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

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 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.

Keywords: Arsenic, Iron, Bioavailability, Phytoextraction, HIDS, EDDS, EDTA, Rice (*Oryza sativa* L.)

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 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; Rahman et al., 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 ºC), 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., 2004; Deng et al., 2010), its effect on DMAA uptake in rice hasn't. 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 ºC 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) ºC.

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 3 times, and the rinsed water was added to the CBE-extracts to make a total of 10 mL.

2.6. Samples 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 ºC 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 ºC for 90 min. After cooling to room temperature, 2 mL of 30% H_2O_2 were added, and heated again at 105

ºC for 30 min. Then, the digests were diluted to 10 mL with DI water for arsenic and iron analysis.

2.7. Chemical analysis

Total arsenic and iron were analyzed in CBE-extract of root surfaces, roots, and shoots of rice seedlings using Total arsenic and iron were analyzed in CBE-extract of root surfaces, roots, and shoots of rice seedlings using Perkin Elmer Zeeman-effect GFAAS (Model- AAnalyst 600) equipped with a transverse heated graphite atomizer (THGA) (Ajtony et al., 2008). Instrumental and working conditions for the determination of arsenic and iron by the GFAAS are summarized in Table 1. For arsenic determination, 10 µL of matrix (5 µg Pd (as nitrate) plus 3 µg $Mg(NO_3)_2$) was added to 20 µL of sample in the THGA as modifier.. At least one reagent blank and two certified standard reference material (1573a, tomato leaf from National Institute of Standards and Technology (NIST), USA) were included in the digestion. Arsenic concentration in certified standard reference material was 0.112±0.004 µg g^{-1} d. wt. while the measured concentration was 0.124±0.057 µg g^{-1} d. wt. All chemical reagents used in this experiment were of analytical grade. Glassware and dishes were washed with detergent and 5 M HCl solution, and rinsed with deionized water before use.

2.8. Data analysis

Data analysis was performed by SPSS 16.0 for windows. The analysis of variance (ANOVA) for arsenic and iron concentrations in roots and shoots of rice was performed using F-statistics. Comparison of means of the treatments was made by Duncan's Multiple Range Test (DMRT). Correlation statistics was calculated by T-test.

3. Results and discussions

3.1. Effect of chelating ligands on arsenic uptake in rice root

Chelating ligands increased arsenate uptake in rice roots while DMAA uptake was not affected by the ligands. The increase of arsenate uptake by chelating ligands was higher in rice seedlings grown with additional iron than those grown without additional iron. HIDS was better for arsenate uptake compared to that of EDDS and EDTA (Figs. 1A, 1B). Previously, Rahman et al. (2008b) reported that EDTA increased arsenate and arsenite uptake in aquatic macrophyte (*Spirodela polyrhiza* L.) significantly while DMAA and monomethylarsonic acid (MMAA) uptake was not affected by EDTA. Rahman et al. (2008a) also reported that chelating ligands increased arsenate uptake in roots of hydroponically grown rice and the trend of effectiveness of the ligands was HIDS > EDTA > EDDS > MGDA ≥ IDS. Results of the present study were in agreement with the previous reports of Rahman et al. (2008a; 2009) suggesting that the effectiveness of chelating ligands in the enhancement of arsenic uptake does not differ whether the plant is grown in hydroponic culture or in soil solution. It is also evident from the results of present and previous studies (Rahman et al., 2008a; Rahman et al., 2009) that HIDS is more effective for arsenic uptake in roots from both water and soil compared to that of other synthetic chelating ligands.

Arsenic concentration on rice root surfaces was negatively correlated with the increase of its concentration in the roots (Rahman et al., 2008a), and arsenate has stronger adsorptive affinity to iron oxides (Pierce and Moore, 1982) than that of DMAA (Lafferty and Loeppert, 2005). Thus, increased arsenate uptake in rice roots was the direct effect of chelating ligands, and the increment of arsenic uptake by the ligands indicate the effectiveness of respective ligand. Additional iron in the soil solution increased the amount of

iron oxides on rice root surfaces which increased the physicochemical adsorption of arsenate on and uptake in the roots.

3.2. Effect of additional iron on arsenic uptake in rice root

DMAA uptake in rice roots was not increased by the additional iron while arsenate uptake was increased. Arsenate uptake in rice roots was 15-20 times higher than that of DMAA when rice seedlings were grown without additional iron (Fig. 1A). In contrast, arsenate uptake was 19-28 times higher than that of DMAA when the seedlings were grown with additional iron in the soil (Fig. 1B). Results indicate that additional iron in the soil solution increased arsenate uptake in rice roots which might be due to the increased physicochemical adsorption of arsenate on Fe-plaque of rice root surfaces (Robinson et al., 2006). Previous studies also showed that the uptake of inorganic arsenic species was much higher than those of methylarsenic species in rice (Odanaka et al., 1987; Rahman et al., 2008b) and in aquatic macrophytes (*Salvinia natans* L., *Spirodela polyrhiza* L.) (Rahman et al., 2008c; Rahman et al., 2008b).

Arsenate has high binding affinity to iron oxides (iron oxides) (Pierce and Moore, 1982). Additional iron in the soil solution increased the amount of Fe-plaque on the roots of rice seedlings, which might facilitate arsenate adsorption on Fe-plaque and uptake in rice roots. Previous studies also showed that arsenate concentration was positively correlated with the amount of iron plaque on roots of *Typha latifolia* (cattail) grown in arsenic contaminated wetland sediments (Blute et al., 2004) and of aquatic plats Taupo Volcanic Zone and Waikato River, New Zealand (Robinson et al., 2006). Thus, arsenate is supposed to be incorporated into iron oxides attached to the surface of the plants. Chen et al. (2005) demonstrated that iron plaques on rice root surfaces not only bound arsenic but also promote its uptake by the roots. According to Robinson et al. (2006) other than the biological mechanisms,

physicochemical adsorption of arsenate on the suspended oxides attached to the roots is an important mechanism for arsenic uptake in aquatic plants. Results of the present study revealed that adsorption of arsenate on the iron plaques of rice root surfaces was much higher then that of DMAA. This was because arsenate strongly adsorbed on iron oxides while DMAA was not appreciably retained by iron oxides (Lafferty and Loeppert, 2005). This phenomenon was also observed by Blute et al. (2004) in roots of wetland plant *Typha latifolia* (cattail). Blute et al. (2004) also observed that the ferric plaques cattail roots were predominantly Fe(III) oxyhydroxide and 80% of the arsenic in it was arsenate.

3.3. Influence of iron on arsenate and DMAA uptake in rice shoot

Arsenate uptake in rice shoots was significantly ($p < 0.01$) higher than that of DMAA. Although arsenate uptake in rice shoots was influenced by chelating ligands and additional iron in the soil solution, DMAA was influenced neither by chelating ligands nor by additional iron (Figs. 1C, 1D). Arsenate concentrations were higher in shoots of rice seedlings grown without additional iron (Fig. 1C) compared to those grown with additional iron (Fig. 1D). Results indicate that arsenate uptake in rice shoots was not affected by its concentrations in roots. Previous studies also showed that arsenic uptake in rice roots was several orders of magnitude higher than that in other parts of the plant (Abedin et al., 2002; Wang et al., 2006; Rahman et al., 2009). Results elucidated that the translocation of arsenic from roots to shoots was limited. This might be because arsenate is rapidly reduced to arsenite inside the root cells, which has a high affinity to the sulphhydryl (–SH) groups of peptides such as glutathione (GSH) and phytochelatins (PCs) (Zhao et al., 2009). *In vitro* studies also showed that GSH and arsenite form a $(GS)_3$-arsenite complex with cysteinyl sulphhydryl as the arsenite binding site (Delnomdedieu et al., 1994). Complexation of arsenite with thiols in roots does not favor transport of arsenic from roots to shoots. Moreover, arsenite is sequestered into vacuoles of

root cells (Zhao et al., 2009). Thus, reduction of arsenate to arsenite and its subsequent complexation with thiols and vacuolar sequestration in root cells decrease arsenic translocation to the shoots (Zhao et al., 2009). It has also been suggested that the Fe-plaque acts as a "buffer" to prevent arsenic translocation from roots to shoots (Chen et al., 2005).

Results of the present study also revealed that chelating ligands increased arsenate uptake in shoots. Compared to control and HIDS treatments, EDTA and EDDS increased arsenic uptake in shoots when the seedlings were grown without additional iron (Fig. 1C). Enhanced uptake of arsenate in shoots of rice seedlings by chelating ligands has also been reported by Rahman et al. (2008a; 2009).

3.4. Effect of chelating ligands, additional iron and arsenic species on iron uptake in rice root

Iron concentrations were determined on root surfaces (CBE-extracts) and in roots of rice seedlings to investigate the effect of EDTA, EDDS and HIDS as well as the influence of additional iron and arsenic species on its uptake in rice roots. Regardless of the chelating ligands, iron uptake was higher in rice roots of seedlings grown with additional iron compared to those grown without additional iron. In addition, irrespective of the additional iron and arsenic species, chelating ligands increased iron uptake in rice roots significantly ($p < 0.05$) compared to the control treatments (Figs. 2B and 2C). The increase of iron uptake in roots of rice seedlings grown with different treatments of arsenic, iron and chelating ligands was related to its concentrations in root surfaces. Correlation analysis showed that iron concentrations in roots were significantly positively correlated with its concentrations in CBE-extracts of the root surfaces (Fig. 3). Therefore, it is evident that the bioavailability and uptake of iron in rice seedlings were increased by the chelating ligands. Hasegawa et al.

(2010) reported that biodegradable chelating ligands increase iron mobility, bioavailability and uptake in radish *Raphanus sativus* L.), and the mobility and bioavailability of iron depends on stability constant and type of the ligand, pH of growth medium, and ligand exposure time (Hasegawa et al., 2011). Hasegawa et al. (2010; 2011) found that HIDS was the most effective ligands studied for the mobility and bioavailability of iron which is in agreement with the results of the present study.

Increasing iron uptake by chelating ligands can be explained by the adsorption of metal-chelants complexes on the Fe-plaques of rice root surfaces and subsequent dissociation of the Fe-chelant complexes in the soil solution (Nowack et al., 1996; Nowack and Sigg, 1997). For example, the dissolution of Fe(III) hydroxides by metal-EDTA complexes occurs by ligand-promoted dissolution process which is initiated by the adsorption of metal-EDTA complexes to the surface and is followed by the dissociation of the complex at the surface and the release of Fe(III)-EDTA in the solution (Nowack and Sigg, 1997). Complexation of metals with strong ligands such as EDTA occurs very often in natural systems. In addition to the complexation, dissolution of iron oxides in the presence of metal-EDTA complexes have been reported to occur in the subsurface environments (Davis et al., 1994). Compared to the uncomplexed EDTA, the dissolution rate is decreased to a great extent if EDTA complexes with metals (Nowack and Sigg, 1997).

Iron uptake in rice roots was also affected by arsenic species. Regardless of the additional iron in the soil solution, iron uptake in rice roots was much higher when the seedlings were grown with DMAA (Figs. 2D, 2E) compared to that with arsenate (Figs. 2B and 2C). Correlation analysis showed that arsenate and iron concentrations in the roots of rice seedlings grown with or without additional iron were related significantly ($r = 0.78$, $p < 0.05$)

while DMAA and iron concentrations in the roots were not related significant (r = -0.16, p > 0.05) (Figs. 4A, 4C). The results indicated that iron uptake in rice roots was inhibited by arsenate due to the increased adsorption of arsenate on iron oxides of root surfaces compared to that of DMAA (Bowell, 1994; Wilkie and Hering, 1996).

3.5. Iron uptake in shoots influenced by chelating ligands, additional iron and arsenic species

Iron concentrations in shoots of rice seedlings were about 23-49 times lower than those in roots. Although iron uptake in the roots of rice seedlings was affected by the additional iron and arsenic species (Fig. 2), its uptake in shoots was not affected significantly by those factors (Fig. 5). Correlation analysis also showed that iron concentrations were correlated neither with arsenate nor with DMAA concentrations in shoots of rice seedlings (Figs. 4B and 4D). Compared to the control treatment, however, chelating ligands increased iron uptake in shoots of rice seedlings grown with arsenate significantly (p < 0.05) (Figs. 5B, 5C). In contrast, iron uptake in shoots was not affected that much when the seedlings were grown with DMAA (Figs. 5D, 5E).

Compared to the roots, lower iron uptake in shoots of hydroponic rice seedlings has been reported by Rahman et al. (2009). It has been reported that soil-grown plants fail to translocate iron from the roots to the aerial parts in iron deficient condition, and iron is usually taken up and used in plant tops once it is made available for transport by the roots (Brown, 1978). But iron uptake in plant roots depends on its mobility and bioavailability in growing medium (Hasegawa et al., 2011), and thus iron uptake in shoots would be related to its availability and concentrations in roots. Chelating ligands have commonly been used to increase iron bioavailability and uptake and to correct iron-chlorosis in plants (Yunta et al.,

2003; Alvarez-Fernandez et al., 2005; Lucena, 2006). In addition to the type of chelating ligands, we found in a recent study (not published) that the concentration and stability constant of the ligands ($\log K_{FeL}$) would be critical determinants for the increase or decrease of iron bioavailability and uptake in plant roots. The results of the present study showed that chelating ligands increase iron uptake in roots as well as in shoots, and HIDS was found to be more effective in increasing iron uptake in rice roots compared to EDTA and EDDS.

4. Conclusion

Chelating ligands increase arsenate uptake in rice roots, and the increment was augmented by additional iron in the soil. In addition, arsenate uptake in rice shoots was increased by the ligands in some cases while DMAA was not in any cases. Among the chelating ligands tested, HIDS increased arsenic uptake in roots. So, the biodegradable HIDS would be a potential ligand for the enhancement of arsenic uptake by plants during phytoremediation. Chelating ligands also increased iron uptake both in roots and shoots of rice seedlings. But arsenate inhibits iron uptake in roots while DMAA does not. In this case, HIDS also found to be more effective for the increase of iron bioavailability and uptake in roots of rice seedlings in most cases. Thus, HIDS would also be a good Fe-fertilizer.

Iron is an important nutrient of plants while arsenic is toxic to plants at high concentration except for hyperaccumulators. Since iron and arsenic, particularly arsenate, have good correlation in plant uptake chelant-enhanced bioavailability of iron and arsenic phytoextraction would be good idea. But if chelating ligands is used for the increase of iron bioavailability to reduce iron-chlorosis in rice plant it can be elucidated from the results of the present study that the ligands not only increase iron bioavailability, but also increase arsenic uptake in rice. Therefore, fertilization of iron-chelants in agricultural soils

contaminated with high level of arsenic for the increase of iron uptake in crop plants should be considered carefully.

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Figure captions

Fig. 1: Influence of chelating ligands and additional iron (in addition to its background concentration in the soil) on arsenic uptake in roots (A, B) and shoots (C, D) of rice seedlings. Control treatments were contained only arsenic but no chelating ligands

and additional iron. Values are mean ± standard deviation (N = 3). In a figure, values having same letter don't differ significantly from each other at 5% level by DMRT.

Fig. 2: Influence of chelating ligands, additional iron (in addition to its background concentration in the soil) and arsenic species on iron uptake in rice roots. Without arsenic (A); arsenate (B, C) and DMAA (D, E). Control treatments did not contain additional iron and chelating ligands. Values are mean ± standard deviation (N = 3). In a figure, values having same letter don't differ significantly from each other at 5% level by DMRT.

Fig. 3: Correlation between iron concentrations in roots and on root surfaces of rice seedlings grown with different treatments of arsenate, additional iron and chelating ligands.

Fig. 4: Correlation between arsenic and iron concentrations in roots (A, C) and shoots (B, D) of rice seedlings. Arsenate (A, B) and DMAA (C, D). CL (chelating ligand).

Fig. 5: Influence of chelating ligands, additional iron (in addition to its background concentration in the soil) and arsenic species on iron uptake in rice shoots. Without arsenic (A); arsenate (B, C) and DMAA (D, E). Control treatments did not contain additional iron and chelating ligands. Values are mean ± standard deviation (N = 3). In a figure, values having same letter don't differ significantly from each other at 5% level by DMRT.

Table 1: Instrumental and working conditions for the determination of arsenic and iron by Perkin Elmer Zeeman-effect GFAAS (AAnalyst 600) equipped with a transverse heated graphite atomizer (THGA).

For arsenic (As)					
Lamp	Electrodeless discharge lamp (EDL)				
Lamp current	380 mA				
Wavelength	193.7 nm				
Slit width	0.7 nm				
Furnace program settings	Drying 1	Drying 2	Pyrolysis	Atomization	Cleaning
Temperature (ºC)	110	130	1200	2000	2450
Ramp time (s)	1	15	10	0	1
Holding time (s)	30	30	20	5	3
Argon flow rate (cm^3 min^{-1})	250	250	250	0	250
For iron (Fe)					
Lamp	Hollow cathode lamp				
Lamp current	30 mA				
Wavelength	248.3 nm				
Slit width	0.2 nm				
Furnace program settings	Drying 1	Drying 2	Pyrolysis 1	Atomization	Cleaning
Temperature (ºC)	110	130	1400	2100	2450
Ramp time (s)	1	15	10	0	1
Holding time (s)	30	30	20	5	3
Argon flow rate (cm^3 min^{-1})	250	250	250	0	250

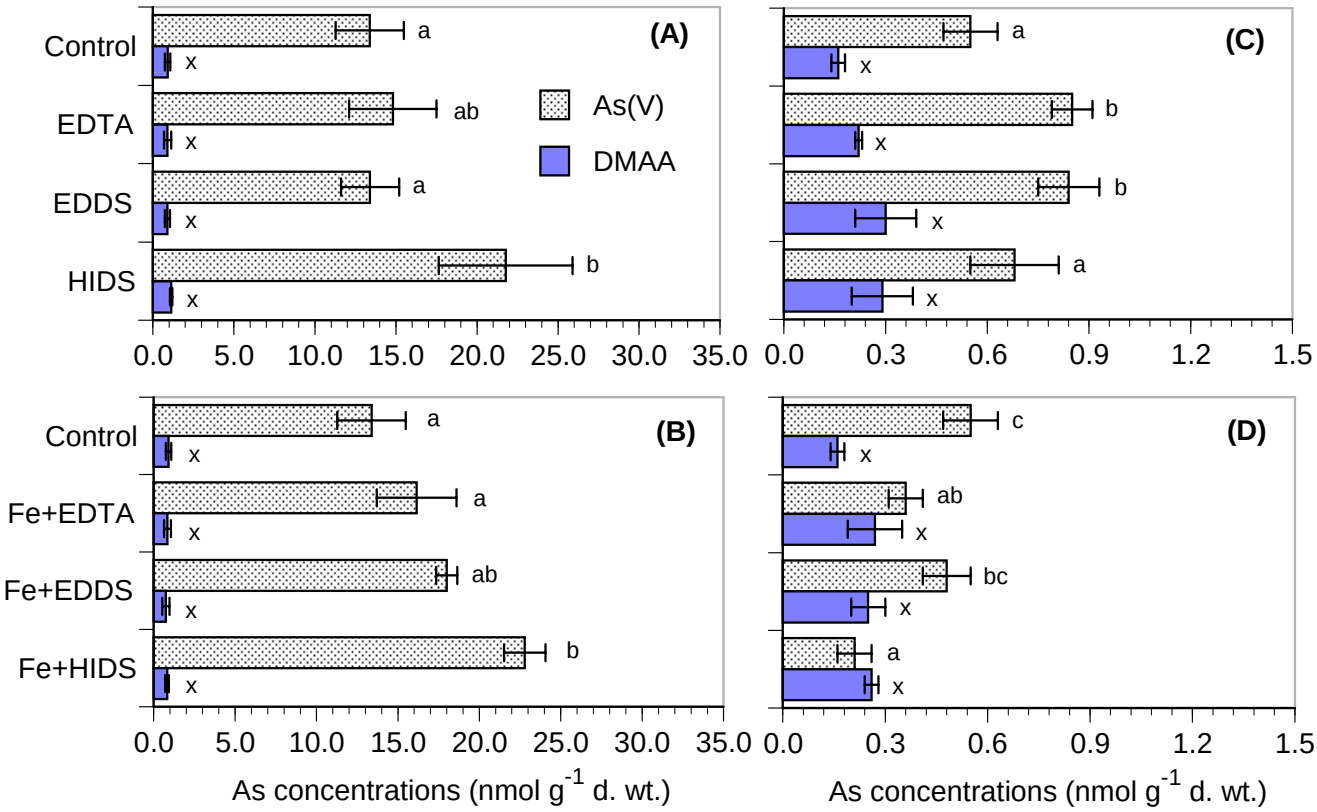

Fig. 1: Influence of chelating ligands and additional iron (in addition to its background concentration in the soil) on arsenic uptake in roots (A, B) and shoots (C, D) of rice seedlings. Control treatments were contained only arsenic but no chelating ligands and additional iron. Values are mean ± standard deviation (N = 3). In a figure, values having same letter don't differ significantly from each other at 5% level by DMRT.

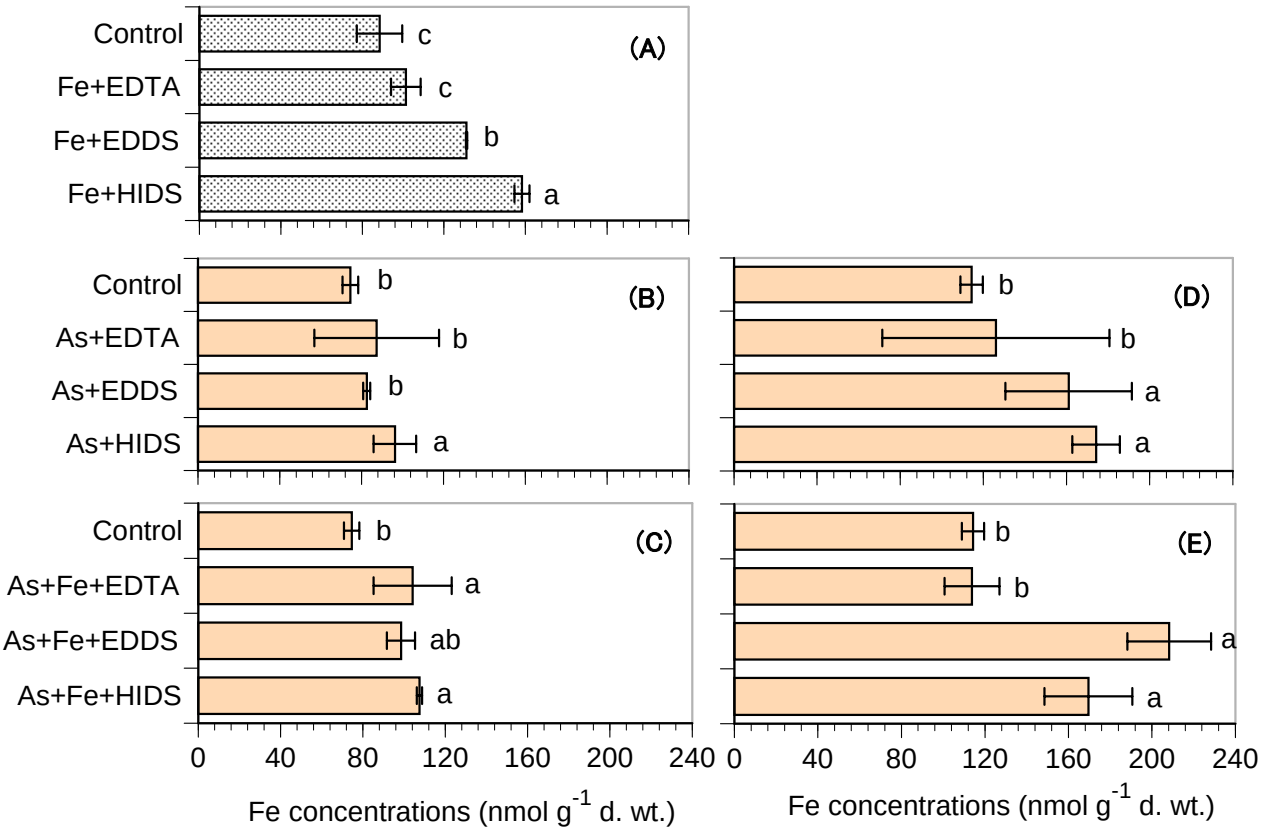

Fig. 2: Influence of chelating ligands, additional iron (in addition to its background concentration in the soil) and arsenic species on iron uptake in rice roots. Without arsenic (A); arsenate (B, C) and DMAA (D, E). Control treatments did not contain additional iron and chelating ligands. Values are mean ± standard deviation (N = 3). In a figure, values having same letter don't differ significantly from each other at 5% level by DMRT.

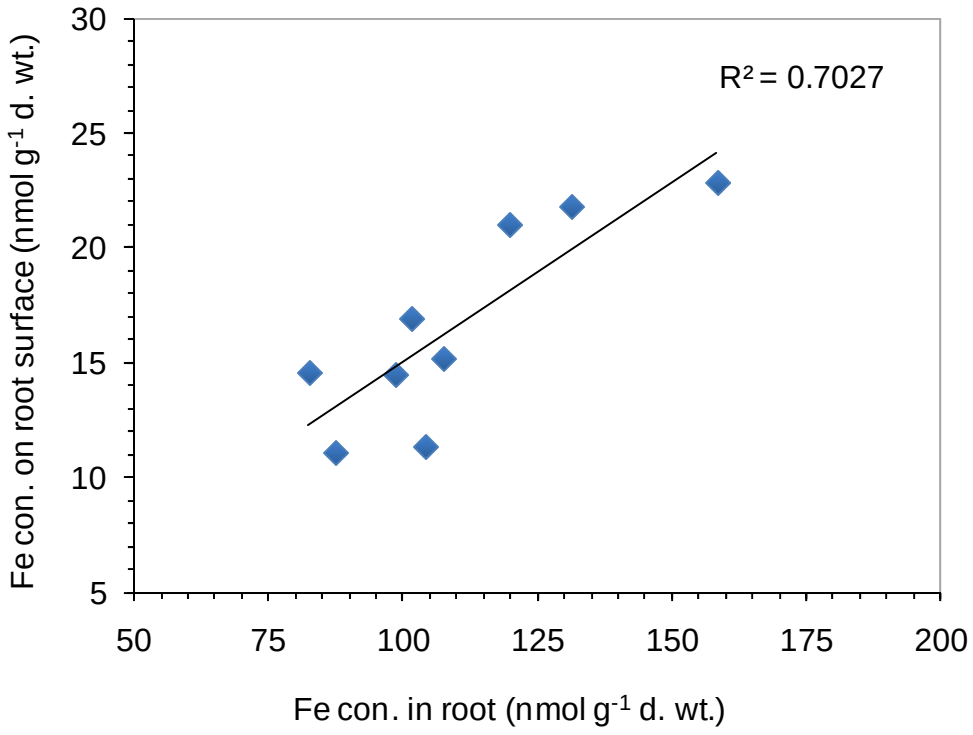

Fig. 3: Correlation between iron concentrations in roots and on root surfaces of rice seedlings grown with different treatments of arsenate, additional iron and chelating ligands.

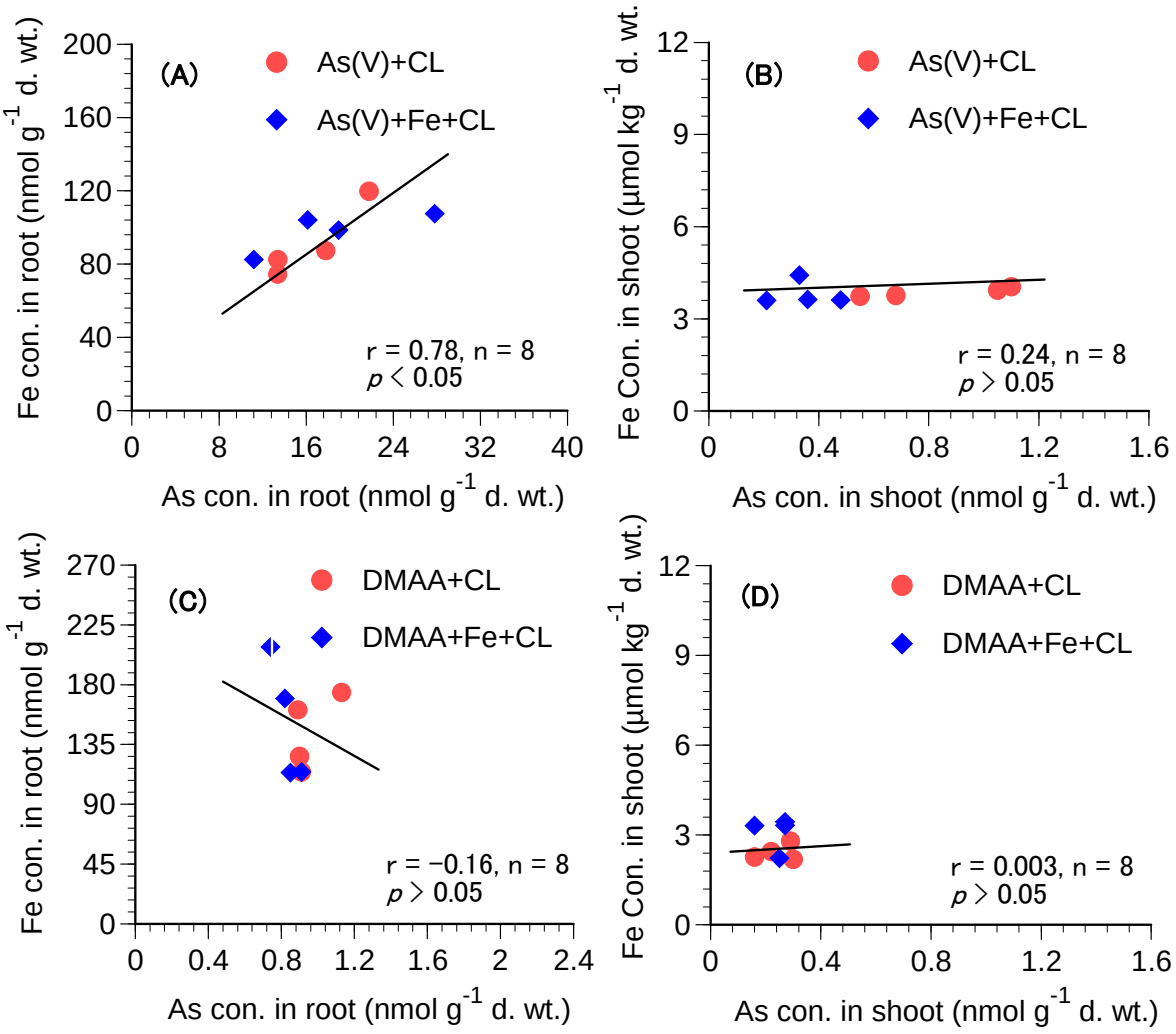

Fig. 4: Correlation between arsenic and iron concentrations in roots (A, C) and shoots (B, D) of rice seedlings. Arsenate (A, B) and DMAA (C, D). CL (chelating ligand).

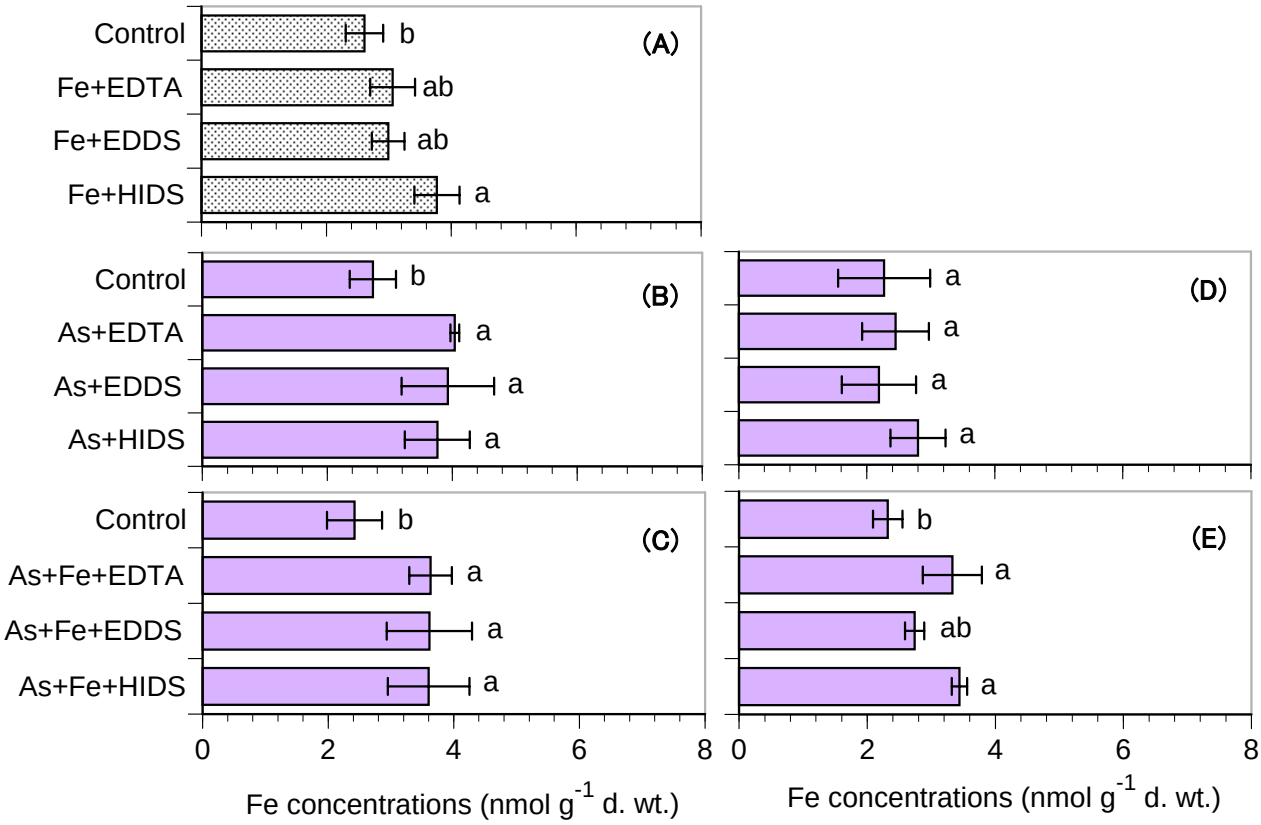

Fig. 5: Influence of chelating ligands, additional iron (in addition to its background concentration in the soil) and arsenic species on iron uptake in rice shoots. Without arsenic (A); arsenate (B, C) and DMAA (D, E). Control treatments did not contain additional iron and chelating ligands. Values are mean ± standard deviation (N = 3). In a figure, values having same letter don't differ significantly from each other at 5% level by DMRT.