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Effect of external iron and arsenic species on chelant-enhanced iron bioavailability and arsenic uptake in rice (*Oryza sativa* L.)

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ABSTRACT

This study was conducted to investigate the effect of external iron status and arsenic species on chelant-enhanced iron bioavailability and arsenic uptake. Rice seedlings (*Oryza sativa* L.) were used as model plant, and were grown in artificially contaminated sandy soils irrigated with Murashige and Skoog (MS) culture solution. Arsenate uptake in roots and shoots of rice seedlings were affected significantly ($p > 0.05$) while dimethylarsinic acid (DMAA) was not by the additional iron and chelating ligand treatments. Regardless of iron concentrations in the soil solution, HIDS increased arsenic uptake for roots more than EDTA and EDDS. Chelating ligands and arsenic species also influenced iron uptake in rice roots. Irrespective of arsenic species, HIDS was found to be more effective in the increase of iron bioavailability and uptake in rice roots compared to other chelants. There was a significant positive correlation ($r = 0.78$, $p < 0.05$) between arsenate and iron concentrations in the roots of rice seedlings grown with or without additional iron indicating that arsenate inhibit iron uptake. In contrast, there was no correlation between iron and DMAA uptake in roots. Poor correlation between iron and arsenic in shoots indicated that iron uptake in shoots was neither affected by additional iron nor by arsenic species. Compared to the control, chelating ligands increased iron uptake in shoots of rice seedlings significantly ($p < 0.05$). Regardless of additional iron and arsenic species, iron uptake in rice shoots did not differed among EDTA, EDDS, and HIDS treatments.

1. Introduction

Although iron is the most abundant nutrient for plants in the mineral solid phase of soils (average of 3.8%), its presence in soil solution is extremely low (Lucena, 2006). Iron forms insoluble ferric hydroxide complexes (Fe-plaque) in the rhizosphere soil at neutral or alkaline pH (Guerinot and Yi, 1994). The formation of Fe-plaque in the rhizosphere soils, however, causes iron deficiency and produces visible symptoms of iron-chlorosis in plants (Pestana et al., 2003). Rhizospheric microbes exude siderophores at the root–plaque interface which solubilize ferric hydroxide in the rhizosphere, render its bioavailability, and plants take up iron by its specific membrane receptors (Romheld and Marschner, 1986). Synthetic iron chelants have also been used to increase iron uptake and correct iron-chlorosis in plants (Hernandez-Apaolaza et al., 1995; Pestana et al., 2003; Alvarez-Fernandez et al., 2005; Lucena, 2006).

Arsenic is one of the widespread toxic environmental pollutants which has chronic and epidemic effects on humans through water and crop contamination reported in Bangladesh (Hossain, 2006) and West Bengal, India (Chowdhury et al., 2000). Arsenic-contaminated groundwater has been used extensively to irrigate paddy rice (*Oryza sativa* L.) in Bangladesh, particularly during the dry season with 75% of the total cropped area given over to rice cultivation (Meharg and Jardine, 2003). Background levels of arsenic in rice paddy soils range from 4 to 8 mg kg^{-1}, which can reach up to 83 mg kg^{-1} in areas where the crop land has been irrigated with arsenic-contaminated groundwater (Abedin et al., 2002). Arsenic-contamination in groundwater has also been reported in some other countries of South and South-East Asia, which is supposed to be a threat to sustainable agriculture in this region (Brammer and Ravenscroft, 2009). Increasing arsenic level in soil leads to elevated arsenic in rice, vegetables and other food crops (Meharg and Jardine, 2003; Williams et al., 2006). Being rice the staple food, elevated arsenic in rice would be a health hazard for the population in this region (Meharg, 2004). Remediation of contaminated soil is important to prevent arsenic deposition in food crops and its subsequent transfer into the humans through the food chains.

Phytoremediation, a plant based green technology, becomes a promising environmentally safe technology for the remediation of environmental pollutants. Solubility and bioavailability is an

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essential prerequisite for arsenic phytoremediation (Fitz and Wenzel, 2002), which may be reduced by adsorption to iron oxides (Pierce and Moore, 1982) and minerals (Goldberg, 2002) at alkaline pH. Chelant-enhanced phytoremediation of heavy metals has received much attention in the past (Luo et al., 2005; Meers et al., 2005; Evangelou et al., 2007; Hernández-Allica et al., 2007; Lestan et al., 2008). This technique aims to cleanse polluted soils by solubilizing the toxic metals, allowing them to be accumulated in plants that would subsequently remove them from the site.

Hydroxyiminodisuccinic acid (HIDS), a novel biodegradable chelating ligands, has been reported to be more effective in increasing iron bioavailability and is expected to be a good choice and alternative to less biodegradable and high persistent EDTA (Rahman et al., 2008a, 2009). The biodegradation rate of HIDS is about 22.4% within 48 h, and it forms complexes with various kinds of metals ions, especially Fe^{3+}, over a wide rage of pH. It also shows high stability in harsh conditions and high temperature (80 °C), and is highly soluble in aqueous alkaline solution (Rahman et al., 2009). We have been interested in HIDS because of high degradation rate and high stability constant with Fe^{3+} (pKaFe^{3+} = 12.5).

Rice plants take up small amounts of dimethylarsinic acid (DMAA) compared to that of inorganic species (As(V) and As(III)) (Odanaka et al., 1987; Rahman et al., 2008b). Although the effect of iron on As(V) uptake in rice has been studied (Liu et al., 2004a; Deng et al., 2010), its effect on DMAA uptake in rice has not. Previously, we investigated the iron bioavailability and arsenate uptake using hydroponic rice (Rahman et al., 2009). Since rice is a wetland plant, studies with soil culture would provide more useful information than the hydroponic experiment. Results of both soil and hydroponic studies would be helpful for the justification and understanding of the facts of the chelating ligands on iron bioavailability in rice. Therefore, the present study was designed to compare the EDTA, EDDS and HIDS as potential soil amendments for iron and arsenic bioavailability and uptake in rice (*Oryza sativa* L.).

2. Materials and methods

2.1. Seed sterilization

Rice seeds of BRRI dhan28 were collected from Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh. The seeds were surface-sterilized before using them in the experiment. For surface sterilization, about 100 g seeds were soaked in 200 mL of 1% methyl-1-butylcarbamoyl-2-benzimidazole carbonate solution for 10 min. Seeds were then washed by deionized (DI) water (using an E-pure system (Barnstead)) and kept in DI water at 20, 45 and 52 °C for 24 h, 2 min and 10 min, respectively.

2.2. Plant growth

Sterilized rice seeds were soaked in DI water for 48 h, and were germinated on pre-sterilized moistened filter paper placed in petri dishes. After 7 d. the germinated seeds produced enough roots and the shoot was about 2 cm. The seedlings were then transplanted into 50-mL polystyrene tubes containing 10 g soil. The composition of the soil was – SiO_2 (95.5%), Al_2O_3 (2.3%), Fe_2O_3 (0.2%), CaO (0.02%), MgO (0.08%). Particle size of the soil was 0.42–0.60 mm (24%) and 0.30–0.42 mm (60%). The experimental soil was irrigated with modified Murashige and Skoog (MS) nutrient solution (Murashige and Skoog, 1962) before transplantation. Phosphate was not included in modified MS nutrient solution to avoid its competition with arsenate for uptake transporter in rice roots, and iron concentration in the solution was 0.36 mM. Four germinated seeds were transplanted in each tube, and the seedlings were allowed to grow for 10 d. Water levels in the tubes were maintained to 1.5 cm above the soil by irrigating with modified nutrient solution every 2 d throughout the experiment. The growth of rice seedlings and subsequent steps of the experiments were performed in a plant growth chamber with conditions of 14:10 h light/dark schedule, 100–125 μE m^{-2} s^{-1} light intensity, and 22(±2) °C.

2.3. Chemical treatments

Treatments of arsenic, iron, and chelating ligands in the soil solution were applied with the MS solution. Stock solution of iron, As(V) and DMAA were prepared from $FeSO_4{\cdot}7H_2O$, $Na_2HAsO_4{\cdot}7H_2O$ and $(CH_3)_2AsO(OH)$, respectively.

Three treatments of iron, arsenic (As(V) or DMAA) and chelating ligands (EDTA, EDDS, or HIDS) were applied to the experimental soil with the modified MS solution as – (i) 2.5 mM chelating ligand and 0.36 mM additional iron (referred as Fe + EDTA, Fe + EDDS, and Fe + HIDS); (ii) 0.6 μM and 2.5 mM arsenic and chelating ligand, respectively, without additional iron (referred as As + EDTA, As + EDDS, and As + HIDS); and (iii) 0.6 μM arsenic, 2.5 mM chelating ligands, and 0.36 mM of additional iron (referred as As + Fe + EDTA, As + Fe + EDDS, and As + Fe + HIDS). One control was also maintained for each of the treatments, and the explanation of control for each treatment is given in the caption of respective figures. The soil solution pH was maintained at 6.5 using 0.1 M HCl or KOH. Replicated (three replications of each treatment) samples were collected after 10 d of the chemical treatments. Rice seedlings were uprooted by hand and washed by deionized water for several times to remove send attached to the roots.

2.4. Chelating ligands and other reagents

Stock solutions of EDTA, EDDS and HIDS were prepared by dissolving ethylenediamine-N,N,N′,N′-tetraacetic acid (Dojindo Molecular Technologies, Japan), ethylenediamine-N, N′-disuccinic acid (Chelest corporation, Japan), and tetrasodium 3-hydroxy-2,2′-iminodisuccinate (Nippon Syokubai, Japan), respectively. Other reagents were of analytical grade or better. All solutions were prepared with DI water.

2.5. CBE-extraction of Fe-plaques

At harvest Fe-plaques from root surfaces were extracted using citrate-bicarbonate-ethylenediaminetetraacetate (CBE)-technique, a modified method of dithionite-citrate-bicarbonate extraction by Taylor and Crowder (1983) to determine the real amount of iron and arsenic contents in rice tissues. The CBE solution was prepared from 0.03, 0.125 and 0.050 M of sodium citrate, sodium bicarbonate, and EDTA, respectively. Roots were treated with 5 mL of CBE solution for 60 min at room temperature. The roots were then rinsed with deionized water for three times, and the rinsed water was added to the CBE-extract to make a total of 10 mL.

2.6. Sample digestion and preparation for chemical analysis

The roots were rinsed by ID water, and blotted dry with tissue paper. The roots were then excised at the basal node and separated from shoots. Roots and shoots were then oven dried at 65 °C for 48 h and dry weights of roots and shoots were measured. The samples were taken into 50-mL polyethylene digestion tubes, and 3 mL of 65% HNO_3 were added and allowed to stand over night. The samples were heated on a heating block at 95 °C for 90 min. After cooling to room temperature, 2 mL of 30% H_2O_2 were added, and heated again at 105 °C for 30 min. Then, the digests were diluted to 10 mL with DI water for arsenic and iron analysis.