Efficiency of Solubilization of Metals by Mugineic Acid from Sodic Soil

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ABSTRACT

Iron has limited solubility in alkaline soils and, consequently, alkaline soils may have insufficient available iron for optimal plant growth. Plants of the family Gramineae secrete phytosiderophores, plant-derived compounds such as mugineic acid (MA) that chelate iron, enabling them to extract iron from deficient soils. In the present study, the efficiency of MA and other chelators to solubilize iron and other metals from sodic soil, an alkaline soil, was studied. We measured the amount of

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solubilized metals from sodic soil, calcareous soil, and andosol. The soils were mixed with the solutions of chelators, incubated with continuous shaking, and centrifuged. The supernatant was filtered and analyzed for metals. In the calcareous soil and andosol, the chelators enhanced solubilization of iron. Although a large amount of iron was solubilized from the sodic soil, the release was independent of the presence of chelators. Similar results were obtained for solubilization of aluminum. When suspended in water, the sodic soil released a considerable amount of silicon, but this was not observed for the other soils. Subsequently, we examined the iron solubilization from salinized or alkalinized andosols by chelators. The results showed that only soil alkalinization enhanced more Fe to be released, and the chelators did not enhance Fe solubilization significantly with increasing soil pH. Because of our results and information of formerly known publications, we suggested that water extractable humic substances caused the iron solubilization from sodic soil. Therefore, we showed the significant information for plant nutrition in sodic soils. Iron, aluminum, and silicon were present in soluble forms in the soil. Further studies are required to characterize precisely the efficiency of MA on metal nutrients for plants in sodic soils. Furthermore, the risk of aluminum toxicity and the usefulness of water-soluble silicon for plant growth are strongly suggested in natural sodic soils.

1. INTRODUCTION

Desertification describes the degradation of land in regions that are historically relatively dry but that become increasingly arid. In recent years, it had been identified as a problem in many parts of the world, including Africa, Asia, North and South America, and Spain [1]. One factor in desertification and soil degradation is the accumulation of salts, particularly Na. About 23% of the cultivated land in the world is saline and about 37% is sodic. Saline and sodic soils cover about 10% of the total arable lands of the world [2]. Soil sodification is one of the contributors of soil alkalization in arid or semi-arid areas [3]. Once soils became sodic, Na_2CO_3 and $NaHCO_3$ accumulate and soil pH increases, accompanied by increased electrical conductivity (EC) and retarded plant growth [3]. Sodic soils are defined as soils with an exchangeable sodium percentage (ESP) greater than 15, an EC of the saturation extract less than 4 dS m^{-1}, and a pH ranging between 8.5 and 10 [4]. Sodic soils are among the most alkaline and barren in the world.

In the alkaline soils, iron precipitates and therefore, Fe deficiency of plants is a trait of the soils [3]. Iron is an essential micronutrient for plants that is important in respiration, many redox reactions, and photosynthesis [3,5,6]. Plants have evolved special mechanisms for acquiring Fe under Fe deficiency, known as Strategy I and Strategy II [3,7].

Strategy I is employed by dicotyledons and monocotyledons that do not belong to the family Gramineae. Strategy I involves the release of reductants into the rhizosphere, increased Fe^{3+}-reducing enzyme activity at the root surface, and an active Fe^{2+}-transporter at the plasma membrane, which involves a potent proton extruding pump. Strategy II operates in Gramineae family, which includes the major crops barley, wheat, rice, maize, and sorghum. Under Fe-deficient growth conditions, these plants release mugineic acid family of phytosiderophores (MAs) from their roots [8,9,10] with a specific efflux transporter (a member of the TOM family of proteins) [11]. MAs solubilize and complexes Fe^{3+} from aerobic soils as Fe^{3+}-MAs. The complex is absorbed through the specific transporter yellow stripe 1 (YS1) in the roots and transported to the xylem [12,13]. Mugineic acid (MA) refers to an amino acid that is a MAs, a small plant compound that can chelate Fe, and MA has other related compounds such as hydrodroxymugineic acid (HMA) or *epi*-HMA [3].

The ability of the major crops to tolerate Fe deficiency and their ability to secrete MAs are positively correlated. Both properties decrease in the following order: barley > wheat and rye > oats > maize > sorghum > rice [3,14,15,16]. Mugineic acid chelates metal ions other than Fe^{3+}, specifically Mn^{2+}, Cu^{2+}, Zn^{2+}, and Al^{3+} [17,18]. Gramineae respond to Zn deficient conditions also by secreting MA and absorbing the MA-chelated Zn^{2+} [19,20,21].

In the sodic soils with a pH greater than 8.5, iron is considered to be precipitated and immobilized

[3]. Therefore, the value of Strategy II in Fe deficiency in sodic soils can be questioned. At present, there has been no research to focus on the effectiveness of MAs for Fe nutrition in sodic soils. Here, we aimed to investigate whether MAs solubilize Fe^{3+} and other metals in sodic soils and if their role is essential for Gramineae to acquire metal nutrients from the rhizosphere of sodic soils.

Although we have shown that the sodic-tolerant *Puccinellia chinampoensis* Ohwi (*P. chinampoensis*) [22,23], a graminaceous plant, responds to sodic conditions by increasing secretion of MAs, in barley, MAs secretion was repressed under the conditions [24]. Furthermore, although we have also shown that MA solubilized Fe^{3+} in a gel preparation, this ability was inhibited by carbonate and bicarbonate ions, which would be present in sodic soils [24]. Despite the ability of carbonate and bicarbonate ions to inhibit MA, it may be that the enhanced ability of *P. chinampoensis* to release MAs permits it to accumulate Fe and enhance its ability to survive sodic environments. There is, however, no evidence for this proposal.

MAs show more effective in solubilizing Fe^{3+} and other metals in calcareous soils, which are high in calcium carbonate, than were microbial siderophores such as desferrioxamine B (FOB) and the artificial chelators ethylenediaminetetraacetic acid (EDTA) and diethylene triamine pentaacetic acid (DTPA) [25]. Specifically, we have compared sodic soils to calcareous soils and andosol.

2. MATERIALS AND METHODS

2.1 Measurements of Chemical Properties of the Soils Utilized in this Study

Three soils were collected and studied: sodic soil in the suburbs of Dàān, Jilin prov., China [26]; calcareous soil in the Konya basin, Turkey [27]; and andosol in the Rokuhara of Kanegasaki, Iwate prefecture, Japan [28].

The soils were air dried and passed through a sieve with 2 mm diameter pores. Then, chemical properties of the soils were measured: soil pH, soil EC, cation exchangeable capacity (CEC), ESP, available P (the amount plants can utilize), the concentrations of soluble carbonates (CO_3^{2-} and HCO_3^-), the amounts of exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), and the total amounts of metals (Fe, Al, Mn, Cu, Zn). The soils were suspended in de-ionized water with a mass ratio of soil: de-ionized water, 1: 2.5 for pH; soil: de-ionized water, 1: 5 for EC. The pH and EC values were measured with a pH conductivity meter (D-54, Horiba Co.). The CEC and the amounts of exchangeable cations were measured by the semi-micro schollenberger method. After extraction in 1.0 M ammonium acetate at pH 7 [29], the amounts of Na, K, Ca, and Mg in the extracted solution were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES; ICPE-9000, Shimadzu, Kyoto, Japan). The amount of available P in the alkaline soils was measured by Olsen P method, and in the andosol by the Truog P method [30]. The ESP was calculated from the values of exchangeable Na^+ and CEC. The concentrations of soluble carbonates were measured by the modified method of Hatano [31] as follows. Ten grams of the soils was added to 50 mL de-ionized water and shaken on a reciprocal shaker for one hour in a thermostatic chamber at 30ºC. Then, the solutions were filtered through No.5C filter paper individually and titrated with 0.02 M H_2SO_4. The concentrations of soluble carbonates were calculated. The amounts of metals were measured by the modified method of Gotoh [32] as follows: One gram of soil was digested with a mixture of HNO_3 and $HClO_4$ (v/v, 5: 1) and the amounts of metals in the digestion samples were analyzed by ICP-OES. Tables 1, 2, and 3 show the chemical properties of the soils.

Table 1. Chemical properties of the soils (data represent mean ± SE; n.d., not detected, the limits of detection were available P: 0.02 mg g^{-1}; soluble carbonates: 0.01 cmol$_c$ kg^{-1})

	Sodic soil	Calcareous soil	Andosol
pH (H_2O)	9.6±0.01	8.4±0.02	4.9±0.05
EC (dS m^{-1})	3.9±0.009	0.71±0.015	0.24±0.001
CEC (cmol$_c$ kg^{-1})	14.1±0.5	13.6±0	43.9±2.1
ESP (%)	88.5±0.320	5.80±0.067	0.561±0.029
available P (mg g^{-1})	0.31±0.04	0.29±0.03	n.d.
soluble CO_3^{2-} (cmol$_c$ kg^{-1})	0.026±0.0001	n.d.	n.d.
soluble HCO_3^- (cmol$_c$ kg^{-1})	2.9±0.005	0.67±0.002	0.22±0

Table 2. Exchangeable cations of the soils

	Sodic soil	Calcareous soil	Andosol
Na^+ ($cmol_c$ kg^{-1})	12.5±0.42	0.790±0.01	0.250±0
K^+ ($cmol_c$ kg^{-1})	0.450±0	0.580±0.01	0.920±0.01
Ca^{2+} ($cmol_c$ kg^{-1})	10.7±0.05	15.3±0.05	2.68±0
Mg^{2+} ($cmol_c$ kg^{-1})	1.25±0.02	3.12±0.01	0.650±0.01

Data represent mean ± SE

Table 3. Total amounts of metals of the soils

	Sodic soil	Calcareous soil	Andosol
Fe (mg g^{-1})	15.8±0.22	22.0±0.23	39.4±1.22
Al (mg g^{-1})	21.1±0.58	32.4±0.83	56.1±2.40
Mn (mg g^{-1})	0.339±0.022	0.469±0.038	0.447±0.016
Cu (mg g^{-1})	0.0069±0.0001	0.0092±0.0006	0.0357±0.0031
Zn (mg g^{-1})	0.022±0.001	0.029±0.0002	0.109±0.0049

Data represent mean ± SE

2.2 Preparation of Mugineic Acid (MA)

The crystalline MA employed in this study was the same which was utilized in the previous work [24]. The MA was isolated by washing the roots of Fe-deficient barley plants (*Hordeum vulgare* L. cv. Minorimugi) according to the method of Takagi et al. [9]. Analysis by Nuclear Magnetic Resonance and Mass Spectrometry indicated that isolated MA did not contain impurities [24].

2.3 Solubilization of Metals from the Soils by Chelators

An aqueous solution containing 0.167 mM of the each chelator (MA, EDTA, DTPA, or FOB) was used to solubilize metals, according Takagi et al. [25]. The efficiency of chelators for metals solubilization from the soils were examined by the modified method of Environment Agency notification No. 46 [33] as follows. One gram of the soils was mixed with 30 mL of the each chelator solution or de-ionized water for control in a centrifugation tube, shaken for six hours on a reciprocal shaker in a thermostatic chamber at 30ºC, and centrifuged at 3000 rpm for 20 min at 20ºC. The supernatant was filtered through

millipore filter with 0.1 µm diameter pores and the amount of Fe, Mn, Cu, Zn, and Al in the filtered solution was analyzed by ICP-OES. The amounts of Si, Ni, Co and Pb in the filtered control were analyzed by ICP-OES and are denoted as water soluble elements.

2.4 Solubilization of Iron from Salinized or Alkalinized Andosols by Chelators

Andosol was air-dried, passed through a sieve with 2 mm diameter pores. Salinization of the andosol was achieved by adding 100 mL of NaCl solutions of varying concentrations (0.02 mM to 0.5 mM) to 50 g of the soil, and air dried. In the alkalinization of the andosol, one-hundred milliliters of NaOH solutions of varying concentrations (from 0.02 mM to 0.5 mM) were added to 50 g of the soil for the adjusted pH of 6, 7, 8, 9, or 10, and the soils were air dried. The control andosol was not treated and had a pH of 4.9. The pH and EC of soils were measured and the results are shown in Tables 4 and 5.

One gram of the salinized or alkalinized andosols was tested for Fe solubilization by MA, EDTA or de-ionized water as described.

Table 4. Soil pH and EC of salinized andosols

	Control	mmol L^{-1}				
		0.02	0.06	0.2	0.3	0.5
pH (H_2O)	4.9±0.05	5.1±0	4.9±0.04	4.6±0.02	4.3±0.21	4.1±0.02
EC (dS m^{-1})	0.24±0.001	7.81±0.15	23.1±0.25	66.6±0.75	95.7±1.55	152.9±3.25

Data are shown as mean ± standard error (SE)

Table 5. Soil pH and EC of alkalinized andosols

	Control	pH 6	pH 7	pH 8	pH 9	pH 10
pH (H_2O)	4.9±0.05	6.2±0.01	7.0±0.02	8.2±0.03	9.0±0.04	10.2±0.01
EC (dS m^{-1})	0.24±0.001	2.56±0.05	3.73±0.18	9.78±0.16	16.7±0.25	38.1±0.15

Data are shown as mean ± standard error (SE)

2.5 Statistical Analyses

Experiments were conducted in triplicate. Data were subjected to an ANOVA using the HP proLiant DL320 G6 computer in Iwate University, Japan [34]. Differences between means were evaluated using the Ryan-Einot-Gabriel-Welsch multiple range test ($p < 0.05$). Different letters were assigned to indicate significant differences. Samples which made the same test were given the same number of prime symbol (', ") and distinguished (A, B, C, D; A', B', C', D'; A", B", C", D").

3. RESULTS

3.1 Chemical Properties of the Soils Utilized in this Study

The chemical properties of soils are shown in Tables 1, 2, and 3. The pH value of the sodic soil was 9.6 and higher than those of the other soils. The EC value and ESP of the sodic soil were also the highest among the soils (EC = 3.9 dS m^{-1}; ESP = 88.5%). These results shows that the sodic soil utilized in this study was true of the definition of sodic soil [4]. In the sodic soil, the amount of exchangeable Na^+ was extremely higher than the other soils although that of K^+ was the lowest. These results proved that much amount of Na_2CO_3 and $NaHCO_3$ accumulate in the sodic soil. In the sodic soil, the total amounts of metals were lowest among the soils.

3.2 Solubilization of Metals from Sodic Soil, Calcareous Soil, and Andosol by Chelators

The soluble Fe content of solutions of the three soils that were collected, with and without chelators, is shown in Fig. 1. Of the three soils, the sodic soil had the most soluble Fe regardless of the presence of chelators, and there was no significant effect of the chelators, including MA ($p < 0.05$). In the calcareous soil (pH 8.4; Table 1), soluble Fe was almost undetectable but MA and DTPA solubilized detectable levels of Fe certainly. In the andosol, it was undetectable in the control but present in measurable quantities when any of the chelators were present. There was significantly more soluble Fe in the presence of MA than when the other chelators were present ($p < 0.05$).

Fig. 2 presents the soluble Mn, Cu, and Zn concentrations in solutions with and without chelators. Mn was solubilized from the sodic soil by all solutions, including the control (Fig. 2a). Mugineic acid did not solubilize significantly more Mn than the control ($p < 0.05$), and solubilized significantly less than the other chelators. The chelators FOB and DTPA solubilized the most. In the calcareous soil, there was no detectable Mn in the control, but all other chelators solubilized detectable levels of Mn, with DTPA being the most effective. In the andosol, manganese was solubilized to the same extent by all chelators and de-ionized water, with the exception of a marked increase in soluble Mn when EDTA was present (Fig. 2a).

Fig. 2b shows the effect of chelators on soluble Cu. In the sodic soil, copper was solubilized by all solutions, but MA and DTPA solubilized significantly more than the control ($p < 0.05$), and MA was more effective than DTPA. In the calcareous soil, no copper was detected in the control or in the FOB solution, but, as was observed for the sodic soil, soluble Cu was detected in the other solutions, and the levels with MA and DTPA were greater, with MA being the most effective. In the andosol, the results were similar to the other soils. Very little Cu was detected in the control or FOB solutions, and the other three chelators increased soluble Cu, with MA the most effective (Fig. 2b).

Zinc was solubilized from the sodic soil by all treatments, but the levels were very low (Fig. 2c). Only EDTA enhanced the Zn solubilization from the soil significantly. In the calcareous soil, MA, EDTA, and DTPA solubilized significantly more Zn than the control or FOB; no soluble Zn was detected in the control or FOB solutions. The chelator EDTA was most effective among the chelators, but all Zn levels were very low. In the andosol, the pattern of Zn solubilization in response to the treatments was similar to that of the calcareous soil (Fig. 2c), but large increases over the control were observed in response to EDTA and DTPA, with EDTA showing the greatest increase.
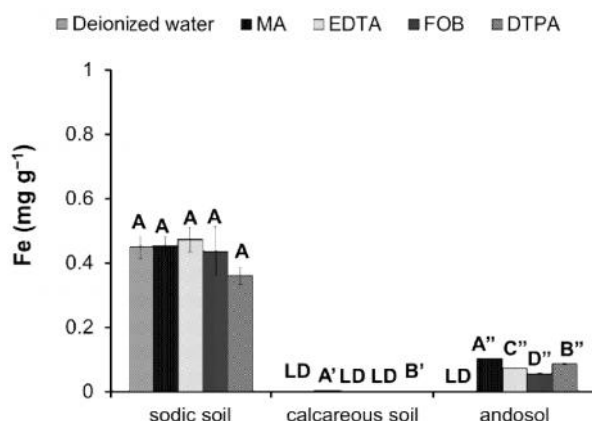
Fig. 1. Concentration of soluble iron from the collected soils in the presence and absence of chelators

Data represent mean ± SD, n = 3; different letters indicate significant differences (p < 0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test. Samples which made the same test were given the same number of prime symbol (', ") and distinguished. The test was performed among the chelates in each soil (sodic soil: A, B, C; calcareous soil: A', B', C'; andosol: A", B", C"). LD indicated that the data was lower than the threshold of detection [0.6 µg g^{-1}]. sodic soil: F = 2.90, calcareous soil: F = 910, p < 0.0001, andosol: F = 3870, p < 0.0001
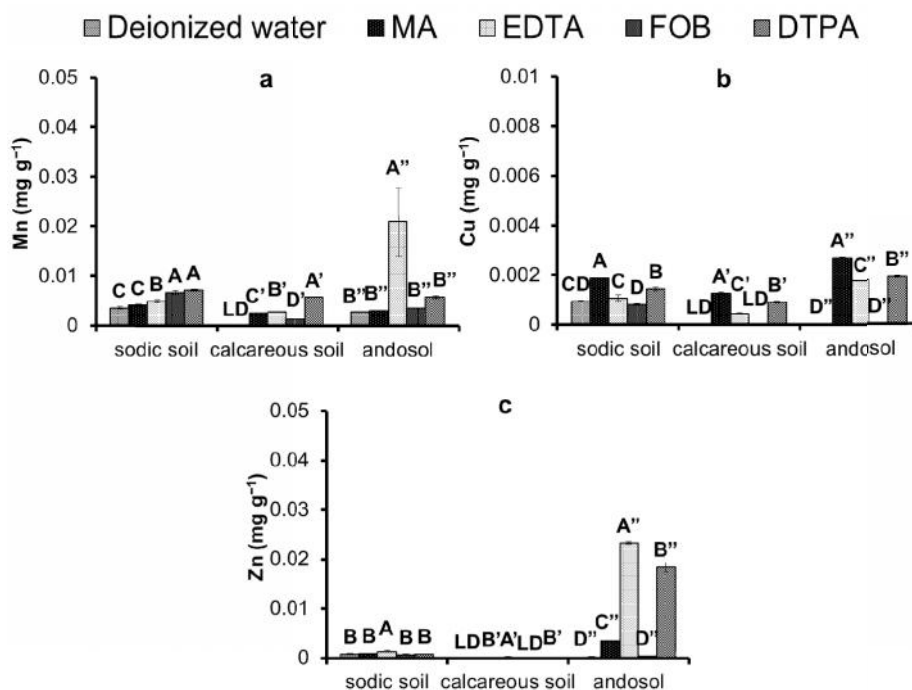

Fig. 2. Concentration of soluble metals from the collected soils in the presence and absence of chelators

Data represent mean ± SD, n = 3; different letters indicate significant differences (p < 0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test. Samples which made the same test were given the same number of prime symbol (', ") and distinguished. The test was performed among the chelates in each soil (sodic soil: A, B, C; calcareous soil: A', B', C'; andosol: A", B", C"). LD indicated that the data was lower than the threshold of detection [0.6 µg g^{-1}]. (a) manganese: sodic soil: F = 101, p < 0.0001, calcareous soil: F = 10313, p < 0.0001, andosol: F = 19.0, p = 0.0001. (b) copper: sodic soil: F = 117, p < 0.0001, calcareous soil: F = 1388, p < 0.0001, andosol: F = 9669, p < 0.0001. (c) zinc: sodic soil: F = 15.5, p < 0.0005, calcareous soil: F = 90.6, p < 0.0001, andosol: F = 1990, p < 0.0001

The results of Al solubilization are shown in Fig. 3. The results were similar to those with Fe. The sodic soil had the most soluble Al among the soils, regardless of the presence of chelators and there were no significant differences among the treatments ($p < 0.05$). In the calcareous soil, of the solutions used, only FOB increased soluble Al, while in the andosol, all treatments solubilized Al. In the andosol, EDTA, FOB, and DTPA were more effective than the control and MA, and EDTA and DTPA were most effective.

Of the water soluble elements that we measured in the soils, Ni, Co, or Pb, were below the threshold of detection, which was 0.6 µg g^{-1} for Ni and Co, and 2.4 µg g^{-1} for Pb. Silicon was the only water soluble element that was solubilized by de-ionized water in the soils (Fig. 4). A large quantity of Si was solubilized from the sodic soil and it was significant higher than the other soils ($p < 0.05$). There was no significant difference in the amount of Si solubilized in the calcareous soil and andosol ($p < 0.05$).

In this experiment, the sodic soil solutions were consistently colored with dark brown (date not shown), while the other soil solutions were transparent.
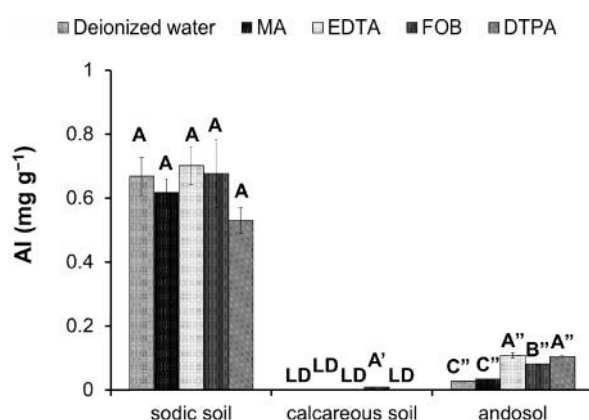

Fig. 3. Concentration of soluble aluminum from the collected soils in the presence and absence of chelators
Data represent the mean ± SD, n = 3; different letters indicate significant differences (p < 0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test. Samples which made the same test were given the same number of prime symbol (', ") and distinguished. The test was performed among the chelates in each soil (sodic soil: A, B, C; calcareous soil: A', B', C'; andosol: A", B", C"). LD indicated that the data was lower than the threshold of detection [2.4 µg g^{-1}]. sodic soil: F = 3.17, calcareous soil: F = 4193, p < 0.0001, andosol: F = 296, p < 0.0001
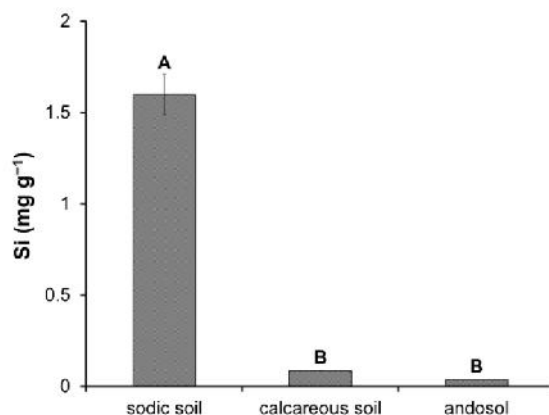

Fig. 4. Concentration of solubilized silicon from the soils in de-ionized water
Data represent mean ± SD, n = 3; different letters indicate significant differences (p < 0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test; F = 573.02, p < 0.0001

3.3 Solubilization of Iron from Salinized or Alkalinized Andosols by Chelators

The effect of salinization on the Fe solubilizing capability of the andosol is presented in Fig. 5a. Very little Fe was released from the andosol in the absence of chelators, regardless of the saline concentration of the soil. The chelators EDTA and MA both caused significantly ($p < 0.05$) more Fe solubilization than the water control at all salt concentrations, but there was no clear dose-response to saline concentration. In contrast, there was a striking effect of alkalization on Fe release by the andosol, as can be seen in Fig. 5b. In the absence of chelators, increased pH caused significantly more Fe to be released ($p < 0.05$) and the enhancement increased with increasing pH. In the control soil (not alkalinized),

as well as soil at pH 6 and 7, the chelators solubilized significantly more Fe than de-ionized water did ($p < 0.05$). At pH 8, mugineic acid solubilized significantly more Fe than de-ionized water, but EDTA did not. The effects of EDTA and MA were not significant at pH 9 and 10 ($p < 0.05$).

4. DISCUSSION

We first wished to compare the efficiency of MAs in the solubilization of Fe^{3+} and other metals in the rhizosphere of sodic soils to their effect in calcareous soil and andosol. The amount of Fe solubilized from the sodic soil in all treatments was much higher than that released from the other soils (Fig. 1). The least amount of solubilized Fe was released
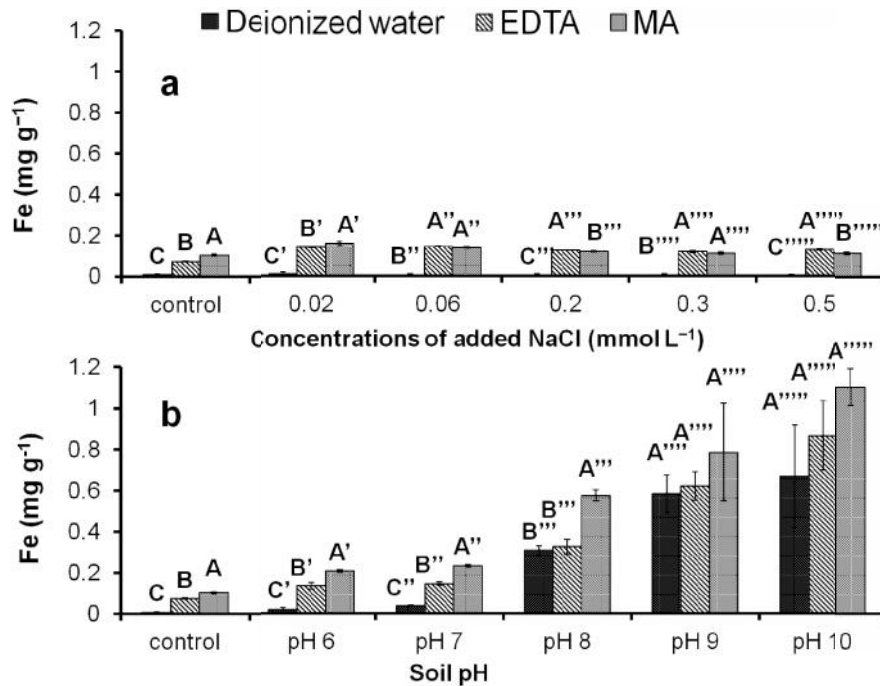

Fig. 5. Concentration of soluble iron solubilized from the soils in the presence or absence of chelators

Data represent mean ± SD, n = 3; different letters indicate significant differences (p < 0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test. Samples which made the same test were given the same number of prime symbol (', ") and distinguished. The test was performed among the chelates in each soil: (a) andosol salinized by NaCl: con; F = 595.26, p < 0.0001, 0.02 mM; F = 448.67, p < 0.0001, 0.06 mM; F = 1415.54, p < 0.0001, 0.2 mM; F = 4371.17, p < 0.0001, 0.3 mM; F = 441.14, p < 0.0001, 0.5 mM; F = 939.84, p < 0.0001. (b) andosols alkalinized by NaOH: con; F = 595.26, p < 0.0001, pH 6; F = 254.38, p < 0.0001, pH 7; F = 777.10, p < 0.0001, pH 8; F = 83.40, p < 0.0001, pH 9; F = 1.49, pH 10; F = 4.24 Each soil was air-dried, passed through a sieve with 2 mm, and 30 mL of an aqueous solution containing 0.167 mM of MA or EDTA was added to the 1 g of the soil.

from the calcareous soil, which was very low even in the presence of the chelators. However, mugineic acid increased the soluble Fe significantly in the calcareous soil, even if the effect was small ($p < 0.05$), in agreement with the results of Takagi et al. [25]. Mugineic acid also increased Fe solubilization in the andosol. In contrast, mugineic acid and the other chelators did not enhance soluble Fe content of the sodic soil.

In this study, we analyzed the Fe in solutions filtered through a 0.1 μm millipore filter. Therefore, the iron species that we measured must have been smaller than 0.1 μm in diameter. One possible reason for the ability of water to solubilize Fe from the sodic soil may have been the absorption of Fe to hydrophilic colloids of clay particles. Generally, it is known that Na^+ on the clay surface weakens bonding strength between clay particles and reduces the overall strength of soil aggregates so that the clay colloids eventually disperse [35], which would release the mineral into the water. The CEC sites on the sodic soil may have been occupied by the large amount of exchangeable Na^+ (Table 2). Supporting this contention, we found that the ESP of sodic soil was 88.5%, the highest among the soils (Table 1). Therefore, dispersion of the clay as hydrophilic colloids may have occurred in the reaction mixture of sodic soil during the experiment. Clay dispersion may also have occurred in the reaction mixture of salinized andosols due to the large amount of NaCl added (Table 4), although the amount of solubilized Fe by water from the salinized andosols was not markedly affected by the amount of soil salinization (Fig. 5a). We consider, then, that few of the clay colloids that absorbed iron did not pass the filter. Thus, it was suggested that the Fe with clay colloids was not an important factor in the Fe solubilization from the sodic soil.

Our last experiment consisted of alkalinizing the andosol (Fig. 5b) and measuring its solubilization of Fe. The amount of Fe solubilized by de-ionized water increased with soil pH up to pH of 10. According to the results in Fig. 1 with the sodic soil, which had a pH of almost 10 (Table 1), high pH solubilizes Fe regardless of the presence of chelators. However, it is known that the solubility of $FeCl_3$ or $Fe(OH)_3$ is much decreased under high pH conditions [36] and at pH 10, the amount of solubilized Fe in the form $Fe(OH)_4^-$ is extremely low. The other possible cause for Fe solubilization by water at high pH is chelation by humic substances, which chelate and solubilize metals such as Fe, Mn, Cu, Zn, or Al [37]. In sodic soils, the organic matter is commonly solubilized, so that extracts of sodic soils have a characteristic dark brown color [4]. In this study, all extracts of sodic soil and alkalinized andosols were dark brown, though the solutions of the other soils were transparent, indicative of a large amount of soluble humic substances. Traditionally, humic and fulvic acids, the soluble fractions of humic substances, are extracted with alkali such as Na_2CO_3 or $NaHCO_3$. The products should be smaller than 0.1 μm [37], so that they will be passed through Millipore filters. We conclude that the high pH of sodic soil and alkalinized andosols caused the extraction of humic substances in the reaction mixture and that a large amount of Fe was solubilized by water from the sodic soil and alkalized andosols due to the chelation of Fe by these substances. Of interest here is our report that MA could solubilize Fe^{3+} from gelled Fe^{3+}, but the activity of MA to solubilize Fe^{3+} was largely repressed by carbonates under sodic condition [24]. Thus, the repression by carbonates and the chelation of Fe by humic substances would obscure chelation by MA or other chelators at high pH.

The question arises as to whether MAs contribute to the assimilation of Fe by plants of the Graminae and if the ability of these plants to secrete MAs under sodic conditions is advantageous to survival. Recently, it has been shown that the Fe complexes that are chelated by soluble humic substances, namely Fe-water-extractable humic substances (WEHS) are of value in Fe nutrition [38]. Strategy I plants utilize Fe-WEHS via reduction of Fe^{3+}-WEHS by the plasma membrane-bound reductase [38,39,40, 41]. Strategy II plants utilize Fe^{3+}-WEHS by an indirect mechanism involving ligand exchange between WEHS and MAs [38,41,42,43]. If MAs can acquire Fe^{3+} from Fe^{3+}-WEHS in the rhizosphere of sodic soils, MAs secretion ability of sodic-tolerant plants, such as *P. chinampoensis,* would be advantageous, explaining both the enhanced MA secreting ability of *P. chinampoensis* under sodic condition [24] and its survival in natural sodic fields [44,45,46]. However, it has not been demonstrated that MAs can acquire Fe^{3+} from Fe^{3+}-WEHS by ligand exchange under sodic conditions, and further work is required to reveal the function of MAs in the mechanisms of Fe^{3+} acquisition of Gramineae adapted to sodic soils.

In the present study, Mn, Cu, and Zn were, like Fe, solubilized by de-ionized water from the

sodic soil (Fig. 2). These metals are precipitated as hydroxides, similar to Fe in the soil, at the high pH of sodic soil. The solubilizaiton of these metals may also be caused by chelation by humic substances. However, the amount of solubilized metals from the sodic soil was not as great as that from the other soils, and solubilization was enhanced by the chelators in the sodic soil (Fig. 2). Solubilization of Ni, Co, and Pb was not observed in this study. It is intriguing to relate these results to the occurrence of these elements in nature and the stability of the complexes formed between them and humic substances. In the metals of the earth's crust, aluminum is the highest amount and Fe is the second highest among the metals [4]. The amounts of Mn, Cu. Zn, Ni, Co, and Pb are much lower. Therefore, it may be that almost all humic substances chelated with Fe or Al in the sodic soil (Figs. 1,2, and 3). Another consideration is that the stability of the metal complexes formed with humic substances is completely different between the species of metals and humic substances and the order of its stability is changed by soil pH [37]. In the sodic soil, with a pH of close to 10, the stability order of the metal complexes formed with humic substances has not been determined, as far as we know. The order of Mn, Cu. Zn, Ni, Co and Pb may be lower than those of Fe and Al. This might explain the results of metals solubilization in the sodic soil. Metal elements acquisition of MAs from WEHS under the sodic conditions has not been intensively studied. How the Gramineae utilize the metals-WEHS is still uncertain. If the plants acquire the metals indirectly by ligand exchange by MAs in sodic soils, further works will be required to demonstrate it.

Aluminum toxicity may be a problem for plants grown in sodic soils [35], partly due to the formation of $Al(OH)_4^-$, reported to be toxic to plant growth in many studies [47,48,49,50]. High levels of Al were solubilized from the sodic soil in this study (Fig. 3) and the pattern of Al and Fe solubilization was similar (Figs. 1 and 3). It may be that Al solubilization is also attributed to chelation by humic substances. Besides, Al would be solubilized as $Al(OH)_4^-$ or other forms at soil pH of approximately 10; at this pH, iron is precipitated. In the rhizosphere of sodic soils, aluminum toxicity would harm plant roots initially and might inhibit synthesis and secretion of MAs. We have hypothesized here that the synthesis and secretion of MAs are essential for Gramineae species to survive in sodic soils. It follows that plants in sodic soils may have dual

tolerance, to Na and Al, synthesizing and secreting MAs without accruing damage from excess Al. The *P. chinampoensis* responds to sodic conditions by increasing secretion of MAs [24]. Thus, sodic tolerant plants such as *P. chinampoensis* may have an Al resistance in sodic conditions. In support of this suggestion, organic acids with the ability to chelate can reduce Al toxicity [51,52,53,54]. The ability of MAs to chelate Al may contribute the detoxification of Al in the rhizosphere of sodic soil. Therefore, the ability of sodic tolerant plants to secrete MAs may be advantageous not only in providing Fe but also in detoxifying Al. Further works are required to reveal the hypothesis and the form of solubilized Al from the sodic soil.

Finally, we showed that a large amount of Si was solubilized from the sodic soil (Fig. 4). To our knowledge, there is little information about Si solubilization in sodic soils. Silicon solubilization in the sodic soil may be due to its chelation by humic substances and the formation of SiO_4^{4-}. It is commonly known that Si is solubilized as SiO_4^{4-} in high pH conditions; approximately 10 [55]. The benefit of Si for plant growth has been reported often [3], especially, amelioration of Al toxicity by Si in acidic conditions [56,57;58,59]. Presumably, silicon would also ameliorate Al toxicity under sodic conditions, and their ability to release Si may be beneficial in this regard. Sodic tolerant plants might have high efficiency of Si utilization under the sodic condition. Further work should reveal the effect on Si on plant growth in sodic soils.

In general, iron is insoluble in alkaline soil. Hence, we investigated the efficiency of MA to solubilize Fe^{3+} from sodic soil. However, the result showed unexpectedly that Fe was soluble in sodic soils. Because of our results and information of formerly known publications, we suggested that humic substances caused the Fe solubilization from sodic soil and it correlated closely with MAs on plants metal nutrition in the soil. Our consideration in this study compiled and summarized the published research works on MAs and humic substances for plant nutrition. Besides, we showed that Al and Si were present in soluble forms in the soil. Therefore, we suggested the Al toxicity and Si usefulness on plant growth in sodic soils. Thus, the present study is scientifically significant and suggests new research areas on plant nutrition in sodic soils as ligand exchange between MAs and humic substances, alkaline Al toxicity, preferential Si absorption of sodic tolerant plants.

Projections are for increases in the human population in the immediate and near future. One of the challenges of an increased population is the need for increased food production. One way to meet this challenge is to increase the land area available for agriculture [60]. Preventing land from degradation, such as soil sodification, is a part of this endeavor and understanding the processes of degradation is essential for ameliorating it. Sodic soils have, to date, largely drawn attention because of the effect of pH on metal availability and saline concentration. We suggest that plant survival in sodic soils should be considered more comprehensively. The effect of humic substances, Al toxicity, and Si usefulness should be considered. Thus, further studies should reveal the nutritive properties of sodic soils and promote their revegetation in the future.

5. CONCLUSIONS

The efficiency of mugineic acid (MA) and other chelators to solubilize the metals (Fe, Mn, Cu, Zn, Al) and Si from sodic soil, calcareous soil, and an andosol was investigated. In the sodic soil, the chelators did not enhance solubilization of Fe and a large amount of Fe was solubilized from the soil. Also, Al and Si were dissolved similarly to Fe and the release of Al was independent of the presence of chelators. The results were not observed for the other soils.

Iron, Al, and Si would be existed as soluble forms in the rhizosphere of sodic soil and may affect to the plant growth there. Further studies are required to characterize the efficiency of MA on Fe solubilization, the usefulness of water-soluble Fe and Si for plant growth, and the risk of Al toxicity in sodic soils.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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