International Journal of Plant & Soil Science
4(4): 297-311, 2015; Article no.IJPSS.2015.031
ISSN: 2320-7035

SCIENCEDOMAIN *international*
www.sciencedomain.org

Iron Solubilization Activity of Mugineic Acid and Secretion of Mugineic Acid Family of Phytosiderophores by Barley and *Puccinellia chinampoensis* Ohwi under Sodic Conditions

T. Yoshida[1*], H. Kudo[2], L. Zhao[3], H. B. Wang[3], A. Sato[4], A. K. Xu[5], M. Q. Zhao[5], B. L. Qi[5], X. M. Guo[6] and S. Kawai[1]

[1]The United Graduate School of Agricultural Sciences, Iwate University, Morioka 020-8550, Japan.
[2]Organization of Revitalization for Sanriku-region, Iwate University, Morioka 020-8550, Japan.
[3]College of Resources and Environment, Jilin Agricultural University, Changchun 130118, China.
[4]Faculty of Bio-resource Science, Akita Prefectural University, Akita 010-1423, Japan.
[5]Grassland Institute, Branch of Animal Husbandry, Jilin Academy of Agricultural Sciences, Gongzhuling 136100, China.
[6]Rice research Institute, Jilin Academy of Agricultural Sciences, Gongzhuling 136100, China.

Authors' contributions

This work was carried out in collaboration among the all authors. Authors TY, HK and SK designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors LZ, HBW, AS, AKX, MQZ, BLQ and XMG gave the seeds of Puccinellia chinampoensis Ohwi and much essential information about the sodic soil in Songnen Plain in northeast China. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2015/13640
Editor(s):
(1) Francisco Cruz-Sosa, Biotechnology Department, Metropolitan Autonomous University, Iztapalapa Campus, Av. San Rafael Atlixco, 186 México City, 09340, México.
Reviewers:
(1) Klára Kosová, Department of Plant Genetics, Breeding and Crop Quality, Crop Research Institute, Prague, Czech Republic.
(2) Anonymous, Ordu University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=776&id=24&aid=6743

Original Research Article

Received 26th August 2014
Accepted 29th September 2014
Published 30th October 2014

ABSTRACT

In order to clarify contribution of the mugineic acid family of phytosiderophores (MAs) to the iron (Fe) nutrient utilization for the survival of grasses grown in sodic soils, the secretion of MAs from the roots of *Puccinellia chinampoensis* Ohwi (*P. chinampoensis*), one of the sodic tolerant grasses,

**Corresponding author: E-mail: tyiwate@yahoo.co.jp;*

was investigated compared with barley (*Hordeum vulgare* L. cv. Minorimugi) as a control plant in Fe-depleted hydroponical cultures. It was clarified that the amount of released MAs of barley which have high MAs secretion ability in their roots, was reduced under sodic conditions. On the other hand, the amount of MAs released by *P. chinampoensis* was increased under the sodic conditions compared with pH 6.5 conditions. It was also shown that MAs release activity of *P. chinampoensis* was subjected to lesser repression by Na_2CO_3/$NaHCO_3$ than that of barley. Subsequently, the activity of MA, one of the MAs, to solubilize Fe^{3+} from gelled Fe^{3+} in various ion compositions was examined. The results showed that Fe^{3+} solubilization activity of MAs was maintained stable in the pH range between 8 and 10 and was much repressed by CO_3^{2-}/HCO_3^- under pH 10 condition. Because of the repression of Fe^{3+} solubilization activity of MA by CO_3^{2-}/HCO_3^-, it was considered that higher ability to secrete MAs of *P. chinampoensis* under sodic conditions will give advantage to the plant for survival in sodic soils. Thus, *P. chinampoensis* is considered to be adapted to sodic conditions. Extensive examination about the behavior of MAs in the rhizosphere of sodic soils and physiological significance of MAs in the sodic tolerant grasses are required in the future.

Keywords: Fe^{3+} solubilization; songnen plain; sodic soil; sodium carbonate/bicarbonate; gelled Fe^{3+}.

1. INTRODUCTION

Iron is one of the essential metal micronutrients and is related to respiration, many redox reactions, and photosynthesis [1-3]. Generally, mineral soils have a total Fe content of about 5%. However, Fe is deposited as Fe $(OH)_3$ in alkaline soils and the total concentration of inorganic Fe species in the soil solution is around 10^{-10}mol/L [3]. Under the conditions of sodic soil of pH 10, Fe is considered to be precipitated and immobilized. Therefore, Fe deficiency is one of the inhibitory factors in plant growth and crop yield in alkaline soils [4]. It is considered that the plants able to be grown there may have high ability to utilize Fe in the soil.

Plants have Fe acquisition systems which are known to be Strategy I and Strategy II functioning under the Fe deficiency [3]. Strategy I is the Fe acquisition system in dicotyledons and non-grasses. Strategy I is comprised of three integral parts, an Fe^{3+}-reducing enzyme system, potent proton extruding pump and secretion of phenolics to rhizosphere. On the other hand, Strategy II is the Fe acquisition system in grasses (e.g. barley, wheat, rice, maize and sorghum). In Strategy II, grasses secrete mugineic acid family of phytosiderophores (MAs) from roots under Fe deficient conditions [5-7], solubilize Fe^{3+} from aerobic soils and carry it into the root cells through a transport system specific to Fe^{3+}-MAs [8-10]. There is a correlation between the degree of Fe efficiency of grasses and the MAs secretion ability of their roots under Fe deficiency, both of these traits being in the order: barley > wheat and rye > oats > maize > sorghum > rice [3,11-13]. Compounds of MAs were proven to be highly efficient in solubilizing Fe^{3+} from calcareous soils than microbial siderophore (Desferrioxamine B), artificial chelators (ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA)) [14]. Thus, MAs secretion may be essential ability for grasses to acquire Fe^{3+} in alkaline conditions.

In recent years, land degradation in arid regions is becoming a more serious problem all over the world, in Africa, Asia, North and South America, and Spain [15]. In cultivated lands in the world, about 23% are saline and 37% are sodic and saline and sodic soils cover about 10% of the total arable lands [16]. Soil sodification is a chemical land degradation which occurs in arid or semi-arid area. Once soil begins to suffer from sodification, Na_2CO_3 and $NaHCO_3$ accumulate in the soil. As a result, the soil physical condition worsens and soil permeability becomes low. Also, the soil pH is raises to around 10 due to HCO_3^- and CO_3^{2-} and soil electrical conductivity (soil EC) also raises due to high concentration of Na^+ [17]. Therefore, the plants grown in sodic soils suffer from damaging effects of alkaline stress and salt stress [18]. In sodic soils, plant growth is repressed strongly as compared with those in saline soil containing neutral salts like NaCl and/or Na_2SO_4 [18]. Thus, sodic soil is one of the most alkaline and barren soil in the world. Yet, there area few studies about plant growth in sodic soils compared with those in calcareous soils. Fe acquisition by plants grown in sodic soils has not been intensively studied, as far as we know. In fact, the plants described above, such as barley and wheat, cannot grow in sodic soils.

In the Songnen Plain in north east China, the increase of sodic soil areas has been becoming a serious problem since the middle of the twentieth century. The causes of soil sodification in the Songnen Plain are due to natural factors such as parent materials, topographic positions, and an arid/semi-arid climate as well as anthropogenic causes such as population pressure, overgrazing, and improper agricultural and economic policies [19-21]. Thus, the Academy of Agriculture Science of Jilin provinces in China has been managing the project to recover the vegetation in the Songnen Plain and the utility for the revegetation of *Puccinellia chinampoensis* Ohwi (*P. chinampoensis*) has been examined due to its high palatability for animal grazing [22,23]. In fact, *P. chinampoensis* is, one of tolerant plants, forms communities in sodic areas in the Songnen Plain. There are some studies about the grass [23,24]. More research about this grass is required.

Demonstration of the superior physiological ability of the grasses grown in the sodic soils such as *P. chinampoensis* will contribute to indicate the utility of the plants for recovering vegetation in the Songnen Plain. Clarification of the relationship between the MAs secretion ability and Fe acquisition of the sodic tolerant grasses is required in order to reveal their superior characteristics for survival in sodic soils.

Therefore, the focus of this study was the MAs secretion ability for Fe nutrient utilization of grasses under sodic conditions. In fact, *P. chinampoensis* can grow without showing Fe chlorosis, which is observed in the plants grown in calcareous soils, in sodic areas of the Songnen Plain. The reason why the plant does not show Fe-chlorosis as a Fe-deficiency symptom remains unknown. It is considered that *P. chinampoensis* can secrete MAs and those MAs may function in the acquisition of Fe^{3+} by grasses in the rhizosphere of sodic soils. At present, however, there has been no research on this topic.

We studied the correlation between the MAs secretion ability of *P. chinampoensis* and the Fe^{3+} solubilization activity of MA, one of the MAs, under Fe-depleted and sodic conditions. The aim of this study is to inspect the contribution of MAs release in Fe nutrient utilization related with the survival activity of grasses grown in sodic soils.

2. MATERIALS AND METHODS

2.1 Plant Growth

Seeds of *P. chinampoensis* were received from A. K. Xu of the Academy of Agriculture Science of Jilin provinces in China. Seeds of barley (*Hordeumvulgare* L. cv. Minorimugi) were obtained from the plants grown in the field of Iwate University. The seeds were placed on a sterilized plastic net that overlay a 1mmol/L $CaCl_2$ solution in a plastic box and germinated. The box was kept in a growth chamber (KG-206HI, Koito Industries Ltd, Tokyo, Japan) with a day-night regime of 14h with 280μmol/m^2·s light at 17°C and 10h dark at 10°C. Both seeds of *P. chinampoensis* and barley in the box were under these conditions for 7 days and 3 days, respectively, in light-shielded conditions and then the seedlings were grown under artificial light. The $CaCl_2$ solution in the seeds box was replaced with 1/5-strength modified Hoagland and Arnon No. 2 medium [25,26]. Twenty one days after the growth in the medium of 1/5 strength medium, the seedlings of *P. chinampoensis* were transplanted into 10L buckets and grown in 1/2-strength modified Hoagland and Arnon No. 2 medium consisting of 3 mmol/L KNO_3, 2mmol/L $Ca(NO_3)_2$, 0.5mmol/L $NH_4H_2PO_4$, 1mmol/L $MgSO_4$, 10μmol/L Fe^{3+}-EDTA, 1.5μmol/L H_3BO_3, 0.25μmol/L $MnSO_4$, 0.1 μmol/L $CuSO_4$, 0.2μmol/L $ZnSO_4$ and 25nmol/L H_2MoO_4 (pH 5.5) with continuous aeration for 7 days. The seedlings of barley were also transplanted into 10L buckets 7 days after growth in 1/5-strength medium and grown in the 1/2-strength medium with continuous aeration for 3 days.

The seedlings of *P. chinampoensis* were again transplanted with 5 plants per bunch to the aerated treatment mediums (described following) and grown for 21 days. Treatment 1 (control): Fe-depleted medium (pH 6.5), Treatment 2 (alkaline conditions): Fe-depleted medium with pH 10 and Treatment 3 (sodic conditions): Fe-depleted medium with pH 10 containing 20mmol/L Na_2CO_3/$NaHCO_3$. The pH of each medium was adjusted by 1mol/L NaOH every day. Root exudates of *P. chinampoensis* were collected at 14 days and 21 days after transfer to the treatment media by the methods of Kawai et al. [25]. The seedlings of barley were transplanted with 3 plants per bunch to the same aerated treatment medium and grown for 14 days. Root exudates of barley were collected at 7 days and

14 days after transfer to the treatment medium, similarly.

SPAD value, an index of the amount of chlorophyll present [27], of the leaves was measured by SPAD-502chlorophyll meter (Minolta Camera Co. Tokyo, Japan). The values were measured in triplicate.

Plants samples of *P. chinampoensis* and barley were harvested at 21 days and 14 days, respectively, after transfer to the Fe-depleted medium. The plants were separated into shoots and roots and dried in an oven at 55°C for 24h. Dry weights of the plant parts were measured.

2.2 Phytosiderophores (MAs) Release of *P. chinampoensis* under Alkaline or Sodic Conditions

The collection of root exudates was performed by a similar method of Kawai et al. [25] as follows. Three samples (five plants of *P. chinampoensis* per sample and three plants of barley per sample) of each plant were rinsed with deionized water immediately at the onset of the light period and soaked in 500mL of deionized water for 4h. After the plants were removed from deionized water, 10mg thymol (Kanto Chemical Co. Tokyo, Japan) was added to the solutions to prevent microbial degradation of MAs. These solutions were denoted as root washing.

Each solution of root washing was introduced individually into a column (1.5cm internal diameter × 15cm) of cation exchange resin (Amberlite IR-120B, Organo Corp. Tokyo, Japan). The resin was washed with 200mL deionized water. The compounds adsorbed onto the resin were eluted with 200mL of 1mol/L NH_4OH and the eluted solution was concentrated under vacuum. These solutions were denoted as root exudate.

A modified method of Fe^{3+}-solubilizing assay of Takagi [5] was employed to estimate the amount of compounds capable of solubilizing Fe^{3+} in the root exudates. In this assay, 10mL of root exudate solution was mixed with 0.5mL of Na-acetate buffer (pH 5.6, 0.5mol/L) and 2mL of aqueous suspension of gelled Fe^{3+} (5mmol/L) which was prepared by neutralizing 0.5mol/L $FeCl_3$ with 0.5mol/L NaOH (pH 7). The mixed solutions were incubated in an oven at 55°C for 120min, with shaking at 40min intervals. The solutions were filtered through No. 5C filter papers (Toyo Roshi Kaisha, Ltd. Tokyo, Japan) and analyzed for Fe by colorimetry with UV-Vis spectrophotometer (UV mini 1240, Shimadzu, Kyoto, Japan). The amount of MAs was calculated based on a regression line made with the amount of Fe^{3+} solubilized from aqueous suspension of gelled Fe^{3+} as a function of amount of the authentic MA.

Root exudates of *P. chinampoensis* were further analysed to identify the type of MAs by cellulose thin-layer chromatography (TLC) according to the modified method of Kawai et al. [11]. The MAs standards employed in TLC were mugineic acid (MA), 2'-deoxymugineic acid (DMA), 3-epi-hydroxymugineic acid (epi-HMA) and hydroxymugineic acid (HMA) [28]. The solvent for TLC consisted of phenol: 28% ammonia: deionized water (15: 2: 4, by volume). The TLC plate was sprayed with 1.5mmol/L $FeCl_3$ in 60% (v/v) acetone/water, exposed to ammonia vapor in a glass chamber for 5min and then soaked in methanol for 20min to remove soluble Fe^{3+} (Fe^{3+} chelated with MAs). Then the plates were dried at room temperature and sprayed with 50% (v/v) ethanol containing 1, 10-*o*- phenanthroline (0.5%) and hydroxylamine-HCl (5%). Upon spraying the solution of the regent the sites where MAs had migrated could be detected as white spots on a reddish-orange colored background.

2.3 Preparation of Mugineic Acid (MA)

Crystalline MA for making MA solutions in assay of the effect of pH or salt in the Fe^{3+} solubilizing activity of MA from gelled Fe^{3+} was isolated from the root washing of Fe-deficient barley (*Hordeumvulgare* L. cv. Minorimugi) that was grown hydroponically according to the method of Takagi et al. [6]. Analysis by Nuclear Magnetic Resonance indicated that isolated MA was pure.

2.4 Iron^{3+} Solubilization Activity of MA from Gelled Fe^{3+} Affected by pH or Salt

2.4.1 Iron^{3+} solubilization by MA under alkaline conditions

For the measurement of Fe^{3+} solubilization activity of MA, modified Takagi's method [5] was employed. 3mL of MA solution (300µmol/L) was added to 12.5mL of deionized water. Then, 2.5mL of buffer solution of 100mmol/L N-cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid (CAPSO) was added to the solution. Then, the pH value of the solutions was adjusted to 8, 9,

10 and 11 by adding 0.1mol/L NaOH or KOH. All samples did not contain CO_3^{2-}/HCO_3^-. Subsequently, 2mL of aqueous suspension of gelled Fe^{3+} (5mmol/L) was added to the solution. The total volume of each mixed solution was adjusted to 30mL with deionized water. The final concentration of MA in these solutions was 30µmol/L. The solutions were incubated in an oven at 55°C for 120 min with shaking at 40min intervals. Then, the solutions were individually filtered through No. 5C filter paper. 1mL of HCl (conc) was added to the filtered solution, which was diluted in a volumetric flask to 50mL. The amount of Fe^{3+} solubilized was measured by ICP-OES (ICPE-9000, Shimadzu, Kyoto).

2.4.2 Iron^{3+} solubilization by MA under sodic conditions

For the measurement of Fe^{3+} solubilization activity of MA, modified Takagi's method [5] was employed. 3mL of MA solution (200µmol/L) was added to 15mL of Na_2CO_3-$NaHCO_3$ or K_2CO_3-$KHCO_3$ buffer (buffers for pH 10) with the following concentrations, 26.7mmol/L, 53.3mmol/L, 80.1mmol/L and 106.7mmol/L. Then, 2mL of suspension of gelled Fe^{3+} (5mmol/L) was added to the solutions. Deionized water was added instead of a buffer solution for control. The value of pH of the solution added with buffer was 10. Value of the pH of the control was 6. The final concentration of MA in each solution was 30µmol/L. The concentrations of CO_3^{2-}/HCO_3^- in the solutions added with buffer were 20, 40, 60 and 80mmol/L. The solutions were incubated in an oven at 55°C for 120min, with shaking at 40min intervals. Subsequently, the solutions were individually filtered through a No. 5C filter paper. 1mL of HCl (conc) was added to the filtered solution, which was diluted in volumetric flask to 50mL. The amount of Fe^{3+} solubilized was measured by ICP-OES (ICPE-9000, Shimadzu, Kyoto).

2.4.3 Iron^{3+} solubilization by MA under saline conditions

For the measurement of Fe^{3+} solubilization activity of MA, modified Takagi's method [5] was employed. 3 mL of MA solution (200µmol/L) was added to 15mL of NaCl with the following concentrations, 80mmol/L, 160mmol/L and 240mmol/L. Subsequently, 3mL of MA solution (200µmol/L) was added to 15mL of Na_2SO_4 with the following concentrations, 40mmol/L, 80mmol/L, and 120mmol/L. Then, 2mL of suspension of gelled Fe^{3+} (5mmol/L) was added to the solutions. Deionized water was added instead of NaCl or Na_2SO_4 solutions for control. Value of pH of the solutions was around 7. Value of the pH of the control was 6. The final concentration of MA in each solution was 30µmol/L. The concentrations of NaCl in the solutions were 60, 120 and 180mmol/L. The concentrations of Na_2SO_4 in the solutions were 30, 60 and 90mmol/L. The following methods were similar to those of the sodic conditions as described above.

2.5 Statistical Analyses

Experiments were conducted in triplicate. Data were subjected to an ANOVA using computer of "HP proLiant DL320 G6" in Iwate university, Japan [29]. Differences between means were evaluated using the Ryan-Einot-Gabriel-Welsch multiple range test ($p< 0.05$).

3. RESULTS

3.1 Plant Growth

The results of the dry weight of the plant parts are shown in Fig. 1. Under the alkaline (pH 10) or sodic condition (pH 10+20mmol/LNa_2CO_3/$NaHCO_3$) with Fe-depleted treatment, both the shoot and root dry weight of *P. chinampoensis* were higher than that of control (pH 6.5) (Fig. 1a). Under the sodic conditions, the shoot dry weight was lower than that of the alkaline conditions and root growth was similar to that of the alkaline condition. In barley (Fig. 1b), the shoot dry weight and root dry weight under the alkaline conditions were higher than those of the other conditions. There was no significant difference in dry weight of the barley plant parts between the sodic conditions and the control.

Table 1 shows the SPAD value of the leaves of the plants. In *P. chinampoensis*, SPAD value of the leaves in alkaline or sodic condition was higher than that of the control. The control plant showed Fe-chlorosis but the plants in alkaline or sodic condition did not show Fe-chlorosis. There was no difference between those of alkaline or sodic conditions. In barley, SPAD value of the leaves in alkaline or sodic condition was higher than that of control. However, the SPAD value of sodic condition was lower than that of alkaline condition. It was shown that the leaves of the plants grown with pH 10 were greener than those of the control.

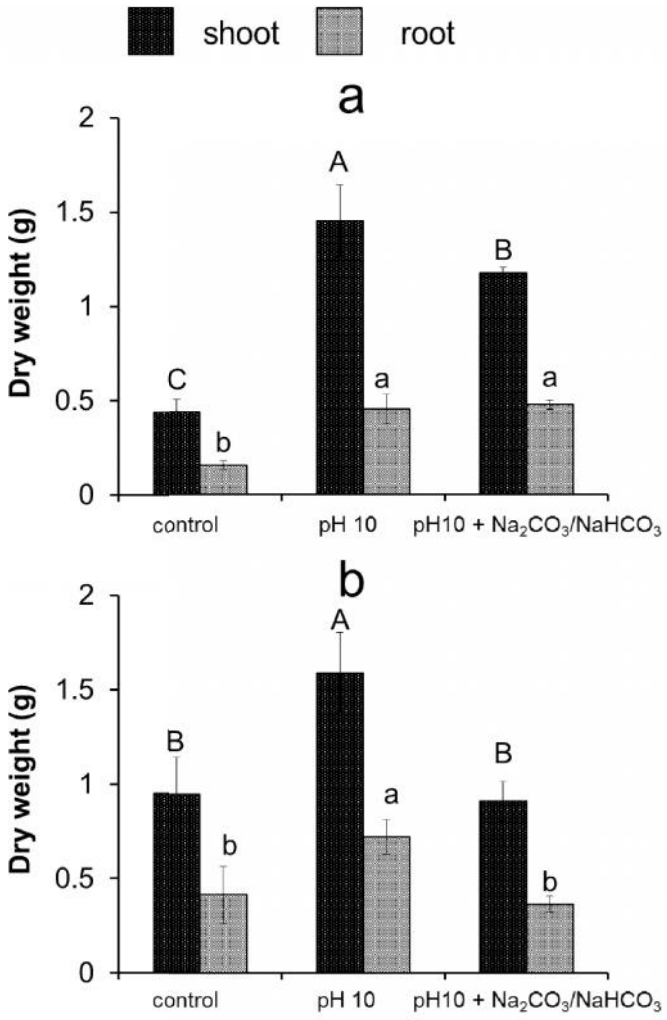

Fig. 1. Shoot or root dry weight of plants grown in each treatments (a) *P. chinampoensis*, (b) barley

Each value represents the mean ± SD (n = 3). Different letters at the top of each column indicate significant differences (p<0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test.
(a) shoot (F = 60.04, p<0.0001), root (F = 54.49, p<0.0001),
(b) shoot (F = 13.37, p<0.01), root (F = 10.05, p<0.05)

Table 1. SPAD value of leaves of each plant under control, alkaline or sodic condition

	P. chinampoensis	Barley
Control	3.80±0.25 b	4.67±0.37 c'
Alkaline condition	33.7±1.61 a	40.7±1.37 a'
Sodiccondition	34.5±4.34 a	20.2±1.38 b'

SPAD value is an indication of the amount of chlorophyll present, Data are shown as mean ± standard error (SE).Different letters indicate significant differences (p< 0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test, P. chinampoensis: F = 42.79, p< 0.0005. barley: F = 251.16, p< 0.0001

3.2 Phytosiderophores (MAs) Release of *P. chinampoensis* and Barley under Alkaline or Sodic Conditions

The amount of MAs released by roots of the plants is shown in Fig. 2. In *P. Chinampoensis* (Fig. 2a), the amount of MAs released by the roots under the alkaline condition (pH 10) was the highest among the conditions at 14 days and 21 days after transfer to Fe-depleted medium. At 14 days, the amount of MAs released by the roots under the sodic condition (pH 10+20mmol/LNa_2CO_3/$NaHCO_3$) was lower than that of alkaline condition and similar to that of

control. At 21 days, the amount of MAs released by the roots under the sodic condition was higher than that of the control, but lower than that of the alkaline condition. It was shown that addition of Na_2CO_3/$NaHCO_3$ reduced MAs release by the roots of the plant as compared with alkaline condition.

In barley (Fig. 2b), the amount of MAs released by the roots were enhanced under the alkaline condition as compared with control at 7 and 14 days after transfer to Fe-depleted medium. However, the amount of MAs under the sodic condition was largely repressed as compared with alkaline condition at 7 and 14 days. At 14 days, MAs release under sodic condition was similar to that of control.

Depressive effect of Na_2CO_3/$NaHCO_3$ on MAs release under the condition of pH 10 was clearly shown in both plants.

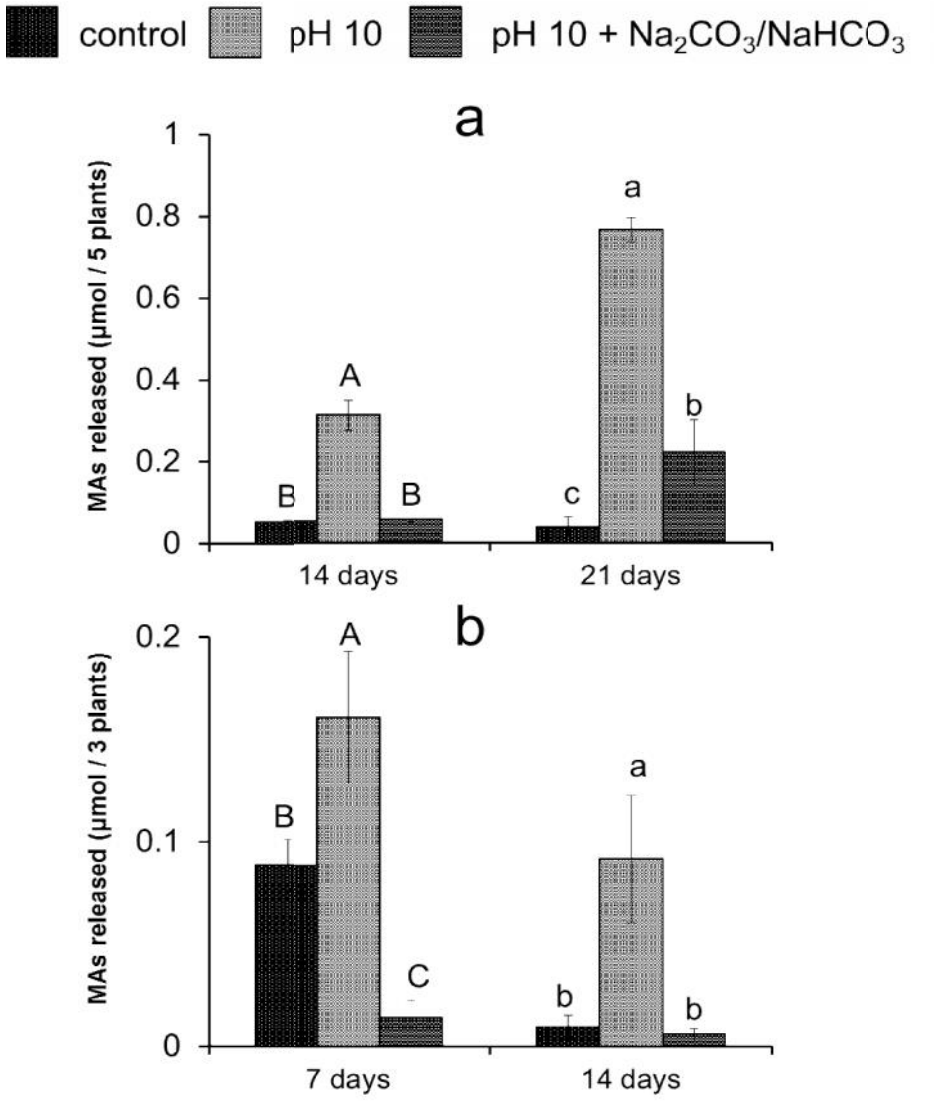

Fig. 2. Amount of mugineic acid family of phytosiderophores (MAs) released by roots of the plants grown in each treatments (a) *P. chinampoensis*, (b) barley

Each value represents the mean ± SD (n = 3). Different letters at the top of each column indicate significant differences (p<0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test, (a)14 days (F = 155.38, p<0.001), 21 days (F = 162.15, p<0.001), (b) 7 days (F = 37.94, p<0.0005), 14 days (F = 21.09, p<0.005)

Thin-layer chromatograms of MAs released by the roots of *P. chinampoensis* grown under the Fe-depleted condition of the control, alkaline and sodic condition are shown in Fig. 3. The prepared roots exudates from *P. chinampoensis* may contain mugineic acid (MA), hydroxymugineic acid (HMA) and a smaller amount of *epi*-hydroxy mugineic acid (epi-HMA).

3.3 Iron^{3+} Solubilization Activity of MA from Gelled Fe^{3+} Affected by pH or Salt

3.3.1 Iron^{3+} solubilization by MA under alkaline conditions

The amount of the Fe^{3+} solubilized by MA under alkaline conditions is shown in Fig. 4. In the solutions whose pH were adjusted by N-cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid (CAPSO) and 0.1mol/L NaOH (Fig. 4a), the amount of solubilized Fe^{3+} was the highest at pH 9. The amount of solubilized Fe^{3+} was similar at pH 8 and pH 10 and was considerably lower at pH 11. In the solutions whose pH were adjusted byN-cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid (CAPSO) and 0.1 mol/L KOH (Fig. 4b), the amount of solubilized Fe^{3+} was almost stable in the range between pH 8-10. At pH 11, the amount of solubilized Fe^{3+} was considerably lower.

3.3.2 Iron^{3+} solubilization by MA under sodic conditions

The amount of the Fe^{3+} solubilized by MA under sodic conditions with a higher concentration of CO_3^{2-}/HCO_3^- is shown in Fig. 5. In the solutions added with Na_2CO_3-$NaHCO_3$ buffer (pH 10) (Fig. 5a), the amount of solubilized Fe^{3+} by MA was much lower than compared with that of the control, and there was no significant difference among the treatments. In the solutions added with K_2CO_3-$KHCO_3$ buffer (pH 10) (Fig. 5b), amount of solubilized Fe^{3+} by MA of the solutions added with alkaline buffer was also much lower compared with that of the control, and there were no significant differences among the treatments.

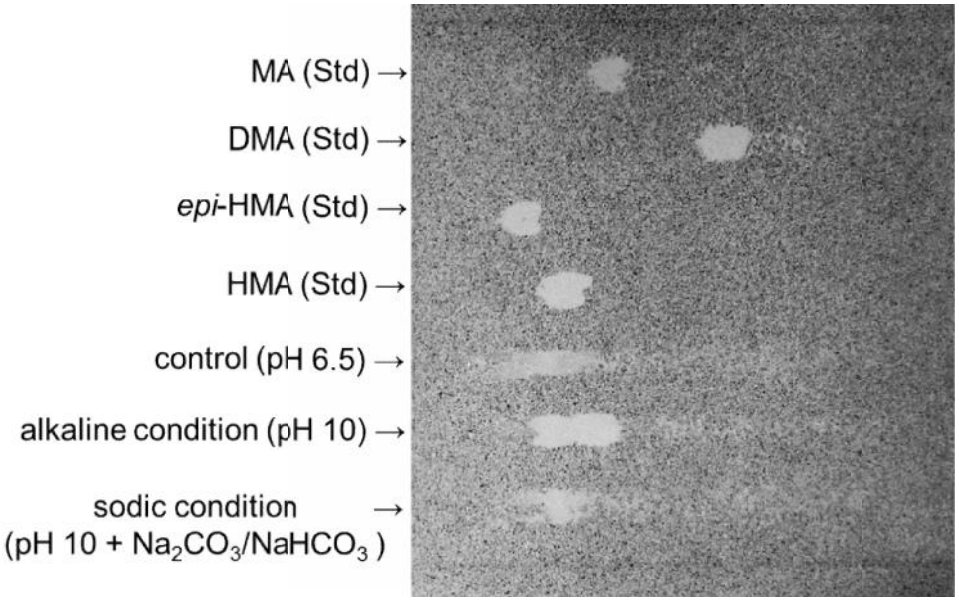

Fig. 3. Thin-layer chromatograms of root exudates released from *Puccinellia chinampoensis* Ohwigrown under Fe-depleted conditions of control (pH 6.5), alkaline condition (pH 10) and sodic condition (pH 10+ 20mmol/L Na_2CO_3/$NaHCO_3$) and compounds of the mugineic acid family of phytosiderophores (MAs) (Std: authentic compound, MA: mugineic acid, DMA: deoxymugineic acid, epi-HMA: epi-hydroxymugineic acid, HMA: hydroxymugineic acid)

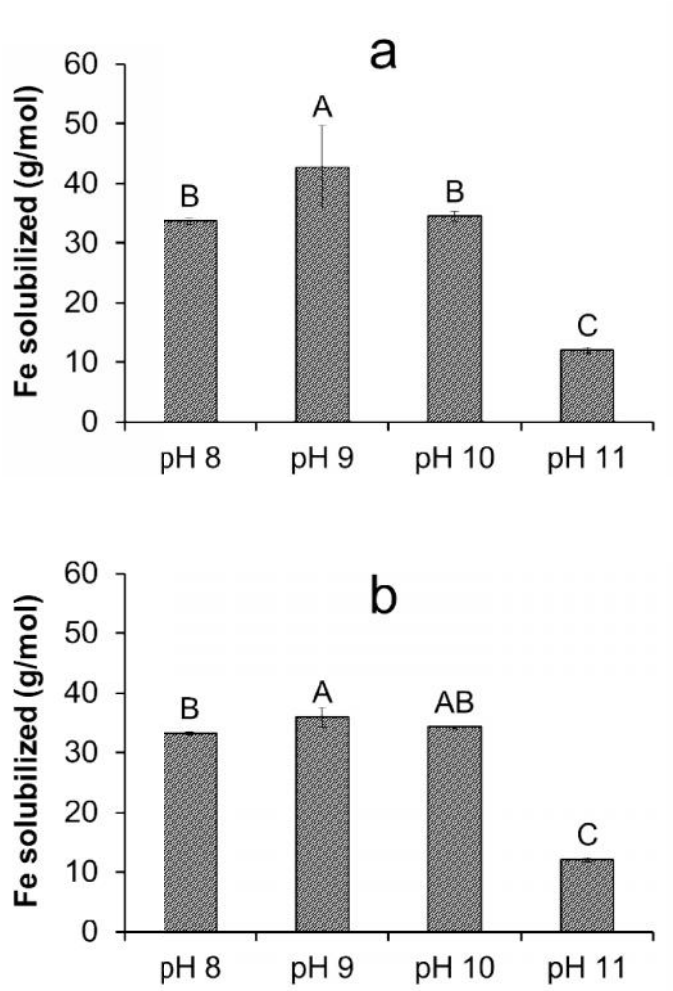

Fig. 4. Amount of Fe^{3+} solubilized by mugineic acid (MA) in the alkaline solutions whose pH was adjusted by N-cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid (CAPSO) and (a) NaOH or (b) KOH

Each value represents the mean ± SD (n = 3). Different letters at the top of each column indicate significant differences (p<0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test.
(a) F = 40.80, p<0.001, (b) F = 510.39, p<0.001

3.3.3 Iron^{3+} solubilization by MA under saline conditions

The amount of the Fe^{3+} solubilized by MA under saline conditions is shown in Fig. 6. In the solutions added with NaCl (pH7) (Fig. 6a), the amount of solubilized Fe^{3+} by MA was similar to that of control and there were no significant differences among the treatments. In the solutions added with Na_2SO_4 (pH7) (Fig. 6b), amount of solubilized Fe^{3+} by MA was similar to that of control and there were no significant differences among the treatments.

4. DISCUSSION

Under the alkaline (pH 10) and sodic condition (pH 10 + Na_2CO_3/$NaHCO_3$) with Fe-depleted treatment, the dry weights of roots and shoots of *P. chinampoensis* were higher than that of the control (pH 6.5) (Fig. 1a). In barley, the growth of roots and shoots under the alkaline condition was higher than those of the other conditions (Fig. 1b) and the growth under the sodic condition was similar to that of control (Fig. 1b). It seems that the alkaline condition could enhance the growth of both plants under Fe-depleted treatment. The reason why growth of the plants was enhanced under pH 10 and Fe-depleted

treatments may be due to greening of the leaves (Table 1), resulting in higher photosynthesis activity. The phenomenon that *P. chinampoensis* did not show Fe-chlorosis was similar to that of the plant grown under sodic condition in the actual field. It was thought that the plants could effectively utilize Fe which was absorbed before Fe-depleted treatment. Fe may be more available in the plant tissues and induce chlorophyll formation when plants are grown under pH 10 conditions. The physiological mechanism of the greening under the condition of pH 10 is not known now, which needs to be investigated in the future.

It was shown that the sodic condition could not promote the growth of barley. But the growth of *P. chinampoensis* was higher under the sodic condition than the control. It seems that presence of Na_2CO_3/$NaHCO_3$ repressed growth under the condition of pH 10, but the growth of the plant was still higher than the control (pH 6.5). Therefore, it is considered that *P. chinampoensis* could adapt to the sodic condition.

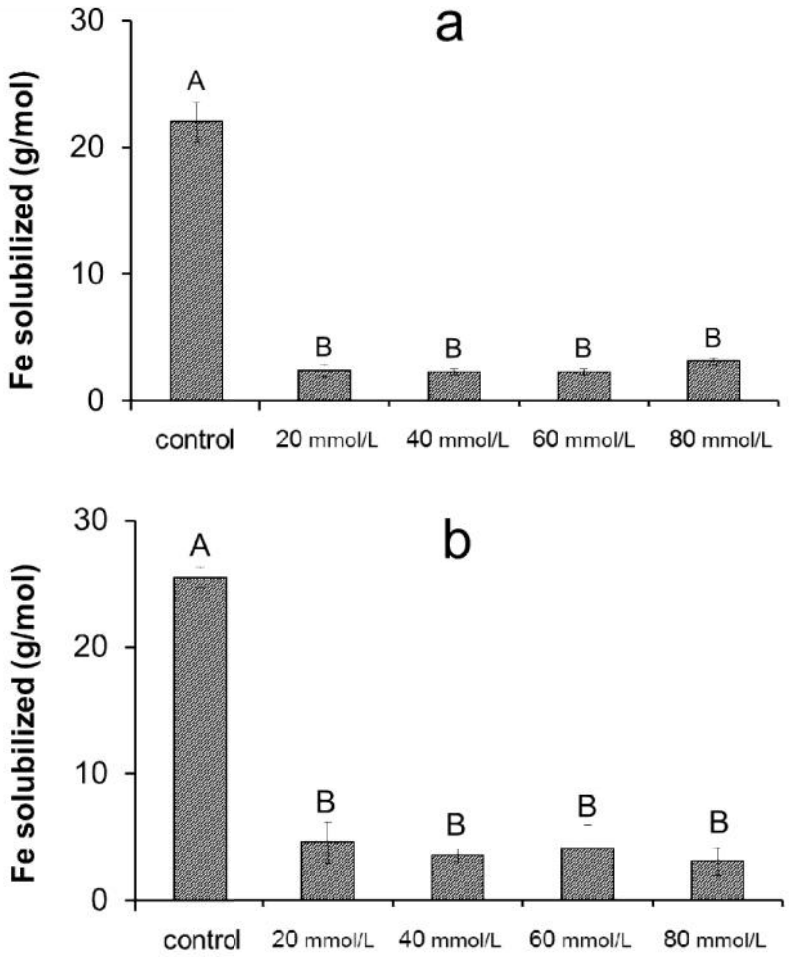

Fig. 5. Amount of Fe^{3+} solubilized by MA in the solutions with varied concentration of (a) Na_2CO_3-$NaHCO_3$ buffer (buffer for pH 10) or (b) K_2CO_3-$KHCO_3$ buffer (buffer for pH 10). Value of pH of the solutions was 10 and that of control was 6

Each value represents the mean ± SD (n = 3). Different letters at the top of each column indicate significant differences (p<0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test
(a) F = 392.73, p<0.001, (b) F = 169.78, p<0.001

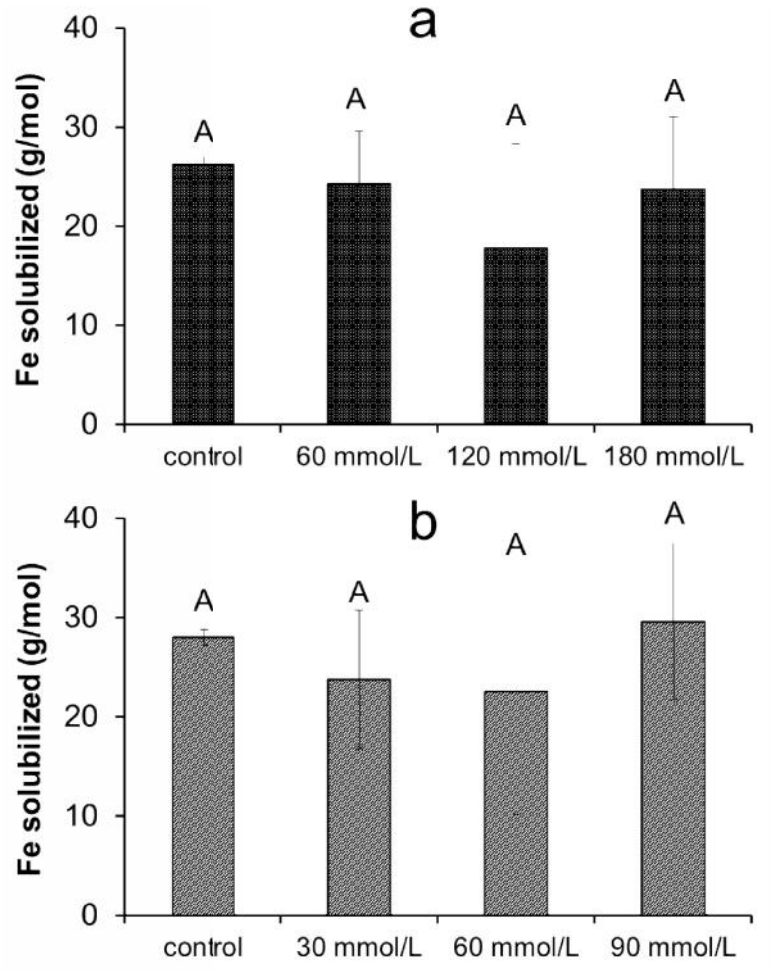

Fig. 6. Amount of Fe^{3+} solubilized by MA in the solutions with varied concentration of (a) NaCl or (b) Na_2SO_4. Value of pH of the solutions was 7 and that of control was 6

Each value represents the mean ± SD (n = 3). Different letters at the top of each column indicate significant differences (p<0.05) according to the Ryan-Einot-Gabriel-Welsch multiple range test (a) F = 0.82, (b) F = 0.52

Under the alkaline condition, *P. chinampoensis* released the highest amount of MAs among the conditions at both 14 days and 21 days (Fig. 2a). At 14 days, the amount of MAs released by the roots of *P. chinampoensis* grown under the sodic condition was similar to that of the control, but it increased at 21 days though the amount of MAs released in the control did not increase (Fig. 2a). In barley, the amount of MAs released by the roots under the alkaline condition was the highest among those of the other conditions at both 7 days and 14 days (Fig. 2b). The amount of MAs released by the roots of barley under the sodic condition was the lowest among the other conditions at both 7 days and 14 days (Fig. 2b). Under the sodic condition, the amount of MAs release in barley was lower than that of the control at 7 days, and it was similar amount to that of control at 14 days (Fig. 2b). It seems that the alkaline condition enhanced MAs secretion in both plants, which is probably due to induction of Fe-deficiency response in the roots. Solubility of Fe^{3+} is extremely lower under pH 10 conditions than pH 6.5 conditions. However, the leaves of the plants showed greening and no chlorosis. It can be considered that the Fe-deficiency responses, the MAs release and Fe-chlorosis, were induced specifically in roots and not in the leaves. As a result, owing to the photosynthate supplied from green leaves, MAs formation and release may be enhanced.

It was shown that the sodic condition enhanced MAs secretion in *P. chinampoensis* and lowered the MAs secretion in barley as compared with the control. It is well known that barley has the higher MAs secretion ability in their roots than the other grasses under the Fe deficiency [3,11,12,13]. It was noticeable that MAs secretion in *P. chinampoensis* was not repressed by the sodic condition though MAs secretion by barley was repressed by the sodic condition. This result shows one of the adaptation mechanisms of *P. chinampoensis* to sodic conditions.

Plants suffer from the damaging effects of both alkaline and salt stress under sodic conditions due to excessive Na^+ and CO_3^{2-}/HCO_3^- [18]. Yousfi et al. [30] suggested that NaCl may inhibit the secretion of MAs of grasses under pH 6.0 condition. Therefore, a large amount of Na^+ may inhibit the secretion of MAs in grasses under sodic conditions. Additionally, high amounts of CO_3^{2-}/HCO_3^- may also interfere with the MAs secretion ability of grasses under sodic conditions. The high pH condition may cause Fe deficiency in roots more severely and activate formation and release of MAs, but Na^+ and CO_3^{2-}/HCO_3^- may reduce formation and/or release activity of MAs under sodic conditions. Probably, biosynthetic pathway and release process of MAs in the plant are not inhibited by high alkaline conditions (pH 10). One or both of biosynthesis and release of MAs may be repressed by Na^+ and CO_3^{2-}/HCO_3^- in barley. However, in *P. chinampoensis*, there may be a defense mechanism to Na^+ and CO_3^{2-}/HCO_3^- in the biosynthetic pathway and release process of MAs.

It was shown by using the method applied to the grasses in the previous report [25,28]. MAs were released by the roots of barley and *P. chinampoensis* by TLC (Fig. 3). *P. chinampoensis* may secrete MAs, such as mugineic acid (MA), hydroxymugineic acid (HMA) and small amount of epi-hydroxymugineic acid (*epi*-HMA) in the Fe-depleted medium (Fig. 3). Therefore, it is considered that *P. chinampoensis* secretes MAs in the rhizosphere of sodic soils and function there for Fe^{3+} acquisition. For precise identification of MAs released by the plant, more analysis is necessary.

The effect of high pH on Fe^{3+} solubilization from gelled Fe^{3+} by MA is shown in Fig. 4, the Fe^{3+} solubilization activity of MA was maintained up to pH 10 and repressed chemically at pH 11, which was consistently shown in Na^+ or K^+ CAPSO buffer solution. In the experiment without use of CAPSO, similar results were obtained (data not shown). However, in the experiment where Na_2CO_3-$NaHCO_3$ or K_2CO_3-$KHCO_3$ buffer (pH 10) was added, the amount of solubilized Fe^{3+} was reduced by adding the buffer solutions (Fig. 5). However, the amount of solubilized Fe^{3+} was not affected by adding NaCl or Na_2SO_4 (pH 7) (Fig. 6). Thus, it was indicated that the Fe^{3+} solubilization activity of MA was largely repressed by CO_3^{2-}/HCO_3^- under the condition of pH 10. Therefore, Fe^{3+} solubilization activity of MA released by the plant roots in the rhizosphere of sodic soils is considered to be repressed by CO_3^{2-}/HCO_3^-. It is known that MAs forms an octahedral configuration complex with Fe^{3+} [31,32]. The hybrid orbital of the Fe^{3+} may bind with orbital of oxygen of CO_3^{2-}/HCO_3^- molecule and the ligand may compete with the orbital of oxygen or nitrogen of MAs molecule, resulting in repression of chelation of Fe^{3+} by MAs. In fact, the formation constant of Fe-HCO_3^- is 20.78 [33], and it is greater than that of Fe^{3+}-MA, 17.7 [34]. This may be the reason why Fe^{3+} solubilized by MA from gelled Fe^{3+} was reduced under the sodic conditions (Fig. 5). Fig. 6 shows the effect of Cl^- or SO_4^{2-}on Fe^{3+} solubilization activity of MAs. There was no substantial effect of the anions to the activity. The results suggested that addition of acids such as HCl or H_2SO_4 to the sodic soils in the actual field would vaporize CO_3^{2-}/HCO_3^- into CO_2 and promote the effectivity of MAs to solubilize Fe^{3+}. The effect will be accompanied with a pH lowering effect by the acids in the rhizosphere of sodic soils.

It has been shown that MAs can solubilize Fe^{3+} though the Fe^{3+} solubilization activity of MAs is much repressed by CO_3^{2-}/HCO_3^- under sodic conditions (Fig. 5). Therefore, it is considered that grasses grown under sodic conditions need to secrete a high amount of MAs for Fe^{3+} acquisition. The MAs secretion ability of *P. chinampoensis* with lower repression by CO_3^{2-}/HCO_3^- than barley is considered to be advantageous to Fe nutrient utilization and survival in sodic soils.

Possibly, *P. chinampoensis* has specific efflux transporter for the release of Fe^{3+}- MAs complex similar to the other grasses (TOM) [10]. At present, it is not known whether the activity of MAs releasing protein of the plant is affected by high pH, Na^+ or CO_3^{2-}/HCO_3^-. Some grasses absorb Fe^{3+}-MAs through the specific transporter (YS1) of roots and convey Fe to the xylem vessel [8,9]. *P. chinampoensis* may have YS1 and

utilize it for Fe^{3+}-MAs transport in the rhizophere of sodic soils. Furthermore, it is reported that Strategy II plants can also uptake Fe^{2+} like Strategy I plants [35]. How such genes are affected by CO_3^{2-}/HCO_3^- has not been investigated. Further study is required to reveal the mechanism how *P. chinampoensis* utilizes Fe under sodic conditions and the contribution of MAs to the Fe nutrient utilization for the survival of grasses grown in sodic soils in the future.

5. CONCLUSION

The secretion of MAs from the roots of *Puccinellia chinampoensis* Ohwi and Fe^{3+} solubilization activity of MA were examined under sodic and Fe-depleted conditions. It was clarified that *P. chinampoensis* can secrete MAs and the amount of released MAs increased under the alkaline or sodic condition as compared with that of the control (pH 6.5). Also, it was suggested that Fe^{3+} solubilization activity of MA was highly repressed by CO_3^{2-}/HCO_3^- under the sodic conditions. Because of the repression of Fe^{3+} solubilization activity of MA by CO_3^{2-}/HCO_3^-, it seems that the grasses grown in sodic soils need to secrete a high amount of the MAs to acquire Fe^{3+}. Therefore, it was considered that plants with higher secretion ability of MAs under sodic conditions will be able to survive in sodic soils. It was also shown that MAs release of *P. chinampoensis* was less reduced by Na^+ and CO_3^{2-}/HCO_3^- than that of barley. Thus, *P. chinampoensis* may have the mechanism to adapt to sodic conditions. It was also suggested that addition of HCl or H_2SO_4 to the sodic soils might have an effect of erasing the repression effect of CO_3^{2-}/HCO_3^- on MAs activity.

It is prospected that the world population will increase in the future. Therefore, optimizing land use for foods production is an important global issue and the land area available for food production needs to be increased [36]. Thus, preventing land from degradation such as soil sodification is an important global issue in the 21st century.

ACKNOWLEDGEMENT

The authors gratefully acknowledge a Grant-in-Aid for Scientific Research (C) from the Japan Society for Promoting of Science (No. 25450078). The authors thank Mr. Mark de Boer of Iwate University for the correction of the English in the manuscript and Dr. Akira Nagasawa of Saitama Unversity for the analysis of mugineic acid by NMR. The first author thanks Mr. Shigeru Kamei for his kind assistance and valuable discussion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Miller GW, Huang IJ, Welkie GW, Pushnik JC. Function of iron in plants with special emphasis on chloroplasts and photosynthetic activity. In: Abadía J, editor. Iron Nutrition in Soils and Plants. Dordrecht: Kluwer Academic Publishers; 1995. ISBN 0-7923-2900-7.
2. Cianzio SR. Strategies for the genetic improvement of Fe efficiency. In: Abadía J, editor. Iron Nutrition in Soils and Plants. Dordrecht: Kluwer Academic Publishers; 1995. ISBN 0-7923-2900-7.
3. Marschner H. Mineral nutrition of higher plants. 2nd ed. London: Academic Press; 1995. ISBN13: 978-0-12-473543-9.
4. Abadía J, Vázquez S, Rellán-Álvarez R, El-Jendoubi H, Abadía A, Álverez-Fernández A, López-Millán AF. Towards a knowledge-based correction of iron chlorosis. Plant Physiol. Biochem. 2011;49: 471-482.
5. Takagi S. Naturally occurring iron-chelating compounds in oat-and rice-root washings. Soil Sci. Plant Nutr. 1976;22(4): 423-433.
6. Takagi S, Nomoto K, Takemoto T. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J. Plant Nutr. 1984;7(1-5): 469-477.
7. Yoshida T, Kawai S, Takagi S. Detection of the regions of phytosiderophore release from barley roots. Soil Sci. Plant Nutr. 2004;50(7):1111-1114.
8. Roberts LA, Pierson AJ, Panaviene Z, Walker EL. Yellow Stripe1. expanded roles for the maize iron-phytosiderophore transporter. Plant Physiol. 2004;135:112-120.
9. Murata Y, Ma JF, Yamaji N, Ueno D, Nomoto K, Iwashita T. A specific transporter for iron (lll)-phytosiderophore in barley roots. The Plant J. 2006;46:563-572.
10. Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK.

Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. J. Biol. Chem. 2011;286(7):5446-5454.
11. Kawai S, Takagi S, Sato Y. Mugineic acid-family phytosiderophores in root-secretions of barley, corn and sorghum varieties. J. Plant Nutr. 1988;11(6-11):633-642.
12. Takagi S. Mugineic acids as examples of root exudates which play an important role in nutrient uptake by plant roots. In: Johansen C, Lee KK, Sahrawat KL, editors. Phosphorus nutrition of grain legumes in the semi-arid tropics. Patancheru: International Crops Research Institute for the Semi-Arid Tropics; 1991.
13. Kawai S, Sasaki O, Hayasaka Y, Takagi S. Biosynthesis of avenic acid A in oat cv. Onward: studies with 14C or 15N labeled compounds. In: Abadía J, editor. Iron Nutrition in Soils and Plants. Dordrecht: Kluwer Academic Publishers; 1995. ISBN 0-7923-2900-7.
14. Takagi S, Kamei S, Yu MH. Efficiency of iron extraction from soil by mugineic acid family phytosiderophores. J. Plant Nutr. 1988;11(6-11): 643-651.
15. Dregne HE. Desertification of arid lands. In: El-Baz F, Hassan MHA, editors. Physics of desertification. New York: Harwood Academic Publishers; 1986. ISBN 3-7186-0168-0.
16. Tanji KK. Salinity in the soil environment. In: Läuchli A, Lüttge U, editors. Salinity: environment-plants-molecules. Dordrecht: Kluwer Academic Publishers; 2002. ISBN 978-1-4020-0492-6.
17. Bear FE. Chemistry of the soil. 2nd ed. New York: Reinhold Publishing Corporation; 1964. ISSN 0065-7751.
18. Yang CW, Jianaer A, Li CY, Shi DC, Wang DL. Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali-resistant halophyte *Chloris virgata*. Photosynthetica. 2008;46(2):273-278.
19. Kawanabe S, Zhu TC. Degeneration and conservational trial of *Aneurolepideum chinense* grassland in Northern China. J Japan Grassl Sci. 1991;37(1):91-99.
20. Bian J, Tang J, Lin N. Relationship between saline-alkali soil formation and neotectonicmovement in Songnen Plain, China. Environ Geol. 2008;55:1421-1429.
21. Wang L, Seki K, Miyazawa T. The causes of soil alkalinization in Songnen Plain of Northeast China. Paddy Water Environ. 2009;7:259-270.
22. Zhao MQ, Lin QB, Chen GG, Kai XA, Chun ZY. Effect of different stage of Nitrogen application on seed and herbage yield of *Puccinellia chinampoensis* Ohwi. Agr Tech. 2007;27(3):59-61. Chinese with english summary.
23. Wang RZ, Yuan YQ. Photosynthesis, transpiration and water use efficiency of two *Puccinellia* species on the Songnen grassland, northeastern China. Photosynthetica. 2001:39(2):283-287.
24. Wang Y, Wang Z, Han Z, Liang Z, Yang F, An F. Enzyme activities of saline-sodic soils under different vegetation types in the Songnen Plain, Northeast China. J Food Agr Environ. 2013;11(3&4):1982-1985.
25. Kawai S, Itoh K, Takagi S. Incorporation of ^{15}N and ^{14}C of methionine into the mugineic acid family of phytosiderophores in iron-deficient barley roots. Physiol. Plant. 1993;88: 668-674.
26. Kudo K, Kudo H, Fujikawa K, Kawai S. The release of copper-induced phytosiderophores in barley plants is decreased by cadmium stress. Botany. 2013;91:568-572.
27. Wallance A, Jones JB Jr. Sample preparation and determination of Iron in biological materials. In: Barton LL, Hemming BC, editors. Iron chelation in plants and soil Microorganisms. San Diego: Academic press; 1993. ISBN: 0-12-079870-0.
28. Takagi S. Production of phytosiderophores. In: Barton LL, Hemming BC, editors. Iron chelation in plants and soil Microorganisms. San Diego: Academic press; 1993. ISBN: 0-12-079870-0.
29. SAS Institute 2011. SAS/STAT 9.3 User's Guide. North Carolina: Cary; 2011.
30. Yousfi S, Wissal M, mahmoudi H, Abdelly C, Gharsalli M. Effect of salt on physiological responses of barley to iron deficiency. Plant Physiol. Biochem. 2007;45: 309-314.
31. Kato M, Izuka S, Fujihara T, Nagasawa A, Kawai S, Tanaka T, Takayanagi T. Electronic structure calculation study of metal complexes with a phytosiderophore mugineic acid. Inorg. Chim. Acta. 2011;370: 304-310.
32. Sugiura Y, Nomoto K. Phytosiderophores structures and properties of mugineic acids and their metal complexes. In: Clarke MJ,

Hill C, Goodenough JB, Ibers JA, Jorgensen CK, Mingos DMP et al, editors. Structure and bonding 58. Berlin: Springer-Verlag; 1984. ISBN: 3-540-13649-5.

33. Geen VA, Robertson AP, Leckie JO. Complexation of carbonate species at the goethite surface: Implications for adsorption of metal ions in natural waters. Geochim. Cosmochim. Acta. 1994;58:2073-2086.
34. Von Wiren N, Khodr H, Hider RC. Hydroxylated phytosiderophore species possess an enhanced chelate stability and affinity for Iron (Ⅲ). Plant Physiol. 2000;124:1149-1158.
35. Kim SA, Guerinot ML. Mining iron: Iron uptake and transport in plants. FEBS Letters. 2007;581: 2273-2280.
36. Eswaran H, Beinroth F, Reich P. Global land resources and population-supporting capacity. Am. J. Alternative Agric. 1999;14:129-136.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=776&id=24&aid=6743