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Selenite modulates the level of phenolics and nutrient element to alleviate the toxicity of arsenite in rice (*Oryza sativa* L.)

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ABSTRACT

Arsenic (As) contamination of paddy rice is a serious threat all over the world particularly in South East Asia. Selenium (Se) plays important role in protection of plants against various abiotic stresses including heavy metals. Moreover, arsenite (AsIII) and selenite (SeIV) can be biologically antagonistic due to similar electronic configuration and sharing the common transporter for their uptake in plant. In the present study, the response of oxidative stress, phenolic compounds and nutrient elements was analyzed to investigate Se mediated As tolerance in rice seedlings during AsIII and SeIV exposure in hydroponics. Selenite (25 μM) significantly decreased As accumulation in plant than As (25 μM) alone treated plants. Level of oxidative stress related parameters *viz.*, reactive oxygen species (ROS), lipid peroxidation, electrical conductivity, nitric oxide and pro-oxidant enzyme (NADPH oxidase), were in the order of As > As + Se > control > Se. Selenium ameliorated As phytotoxicity by increased level of phenolic compounds particularly gallic acid, protocatechuic acid, ferulic acid and rutin and thiol metabolism related enzymes *viz.*, serine acetyl transferase (SAT) and cysteine synthase (CS). Selenium supplementation enhanced the uptake of nutrient elements *viz.*, Fe, Mn, Co, Cu, Zn, Mo, and improved plant growth. The results concluded that Se addition in As contaminated environment might be an important strategy to reduce As uptake and associated phytotoxicity in rice plant by modulation of phenolic compounds and increased uptake of nutrient elements.

1. Introduction

Arsenic (As) is an environmental toxin and class I human carcinogen. Arsenic contamination of groundwater particularly in South and South-East Asia has now become a major environmental concern due to its natural geogenic origin (Chakraborti et al., 2013). Approximately 150 million people are at the risk of As related health issues in this region (Ravenscroft et al., 2009). Rice is the dietary staple for half of the world's population and is specifically a problem for the entry of As into the food chain due to the anaerobic growing conditions and specific plant physiological characteristics.

Arsenic occurs in both organic and inorganic forms in the environment. Inorganic forms of As *viz.*, arsenate (AsV) and arsenite (AsIII) predominantly occur in water and soil and finds its way into plants through transporters of essential elements (Tripathi et al., 2007). Arsenate is taken up through phosphate transporter, while AsIII enters *via* the NIP (Nodulin 26-type intrinsic protein) transporter of aquaporins family in rice (Zhao et al., 2009). Arsenic exerts toxicity to plants in various ways and hamper plants growth. Arsenate may replace P in DNA and phosphorylation reactions and interferes with the energy and phosphate metabolism, while AsIII binds to the sulphydryl groups of peptides and proteins and hamper their activity (Finnegan and Chen, 2012; Mishra et al., 2016). Increased production of reactive oxygen species (ROS) and oxidative damage to plants are apparent symptoms of As toxicity (Hartley-Whitaker et al., 2001).

Selenium (Se) is an essential nutrient to human beings. It is also considered beneficial to plants as an antioxidant at low dosages, but a pro-oxidant at high dosages (Hartikainen et al., 2000; Mora et al., 2008). Selenium acts as a protectant against various abiotic stresses in plants. Selenium is involved in the detoxification of heavy metals by alleviating the oxidative stress and antagonizing the uptake of heavy metals (Zhu et al., 2009). Due to the similar electronic configuration, SeIV and AsIII can be antagonistic to each other. Further, transporters belonging to the NIP subfamily of aquaporins (*OsNIP* 2;1) are reported to facilitate the uptake of both AsIII and SeIV in rice (Ma et al., 2008; Zhao et al., 2010). Besides, SeIV uptake is also reported through the

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OsPT-2 transporter (a rice phosphate transporter) in rice (Zhang et al., 2014).

Plants respond against As induced oxidative stress through synthesis of enzymatic and non-enzymatic antioxidants and increased sulfur assimilation (Dwivedi et al., 2010; Tripathi et al., 2012, 2013). Phenolic compounds constitute one of the non-enzymatic antioxidants and can scavenge harmful ROS in plants. In higher plants, these compounds are synthesized as secondary metabolites, which are also implicated in the plant defense-related machinery (Boudet, 2007; Gill and Tuteja, 2010). Under heavy metal stress, the antioxidant activity of phenolics increases due to its ability to chelate transition metal ion, to inhibit superoxide-driven Fenton reaction (Rice-Evans et al., 1997; Arora et al., 1998) and to stabilize membranes by decreasing membrane fluidity (Blokhina et al., 2003). Several studies have been done to investigate the role of antioxidative defense in plants including rice during As stress. However, to the best of our knowledge the effect on phenolic compound and their role in As mitigation during As/ As + Se exposure has not been studied in rice.

Rice is a good accumulator of both Se and As. In the recent past, a few studies have analyzed the effects of Se supplementation on As uptake and antioxidant responses (Malik et al., 2012) and found that rice varieties have antagonistic accumulation profile of Se and As during hydroponic as well as field conditions (Kumar et al., 2013, 2014; Tripathi et al., 2015). However, deeper understanding of Se mediated amelioration of As toxicity is still lacking. Our knowledge is limited on the systematic studies with respect to observations in responses of phenolic compounds, nutrient elements and reactive oxygen species (ROS) generation during Se and As interaction in rice. It was hypothesized that As toxicity amelioration through Se supplementation may involve sustained balance of phenolic compounds and nutrient elements. Therefore, the present study was designed to investigate the impact of Se on As tolerance in rice seedlings by analyzing growth, As and nutrient elements accumulation and phenolic compounds to further deepen our knowledge of protective role of Se against As toxicity.

2. Material and methods

2.1. Plant material and experimental conditions

Seeds of rice variety (Triguna), was procured from Rice Research Station Chinsurah, West Bengal, were disinfected in 0.1% $HgCl_2$ solution for 30 s followed by thorough washing with deionized water and soaking in Milli-Q for 24 h. The imbibed seeds were then transferred to Petri dishes (3–4 days) kept in culture room at 26 °C in dark for proper germination. Germinated seedlings having uniform length were transplanted in trays having fixed PVC cups (4 cm diameter and 5 cm high, ten plants per cup) and grown in modified Hewitt nutrient medium (Liu et al., 2004) under hydroponic conditions for 10 days (for acclimatization). Then, seedlings were exposed to As and Se for 15 days using sodium arsenite (AsIII, $NaAsO_2$) and sodium selenite (SeIV, Na_2SeO_3). The experiment was carried out in a controlled environment growth chamber with 14-h light period (260–350 μE $m^2 s^{-1}$) and temperatures of 28 °C day and 20 °C night with 70% relative humidity maintained by humidifier. All nutrient solutions were changed twice per week, and pH was adjusted to 5.5 using 0.1 M KOH or HCl. All experiments were conducted twice comprising different SeIV levels (0, 5, 10 and 25 μM) in combination with 25 μM AsIII. Root and shoot length were measured by metric scale. After the treatment, seedlings were harvested, washed with Milli-Q, blotted gently, freezed properly in liq.N_2 and stored at −80 °C for the comparative study of various physiological and biochemical parameters.

2.2. Arsenic and nutrient elements quantification and quality control

For estimation of total As and nutrient elements, dried plant samples (0.2 g) were powdered and digested in 3 mL HNO_3 at 120 °C for 6 h (Dwivedi et al., 2010). The level of As, Mn, Fe, Co, Cu, Zn, Mo and Se was quantified with the help of inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500 cx). The multielement standard solution (Agilent, Part # 8500-6940) was used for the calibration and quality assurance for each analytical batch. Known concentrations of spiked samples were prepared to check the As and Se recovery. Selenium and As were recovered about 93.5% (± 2.3; n =5) and 92.5% (± 3.1; n =5), respectively, while, rice flour NIST 1568a was also used as a reference material for total As and recovered about 95.3% (± 2.8; n =5). The detection limit of As and nutrient elements was 1 μg L^{-1}. Rhodium (Rh) and stannous (Sn) were used as internal calibration standards.

2.3. Histochemical detection of superoxide radicals and hydrogen peroxide

In vivo $O_2^{\bullet-}$ were detected by nitro blue tetrazolium (NBT) staining according to Unger et al. (2005).H_2O_2 accumulation was analyzed using 3,3′-diaminobenzidine (DAB) according to Thordal-Christensen et al. (1997). The stained roots and shoots were observed with MZ 16 Leica stereomicroscope (4X) and NBT stained roots were photographed.

2.4. Quantification of phenolic compounds by HPLC

2.4.1. Extraction for phenolic estimation

The powdered rice shoot and root (1g) was successively extracted three times with 50% methanol (10 mL) on orbital shaker for 2 h at room temperature. The pooled plant extract (30 mL) was centrifuged (8000 × *g*), concentrated to half of the volume (15 mL) on a rotary evaporator (Buchi, USA) under reduced pressure, and fractionated by ethyl acetate (15 mL) three times. All the fractions of ethyl acetate (45 mL) were pooled and again concentrated under reduced pressure on a rotary evaporator. All residues obtained were freeze dried (Freezone 4.5, Labconco, USA) under high vacuum (133×10^4 mBar) at −40 ± 2 °C. The residues were dissolved in 2 mL methanol before injecting in HPLC-PDA and Mass Spectrometry. Content of the phenolic compounds in shoots and roots were calculated in mg g^{-1} by comparison of peak areas of the samples with those of standards. A simple mobile phase was used as a control to see the blank peaks (Niranjan et al., 2011).

2.4.2. HPLC MS/MS analysis

Qualitative and quantitative analysis of phenolic compounds was performed by HPLC-PDA with a LC-10 (Shimadzu, Japan) system comprising of LC-10AT dual pump, an SPD-M20A PDA detector, and rheodyne injection valve furnished with a sample loop (20 μL). Molecules were separated on a RP-C18 column (Merck) of 250 mm × 4.6 (i.d.), 5 mm pore size, protected by guard column. A gradient of 0.5% (v/v) phosphoric acid in (component A) and methanol (component B) was used 25–50% B in 0–3 min, 50–80% B in 3–18 min, 80 to 25% B in 25 min, and 25% B in 30 min) with a flow rate of 0.8 mL/min. The solvents were filtered through 0.45-mm nylon filters (Millipore, USA) and deaerated in an ultrasonic bath before use and samples were also filtered before injection (20 μL). Shimadzu class VP series software was used for the integration of the data and quantification was carried out by comparison with standards.

The method was also validated for linearity, range, specificity, sensitivity, precision, limit of detection (*LOD*), limit of quantification (*LOQ*), and system suitability. Calibration plots of peaks were constructed after triplicate analysis of solutions at seven different concentrations ranging from 0.5 to 50 μg mL^{-1}. Peaks were identified by comparing the retention times and mass spectra of standards and samples. The phenolic compounds used as standards in the study were gallic, protocatechuic, chlorogenic, caffeic, ferulic acids, rutin, quercetin, and kaempferol. In plant extracts the amount of each standard was calculated by linear regression of peak areas in chromatograms within the linear range of the detector. The *LOD* of the method was defined as the amount in a sample for which the signal was three times the