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Selenate mitigates arsenite toxicity in rice (*Oryza sativa* L.) by reducing arsenic uptake and ameliorates amino acid content and thiol metabolism

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ABSTRACT

Arsenic (As) is a toxic element with the potential to cause health effects in humans. Besides rice is a source of both amino acids (AAs) and mineral nutrients, it is undesired source of As for billions of people consuming rice as the staple food. Selenium (Se) is an essential metalloid, which can regulate As toxicity by strengthening antioxidant potential. The present study was designed to investigate As^{III} stress mitigating effect of Se^{VI} in rice. The level of As, thiolic ligands and AAs was analyzed in rice seedlings after exposure to As^{III}/Se^{VI} alone and $As^{III}+Se^{VI}$ treatments. Selenate supplementation (As^{III} 25 μM+Se^{VI} 25 μM) decreased total As accumulation in both root and shoot (179 & 144%) as compared to As^{III} alone treatment. The $As^{III}+Se^{VI}$ treatment also induced the levels of non-protein thiols (NPTs), glutathione (GSH) and phytochelatins (PCs) as compared to As^{III} alone treatment and also modulated the activity of enzymes of thiol metabolism. The content of amino acids (AAs) was significantly altered with Se^{VI} supplementation. Importantly, essential amino acids (EAAs) were enhanced in $As^{III}+Se^{VI}$ treatment as compared to As^{III} alone treatment. In contrast, stress related non-essential amino acids (NEAAs) like GABA, Glu, Gly, Pro and Cys showed enhanced levels in As^{III} alone treatment. In conclusion, rice supplemented with Se^{VI} tolerated As toxicity with reduced As accumulation and increased the nutrition quality by increasing EAAs.

1. Introduction

Arsenic (As) is classified as class I carcinogen for humans. It is a non-essential and toxic element and is reported to interfere with various metabolic and physiological processes of plants (Meharg and Hartley-Whitaker, 2002; Kumar et al., 2015a). It exists in two main inorganic forms *viz.,* arsenate (As^{V}) and arsenite (As^{III}) in environment (Zhao et al., 2009). Arsenite is the predominant form under anaerobic conditions while As^{V} under aerobic conditions. Widespread As poisoning in South East Asia has become an issue of concern for public health and agricultural yield and produce quality. The West Bengal in India is one of most severely affected regions with reported As concentrations in water being up to 3200 μg L^{-1} (McCarty et al., 2011). Rice is main agrarian crop in South East Asia and is also an efficient accumulator of As among crop plants. Hence, rice serves as main entry route of dietary exposure of As for humans.

Selenium (Se) is a metalloid associated with the enhancement of antioxidant activity in plants, animals and humans (Rayman, 2002). Various studies have reported protective role of Se against various abiotic stresses (Hartikainen et al., 2000; Mallick et al., 2012; Kumar et al., 2013, 2014a). In recent studies, Se has been reported to impart tolerance to As in rice (Kumar et al., 2014a, 2015b). Therefore, Se application is a prospective strategy that can be applied at field scale to deal with As contamination. However, As stress is also known to alter the quality of rice grains in terms of amino acid (AA) levels (Dwivedi et al., 2010b, 2012; Kumar et al., 2014b). It is therefore desirable to understand the As-Se interaction in terms of quality parameters for growth.

Amino acids are the building blocks of proteins and precursors or activators of several phytohormones and growth substances. Amino acids also play vital role in plant stress tolerance by regulating intracellular pH and ion transport, modulating stomatal conductance and detoxification of reactive oxygen species (ROS) (Szabados and Savoure, 2010; Choi et al., 2011). Free amino acids

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accumulation serves as storage for nitrogen and carbon (Winter et al., 2015) and also helps of removal free radicals, and stabilization of macromolecules and organelles (Bohnert and Jensen, 1996; Bray et al., 2000; Szabados and Savoure, 2010). The role of histidine (His) and asparagine (Asp) plays a significant role in heavy metal induced stress (Kerkeb and Krämer, 2003). A study by Uroic et al. (2012) demonstrated significant impact of As^{V} on production and metabolite profile of xylem sap and down regulation of isoleucine (Iso), which was supposed to play important role in As stress.

In this backdrop, the present study was planned to assess the impact of Se^{VI} supplementation on responses of rice seedlings to As^{III} exposure. The study reveals significant positive impact of Se^{VI} for combating As^{III} stress by rice.

2. Materials and methods

2.1. Plant growth conditions and treatments

Seeds of rice (*Oryza sativa* L.) variety Sarju-52 were obtained from Rice Research Station, Chinsurah, West Bengal. Seeds were surface sterilized in 0.01% $HgCl_2$ solution for 30 s, followed by washing with deionized water and soaking in milli-Q for 24 h. The plants were grown in modified Hewitt medium (Liu et al., 2004) for 10 d before treatment and then exposed to different concentrations of As^{III} (0 and 25 μM) using sodium arsenite ($NaAsO_2$) supplemented with Se^{VI} (0, 10, 25 μM) using sodium selenate (Na_2SeO_4) for 7 days. The hydroponic culture and all experiments were conducted inside a controlled environment growth chamber under the following conditions: 16-hour light period with a light intensity of 350 μmol m^{-2} s^{-1}; 25/20 oC day/night temperatures; and 60% relative humidity. Plants were then harvested, washed with milli-Q, separated into roots and shoots, and used for the study of various parameters. All the samples were ground in liquid N_2 and stored at −80 °C till further use. Root and shoot lengths were measured by using a metric scale. Total fresh weight of plants was also noted.

2.2. Arsenic and selenium quantification and quality control

Estimation of total As and Se, 0.5 g oven dried tissue was taken and digested in 3 ml of HNO_3 as detailed in Dwivedi et al., (2010a). The As and Se were quantified with the help of Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7500 cx). The standard solution material of As and Se (Agilent, Part # 8500–6940) was used for the calibration and quality assurance for each analytical batch. Recovery of Se was found to be more than 93.5% as determined by spiking samples with a known amount of Se while, for As, rice flour NIST 1568a was used as a reference material with known spiked samples and recovery of total As were 95.3% (± 2.8; $n=5$) and 92.5% (± 3.1; $n=5$) respectively. The detection limit of As and Se was 1 μg L^{-1}.

2.3. Amino acid profiling

The pico tag method (Bidlingmeyer et al., 1984) was followed for the estimation of AAs on High Performance Liquid Chromatography (HPLC) system. 200 mg of homogenized rice plant sample were hydrolyzed in 10 ml 6 N HCl for an hour at 150 °C in an oven. Samples were then filtered for further analysis. 10 μl of samples and standard (2.5 μmole ml^{-1} in 0.1 N HCl) were dried in a vacuum oven at 55 °C for 30 min at 75 milli torr. This was redried twice by adding 20 μl of redrying solution (Ethanol: Triethylamine: Water, 2:1:2). Then samples were derivatizated by adding 20 μl of derivatization reagent (Ethanol: Triethylamine: Water: Phenyoisothiocynate, 7:1:1:1) and again vacuum dried. These samples were diluted to 1 ml with pico tag sample diluent, filtered (0.22 μm syringe filters). The separation was carried out at 40 °C using a Pico Tag amino acid C18 column (3.9 × 300 mm). For each sample, 20 μl of extract was injected and the column was eluted at 1 ml min^{-1}, with an optimized gradient established using solvents A (0.14 M sodium acetate, containing 0.05% triethylamine and 6% acetonitrile, pH 6.40) and B (60% acetonitrile in water). A step-by-step gradient was used with an increase of proportion of solvent B until it reached 46% during 10 min, followed by an increase up to 100% in 5 min, with a flux of 1 ml min^{-1}. The column was then cleared and optimized to 100% A for 8 min at 1 ml min^{-1}. The amino acids analyzed were Ile, Leu, Lys, Met, Phe, Thr, Val, Asp, Glu, Ser, Gly, His, Arg, Ala, Pro, Tyr, Trp, Cys, gamma-aminobutyric acid (GABA), β-Ala, Cyst and Hypro. Asn and Gln cannot be analyzed by this procedure, as these are heat labile. Chromatograms were integrated using Empower 2 HPLC software v 6.0. Amino acid content was expressed in mg kg^{-1} fw.

2.4. Assay of thiols and enzymes of thiolic metabolism

The estimation of non-protein thiol (NPT) and reduced and oxidised glutathione (GSH and GSSG, respectively) was done according to protocols given in Kumar et al., (2014a). The concentration of phytochelatins (PCs) was calculated as PCs = NPT-(GSH + GSSG) (Duan et al., 2011). The assay of serine acetyltransferase (SAT, EC 2.3.1.30) activity was performed following Blaszczyk et al. (2002). Reaction mixture contained 63 mM Tris–HCl (pH 7.6), 1.25 mM EDTA, 1.25 mM DTNB (5,5′-dithiobis-2-nitrobenzoic acid), 0.1 mM acetyl-CoA, 1 mM L-serine and suitable aliquot of extract. The rate of reaction was followed at 412 nm. A unit of enzyme is the amount of enzyme catalyzing the acetylation of 1 nmole of L-serine per 5 min. The assay of cysteine synthase (CS; EC 2.5.1.47), γ-glutamyl cysteine synthase (γ-ECS; EC 6.3.2.2), γ-glutamyltransferase (γ-GT; EC 2.3.2.2) and glutathione-S-transferase (GST; EC 2.5.1.18) was done by following the protocols of Saito et al. (1994); Seelig and Meister (1984); Orlowski and Meister, (1973) and Habig and Jacoby, (1981), respectively, as detailed previously (Mishra et al., 2008).

2.5. Statistical analysis

Analysis of variance (ANOVA), Duncan's multiple range test (DMRT) and correlation analysis were performed to determine the significant difference between treatments by using SPSS 17.0 software.

3. Results

3.1. Selenate supplementation improved growth of arsenite exposed rice seedlings

Growth parameters were measured in terms of root and shoot length and biomass. Initial experiments were carried out with two As^{III} concentrations (10 and 25 μM). However, no significant difference in growth was observed with 10 μM As^{III}. Hence, further experiments were performed with 25 μM As^{III} along with two doses of Se^{VI} (10 and 25 μM). A significant decrease in growth parameters was observed in As^{III} (25 μM) exposed rice plants after 7 d exposure (Fig. 1). Both the doses of Se^{VI} (10 and 25 μM) improved growth of plants, although higher dose of Se^{VI} (25 μM) supplementation showed better response. Supplementation of 25 μM Se^{VI} increased the root and shoot length by 59% and 45%, respectively, while root and shoot biomass were increased by 94% and 45%, respectively as compared to As^{III} alone exposed plants

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