiol complex in sulfur-containing amino acids like cysteine and methionine (Lombi et al., 2009).

In the present study, a hydroponic system was used to investigate the potential roles of Se in the uptake, translocation, and distribution of As in rice plants. An objective of this study was to develop strategies for the reduction of As toxicity and the maintenance of sustainable crop production.

2. Materials and methods

2.1. Rice culture conditions

Rice (*Oryza sativa* L., Fengyuanyou 299) seeds were surface-sterilized by subjecting them to 30% v/v hydrogen peroxide (H_2O_2) for 15 min. They were then rinsed with distilled water and soaked overnight in a saturated $CaSO_4$ solution at 25 ± 2 °C in the dark. The seeds were germinated at 25 °C on pre-sterilized floating plastic sheet nets moistened with deionized water. After 7 d, seedlings more or less equal and uniform in size were selected and transferred to 2.5-L plastic pots (four plants per pot) containing half-strength Kimura solution. They remained there for 35 d. The composition of the nutrient solution was (in mM): KNO_3, 0.091; $Ca(NO_3)_2{\cdot}4H_2O$, 0.183; $MgSO_4{\cdot}7H_2O$, 0.274; KH_2PO_4, 0.1; $(NH_4)_2SO_4$, 0.183; $MnSO_4{\cdot}H_2O$, 1×10^{-3}; H_3BO_3, 3×10^{-3}; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1×10^{-3}; $ZnSO_4{\cdot}7H_2O$, 1×10^{-3}; $CuSO_4{\cdot}5H_2O$, 2×10^{-4}; and Fe(III)-EDTA, 6×10^{-2}. The pH was adjusted to 5.5 using KOH or HCl. Each treatment was carried out in triplicate. The nutrient solution was renewed twice per week (i.e., after every 4 d). Plants were grown in a greenhouse at 25 ± 4 °C/20 ± 2 °C day/night temperatures and 60–70% relative humidity (RH), under a 14-h photoperiod and 240–350 μmol·$(m^2{\cdot}s)^{-1}$ light intensity.

2.2. As and Se treatment

In this report, arsenite ($NaAsO_2$), arsenate (Na_2HAsO_4), selenite (Na_2SeO_3), and selenate (Na_2SeO_4) are abbreviated as As(III), As(V), Se(IV), and Se(VI), respectively. The objective of the first experiment was to investigate the effect of selenite or selenate on arsenite and arsenate uptake during different stages of seedling cultivation. At 42 d, uniform seedlings were transferred to pots (two plants per pot) each containing 2.5 L nutrient solution (pH 5.5) to which Se (5 μM) or As (5 μM) was added (Wan et al., 2016). Six seedling groups were replicated in three pots. Each was treated with one of the following combinations: As(III), As(V), As(III) + Se(IV), As(III) + Se(VI), As(V) + Se(IV), or As(V) + Se (VI). All other nutrients present were the same as those found in normal nutrient solutions. The plants were harvested 2 h, 6 h, 30 h, 48 h, 72 h, 100 h, and 150 h after treatment. For this experiment, a total of 63 pots were used.

The purpose of the second experiment was to determine arsenite uptake in rice seedlings exposed to various Se levels. After 42 d, uniform seedlings were transferred to pots (two plants per pot) containing 2.5 L nutrient solution (pH 5.5) to which 5 μM As(III) and one of three different selenite or selenate concentrations (1 μM, 3 μM, or 5 μM) were added. All other nutrients present were the same as those found in conventional nutrient solutions. The plants were harvested at 2 d and 7 d after treatment, and each combination was replicated in three pots.

2.3. Sample preparation and chemical analysis of rice plants

Plants were harvested after their predetermined As exposure times and rinsed with deionized water. Their roots were immersed for 15 min in an ice-cold desorption solution containing 0.5 mM $Ca(NO_3)_2$, 5 mM MES (pH 5.5), and 1 mM K_2HPO_4 (Hu et al., 2014) to remove any As adhering to the root surfaces. The roots and shoots were separated, dried, weighed, and powdered. About 0.25 g each of the root and shoot powders were digested in 8 mL nitric acid (HNO_3). Digestion tubes were left overnight at room temperature. The next day, the samples were extracted in a microwave oven (MARS5, CEM Corp., Matthews, NC, USA). After digestion, the supernatants were cooled, diluted to 50 mL with deionized water, and passed through a 0.45 μm filter before being analyzed by an atomic fluorescence spectrometer (AFS-920, Beijing Jitian Instruments Co., Ltd., Beijing, China). The standard reference material GBW10049 (GSB-27) was included in the digestion process to verify the accuracy and precision of the extraction procedure and sample analysis. The range of recovery of the certified reference material was 85–110%.

2.4. Data analysis

The As contents ($C_{root-As}$, $C_{shoot-As}$) in the roots and shoots were calculated on a dry weight basis. The total As (T_{As}), the proportion of As distributed to the roots and shoots (Root-As %, Shoot-As %) and the transfer factor (TF) were calculated as follows:

$$T_{Root-As}C_{Root-As} \times Root_{Drybiomass} \quad (1)$$

$$T_{Shoot-As}C_{Shoot-As} \times Shoot_{Drybiomass} \quad (2)$$

$$T_{As}T_{Root-As} + T_{Shoot-As} \quad (3)$$

$$As\ uptake\ T_{As}/Root_{Drybiomass} \quad (4)$$

$$Root\text{–}As\,(T_{Root-As}/T_{As}) \times 100 \quad (5)$$

$$Shoot\text{–}As\,(T_{shoot-As}/T_{As}) \times 100 \quad (6)$$

$$TFC_{shoot-As}/C_{Root-As} \quad (7)$$

2.5. Statistical analysis

The statistical analysis was run with SPSS v. 20.0 for Windows and Microsoft Excel 2010. One-way ANOVA and Tukey's test were performed to compare the means among different treatments and to identify significant effects at the acceptance level of $P < 0.05$.