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REGULAR ARTICLE

Effects of silicon nutrition on cadmium uptake, growth and photosynthesis of rice plants exposed to low-level cadmium

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Abstract The effect of silicon (Si) nutrition on low-level cadmium (Cd) toxicity symptoms was investigated in hydroponically-grown rice seedlings (*Oryza sativa* L.). Silicon (0.0, 0.2, or 0.6 mM) was added when seedlings were 6 or 20 days old representing early (Si^E) or late (Si^L) Si treatment, respectively. Cadmium (0.0 or 2.5 μM) was added when seedlings were 6 days old. Measurements included generation of CO_2 and light response curves; chlorophyll fluorescence analysis; growth; and tissue-element content analysis. Our results showed that low-level Cd treatment generally inhibited growth and photosynthesis. However, the addition of 0.2 or 0.6 mM Si^E or Si^L significantly reduced root- and leaf-Cd content. Consequently, the addition of 0.6 mM Si^L significantly alleviated low-level Cd-induced inhibition of growth. Furthermore, 0.2 mM Si treatment significantly reduced g_s compared to 0.0 or 0.6 mM Si without inhibiting A, especially in +Cd plants, suggesting an increase in instantaneous water-use-efficiency (IWUE). Additionally, in +Cd plants, the addition of 0.6 mM Si^E significantly reduced F_o but increased F_v/F_m, while treatment with 0.2 mM Si^L significantly increased q_P, suggesting an increase in light-use-efficiency. We thus, propose that 0.6 mM Si^L treatment is required for the alleviation of low-level Cd-mediated growth inhibition. Furthermore, we suggest that 0.2 mM Si concentration might be close to the optimum requirement for maximum Si-induced increase in IWUE in rice plants, especially when under low-level Cd-stress. Our results also suggest that Si alleviates low-level Cd toxicity by improving light-use-efficiency.

Keywords Chlorophyll fluorescence · Instantaneous water-use-efficiency · Low-level cadmium · Silicon · Stomatal conductance

Abbreviations

A	net CO_2 assimilation rate
A_{max}	maximum net CO_2 assimilation rate
C_a	ambient CO_2 concentration
C_E	carboxylation efficiency
C_i	intercellular CO_2 concentration
E	transpiration rate
F_m	maximum chlorophyll fluorescence yield in a dark-adapted state
F_o	minimum chlorophyll fluorescence yield in a dark-adapted state
F_o/F_m	basal quantum yield of non-photochemical processes in PS2 in a dark-adapted state
F_v	maximum variable fluorescence yield in a dark-adapted state
F_v/F_m	quantum efficiency of open PS2 centers in a dark-adapted state

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g_s	stomatal conductance rate
g_{smax}	maximum stomatal conductance rate
I_s	photosynthetic light-saturation point
IWUE	instantaneous water-use-efficiency
PS	photosystem
q_N	non-photochemical quenching coefficient in a light-adapted state
q_P	photochemical quenching coefficient in a light-adapted state
RUBISCO	ribulose-1, 5-bisphosphate carboxylase/oxygenase
Si^E	Si was added (early) when plants were 6 days old
Si^L	Si was added (late) when plants were 20 days old

Introduction

Among the well-known heavy metals in the environment, cadmium is of considerable importance due to its high water solubility, relative mobility, and phytotoxicity even in minute amounts (Wagner 1993; Ralph and Burchett 1998; Sanità di Toppi and Gabrielli 1999). The high water solubility of cadmium salts compared to those of other heavy metals makes the study of its effects on rice important, especially since rice is grown in flooded or marshy soils and is the staple food for the majority of the world's population (Wang et al. 2000). It was reported that the consumption of rice grown in cadmium-polluted soils in Japan in the mid-1950s and mid-1960s was responsible for the human *itai itai* disease with symptoms that included joint pains and kidney malfunction (Herawati et al. 2000).

Symptoms of Cd toxicity in plants have been associated with leaf chlorosis, growth inhibition and the disruption of key physiological processes including photosynthesis (Atal et al. 1991; Moya et al. 1995; Das et al. 1998; Chugh and Sawhney 1999; Kukier and Chaney 2002). Plants have evolved means of alleviating and tolerating heavy metal stress. Studies have shown that plant responses to Cd-stress involve: exclusion, compartmentalization and synthesis of phytochelatins, metallothioneins, stress proteins and ethylene (Sanità di Toppi and Gabrielli 1999; Clemens 2006). Cadmium toxicity in plants has also been shown to be alleviated by interaction with other elements, such as zinc (Kukier and Chaney 2002) and silicon (Si; Neumann et al. 1997; Wang et al. 2000; Liang et al. 2005; Ma et al. 2001; Neumann and Zur Neiden 2001; Iwasaki et al. 2002).

Silicon is not considered to be an essential element for plants although recent studies have revisited this issue (Epstein 1999; Ma and Takahashi 2002b). Silicon is the second most abundant element in soils and is available to plants in the form of silicic acid. Rice, like other members of the poaceae family, is the most effective known Si-accumulating plant, taking up about 10% of its dry weight as Si and having a Si/Ca ratio of over 17 (Ma and Takahashi 2002a).

Although the described effects of Si vary among plants species, Si has been shown to improve disease resistance, light interception, water-use-efficiency (WUE) and photosynthesis, as well as remediating nutrient imbalance in plants (Agarie et al. 1998; Epstein 1999; Ma and Takahashi 2002b; Gao *et al.* 2006; Romero-Aranda et al. 2006; Liang et al. 2007). The alleviation of aluminum (Al) toxicity in maize (*Zea mays* L.) was attributed to silicon-induced exudation of flavonoid-type phenolics, especially catechin (Kidd et al. 2001). The detoxification of zinc (Zn) as zinc silicate in known Zn hyperaccumulators such as Irish moss (*Minuartia verna* L.) and *Cardaminopsis halleri* (L.) was attributed to high Si concentrations in the vacuoles, cell walls and intercellular spaces (Neumann et al. 1997; Neumann and Zur Neiden 2001). Wang et al. (2000) showed Si-induced apoplastic exclusion of Cd from leaf and root cells and also a reduction in Cd transport from root to shoot and proposed a possible sequestration of cadmium in root cell walls by the formation of colloidal high-absorbing silica as a means whereby silicon ameliorates cadmium uptake in rice.

However, the effect of Si on growth and physiological processes such as photosynthesis and chlorophyll fluorescence is not well understood yet sparsely investigated. A study by Ma et al. (1989) showed that a late addition of Si nutrition to growing rice plants increased productivity more than an early addition of Si nutrition. In another study, Ma and Takahashi (2002b) reported that treatment of rice plants with Si increased net CO_2 assimilation per individual plant but not per unit leaf area. Furthermore, a "window hypothesis" was suggested for Si, whereby Si in the form of silica bodies deposited in leaf epidermal cells acts as a "window" that could enhance light-use-

efficiency by facilitating the transmission of light to the photosynthetic mesophyll tissue (Kaufman et al. 1979). However, there has been little evidence to support this hypothesis (Ma and Takahashi 2002b). Additionally, recent studies showed significant Si-induced enhancement of photosynthesis and/or chlorophyll fluorescence parameters in salt-stressed tomato (*Lycopersicon esculentum*; Al-aghabary et al. 2004), drought-stressed sorghum (*Sorghum bicolor*; Hattori et al. 2005), and drought-stressed maize plants (*Zea mays*; Li et al. 2007), but described minimal Si-induced effects on these same parameters in healthy unstressed plants. The conclusion from these studies is that although the role of Si on photosynthesis and chlorophyll fluorescence parameters in healthy unstressed plants is minimal or not well understood, Si seems to play a significant role in improving these same parameters in stressed plants.

Dose-response relationships indicate that plant response to Cd exposure is a very complex phenomenon and not yet fully resolved (Simonova et al. 2007). Furthermore, studies on Cd toxicity in plants have involved mostly the exposure of plants to high levels of Cd for short periods of time (Wagner 1993; Prasad 1995; Sanità di Toppi and Gabrielli 1999). However, the concentrations used in these high-level Cd studies are far from realistic since agricultural plants are typically exposed to much lower Cd concentrations for much longer periods (Sanità di Toppi and Gabrielli 1999). Thus, in order to obtain a broader understanding of the dynamics of Cd toxicity in plants, more research is needed on the more realistic scenario of plant exposure to long-term low-level Cd (Wagner 1993; Sanità di Toppi and Gabrielli 1999; Clemens 2006).

In this study we examined the importance of concentration of Si nutrition and time of Si addition on the alleviation of low-level Cd-induced toxicity effects on growth and physiological processes in plants. The main objectives of this study were: (1) to determine the toxicity symptoms of low-level Cd exposure on plant growth, photosynthetic and chlorophyll fluorescence parameters; (2) to investigate the role of Si nutrition in plants, with respect to concentration and time of addition, on the alleviation of low-level Cd toxicity effects on Cd uptake, growth, photosynthesis, stomatal conductance, and chlorophyll fluorescence parameters. It is hoped that the information garnered hereunder would aid in the development of crop production techniques aimed at reducing the risks associated with growing plants in soils contaminated with Cd.

Materials and methods

Plant material and growth conditions

Rice (*Oryza sativa* L. var. Jefferson) seeds obtained from USDA-ARS Rice Research Center (Beaumont, Texas) were soaked in 10% bleach for 10 min, rinsed in double-distilled water, and germinated on four layers of autoclave-sterilized paper towels pre-soaked in deionized, autoclave-sterilized water. Six days after the beginning of germination, seedlings were transferred to aerated nutrient solutions. Nutrient solutions were prepared after Kukier and Chaney (2002) using deionized-nanopure water as follows; 0.3 mM $CaCl_2$, 2.0 mM KNO_3, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.5 mM $(NH_4)_2SO_4$, 20.0 μM FeHEDTA, 0.1 μM Na_2MoO_4, 20.0 μM H_3BO_3, 1.0 μM $MnCl_2$, 2.0 μM $CuSO_4$, 2.0 μM $ZnSO_4$. The final pH after the addition of treatments was adjusted to 5.5 using 1 N NaOH or HNO_3 and monitored throughout the experimental period. Growth solutions were changed every 5 days for the first 20 days, and then every 3 days for the remainder of the experiment. The plants were grown in a greenhouse at Miami University, Oxford, Ohio, from June–July 2004 with ambient temperature and relative humidity maintained at between 25–35°C and 50–70%, respectively. Ambient light was supplemented with an electrodeless sulfur lamp (Fusion Lighting, Rockville, Maryland, USA) yielding a total photosynthetic photon flux density (PPFD) of 500–1,200 μmol m^{-2} s^{-1} throughout the experimental period. The lamp was set to a 14:10 day/night hour period.

Treatments

Silicon treatments included either 0.0, 0.2 or 0.6 mM Si; and Cd treatments included either 0.0 or 2.5 μM Cd. Silicon treatments were introduced when seedlings were 6 days old or 20 days old representing early Si treatment (Si^E) or late Si treatment (Si^L), respectively. Cadmium treatment was initiated when seedlings were 6 days old. Silicon was added as sodium silicate ($Na_2Si_3O_7$) solution (27% SiO_2 in

14% NaOH), while Cd was added as cadmium sulfate ($CdSO_4$). An equivalent amount of Na (as NaCl) was added to the 0.0 or 0.2 mM Si-treated plants to compensate for the Na content of 0.6 mM Si-treated plants. About 2.5 or 6.5 ml of 1 N HNO_3 was added to 5 gal solutions containing 0.2 or 0.6 mM Si concentration in order to adjust the pH to 5.5. The treatments were arranged factorially in a randomized complete block design with five replicate seedlings per treatment. Due to space limitations, $-Cd\ +Si^L$ treatment was excluded because our preliminary studies showed that its effects were generally not significantly different from that of $-Cd\ +Si^E$ treatment for all the parameters measured in this study (data not shown).

Gas exchange measurements

Gas exchange measurements commenced 43 days after seedlings were transplanted and was conducted on the third fully-expanded leaf from top using an infrared gas analyzer (*LI-6400*, Licor, Lincoln, NE) as previously described (McDermitt et al. 1989). All gas exchange measurements were taken with the relative humidity and ambient temperature conditions in the *LI-6400* leaf chamber maintained at 68±3% and 32±2°C, respectively. Leaves were subjected to a set series of varying ambient CO_2 concentrations (C_a) as follows: 400, 300, 200, 100, 50, 400, 400, 600, and 800 μmol mol^{-1} by employing the auto programs provided by the *LI-6400*. The net CO_2 assimilation (A) and transpiration rates (E) were recorded at each C_a value. The *LI-6400* software automatically calculated stomatal conductance rate (g_s) and intercellular CO_2 concentration (C_i) values from observed A and E values. The maximum CO_2 fixation capacity (A_{max}) and maximum stomatal conductance rate (g_{smax}) were recorded as the highest attained A and g_s values, per plant, observed on the A/C_i and g_s/C_i curves, respectively. The initial slope of the A/C_i curve was used to determine the carboxylation efficiency (C_E) of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RUBISCO). Light response curves (A/PPFD) were generated under a constant C_a of 400 μmol mol^{-1} by measuring A against a descending PPFD series of 1,500, 1,000, 600, 400, 200, 100, 0 μmol m^{-2} s^{-1}. The photosynthetic light saturation point (I_s) was recorded as the highest attained A value, per plant, observed on the A/PPFD curve.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured 47 days after seedlings were transplanted and was conducted on the adaxial surface of the third fully-expanded leaf from top using a modulated fluorimeter (OS5-FL, Opti-Science Inc., Tyngsboro, MA). Plants were allowed to dark-adapt for 2 h after sunset or 2 h after the automated supplemental light shut off, whichever occurred last. The minimal fluorescence (F_o) was measured with a weak modulated λ_{660}-irradiation (<0.1 μmol m^{-2} s^{-1}). A 0.8-s saturating λ_{690} flash (>9,000 μmol m^{-2} s^{-1}) was then applied to measure the maximum fluorescence yield (F_m). Immediately after the initial saturating flash, the leaf was irradiated with a continuous actinic beam (~550 μmol m^{-2} s^{-1}) supplied by a halogen lamp (PAR clip with external illuminator, Opti-Science Inc., Tyngsboro, MA). Fifteen seconds after the actinic light was switched on, the leaf was subjected to a series of ten 0.8-s saturating flash pulses (>9,000 μmol m^{-2} s^{-1}) with a 15-s interval between each flash. The OS5-FL recorded the minimum and maximum fluorescence yields in a light-adapted state or F_s and F_{ms} values, respectively, from which the photochemical (q_P) and non-photochemical quenching (q_N) coefficients were auto-calculated at each post-actinic saturating flash pulse. The curves of q_P or q_N vs. time were generated and a best fit trend line was applied. Our final q_P or q_N value was recorded as the value of q_P or q_N at 150 s (post actinic), at which point the photosystems (PS) had attained steady-state values of q_P or q_N. The actinic light source used had a dichroic heat filter preventing irradiation of wavelengths >700 nm from reaching the leaves. The OS5-FL automated "kinetic mode" test was employed for all measurements and the average leaf temperature during measurements was 33±0.15°C. Calculations used were as follows (Maxwell and Johnson 2000; Roháček 2002): $F_v = F_m - F_o$; $F_v/F_m = (F_m - F_o)/F_m$; F_o/F_m; $q_P = (F_{ms} - F_s)/(F_{ms} - F_o)$; $q_N = (F_m - F_{ms})/(F_m - F_o)$. Symbols and equations not previously described are as follows: F_v is the maximum variable chlorophyll fluorescence yield in a dark-adapted state; F_o/F_m represents the basal quantum yield of non-photochemical processes in PS2 in a dark-adapted state; F_v/F_m denotes the quantum efficiency of open PS2 centers in a dark-adapted state.

Growth parameter measurements

The growth period was ended 48 days after seedlings were transplanted. Shoot and root lengths were measured to the nearest centimeter. Thereafter, shoots and roots were separated, oven-dried to a constant dry weight for 2 days at 75°C and then weighed. Prior to drying of shoots, all leaves were removed from each plant and total leaf area was determined with a leaf area meter (LI-3000, Licor, Lincoln, NE).

Cadmium and silicon content determination

Plant roots were rinsed in three consecutive batches of half strength Hoagland solution (excluding KH_2PO_4) to desorp Cd, followed by rinsing in deionized water and blotting with paper towels (Kukier and Chaney 2002) before drying. Dried tissue samples were ground in a stainless steel mill to pass a 0.5 mm mesh screen (ZM 100, Retsch, Germany). Tissue-Cd content was determined by atomic absorption spectrometry (AAS; Jorhem et al. 2001), and tissue-Si content was determined by the colorimetric molybdenum blue method (Elliot and Snyder 1991).

Statistical analysis

Linear regression analysis (Microsoft Excel 2003) was used to determine the slope of the initial portion of the A/C_i curves and a logarithmic regression line was used to estimate our final q_N and q_P values. All data were subjected to analysis of variance (ANOVA) using the SigmaPlot with SigmaStat integrated statistical software version 9.01 (Systat Software, Inc., Point Richmond, California, USA). Means ($n=5$) were separated using the Fischer least significant difference (FLSD) test at 95% ($P<0.05$) confidence interval.

Results

Cadmium and Si content of tissues

Our results showed that the addition of Si significantly increased root- and leaf-Si content in a linear fashion and there was significantly more Si in the leaves than roots (Fig. 1a,b). Additionally, there was no difference in tissue-Si content between −Cd +Si^E

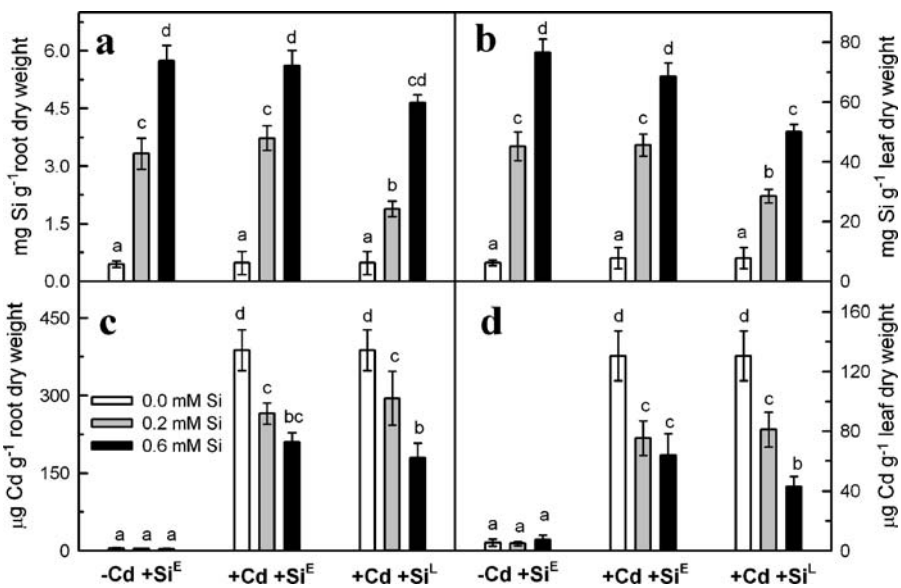

Fig. 1 Effects of different levels of Si nutrition on tissue-element concentrations (**a** root-Si concentration; **b** leaf-Si concentration; **c** root-Cd concentration; **d** leaf-Cd concentration) of rice seedlings in the absence (−) or presence (+) of Cd. Plants were 6 d old when exposed to 2.5 µM Cd for 48 days. Silicon nutrition was initiated when seedlings were 6 or 20 days old, representing early (+Si^E) or late (+Si^L) Si treatment, respectively. Bars represent means ± SE of five replications. *Bars* with the same *lower case letter* within a parameter are not significantly different

and +Cd +Si^E plants (Fig. 1a,b). However, 0.2 mM Si^L-treated plants had about 38% and 49% less Si in their roots and leaves, respectively, compared to 0.2 mM Si^E-treated plants. Additionally, 0.6 mM Si^L-treated plants had about 27% and 17% less Si in their roots and leaves, respectively, compared to 0.6 mM Si^E-treated plants (Fig. 1a,b). Our results also showed that treatment with Cd significantly increased root- and leaf-Cd content but there was significantly more Cd in the roots than leaves (Fig. 1c,d). Additionally, in +Cd plants, there was an inverse relationship between Si treatment concentration and tissue-Cd content (Fig. 1c,d). Consequently, we observed that addition of 0.2 or 0.6 mM Si to +Cd plants reduced root-Cd content by about 28% or 45%, respectively (Fig. 1c) and also reduced leaf-Cd content by about 41% or 60%, respectively (Fig. 1d).

Effects of Cd and Si on plant morphology

We observed that treatment with Cd significantly inhibited growth regardless of Si nutrition, resulting in a concerted decrease in shoot length, root length, shoot dry weight, root dry weight and total leaf area (Table 1). Interestingly, in +Cd plants, the addition of 0.6 mM Si^L resulted in a significant increase in all growth parameters compared to all other Si treatments (Table 1).

Effects of Cd and Si on gas exchange parameters

Our results showed that treatment with Cd significantly reduced A_{max}, (Fig. 2a–c), C_E (Fig. 3), and I_s (Fig. 4a–c), but did not significantly affect C_i (data not shown). Treatment with Si, whether applied early or late, had no significant effect on these same parameters. Additionally, in −Cd plants, Si treatment generally reduced ($P>0.05$) g_{smax} by about 10%, but interestingly, in these same plants, only 0.2 mM Si treatment significantly reduced g_s at high C_i values [>500 μmol (CO_2) mol^{-1}] when compared to Si-deficient plants (Fig. 2d). Furthermore, treatment with Cd significantly reduced g_{smax}, and the g_s of +Cd plants remained almost constant regardless of C_i or Si treatment suggesting a loss of stomatal control in these plants (Fig. 2e,f). However, it is interesting to note that the reduction of g_s due to Cd treatment was greater with the addition of Si (Fig. 2e,f), and the g_s of 0.2 mM Si-treated plants was significantly lower compared to that of 0.6 mM Si-treated plants (Fig. 2e,f). We also wish to mention that our plants generally

Table 1 Effects of different levels of Si nutrition on growth parameters of rice seedlings in the absence (−) or presence (+) of Cd

Growth parameter	Silicon concentration (mM)	Cd treatment and time of Si addition		
		−Cd +Si^E	+Cd +Si^E	+Cd +Si^L
Shoot length (cm)	0.0	77.6±0.93 c	23.9±2.69 a	23.9±2.69 a
	0.2	73.0±1.05 c	24.2±5.07 a	24.4±3.12 a
	0.6	73.4±3.86 c	25.4±4.45 a	35.6±4.98 b
Root length (cm)	0.0	45.8±1.77 d	23.9±2.91 a	23.9±2.69 a
	0.2	47.6±1.50 d	28.6±4.98 ab	29.6±2.52 a
	0.6	40.2±4.75 cd	25.5±5.37 a	37.8±3.58 bc
Shoot dry weight (g)	0.0	8.29±0.32 c	0.33±0.11 a	0.33±0.11 a
	0.2	9.05±0.38 c	0.45±0.25 a	0.34±0.11 a
	0.6	8.03±0.43 c	0.28±0.09 a	1.02±0.41 b
Root dry weight (g)	0.0	1.22±0.09 c	0.13±0.02 a	0.33±0.02 a
	0.2	1.31±0.16 c	0.15±0.04 a	0.14±0.02 a
	0.6	1.14±0.43 c	0.13±0.02 a	0.27±0.07 b
Total leaf area (cm^2)	0.0	683.0±31.7 c	73.2±18.6 a	73.2±18.6 a
	0.2	713.3±40.6 c	95.4±41.3 a	72.8±16.5 a
	0.6	716.4±38.8 c	65.3±15.6 a	179.5±58.3 b

Plants were 6 days old when exposed to 2.5 μM Cd for 48 days. Silicon nutrition was initiated when seedlings were 6 days old or 20 days old, representing early (+Si^E) or late (+Si^L) Si treatment, respectively. Data represents means ± SE of five replications. Values with the same lower case letter within a parameter are not significantly different.

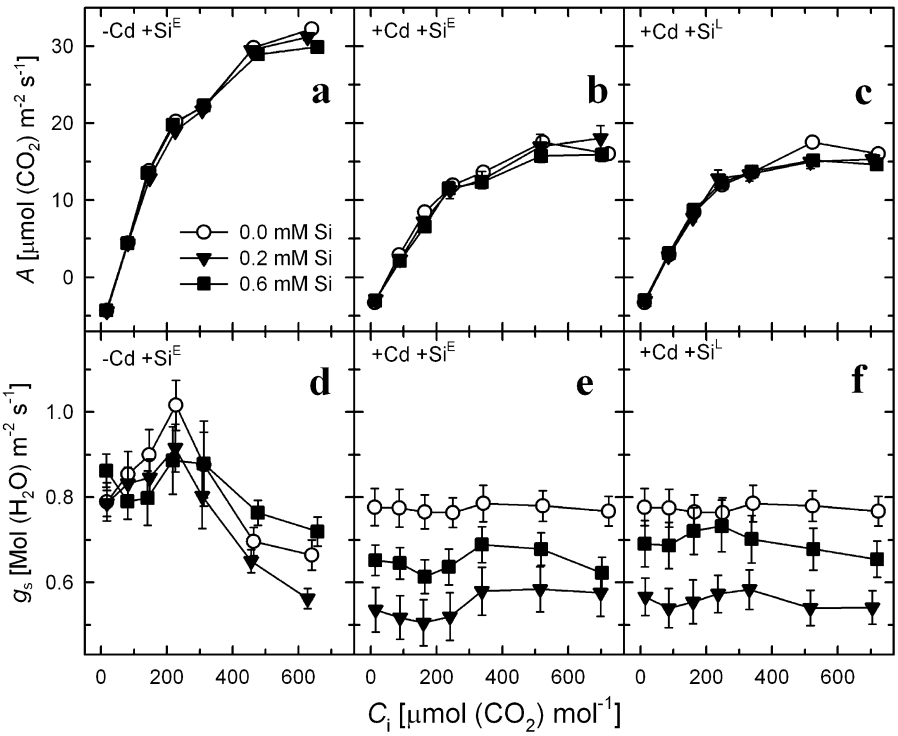

Fig. 2 Effects of different levels of Si nutrition on net CO_2 assimilation rate (A) and stomatal conductance (g_s) at varying intercellular CO_2 concentrations (C_i) of rice seedlings in the absence (−) or presence (+) of Cd. Plants were 6 days old when exposed to 2.5 μM Cd for 48 days. Silicon nutrition was initiated when seedlings were 6 or 20 days old, representing early (+Si^E) or late (+Si^L) Si treatment, respectively. Means ± SE of five replications are shown

attained their g_{smax} values when the value of C_i was close or similar to what is expected under normal growth conditions (Chen and Huerta 1997).

Effects of Cd and Si on chlorophyll fluorescence parameters

We observed that treatment with Cd significantly reduced the fluorescence yields of F_o, especially between −Cd +Si^E and +Cd +Si^E plants (Fig. 5a). Additionally, in +Cd plants, the addition of 0.6 mM Si^E resulted in the lowest F_o value compared to almost all other treatments (Fig. 5a). Furthermore, treatment with Cd significantly reduced the values of F_m and F_v/F_m, but increased F_o/F_m, regardless of the addition of Si (Fig. 5b–d). However, in +Cd plants, the addition of 0.6 mM Si^E induced a significant increase in and F_v/F_m compared to Si-deficient plants (Fig. 5c). Furthermore, in +Cd plants, the addition of 0.2 mM Si^L induced a significant increase in q_P compared to all other treatments (Fig. 5e). Our analysis did not detect any significant effects on q_N due to Cd and/or Si treatments (Fig. 5f).

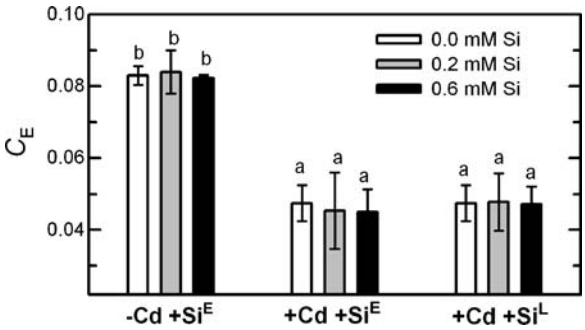

Fig. 3 Effects of different levels of Si nutrition on carboxylation efficiency (C_E) of rice seedlings in the absence (−) or presence (+) of Cd. Plants were 6 days old when exposed to 2.5 μM Cd for 48 days. Silicon nutrition was initiated when seedlings were 6 or 20 days old, representing early (+Si^E) or late (+Si^L) Si treatment, respectively. Bars represent means ± SE of five replications. *Bars* with the same *lower case letter* are not significantly different

Discussion

Our study showed that plants treated with Si accumulated significantly more Si in their shoot than roots (Fig. 1a,b). This is typical for Si-accumulating plants like rice, and is in agreement with a recent demonstration that Si uptake in rice is an active process (Ma et al. 2006, 2007). Active Si uptake has also been suggested in wheat (*Triticum aestivum*; Rains et al. 2006). On the other hand, Cd uptake is

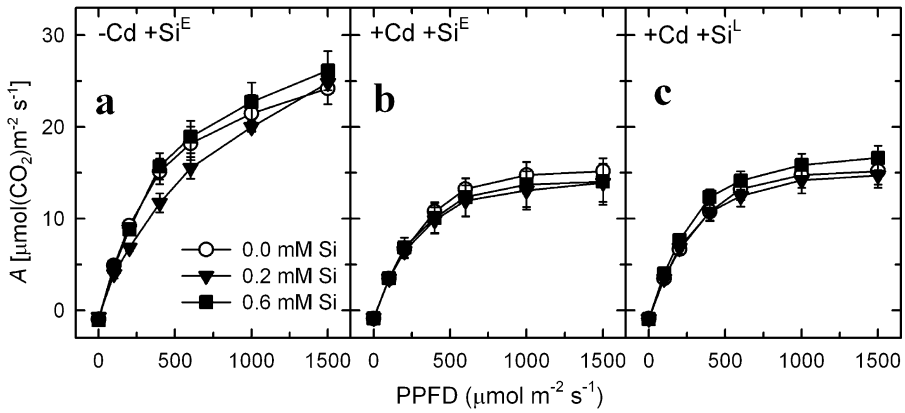

Fig. 4 Effects of different levels of Si nutrition on net CO_2 assimilation (A) rate at varying photosynthetic photon flux densities (PPFD) of rice seedlings in the absence (−) or presence (+) of Cd. Plants were 6 days old when exposed to 2.5 μM Cd for 48 d. Silicon nutrition was initiated when seedlings were 6 or 20 days old, representing early ($+Si^E$) or late ($+Si^L$) Si treatment, respectively. Means ± SE of five replications are shown

typically taken up apoplastically by plants (Clemens 2006), but can also be taken up symplastically as an opportunistic cation, imported through selective cation channels (Kim et al. 2002; Shi et al. 2005). Thus, except for Cd-hyperaccumulating plants, plants are generally known to accumulate significantly more Cd in their root than shoot (Kirkham 2006), as shown in our study (Fig. 1c,d).

Silicon-induced inhibition of Cd-uptake in plants as observed in this study (Fig. 1c,d), has also been previously demonstrated. Wang et al. (2000) showed that cell wall-bound silica has a strong affinity for

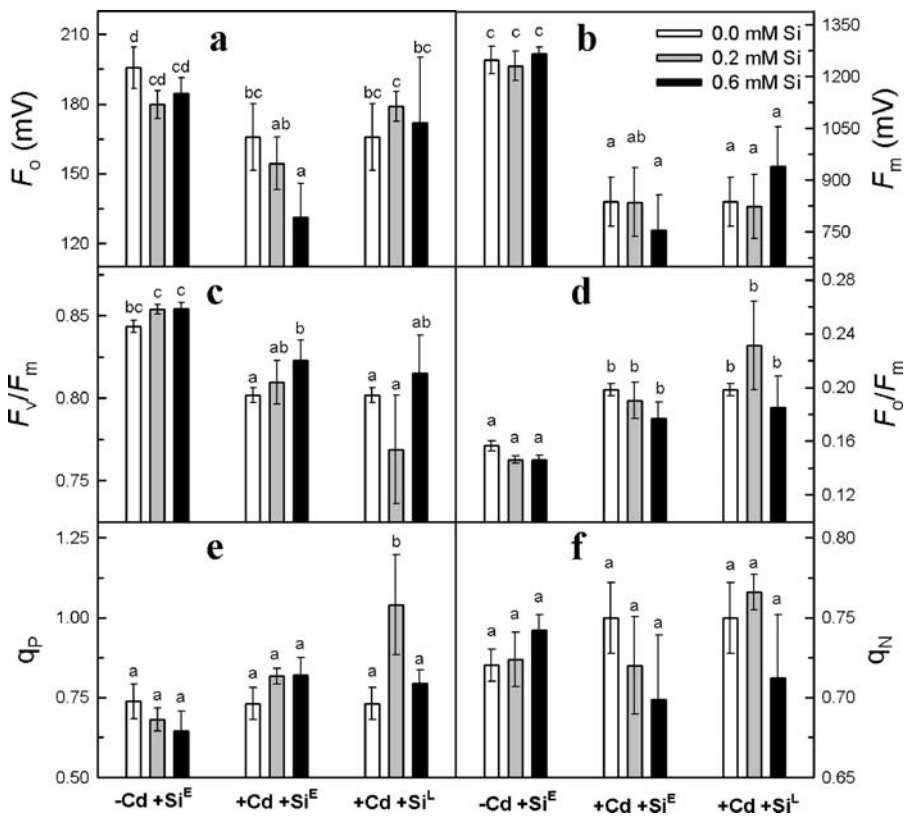

Fig. 5 Effects of different levels of Si nutrition on chlorophyll fluorescence parameters (**a** F_o, **b** F_m, **c** F_v/F_m, **d** F_o/F_m, **e** q_P, and **f** q_N) of rice seedlings in the absence (−) or presence (+) of Cd. Plants were 6 days old when exposed to 2.5 μM Cd for 48 days. Silicon nutrition was initiated when seedlings were 6 or 20 days old, representing early ($+Si^E$) or late ($+Si^L$) Si treatment, respectively. Bars represent means ± SE of five replications. *Bars* with the same *lower case letter* within a parameter are not significantly different

Cd. Thus, the elevated concentration of Si in Si-accumulating plants, such as rice, can significantly inhibit apoplastic Cd uptake by covalently bonding with and trapping Cd as it diffuses through the cell wall and extracellular spaces. Furthermore, Neumann and Zur Neiden (2001) showed Si-induced inhibition of symplastic heavy metal transport and suggested that intracellular Si is able to form unstable silicates with heavy metals in the cytoplasm. They stated that these silicates are then slowly degraded to SiO_2 and retained, whereas the heavy metals are sequestered and bound in the vacuole by organic acids (Neumann and Zur Neiden 2001). Shi et al. (2005) showed a restriction of Cd transport by Si but observed that Si did not change the distribution ratio of symplastic or apoplastic bound Cd. Silicon has also been shown to alleviate Al toxicity via external precipitation of Al or external immobilization of Al by the formation of Al–Si complexes in the external solution (Liang et al. 2001). Additionally, Si-induced alleviation of Mn toxicity in barley (*Hordeum vulgaris* L.) was shown to be due to a more homogenous distribution of Mn in leaves as opposed to high concentrations of Mn at necrotic spots of Si-deficient plants (Horigchi and Morita 1987). Thus, any of the above scenarios could be responsible for the observed reduction in the tissue-Cd content of +Cd plants due to the addition of Si (Fig. 1c,d).

Furthermore, Cd toxicity in plants has been shown to be mediated by the inhibition of antioxidases resulting in lipid peroxidation and superoxide anion generation (Chien et al. 2001; Shah et al. 2001), which are factors that can significantly inhibit growth and physiological processes in plants. Whereas, Si treatment has been shown to increase the activities of antioxidases (Liang 1999; Liang et al. 2003; Gong et al. 2005). Thus, the significant reduction of Cd-availability by Si in plant tissues would be complementary to the overall alleviation of Cd-induced inhibition of growth and photosynthesis, which are further discussed.

The inhibition of growth in plants treated with Cd, as observed in this study (Table 1), has been suggested to be due to reduced cell expansion mediated by increased cross-linking of pectin in the middle lamellae (Poschenrieder et al. 1989). Cadmium-induced inhibition of growth has also been demonstrated to result from the accumulation of storage products at the expense of cell growth and elongation (Moya et al. 1995; Verma and Dubey 2001). Furthermore, Chen and Huerta (1997) suggested that Cd-induced growth inhibition is due to an inhibition of photosynthesis. Thus, based on our results we suggest that the inhibition of growth due to low-level Cd-stress is most likely coupled to a reduction in photosynthesis (Figs. 2a–c, 3 and 4). However, Cd-induced reduction in cell expansion and/or increased lipid peroxidation as well as increase in the activities of reactive oxygen species, as earlier suggested, could as well be responsible for the observed reduction in growth due low-level Cd-stress. Furthermore, Ma et al. (1989) studied the effect of time of addition of Si nutrition on rice plants and showed that plants whose Si treatments were initiated late (only during the reproductive growth stage) showed significantly higher productivity compared to plants treated early (only during the vegetative growth stage). Additionally, Hossain et al. (2002) demonstrated that Si did not promote growth during the early stages of organ development and suggested that Si-induced growth promotion in rice plants is due to an increase in cell wall extensibility, which was also supported in a study by Hattori et al. (2003). These studies are in agreement with our results particularly for +Cd plants treated with 0.6 mM Si^L (Table 1).

It is interesting that under Cd-stress, only 0.6 mM Si^L-treated plants showed a significant increase in growth (Table 1), even when their tissues had significantly less Si compared to 0.6 mM Si^E-treated plants (Fig. 1a,b). We suggest that this could be due to a threshold requirement of Si treatment for the alleviation of low-level Cd-induced inhibition of growth. Several mineral nutrition studies have presented similar nutrient threshold requirements in plants. A study on Si nutrition requirement by Datnoff et al. (1997) suggested that the minimum tissue concentrations of Si required for disease control in rice is between 3–5%. Another study on Si and P requirements showed that in the presence of Si, treatment of rice plants with 0.014 or 0.7 mM P both increased the dry weight of rice plants more than treatment with 0.21 mM P, but that in the absence of Si, shoot dry weight was highest at 0.21 mM P compared to the 0.014 or 0.7 mM P treatments (Ma and Takahashi 1990). Furthermore, in a general study on mineral nutrition requirement in plants, Chaudhari and Singh (2006) demonstrated that increasing N, P, K, Ca, and S levels significantly increased plant height, dry weight, chlorophyll content, pod yields, and *E* in ground nut plants (*Arachis hypogaea*) up to a

certain threshold value, and the actual nutrient concentration responsible for achieving that threshold value represented the optimum nutrient requirement for that parameter. However we observed that in +Cd plants, the addition of 0.2 mM Si^E induced about the same tissue-Si content as observed in 0.6 mM Si^L (Fig. 1a,b), but showed no improvement in growth (Table 1). We suggest that this could be due to the significant reduction in g_s in the former (Fig. 2e). Plants depend on the transpiration pull to maximize the uptake of other essential nutrients, besides Si, that are necessary for growth (Silberbush et al. 2005; Henriet et al. 2006). Thus, putting all these in context, we propose that 0.6 mM Si^L is required for the alleviation of low-level Cd-mediated growth inhibition.

Cadmium-induced inhibition of photosynthesis is typically attributed to an inhibition of the activities of key enzymes of the Calvin cycle and the photosynthetic electron transport chain. Stiborova (1988) showed a Cd-induced inhibition of Rubisco in barley, while Chugh and Sawhney (1999) found that Cd inhibited not only Rubisco, but also fructose-1, 6-bisphosphatase and NADP-glyceraldehyde-3-phosphate dehydrogenase in garden pea (*Pisum sativum*). Cadmium has also been shown to negatively affect photosynthetic efficiency by disrupting thylakoid organization in maize (Souza et al. 2005). Furthermore, a reduction in A_{max} and C_E due to Cd treatment has been associated with the inhibition of the activities of RUBISCO (Chen and Huerta 1997; Panković et al. 2000). However, studies have also suggested that Cd inhibited photosynthesis by inducing stomatal closure (Souza et al. 2005), which would consequently inhibit g_s (Flexas and Medrano 2002) and/or C_i (Vassilev et al. 2002). Thus, since our study showed that treatment with Cd resulted in an inhibition of A_{max} (Fig. 2a–c), C_E (Fig. 3), and I_s (Fig. 4) without a reduction in C_i (Fig. 5), we conclude that the low-level Cd-induced inhibition of photosynthesis observed in our rice seedlings seems to be less associated to a reduction in CO_2 diffusion into the leaves, but more likely due to an inhibition of Calvin cycle enzymes and/or an inhibition of the photosynthetic electron transport chain (Jiang et al. 2006).

Our results showed a significant loss in stomatal control due to Cd treatment, especially in Si-deficient plants (Fig. 2d–f), which has also been demonstrated in previous studies. Souza et al. (2005) showed severely damaged guard cells of maize plants due to Cd-treatment, while Perfus-Barbeoch et al. (2002) reported that Cd inhibited guard cell regulation in an ABA-independent manner.

Silicon-treated plants generally showed a reduction in g_s without inhibiting A (Fig. 2), suggesting a Si-induced increase in instantaneous WUE (IWUE) as has been previously demonstrated (Agarie et al. 1998; Ma and Takahashi 2002b; Gao et al. 2006; Romero-Aranda et al. 2006). Silicon is taken up by rice plants in the monosilicic acid form but is predominantly stored in leaves in special silica cells, forming a Si-cuticle double layer (Yoshida et al. 1962; Mitani et al. 2005). This Si-cuticle double layer is suggested to be responsible for Si-induced improvement of IWUE by decreasing the rate of water loss from leaves without inhibiting A. Interestingly, in −Cd plants, although we observed a general Si-induced reduction in g_{smax}, only the plants supplied with 0.2 mM Si maintained significantly lower g_s when C_i was high [>500 μmol (CO_2) mol^{-1}] compared to Si-deficient plants (Fig. 2d). Additionally, in +Cd plants, all the addition of Si generally reduced g_s, treatment with 0.2 mM Si significantly reduced g_s more than treatment with 0.6 mM Si (Fig. 2e,f). These observations are in contrast with those of previous studies, which have generally shown an inversely linear relationship between Si treatment concentration and g_s values in rice (Ma and Takahashi 2002b). However, most of the studies involving the effect of Si on g_s in rice plants have been limited to Si nutrition concentrations not exceeding 0.1 mM (Agarie et al. 1998; Ma and Takahashi 2002b). As such we propose that there is also a threshold Si treatment requirement for Si-induced effects on photosynthesis. Thus since the addition of 0.2 mM Si compared to 0.0 or 0.6 mM Si, induced significantly reduced g_s without inhibiting A (Fig. 2), we suggest that a concentration of 0.2 mM Si is close to the optimum requirement for maximum Si-induced decrease in g_s without an inhibition of A, thus increasing IWUE in rice plants, especially under elevated C_i or low-level Cd-stress conditions.

Chlorophyll fluorescence parameters are frequently used to detect stress-induced effects on light utilization efficiency of the photosynthetic machinery (Maxwell and Johnson 2000; Roháček 2002). Studies have shown that changes in the value of F_o could be interpreted in three ways. Firstly, F_o often represents an estimate of the relative size of the antenna pigments of the PS2 complex (Huang et al. 2004). Secondly, an

increase in F_o has been shown to be a symptom of damage to the PS2 reaction center resulting in a decrease in absorbed light and subsequent increase in un-used emitted light (Schnettger et al. 1994). Thirdly, under normal conditions, dissipation of absorbed light energy by chlorophyll fluorescence is mainly through fluorescence of the chlorophyll molecules associated with PS2 (Krause and Weis 1991). However, under stress conditions, F_o levels can increase due to a contribution of chlorophyll fluorescence emission from PS1 (Pfundel 1998). Thus, with all three points in mind, together with our growth and gas exchange results, we suggest that the high F_o values observed in our −Cd plants compared to +Cd plants (Fig. 5a), is most likely due to a larger size of antenna pigments in −Cd plants compared to +Cd plants. Furthermore, we suggest that the decrease in F_o of Cd-stressed plants due to Si^E treatment (Fig. 5a) is most likely due to a Si-induced alleviation of Cd-mediated damage to PS2 components and not due to reduced chlorophyll content because all Cd-treated plants showed relatively similar extents of chlorosis. Additionally, in +Cd plants, the high F_o values of Si^L-treated plants compared to Si^E-treated plants could be due to an increased contribution of chlorophyll fluorescence from PS1 and/or more damaged PS2 centers in Si^L-treated plants compared to Si^E-treated plants. This suggests that an early addition of Si to low-level Cd-stressed plants could be required for the alleviation of Cd-induced inhibition of F_o.

Furthermore, we observed that treatment with Cd reduced F_m and F_v/F_m but increased F_o/F_m (Fig. 5b–d). Atal et al. (1991) also showed a reduction in F_m and F_v in wheat seedlings (*Triticum aestivum* L.) due to Cd treatment. Ralph and Burchett (1998) suggested that the Cd-induced decline in F_m observed in seagrass (*Halophila ovalis*) was due to a change in the ultrastructure of the thylakoid membrane, negatively affecting the electron transport rate. Vassilev and Manolov (1999) suggested that Cd reduced the value of F_v/F_m in barley plants by inducing a down regulation of PS2 in order to avoid an over-reduction of QA, thus reducing the load on the electron transport chain. Additionally, a reduction in F_v/F_m ratio, especially under stress conditions, is often an indicator of photoinhibition or other kind of injury to PS2 components (Roháček 2002). Furthermore, under high irradiance, xanthophylls localized in the light harvesting complex of photosystems are thought to be involved in a non-radiative de-excitation of excess light energy in order to protect plants from photodamage, a process known as non-photochemical quenching (Larsson et al. 1998). Therefore, an increase in non-photochemical quenching would be expected under Cd-stress as a result of a decrease in the utilization of light energy due to Cd-induced reduction in PS2 efficiency or F_v/F_m (Fig. 5c). This might explain the increase in the value of F_o/F_m in +Cd plants (Fig. 5d). Several studies have reported stress-induced increases in the value of F_o/F_m (Bilger et al. 1987; Horton and Ruban 1992).

However, in +Cd plants, the addition of 0.6 mM Si^E significantly increased the value F_v/F_m (Fig. 5c), while the addition of 0.2 mM Si^L significantly increased the value of q_P (Fig. 5e). Kaufman et al. (1979) proposed a "window hypothesis" for Si by suggesting that Si in the form of silica bodies deposited in leaf epidermal cells could act as a "window" that enhances light-use-efficiency by facilitating the transmission of light to the photosynthetic mesophyll tissue. Consequently, Si-induced enhancement of chlorophyll fluorescence parameters was recently demonstrated in salt-stressed tomato (Al-aghabary et al. 2004) and drought-stressed maize plants (Li et al. 2007). Thus, the observed Si-induced increase in F_v/F_m and q_P in rice plants, especially in +Cd plants, can be broadly interpreted as an increase in the application of light energy towards sustaining photochemical reactions, which denotes an increase in light-use-efficiency (Krause and Weis 1991). However, considering the fact that we observed a significant Cd-induced inhibition of A_{max} (Fig. 2a–c), C_E (Fig. 3), and I_s (Fig. 4), we suggest that our inability to detect a significant Cd-induced effect on q_P or q_N (Fig. 5e,f) might be dependent on the relatively low intensity of our actinic light source (~550 μmol m^{-2} s^{-1}) as demonstrated by Vassilev and Manolov (1999).

In summary, this study suggests that the effect of Si nutrition on Cd-uptake, growth and photosynthesis in rice plants under low-level Cd-stress is dependent on Si treatment concentration as well as time of Si addition. Thus, with respect to tissue-Cd content, there was an inversely linear relationship between Si treatment concentration and tissue-Cd content. Additionally, 0.6 mM Si^L seems to be required for Si-induced alleviation of low-level Cd-mediated growth inhibition. Furthermore, gas exchange analysis showed that, a concentration of 0.2 mM Si could be close to the optimum requirement

for maximum Si-induced reduction in g_s without inhibiting A, thus increasing IWUE. Our chlorophyll fluorescence analysis suggests that Si alleviates low-level Cd toxicity by reducing F_o while increasing F_v/F_m, and q_P, thus improving light-use-efficiency.

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