



## High Phosphorus Utilization and Silicon Amelioration of Aluminium Toxicity by *Puccinellia chinampoensis* Ohwi under Sodic Conditions

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### Authors' contributions

This work was carried out in collaboration among the all authors. Authors TY, HK, KS, S. Kamei and S. Kawai designed the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors LZ, HBW and AS gave the much essential information about the sodic soil in Songnen plain in northeast China. All authors read and approved the final manuscript.

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### ABSTRACT

In the rhizosphere of sodic soil, aluminium (Al) and silicon (Si) are present in soluble forms. Hence, plants growing in sodic soils suffer Al toxicity, which can be ameliorated by Si. The aim of this study was to investigate the effect of Al and Si on the growth of *Puccinellia chinampoensis* Ohwi (*P. chinampoensis*), a sodic-tolerant grass, under sodic conditions in hydroponic cultivation. Barley (*Hordeum vulgare* L. cv. Minorimugi) was used as a control. Plant growth under alkaline conditions

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[pH 10 without Sodium carbonate/bicarbonate ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ )] and sodic conditions (pH 10 with  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ ) in the presence and absence of added Al and Si were compared. Phosphorus (P), sulphur (S), Si and potassium (K) contents were then analysed by particle induced X-ray emission. Under sodic conditions, P, S, Si and K uptakes of *P. chinampoensis* were enhanced, and Al toxicity was alleviated. In addition, Si amelioration of Al toxicity in *P. chinampoensis* was enhanced under sodic conditions. On the other hand, the uptake of P, S, Si and K of barley was repressed under sodic conditions, and Si amelioration of Al toxicity was weakened. Phosphorus, S, Si, and K uptake in barley was not repressed under alkaline conditions (pH of around 10 and no  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  added). The results suggested that sodium ion ( $\text{Na}^+$ ) and carbonate/bicarbonate ions ( $\text{CO}_3^{2-}/\text{HCO}_3^-$ ) repressed barley uptake of ions, such as potassium ion ( $\text{K}^+$ ), phosphate ion ( $\text{PO}_4^{3-}$ ), sulfate ion ( $\text{SO}_4^{2-}$ ) or silicate ion ( $\text{SiO}_4^{4-}$ ), and that Al toxicity was enhanced under sodic conditions. In *P. chinampoensis*, the uptake of ions was activated by  $\text{Na}^+$  and  $\text{CO}_3^{2-}/\text{HCO}_3^-$ , resulting in increased Si amelioration of Al toxicity and Al tolerance by high nutrient uptake. Thus, *P. chinampoensis* appears to be well adapted to sodic conditions. Further studies of the nutritive properties of sodic-tolerant plants are needed to enable their use in the promotion of revegetation of sodic soils in the future.

**Keywords:** *Puccinellia chinampoensis* Ohwi; sodic soil; sodium carbonate/bicarbonate; aluminum toxicity; silicon amelioration; surfur; phosphorus; potassium; particle induced X-ray emission.

## 1. INTRODUCTION

Land desertification in dry regions is an increasing problem [1]. In recent years, soil degradation has been identified as a problem in both arid and semi-arid areas of the world, including Africa, Asia, North and South America and Spain [1]. One factor involved in soil degradation is salt accumulation, particularly sodium (Na). About 10% of the total arable land in the world consists of saline and sodic soils [2]. In terms of cultivated land, about 23% is saline, and 37% is sodic [2]. Soil sodification refers to the process of soil alkalization in arid or semi-arid areas. Accumulated sodium carbonate/bicarbonate ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ ) increases the soil pH and Electrical Conductivity (EC), thereby retarding plant growth [3]. Among soil types, sodic soil is one of the most alkaline and barren and restricts agricultural production worldwide [2-4]. The prevention of soil sodification is an important global issue in the 21st century. Revealing characteristics of plant nutrition in sodic soils is essential for revegetation of sodic soils.

A sodic soil is defined as one with an exchangeable sodium percentage (ESP) greater than 15, EC of saturation extract less than  $4 \text{ dS m}^{-1}$  and a pH range between 8.5 and 10 [4]. Sodic soils are often classified based on their soil pH, soil EC and ESP [4]. However, in previous work, we concluded that soil factors other than soil pH, soil EC and ESP (i.e. solubilization of metals) characterized sodic

soils [5]. Generally, plant metal micronutrients, such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn), are considered to be precipitated and immobilized in alkaline soils, resulting in low levels of solubility of plant metal micronutrients [3]. Thus, phytosiderophores, such as those belonging to the mugineic acid family (MAs), are important in the uptake of metal micronutrients by graminaceous plants to enhance the solubilization of metal micronutrients [3, 6-14]. Previous work revealed that the efficiency of MAs was repressed under sodic conditions [15]. Research also revealed that metal micronutrients dissolved into water in the rhizosphere of sodic soils and that a large amount of Fe, Al and Si existed in water-soluble forms in the rhizosphere [5]. The same study concluded that chelation by water-soluble humic substances and the formation of aluminate ion [ $\text{Al}(\text{OH})_4^-$ ] and silicate ion ( $\text{SiO}_4^{4-}$ ) under high pH conditions (i.e. pH of around 10) were responsible for solubilization of specific metals.

In the Songnen Plain in northeast China, the expansion of sodic areas has been a serious problem since the middle of the 20<sup>th</sup> century [16, 17]. The expansion is due to natural factors, such as parent materials, topographic positions and the region's arid/semi-arid climate [16,17]. Anthropogenic factors, such as population pressure, overgrazing and poorly considered agricultural and economic policies, have contributed to the expansion of sodic areas in the region [16,17]. The Academy of Agriculture Science of Jilin Provinces in China has been

managing the Songnen Plain, using *Puccinellia chinampoensis* Ohwi, a sodic-tolerant gramineous plant, as revegetation [18].

Generally, plants grown in sodic soils are thought to suffer alkaline and saline stress due to the soil's high soil pH and EC [19-27]. Previous research revealed that *P. chinampoensis* showed superior tolerance to both alkaline and saline stress [28,29]. *P. chinampoensis* also exhibited high selectivity for metal macronutrients (potassium [K<sup>+</sup>], calcium [Ca<sup>2+</sup>] and magnesium [Mg<sup>2+</sup>]) over sodium (Na<sup>+</sup>) under sodic conditions (i.e. a soil pH greater than 10 by Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>) [28,29]. In addition, *P. chinampoensis* showed high root growth ability under sodic conditions [15]. Thus, repression of MA secretion by *P. chinampoensis* under sodic conditions was much lower than MA secretion by barley [15].

Sodic-tolerant plants, such as *P. chinampoensis*, are thought to have particular properties that enable them to survive in sodic soil. High concentrations of Al and Si in the rhizosphere of sodic soil are assumed to affect plant growth [5]. The results of previous studies pointed to Al toxicity of crops grown in alkaline soil [30-34]. Thus far, there have been no studies of Si solubilization in the rhizosphere of sodic soil. Furthermore, to the best of our knowledge, no studies have examined the correlation between Al toxicity and Si amelioration and subsequent effects on plant growth in sodic soil.

We hypothesized that sodic-tolerant plants would show specific responses to Al and Si in soil. The aim of this study was to investigate the effect of Al and Si on plant growth under sodic conditions. With this aim in mind, we examined the absorption of elements by *P. chinampoensis*, a sodic-tolerant plant, and subsequent effects on Al toxicity and Si amelioration of Al toxicity under sodic conditions.

## 2. MATERIALS AND METHODS

### 2.1 Pre-treatments of Plants

Seeds of *P. chinampoensis* were obtained from A. K. Xu of the Academy of Agriculture Science of Jilin provinces in China. Seeds of barley (*Hordeum vulgare* L. cv. Minorimugi) were obtained from plants grown in fields belonging to Iwate University.

*P. chinampoensis* plants were grown as described in a previous study [15]. The seeds

were placed on a sterilized plastic net, overlaid with 1 mmol/L of CaCl<sub>2</sub> solution in a plastic box and germinated. The box containing the seed was then placed in a growth chamber (KG-206HI, Koito Industries Ltd., Tokyo, Japan) with a day-night regime of 14 h of 280 μmol/m<sup>2</sup>·s<sup>-1</sup> light at 17°C and 10 h dark at 10°C. *P. chinampoensis* and barley seeds remained under these conditions for 7 days and 3 days, respectively, in light-shielded conditions. The seedlings were then grown under artificial light. The CaCl<sub>2</sub> solution in the seed box was replaced with 1/5-strength modified Hoagland and Arnon No. 2 medium [35,36]. Twenty-one days later, *P. chinampoensis* seedlings were transplanted into 10 L buckets and grown in 1/2-strength modified Hoagland and Arnon No. 2 medium, consisting of 3 mmol/L of KNO<sub>3</sub>, 2 mmol/L of Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mmol/L of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 1 mmol/L of MgSO<sub>4</sub>, 10 μmol/L of Fe<sup>3+</sup>-EDTA, 1.5 μmol/L of H<sub>3</sub>BO<sub>3</sub>, 0.25 μmol/L of MnSO<sub>4</sub>, 0.1 μmol/L of CuSO<sub>4</sub>, 0.2 μmol/L of ZnSO<sub>4</sub> and 25 nmol/L of H<sub>2</sub>MoO<sub>4</sub> (pH 5.5), with continuous aeration for 7 days. The barley seedlings were also transplanted into 10 L buckets 7 days after growth in 1/5-strength medium and grown in 1/2-strength medium, with continuous aeration for 3 days.

### 2.2 Plant Growth with Added Al or Si under Sodic Conditions

*P. chinampoensis* seedlings were transplanted again, with five plants per bunch transplanted to an aerated treatment medium for 28 days. Barley seedlings (three plants per bunch) were transplanted, to the same type of aerated treatment medium for 21 days. The treatment medium (1/2-strength modified Hoagland and Arnon No. 2 medium) was prepared under both alkaline conditions and sodic conditions. Al or Si was added as follows: Treatment 1 (control), 1/2-strength medium (pH 10), alkaline conditions (pH 10); Treatment 2, alkaline and Al conditions, 1/2-strength medium, with 300 μmol/L of AlCl<sub>3</sub>·6H<sub>2</sub>O (pH 10) added; Treatment 3, alkaline and Si conditions, 1/2-strength medium, with 500 μmol/L of Na<sub>2</sub>SiO<sub>3</sub> (pH 10) added; and Treatment 4, alkaline, Al and Si conditions, 1/2-strength medium, with 300 μmol/L of AlCl<sub>3</sub>·6H<sub>2</sub>O and 500 μmol/L Na<sub>2</sub>SiO<sub>3</sub> (pH 10) added. Under the sodic conditions (pH 10 with HCO<sub>3</sub><sup>-</sup>/CO<sub>3</sub><sup>2-</sup>): the treatments were as follows: Treatment 1 (control), 1/2-strength medium, with pH 10 containing 20 mmol/L of Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>; Treatment 2 (sodic and Al conditions), 1/2-strength medium, with 300 μmol/L of AlCl<sub>3</sub>·6H<sub>2</sub>O

(pH 10) containing 20 mmol/L of  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  added; Treatment 3 (sodic and Si conditions), 1/2-strength medium, with 500  $\mu\text{mol/L}$  of  $\text{Na}_2\text{SiO}_3$  with pH 10 containing 20 mmol/L of  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ ; and Treatment 4 (sodic, Al and Si conditions), 1/2-strength medium, with 300  $\mu\text{mol/L}$  of  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  and 500  $\mu\text{mol/L}$  of  $\text{Na}_2\text{SiO}_3$  with pH 10 containing 20 mmol/L of added  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ . The pH of each medium was adjusted by 1 mol/L of NaOH every day.

### 2.3 Analysis of Elements

Plants samples of *P. chinampoensis* and barley were harvested 28 days and 21 days, respectively, after transfer to the media. The SPAD value, an index of the amount of chlorophyll present in the leaves, was measured using a SPAD-502 chlorophyll meter (Minolta Camera Co., Tokyo, Japan) before the plants were harvested [37]. The SPAD value of each plant was measured in the middle point of the longest leaf, second longest leaf and third longest leaf. The plants were then separated into shoots and roots for drying in an oven at +80°C to a constant weight for 24 h. The dry weights of the plant parts were then measured. The plant parts were digested with a mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$  (v/v, 5: 1), and the contents of Si, sulphur (S), K and P were measured by particle induced X-ray emission [38-41].

### 2.4 *P. chinampoensis* Growth with Added Si and Different Fe Concentrations

*P. chinampoensis* seedlings were transplanted (five plants per bunch) again to an aerated treatment medium for 28 days and grown under alkaline conditions, with Si added and different concentrations of Fe as follows: Treatment 1 (control), alkaline conditions (pH 10), 1/2-strength medium (pH 10); Treatment 2 (alkaline and Si conditions), 1/2-strength medium, with 500  $\mu\text{mol/L}$  of  $\text{Na}_2\text{SiO}_3$  (pH 10) added; Treatment 3 (alkaline, Si and Fe  $\times$  5 conditions), 1/2-strength medium, with 500  $\mu\text{mol/L}$  of  $\text{Na}_2\text{SiO}_3$  and 40  $\mu\text{mol/L}$  Fe-EDTA (pH 10) added; and Treatment 4 (alkaline, Si and Fe  $\times$  8 conditions), 1/2-strength medium, with 500  $\mu\text{mol/L}$  of  $\text{Na}_2\text{SiO}_3$  and 70  $\mu\text{mol/L}$  of Fe-EDTA (pH 10) added. The pH of each medium was adjusted by 1 mol/L NaOH every day. The SPAD value of *P. chinampoensis* was measured using a SPAD-502 chlorophyll meter 28 days after transfer to the media. The SPAD value was measured in the middle point of the longest leaf, second longest leaf and third longest leaf.

### 2.5 Statistical Analysis

All experiments were conducted in triplicate. Data were subjected to an analysis of variance using the HP proLiant DL320 G6 at Iwate university, Japan [42]. Differences between means were evaluated using the Ryan-Einot-Gabriel-Welsch multiple-range test ( $P = .05$ ). In Figs. 1-8, each value represents the mean  $\pm$  SD ( $n = 3$ ). The different letters or an asterisk at the top of each column in the figures indicate a significant difference ( $P = .05$ ) according to the Ryan-Einot-Gabriel-Welsch multiple-range test.

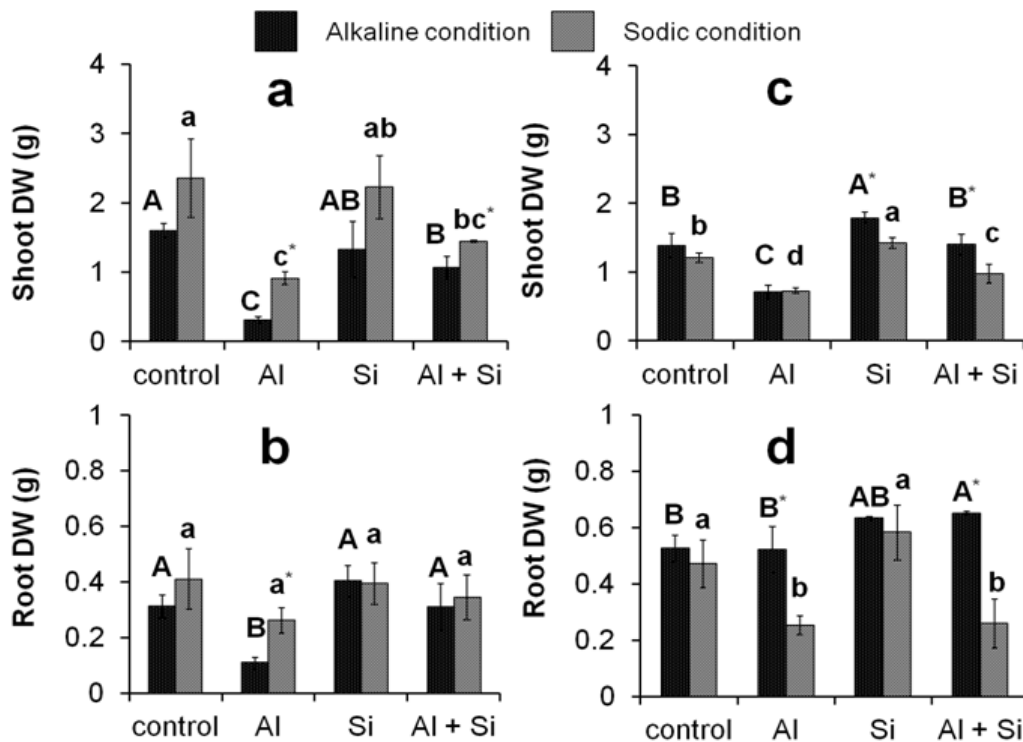
## 3. RESULTS AND DISCUSSION

A previous study revealed that a large amount of Al and Si existed as a water-soluble form in the rhizosphere of sodic soil [5]. The aim of this study was to investigate the effects of Al and Si on plant growth under sodic conditions. Various studies reported that Al, such as  $\text{Al}(\text{OH})_4^-$ , was generally toxic to plant growth under high pH conditions [31-34]. Research showed that the toxic actions of Al were primarily root related, with the root system becoming stubby as a result of the inhibition of elongation of the main axis and lateral roots [3]. Various studies demonstrated beneficial effects of Si on plant growth, including the role of Si in amelioration of Al toxicity under acidic conditions [3, 43-46]. In the present study, the results showed that the addition of Si in the Al treatment enhanced plant growth both *P. chinampoensis* and barley under alkaline and sodic conditions (Fig. 1). Thus, Al toxicity influenced plant growth under high pH conditions (i.e. around 10), and Si ameliorated this toxicity.

The effect of Al and Si on plant growth differed in the presence of  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ . Under alkaline conditions, Al repressed the shoot growth of plants, and Si significantly ameliorated Al toxicity (Figs. 1a and 1c). Regarding root growth of *P. chinampoensis*, Al repressed growth, and Si ameliorated it under alkaline conditions (Figs. 1b and 2a). For barley, Al decreased the density of long roots and increased the amount of stubby roots (Figs. 1d and 2b). Though the effect of Al on the dry weight and length of roots in barley under alkaline conditions was obscure, Al toxicity and Si amelioration of root growth had a strong effect under alkaline condition. However, under sodic conditions in the presence of  $\text{Na}_2\text{CO}_3$  and  $\text{NaHCO}_3$ , the plants showed different growth responses to added Al and Si. Sodium carbonate/bicarbonate ameliorated Al toxicity of *P. chinampoensis*, and they enhanced Si

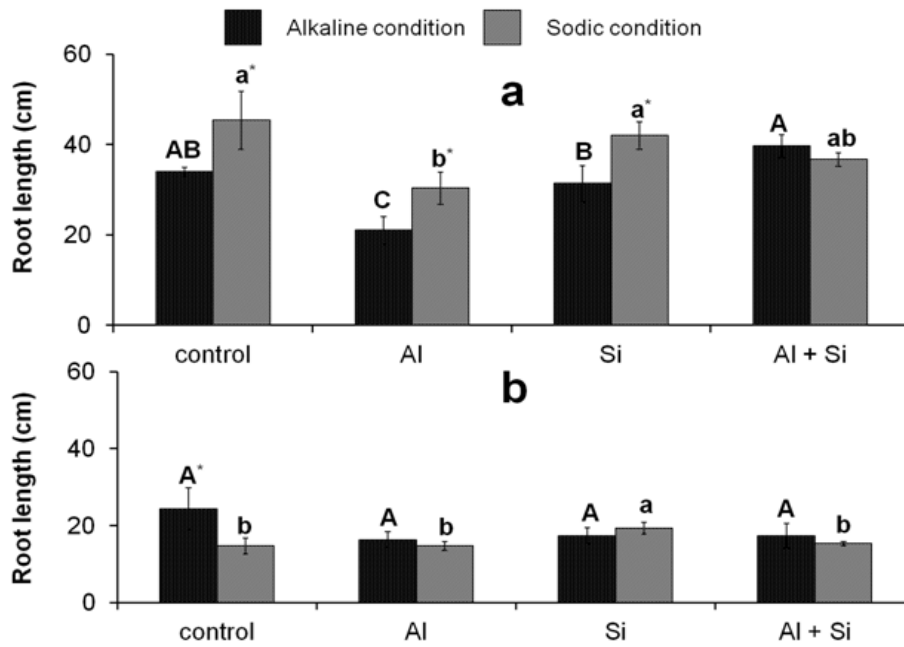
amelioration of Al toxicity under sodic conditions (Figs. 1a and 3a). The shoot dry weight in Al or Al + Si treatment increased by  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  as compared with those treatments under alkaline condition (Fig. 1a). Leaves of *P. chinampoensis* showed greening and no chlorosis in the Al and Si treatments (Fig. 3a). In addition, in Al treatment with  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ , the root dry weight and length increased as compared with that treatment under alkaline condition (Fig. 1b and 2a). However,  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  did not have a positive effect on shoot growth of barley. Under sodic conditions, the shoot dry weight of barley in the Al + Si treatment decreased, indicating that  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  repressed Si-induced amelioration of Al toxicity (Fig. 1c). Furthermore, barley leaves did not show greening in the  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  and Al treatment (Fig. 3b). Sodium carbonate/bicarbonate enhanced Al toxicity of root dry weight of barley (Fig. 1d), and  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  repressed Si amelioration of Al toxicity and adversely affect root growth (Fig. 1d).

In the rhizosphere of sodic soil, Al, Si and  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  exist in soluble form [5]. Therefore, the results of Al + Si treatment under sodic conditions are similar to those of the rhizosphere of sodic soil in nature. In Al + Si treatment under sodic conditions, *P. chinampoensis* showed better growth than barley, especially root growth. Previous research reported that *P. chinampoensis* was well adapted to survive under sodic conditions and that it showed high root growth ability under these conditions [15,29]. The findings of the present study and those in the literature show that the high adaptation ability of *P. chinampoensis* under sodic conditions can help prevent Al toxicity and improve Si amelioration of Al toxicity. Furthermore, they indicate that *P. chinampoensis* can tolerate Al toxicity only under sodic conditions. Thus, *P. chinampoensis* can survive in sodic soils under natural conditions in the field, whereas barley, an alkaline-tolerant plant, cannot.

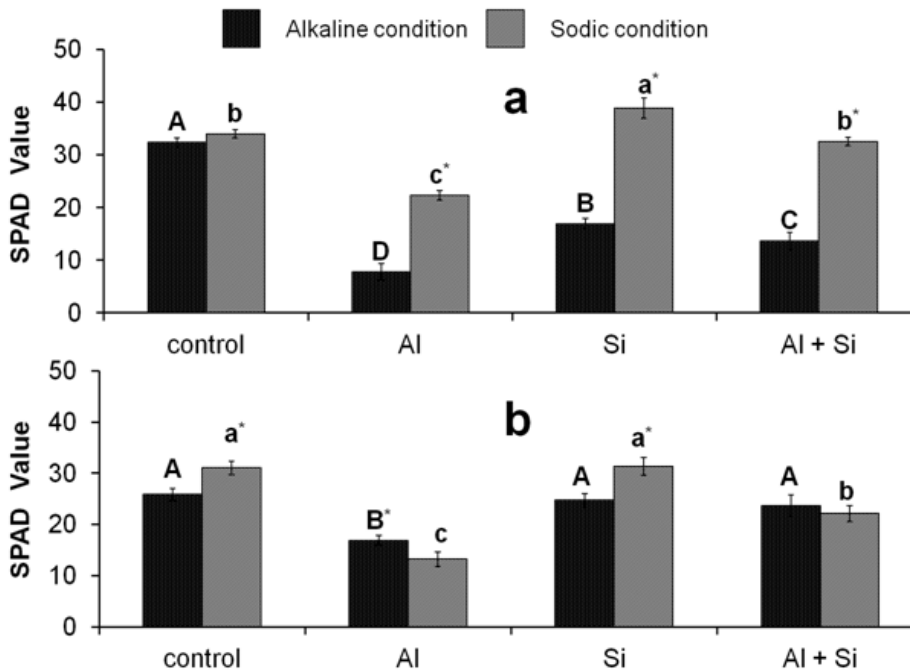


**Fig. 1. Dry weight (DW) of plants under alkaline and sodic conditions**

(a) Dry shoot weight of *P. chinampoensis* (alkaline conditions:  $F = 18.32, P < 0.001$ ; sodic conditions:  $F = 10.31, P < 0.005$ ). (b) Dry root weight of *P. chinampoensis* (alkaline conditions:  $F = 15.39, P < 0.005$ ; sodic conditions:  $F = 2.05, P > 0.05$ ). (c) Dry shoot weight of barley (alkaline conditions:  $F = 35.40, P < 0.0001$ ; sodic conditions:  $F = 36.06, P < 0.0001$ ). (d) Dry root weight of barley (alkaline conditions:  $F = 5.86, P < 0.05$ ; sodic conditions:  $F = 12.64, P < 0.005$ )



**Fig. 2. Root lengths of plants under alkaline and sodic conditions**  
 (a) Root length of *P. chinampoensis* (alkaline conditions:  $F = 22.44$ ,  $P < 0.0005$ ; sodic conditions:  $F = 7.94$ ,  $P < 0.01$ ). (b) Root length of barley (alkaline conditions:  $F = 3.32$ ,  $P > 0.05$ ; sodic conditions:  $F = 7.25$ ,  $P < 0.05$ )



**Fig. 3. SPAD value of plants under alkaline and sodic conditions**  
 (a) SPAD value of *P. chinampoensis* (alkaline conditions:  $F = 187.61$ ,  $P < 0.0001$ ; sodic conditions:  $F = 99.7$ ,  $P < 0.0001$ ). (b) SPAD value of barley (alkaline conditions:  $F = 21.78$ ,  $P < 0.0005$ ; sodic conditions:  $F = 94.83$ ,  $P < 0.0001$ )

Al toxicity interferes directly with active ion uptake processes, with many studies reporting repression of P uptake in particular [3,47,48]. As shown in Fig. 4, *P. chinampoensis* appeared to repress Al toxicity under sodic conditions by high P uptake (Fig. 4). Under alkaline conditions, Al toxicity and Si amelioration of Al toxicity repressed P uptake by plant roots (Fig. 4b and 4d). The Al also repressed P transfer to shoots of *P. chinampoensis* (Fig. 4a). In the case of barley, the P level in shoots remained high, with or without Al, and Si amelioration of Al toxicity was not shown clearly (Fig. 4c). However, under sodic conditions, *P. chinampoensis* showed high P uptake of roots in the Al treatment, and there was no significant difference in P uptake as compared with that in the Al treatment, Al + Si treatment or control (Fig. 4b). In addition, Al toxicity repressed shoot P levels of *P. chinampoensis* under sodic condition, whereas  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  ameliorated Al toxicity as compared with that under alkaline conditions (Fig. 4a). In *P. chinampoensis*,  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  had no effect on the P level in Al + Si treatment, whereas  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  repressed P uptake in barley in Al + Si treatment (Fig. 4c and 4d). In barley, P uptake was not ameliorated under sodic conditions (Fig. 4c and 4d). The P level in roots was reduced by  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ , and the reduction seemed to be promoted by Al, although there was no significant difference in P levels between control and Al treatment (Fig. 4d). The addition of Si significantly reduced P uptake of barley under sodic condition (Fig. 4d). Shoot P levels of barley remained low under sodic conditions and were unaffected by the presence of Al or Si (Fig. 4c).

*P. chinampoensis* did not show the reduction of P uptake capacity under sodic conditions (Fig. 4a and 4b). In the present study, other than the presence of  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ , there was no difference between the alkaline and sodic conditions. All the treatments proceeded under high pH conditions (i.e. around 10). The effect of  $\text{Na}^+$  on plant nutrients is mainly competition for uptake of metal macronutrients, such as  $\text{K}^+$ , and  $\text{Na}^+$  will not compete to anions due to their cationic power [28,29]. Therefore, we suggest that by  $\text{CO}_3^{2-}/\text{HCO}_3^-$  is responsible for repression of P uptake. Under sodic conditions, the inhibition by  $\text{CO}_3^{2-}/\text{HCO}_3^-$  of P uptake may be due to ionic competition between  $\text{PO}_4^{4-}$  and  $\text{CO}_3^{2-}/\text{HCO}_3^-$  in roots. The radii of  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  are 178 pm and 156 pm, respectively, whereas the radius of  $\text{PO}_4^{4-}$  is smaller (238 pm) [49]. Under sodic conditions, as the  $\text{CO}_3^{2-}/\text{HCO}_3^-$

content increases, these ions may inhibit anion uptake in a manner similar to cation competition in the interaction between  $\text{Na}^+$  during  $\text{K}^+$  uptake. Under sodic conditions, *P. chinampoensis* may activate P uptake across the root-cell plasma membrane by  $\text{CO}_3^{2-}/\text{HCO}_3^-$ . Activated P uptake may help the plant tolerate Al toxicity under sodic conditions. In barley, P uptake did not improve under sodic conditions, showing that barley does not have the ability to activate P uptake by  $\text{CO}_3^{2-}/\text{HCO}_3^-$ . As a result, the P level in barley decreased under sodic conditions. Previous research reported that *P. chinampoensis* exhibited high selectivity for  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  over  $\text{Na}^+$  [28,29]. The findings of the present study indicate that sodic-tolerant plants, such as *P. chinampoensis*, seem to show not only cation selectivity but also anion selectivity for nutrient uptake in the rhizosphere of sodic soil.

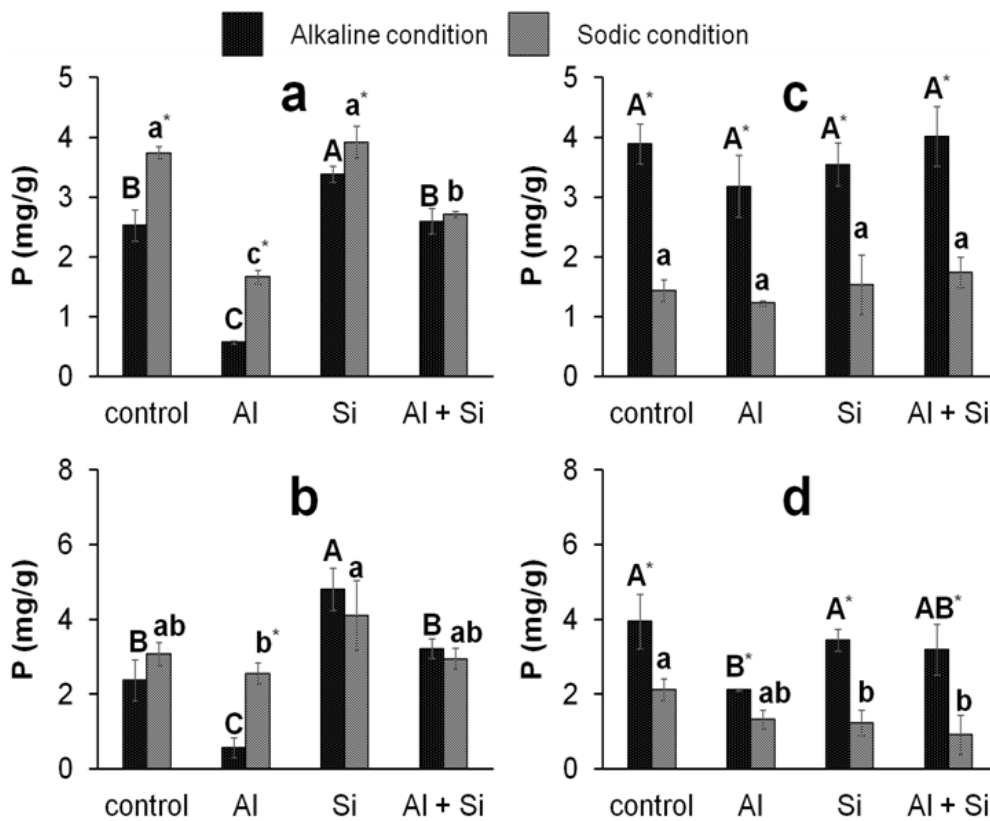
In the current study, S and P levels of plants were very similar, thereby providing support for our hypothesis about anion uptake competition under sodic conditions (Fig. 5). Under sodic conditions, *P. chinampoensis* did not show the reduction of S uptake capacity in root (Fig. 5b), whereas the S level in barley remained low in all the treatments (Fig. 5c and 5d). The radius of  $\text{SO}_4^{2-}$  is 258 pm and bigger than that of  $\text{CO}_3^{2-}/\text{HCO}_3^-$  [49]. Therefore, anion competition by  $\text{CO}_3^{2-}/\text{HCO}_3^-$  may explain the low S uptake of barley. In Al + Si treatment under sodic conditions, the S level of barley was lower than under alkaline conditions same as P level. This result suggested that in major food crops, such as barley, both P and S uptake are prevented by  $\text{CO}_3^{2-}/\text{HCO}_3^-$  in the rhizosphere of sodic soil. In contrast, for *P. chinampoensis*, S uptake increased under sodic conditions, and  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  ameliorated the effect of Al toxicity on S uptake. These findings suggested that activation of the S uptake process by *P. chinampoensis* may occur in a similar manner to P uptake (i.e. via  $\text{CO}_3^{2-}/\text{HCO}_3^-$ ). Under sodic conditions, the addition of Si reduced Al-induced repression of S transfer to shoots in *P. chinampoensis*, whereas Si did not enhance S transfer to shoots in barley in the all treatments (Fig. 5a and 5c). This result suggested that sodic-tolerant plants can transfer and use S more efficiently than nonsodic-tolerant plants in the rhizosphere of sodic soil.

Both P and S are essential for plant growth-related functions, such as energy transfer by ATP and the synthesis of nucleic acids, amino

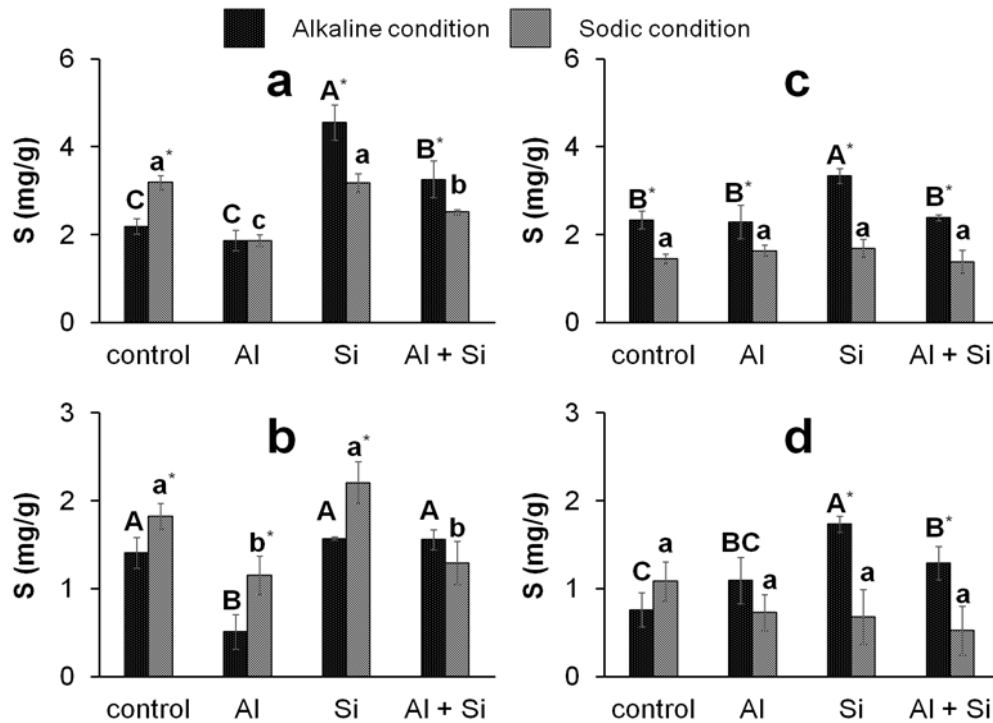
acids, proteins and coenzymes [3]. The results of the present study suggest that plants grown in sodic soil suffer not only saline stress and alkaline stress but also Al toxicity, P deficiency and S deficiency. As noted earlier, sodic-tolerant plants, such as *P. chinampoensis*, show selectivity for P and S uptake over  $\text{CO}_3^{2-}/\text{HCO}_3^-$ , enabling roots of sodic-tolerant plants to become established in the rhizosphere of sodic soils. Due to their high root growth capacity, sodic-tolerant plants show Al tolerance, cation selectivity, high secretion of MAs and high uptake of metal micronutrients. *P. chinampoensis* seems to be well adapted to survive in sodic soil.

Generally, K uptake of plant is inhibited by Al [50]. Previous studies showed that *P. chinampoensis* had a high root growth capacity and that it exhibited high selectivity for metal macronutrients, such as  $\text{K}^+$  over  $\text{Na}^+$

[15,28,29]. In this study, *P. chinampoensis* showed high K uptake capacity and inhibition of Al toxicity under sodic conditions (Fig. 6). In Al treatment, under sodic conditions, the addition of Al did not affect the K content in roots of *P. chinampoensis*, and the shoot K content was ameliorated by  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ . Potassium uptake of roots was enhanced by the addition of Si under sodic conditions, with or without Al. In *P. chinampoensis*, optimum K transfer to shoots occurred in Al + Si treatment (Fig. 6a and 6b). The uptake of K in barley was similar to that of P and S (Fig. 6c and 6d). The K content of barley under sodic conditions was lower than that under alkaline conditions, and the root K content decreased in the treatments with Al or Si added. These results suggest that the high K selectivity of *P. chinampoensis* allows it to exploit the rhizosphere of sodic soil, which contains soluble Al, Si and  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ .



**Fig. 4. Phosphorus (P) content of the plants under alkaline and sodic conditions**  
 (a) P content in shoots of *P. chinampoensis* (alkaline conditions:  $F = 130.34$ ,  $P < 0.0001$ ; sodic conditions:  $F = 130.82$ ,  $P < 0.0001$ ). (b) P content in roots of *P. chinampoensis* (alkaline conditions:  $F = 49.94$ ,  $P < 0.0001$ ; sodic conditions:  $F = 4.67$ ,  $P < 0.05$ ). (c) P content in shoots of barley (alkaline conditions:  $F = 2.25$ ,  $P > 0.05$ ; sodic conditions:  $F = 1.54$ ,  $P > 0.05$ ). (d) P content in roots of barley (alkaline conditions:  $F = 6.66$ ,  $P < 0.05$ . sodic conditions:  $F = 5.91$ ,  $P < 0.05$ )



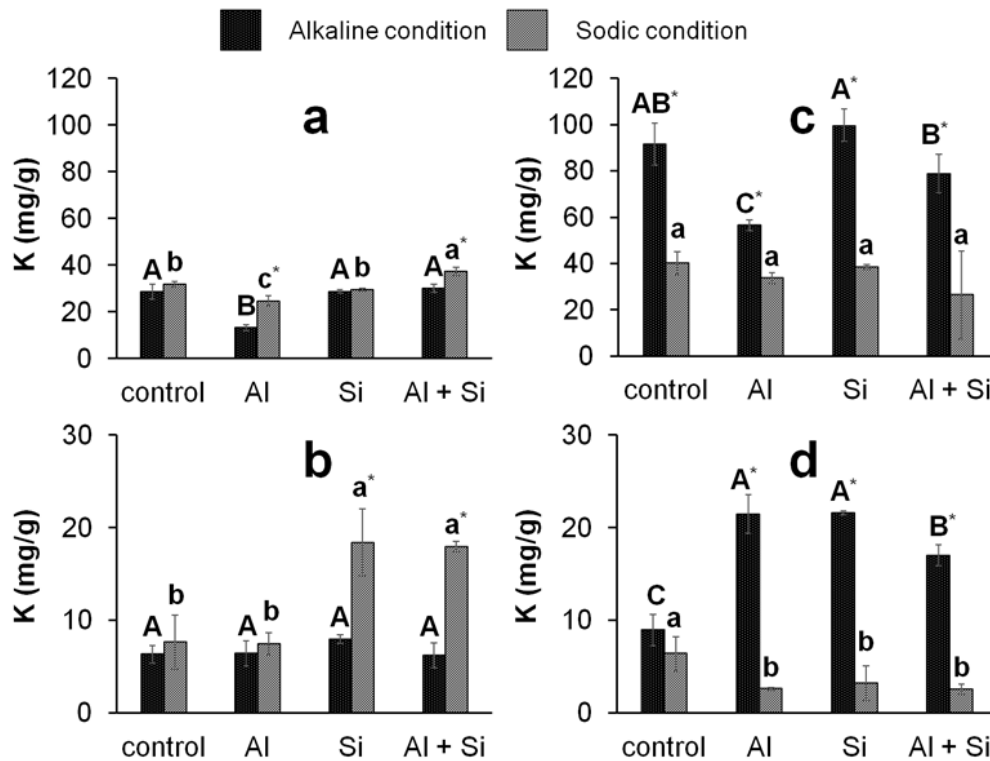
**Fig. 5. Sulphur (S) content of plants under alkaline and sodic conditions**

(a) S content in shoots of *P. chinampoensis* (alkaline conditions:  $F = 42.2$ ,  $P < 0.0001$ ; sodic conditions:  $F = 50.95$ ,  $P < 0.0001$ ). (b) S content in roots of *P. chinampoensis* (alkaline conditions:  $F = 37.49$ ,  $P < 0.0001$ ; sodic conditions:  $F = 15.26$ ,  $P < 0.005$ ). (c) S content in shoots of barley (alkaline conditions:  $F = 13.76$ ,  $P < 0.005$ ; sodic conditions:  $F = 1.83$ ,  $P > 0.05$ ). (d) S content in roots of barley (alkaline conditions:  $F = 12.93$ ,  $P < 0.005$ ; sodic conditions:  $F = 2.53$ ,  $P > 0.05$ )

In addition to P, S and K uptake, *P. chinampoensis* showed specific uptake of Si (Fig. 7). Si uptake was enhanced in roots *P. chinampoensis* in Al + Si treatment under sodic conditions. In contrast, Si uptake did not increase significantly in barley under sodic conditions (Fig. 7b and 7d). The radius of  $\text{SiO}_4^{4-}$  is 240 pm and interferes with plant uptake by anion competition under sodic conditions [49]. Hence,  $\text{CO}_3^{2-}/\text{HCO}_3^-$  may repress Si uptake of plants. *P. chinampoensis* may activate Si uptake by  $\text{CO}_3^{2-}/\text{HCO}_3^-$ , and this high utilization capacity of Si in *P. chinampoensis* may be beneficial for the plant's survival in sodic soil.

Under sodic conditions, the SPAD values of *P. chinampoensis* grown in the Al and Si treatments were significantly higher than those of plants grown under alkaline conditions (Fig. 3a). The SPAD values of barley in the control and Si treatment were significantly higher under sodic conditions than alkaline conditions (Fig. 3b). The recovery of chlorosis in barley will be caused by the correlation of P level and Fe deficiency about

the P regulation of Fe mobilization in plant. Previous reports suggested that a low P content prevented immobilization of Fe in the form of phosphate-iron complexes in root cells and enhanced Fe mobilization to shoots, resulting in leaf greening [51,52]. In the present study, greening of barley occurred in the presence of a low P content under sodic conditions (Fig. 4c). This result suggests that major food crops cultivated in sodic soil seem to grow well, despite P deficiency. In contrast, P uptake by *P. chinampoensis* was not repressed under sodic conditions though the greening occurred (Figs. 3a and 4a). We attribute the greening of *P. chinampoensis* to high Fe utilization capacity by MAs. In a previous report, we demonstrated high MA secretion ability of *P. chinampoensis* under sodic conditions, whereas MA secretion was repressed in barley under these conditions [15]. We also suggest that *P. chinampoensis* will show high transportation of  $\text{Fe}^{3+}$ -MAs from roots to shoots and that it utilized it efficiently for photosynthesis.



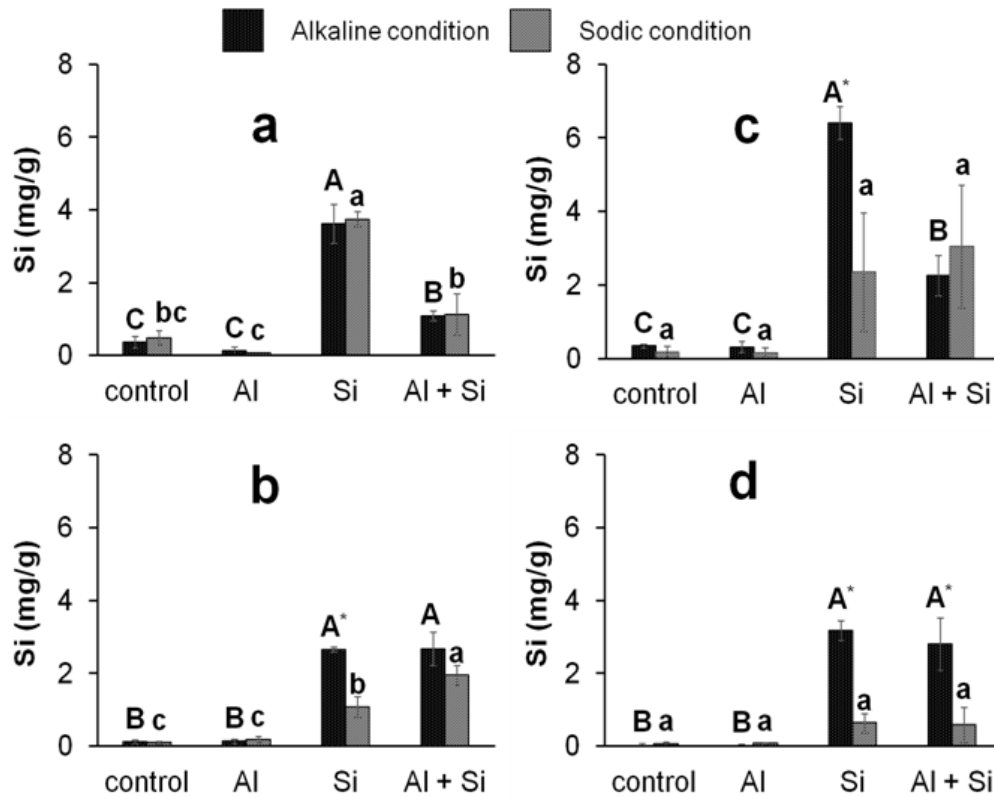
**Fig. 6. Potassium (K) content of plants under alkaline and sodic conditions**

(a) K content in shoots of *P. chinampoensis* (alkaline conditions:  $F = 46.43$ ,  $P < 0.0001$ ; sodic conditions:  $F = 37.21$ ,  $P < 0.0001$ ). (b) K content in roots of *P. chinampoensis* (alkaline conditions:  $F = 1.68$ ,  $P > 0.05$ ; sodic conditions:  $F = 19.48$ ,  $P = 0.0005$ ). (c) K content in shoots of barley (alkaline conditions:  $F = 20.58$ ,  $P < 0.0005$ ; sodic conditions:  $F = 1.16$ ,  $P > 0.05$ ). (d) K content in roots of barley (alkaline conditions:  $F = 50.39$ ,  $P < 0.0001$ ; sodic conditions:  $F = 5.47$ ,  $P < 0.05$ )

In the present study, in Si treatment, chlorosis occurred in *P. chinampoensis* under alkaline conditions (Fig. 3a), and chlorosis recovered following the addition of Fe (Fig. 8), suggesting that chlorosis was due to Fe deficiency induced by Si. To the best of our knowledge, there have been no studies of Fe deficiency of sodic-tolerant plants induced by Si. This phenomenon occurred only in the high pH conditions, without  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ . As a large amount of  $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$  exists in the rhizosphere of sodic soil, Si-induced Fe deficiency will not occur in sodic soil in the field. The high uptake capacity of *P. chinampoensis* for nutrients, such as P or Si, may explain Fe deficiency of sodic-tolerant plants induced by Si. Further investigations are needed to identify the mechanism underlying this physiological characteristic of sodic-tolerant plants.

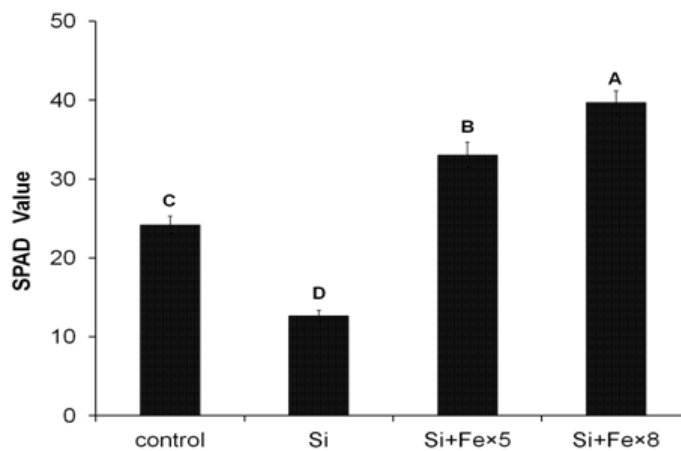
This study showed that plants grown in sodic soil suffered not only salt stress and alkaline stress

but also Al toxicity (ameliorated by Si) and  $\text{CO}_3^{2-}/\text{HCO}_3^-$  stress. Therefore, sodic-tolerant plants must possess specific mechanisms for nutrient uptake in the rhizosphere of sodic soil to survive. Previous studies reported that *P. chinampoensis* exhibited high selectivity for metal macronutrients, such as  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , over  $\text{Na}^+$  under sodic conditions [28,29]. *P. chinampoensis* responded to sodic conditions by increasing the secretion of MAs, and this response contributed to uptake of metal micronutrients, such as Fe, Mn, Cu and Zn [15]. In this study, *P. chinampoensis* showed high uptake capacity of P, S and Si, with uptake enhanced under sodic conditions. As a result of its nutrient uptake capacity, *P. chinampoensis* exhibited high root growth capacity, in addition to high tolerance to salt stress, alkaline stress, Al toxicity and other stresses, in the rhizosphere of sodic soil. Thus, there is no doubt that *P. chinampoensis* is well adapted to survive in sodic soil.



**Fig. 7. Silicon (Si) content of plants under alkaline and sodic conditions**

(a) Si content in shoots of *P. chinampoensis* (alkaline conditions:  $F = 88.20$ ,  $P < 0.0001$ ; sodic conditions:  $F = 81.78$ ,  $P < 0.0001$ ). (b) Si content in *P. chinampoensis* roots (alkaline conditions:  $F = 116.95$ ,  $P < 0.0001$ ; sodic conditions:  $F = 53.98$ ,  $P < 0.0001$ ). (c) Si content in barley roots (alkaline condition:  $F = 188.90$ ,  $P < 0.0001$ ; sodic conditions:  $F = 4.93$ ,  $P < 0.05$ ). (d) Si content in barley roots (alkaline conditions:  $F = 58.48$ ,  $P < 0.0001$ ; sodic conditions:  $F = 3.74$ ,  $P > 0.05$ )



**Fig. 8. SPAD value of *P. chinampoensis* with added Si under alkaline conditions with different Fe concentrations**  
( $F = 252.1$ ,  $P < 0.0001$ )

Population increases worldwide have resulted in the need for increased food production [53]. One way to meet this challenge is to increase the land area available for agriculture [53]. Preventing land from degradation (e.g. soil sodification) is part of this endeavour, and understanding the processes of degradation is essential for preventing degradation. We suggest that sodic-tolerant plants may have unknown physiological mechanisms that enable them to grow in sodic soils. Elucidation of the properties of plants that allow them to survive in sodic soils could help promote the revegetation of soil in the future.

#### 4. CONCLUSION

This study investigated Al toxicity in plants under sodic conditions and Si-induced amelioration of Al toxicity. *P. chinampoensis*, a sodic-tolerant plant, showed high selectivity for P, S, K and Si uptake over Al, as compared with barley under sodic conditions. Si enhanced nutrient uptake under sodic conditions and efficiently alleviated Al toxicity. The Al, Si and Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> exist as soluble forms in the rhizosphere of sodic soil and affect plant growth. We suggest that sodic-tolerant plants, such as *P. chinampoensis*, have a high nutrient uptake capacity in sodic soil and are well adapted to survive there. Further studies are required to shed light on the characteristics of plants that allow them to survive in sodic soils.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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