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Silicon-Mediated Amelioration of Fe^{2+} Toxicity in Rice (*Oryza sativa* L.) Roots*[1]

FU You-Qiang[1], SHEN Hong[1,*2], WU Dao-Ming[1] and CAI Kun-Zheng[2]

[1] *College of Resources and Environment, South China Agricultural University, Guangzhou 510642 (China)*
[2] *College of Agronomy, South China Agricultural University, Guangzhou 510642 (China)*

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ABSTRACT

Silicon (Si) can enhance the resistance of plants to many abiotic stresses. To explore whether Si ameliorates Fe^{2+} toxicity, a hydroponic experiment was performed to investigate whether and how Si detoxifies Fe^{2+} toxicity in rice (*Oryza sativa* L.) roots. Results indicated that rice cultivar Tianyou 998 (TY998) showed greater sensitivity to Fe^{2+} toxicity than rice cultivar Peizataifeng (PZTF). Treatment with 0.1 mmol L^{-1} Fe^{2+} inhibited TY998 root elongation and root biomass significantly. Reddish iron plaque was formed on root surface of both cultivars. TY998 had a higher amount of iron plaque than PZTF. Addition of Si to the solution of Fe treatment decreased the amount of iron plaque on root surface by 17.6% to 37.1% and iron uptake in rice roots by 37.0% to 40.3%, and subsequently restored root elongation triggered by Fe^{2+} toxicity by 13.5% in the TY998. Compared with Fe treatment, the addition of 1 mmol L^{-1} Si to the solution of Fe treatment increased xylem sap flow by 19.3% to 24.8% and root-shoot Fe transportation by 45.0% to 78.6%. Furthermore, Si addition to the solution of Fe treatment induced root cell wall to thicken. These results suggested that Si could detoxify Fe^{2+} toxicity and Si-mediated amelioration of Fe^{2+} toxicity in rice roots was associated with less iron plaque on root surface and more Fe transportation from roots to shoots.

Key Words: cell wall, Fe transportation, iron plaque, xylem sap

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INTRODUCTION

In humid tropical and subtropical area such as South Asia, Fe^{2+} toxicity is one of the major physiological diseases that limit rice growth, especially in early spring time (Li *et al.*, 2001; Shimizu *et al.*, 2005; Olaleye *et al.*, 2009; Zhang *et al.*, 2011). Fe^{2+} toxicity injures plants by inhibiting the elongation of rice roots. Batty and Younger (2003) indicated that iron plaque on the surface of rice roots was harmful to the roots, which decreased root activity and inhibited nutrient uptake (Zhong *et al.*, 2010). Moreover, the epidermal and cortex cells within rice roots died when iron plaque was formed (Zhang *et al.*, 2011). However, Liang *et al.* (2006) indicated iron plaque could act as a nutrient reservoir and enhance the growth of rice roots. The contrasting results are probably related to different rice cultivars or genotypes, Fe^{2+} concentration, durations, *etc.* (Shimizu *et al.*, 2005; Liang *et al.*, 2006; Olaleye *et al.*, 2009; Zhang *et al.*, 2011). The overall effects of iron plaque on the growth of plant may depend on the amount and thickness of iron plaque (Otte *et al.*, 1989) and the status of the nutrients (Zhang *et al.*, 1999; Liang *et al.*, 2006).

Silicon (Si) is a beneficial element for crop growth, which plays an important role in the growth and development of crop, especially for gramineae crops (Epstein, 1999; Hodson *et al.*, 2005). Si could alleviate the toxicity of metals in metal-contaminated soils, such as aluminum, manganese, cadmium, and zinc (Liang *et al.*, 2001; Neumann and zur Nieden, 2001; Iwasaki *et al.*, 2002; Ryder *et al.*, 2003; Liang *et al.*, 2005; Vieira da Cunha, 2008; Doncheva, 2009; Song, 2009). Most of the beneficial effects of Si are realized through Si deposition in cell walls of the epidermal surfaces of leaves, stems, and hulls (de Melo *et al.*, 2010). Deposition of Si enhances the strength and rigidity of cell walls and thus increases the resistance of plants to various stresses (Ma *et al.*, 2004). Up to now, it remains unclear whether Si has ameliorating effects on Fe^{2+}

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*[2]Corresponding author. E-mail: hshen@scau.edu.cn.

toxicity. If so, how does Si ameliorate the toxicity of Fe^{2+} to rice roots? In this study, two rice varieties differing in Fe^{2+} sensitivity were used to examine the ameliorating effects of Si on Fe^{2+} toxicity. The mechanisms of Si-mediated amelioration of Fe^{2+} toxicity are also discussed.

MATERIALS AND METHODS

Plant materials and growth conditions

Two rice (*Oryza sativa* L.) cultivars, *i.e.*, Tianyou 998 (TY998) and Peizataifeng (PZTF), were purchased from the Rice Institute, Guangdong Academy of Agricultural Sciences, Guangdong, China. After sterilizing in 10% (v/v) H_2O_2 for 10 min and washing in deionized water thoroughly, rice seeds were germinated on a piece of nylon mesh that was floated on 0.5 mmol L^{-1} $CaCl_2$ (pH 5.5) solution at 30 °C. After 5-d germination, rice seedlings of similar size were selected and transplanted to a 10 L plastic container (30 cm length, 20 cm width and 14 cm height). The seedlings were grown in a 0.5-strength nutrient solution for 3 d and thereafter in full strength solution for a further 21 d. The nutrient solution formula was modified slightly according to Yoshida *et al.* (1976). The nutrients in the solution were as follows: the macronutrients: NH_4NO_3, 0.429 mmol L^{-1}; $Ca(NO_3)_2{\cdot}4H_2O$, 1 mmol L^{-1}; $MgSO_4{\cdot}7H_2O$, 1.667 mmol L^{-1}; KH_2PO_4, 1 mmol L^{-1}; and K_2SO_4, 0.513 mmol L^{-1}; and the micronutrients: Fe(III)-EDTA, 50 μmol L^{-1}; $MnSO_4$, 9.1 μmol L^{-1}; $ZnSO_4$, 0.15 μmol L^{-1}; $CuSO_4$, 0.16 μmol L^{-1}; $(NH_4)_4MoO_{24}{\cdot}4H_2O$, 0.52 μmol L^{-1}; and H_3BO_3, 19 μmol L^{-1}. The pH of nutrient solution was adjusted to 5.5 using 0.1 mol L^{-1} HCl. The volume of solution was restored daily with the tap water and renewed every 3 d.

After 21-d growth, the seedlings of the similar size were selected and transplanted in a PVC container (7.5 cm diameter × 14 cm height, one seedling per pot) containing 0.5 L of P-deficient nutrient solution for 2 d. After P starvation, rice seedlings were exposed to 3 treatments as follows: 1) control; 2) 0.1 mmol L^{-1} $FeSO_4$ (Fe); and 3) 0.1 mmol L^{-1} $FeSO_4$ and 1 mmol L^{-1} Na_2SiO_3 (Fe+Si). pH value in the treatment solution was set at 5.5. Treatment duration was 2 d. Rice seedlings were placed in a growth chamber and kept in a controlled environment (180 μmol m^{-2} s^{-1}, 30 °C/20 °C day/night temperatures and 14 h/10 h light/dark cycles).The light intensity and relative humidity were 500 μmol m^{-1} s^{-1} and 70%, respectively. Treatments were arranged with 4 replicates in a completely randomized design.

DCB (dithionite-citrate-bicarbonate) extraction of iron plaque

After treatment, the primary root length was measured by a ruler, and the number of roots was counted. Fresh roots were washed in tap water followed by deionized water, and then dried by filter paper. Iron plaque on the root surfaces was extracted with a cold DCB solution according to Taylor and Crowder (1983) and Otte *et al.* (1989). The whole root system was mechanically agitated at room temperature (25 °C) for 3 h in the mixture of 40 mL of 0.3 mol L^{-1} sodium citrate, 5 mL of 1.0 mol L^{-1} sodium bicarbonate, and 3 g of sodium dithionite. Roots were then rinsed three times in deionized water. The resulting solution was made up to 100 mL with deionized water for further Fe assay.

Measurement of Fe^{2+} and Fe^{3+}

Fe^{2+} and Fe^{3+} in iron plaque of rice roots were measured with ethylenediamine tetra-acetic acid disodium-bathophenanthroline disulfonate (EDTA-BPDS). Rice roots were firstly rinsed in deionized water for 6 h, then extracted in a mixed solution containing 1.0 mmol L^{-1} of EDTA and 0.3 mmol L^{-1} of BPDS for 5 h in shake with the speed of 125 r min^{-1} (rotation per minute) at room temperature. After that, the extraction solution was collected for Fe analysis. In case of Fe analysis, firstly, Fe^{2+} in the solution was measured by using a UV-visible spectrophotometer at the 535 nm wavelength. Then the Fe^{3+} in the solution was reduced into Fe^{2+} by 30 mmol L^{-1} NaS_2O_4. The total Fe^{2+} in the solution was measured by using a UV-visible spectrophotometer at the 535 nm wavelength. The difference between the total Fe^{2+} concentration and the first Fe^{2+} concentration indicated Fe^{3+} concentration (Wang and Peverly, 1998).

Collection of xylem sap

Rice seedlings were firstly treated in a P-deficient nutrient solution containing 100 μmol L^{-1} Fe^{2+} with or without 1 mmol L^{-1} Si for 2 d. Then the shoot of rice seedling was excised at 2 cm above the point of root-shoot conjunction. Xylem sap was collected by a micropipette (Guo *et al.*, 2009). The total collection time is 5 h from 18:00 to 23:00. The xylem sap was diluted to 4 mL with distilled water for Si and Fe measurements.

Analysis of Fe and Si concentrations

After DCB extraction, both roots and shoots were

oven-dried at 70 °C for 72 h. Dried shoots and roots were finely ground into powder and 0.5 g of tissue powder was digested. After digestion, the solutions were cooled and diluted into 25 mL with ultrapure water. 1 mL of 22.5 mol L^{-1} HF solution was added to the flasks. The solution was shaken overnight. A reagent blank and a standard reference sample were used to ensure the accuracy and precision of digestion and subsequence analysis. Concentrations of Si and Fe in the digestion solution and xylem sap were analyzed using the colorimetric molybdenum blue method described by van der Vorm (1987) and AAS (atomic absorption spectrophotometer: Hitachi Z-5700, Japan) method, respectively.

Microscopic observation

After treatment, the base roots were excised into 2- to 3-cm segments in a solution of 0.5 mmol L^{-1} $CaCl_2$. The root segments were washed 3 times in 0.5 mmol L^{-1} $CaCl_2$ solution and then transferred to a 5% (v/w) agarose solution at the temperature of 45 to 50 °C. The root segments were fixed in agarose medium at 4 °C for over 2 h. The sections of root segments were obtained by a vibrating microtome (Microsom HM-650 V). The thickness of root section was 70 μm. The observation was performed directly under a light microscope and the typical pictures were obtained.

Transmission electron microscopy observation

After treatment, rice roots of cultivar TY998 were washed and prepared for ultra-structural and microscopic observation. Briefly, samples of root segments (1 cm) were fixed in a mixture of 3% (v/v) glutaraldehyde and 2% (w/v) paraformaldehyde in 0.1 mol L^{-1} phosphate buffer (pH 7) for overnight at room temperature. Samples were subsequently postfixed with 1% (w/v) osmium tetroxide in the same buffer for 1 h at 4 °C and dehydrated in a graded ethanol series. Ultrathin sections (0.1 μm) were cut by using a UCT ultramicrotome (Leica, Germany), and then stained with uranyl acetate and lead citrate. The observation was performed under a Tecnai 12 transmission electron microscope (FEI, the Netherlands).

Statistical analysis

All experimental data shown in the tables and figures were examined statistically by analysis of variance. Their mean values were subjected to Duncan's new multiple range test at $P < 0.05$ using SPSS 17.0.

RESULTS

Growth of rice seedlings

Results from Table I indicated that two rice cultivars showed different responses to Fe treatment. Fe treatment decreased the biomass and root number of rice cultivar TY998 significantly, while influenced rice cultivar PZTF slightly, suggesting that TY998 was more sensitive to Fe^{2+} toxicity than PZTF. Fe+Si treatment increased the biomass and root numbers of TY998 and ameliorated the toxic effect of Fe^{2+} on this cultivar obviously (Table I). Root biomass and root number of TY998 under Fe+Si treatment were 123.1% and 119.0%, respectively, of those under Fe treatment. Fe treatment did not reduce the biomass of PZTF. Interestingly, Fe+Si treatment increased shoot biomass of PZTF significantly. The biomass and root numbers of TY998 were higher than those of PZTF under the control and Fe treatment, while the resistance of TY998 to Fe^{2+} toxicity was lower than that of PZTF. The reason needs to be elucidated.

Formation of iron plaque on root surface

Liu *et al.* (2008) indicated that P starvation-induced formation of iron plaque on root surface was

TABLE I

Biomasses, root lengths and root numbers of the rice seedlings tested

Rice cultivar	Treatment	Dry biomass		Primary root length	Root number
		Root	Shoot		
		mg $plant^{-1}$		cm	$plant^{-1}$
PZTF	Control	93.8±3.5[a)]a[b)]	402.5±25.0b	24.5±1.8a	15.7±1.8a
	Fe	93.1±3.4a	419.2±24.3b	24.8±1.4a	12.7±2.4a
	Fe+Si	95.1±2.5a	462.5±13.8a	24.5±0.8a	13.7±0.7a
TY998	Control	120.0±7.3a	570.0±12.2b	24.8±1.0a	25.3±1.2a
	Fe	101.8±4.1b	522.5±20.4c	22.3±0.8b	21.0±1.5b
	Fe+Si	125.3±6.8a	612.5±12.2a	25.3±0.5a	25.0±1.7a

[a)] Means±standard errors ($n = 4$).

[b)] Values followed by the same letter within a column for the same cultivar are not significantly different at $P < 0.05$, according to Duncan's new multiple range test.

the results of bio-chemical reactions occurred on rice root surface. In this study, after 2-d P starvation, rice roots was then treated with 0.1 mmol L^{-1} Fe^{2+} for 2 d, and reddish iron plaque was appeared on root surface of rice seedlings (Fig. 1b, c). They showed an uneven distribution along different root segments. The basal roots had a higher amount of iron plaque, while the apical roots had less iron plaque. Some new-born roots did not have iron plaque (Fig. 1b). Interestingly, iron plaque was also formed on the surface of root hairs. To explore the cross distribution of iron plaque, root cross section was made by a vibrating microtome and observed under a microscope. Results indicated that iron plaque was mainly distributed on the surface area of epidermal cells (Fig. 1b′). The thickness of iron plaque was about 20 to 30 μm from epidermal cell surface. Addition of Si to Fe^{2+} solution decreased the formation of iron plaque (Fig. 1c′). The color of reddish iron plaque became much lighter under Si+Fe treatment (Fig. 1c′). The results suggested that Si amendment could reduce the precipitation of reddish iron plaque on the surface of epidermal cells and root hairs (Fig. 1b′, c′).

Si and Fe concentrations in iron plaque

To examine how Si reduces iron plaque on root surface of rice seedlings, Fe and Si concentrations in iron plaque were measured by DCB method. Results indicated that rice roots under the control had very little iron plaque compared with those under Fe or Fe+Si treatment (Fig. 2). Fe and Si concentrations on root surface of rice seedlings under the control were 0.26 to 0.54 g kg^{-1} and 42.37 to 195.38 mg kg^{-1}, respectively. Rice cultivar TY998 had a higher Fe concentration in its iron plaque than cultivar PZTF under the control condition. Fe concentration in iron plaque reached 5.64 to 6.91 g kg^{-1}. Addition of Si to Fe^{2+} solution decreased Fe concentration in iron plaque obviously in both PZFT and TY998 (Fig. 2). Si addition increased the concentration of Si in iron plaque significantly (Fig. 2).

Fe form in iron plaque

Except for the concentration of Fe, Fe^{2+} and Fe^{3+} in iron plaque was also examined, as shown in Table II. Fe in iron plaque was mainly presented as the form

Fig. 1 Distribution of reddish iron plaque on the surface of rice roots under treatments of control (a and a′), 0.1 mmol L^{-1} Fe^{2+} (b and b′) and 0.1 mmol L^{-1} Fe^{2+} + 1 mmol L^{-1} Si (c and c′). After 21-d growth with another 2-d P starvation, rice roots were submitted to different treatments for 2 d, and then washed in deionized water for taking pictures. For a′–c′, the root sections (2 cm from the root base) were prepared by a vibrating microtome. Each treatment had 8 replicates.

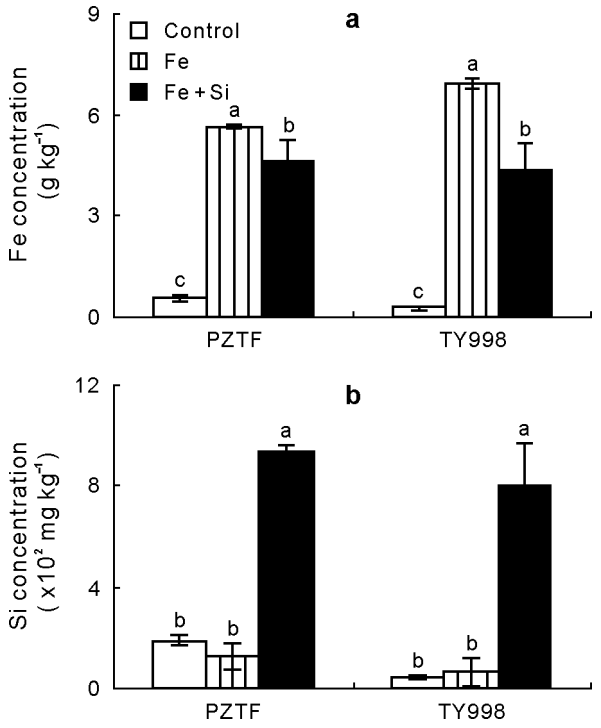

Fig. 2 Fe (a) and Si (b) concentrations in iron plaque on root surface of rice cultivars TY998 and PZTF under treatments of control, 0.1 mmol L^{-1} Fe^{2+} (Fe) and 0.1 mmol L^{-1} Fe^{2+} + 1 mmol L^{-1} Si (Fe+Si). Rice seedlings were grown in normal nutrient solution for 21 d and another 2-d P starvation, and then transferred to the solution of different treatments for 2 d. Error bars represent the standard errors of the means ($n = 4$). Values marked by the same letter for a given cultivar and a given element concentration are not significantly different at $P \leq 0.05$.

TABLE II

Concentrations of Fe^{3+} and Fe^{2+} in iron plaque

Treatment	Fe^{2+}	Fe^{3+}	$Fe^{3+}/(Fe^{3+} + Fe^{2+})$
	mg kg^{-1}		%
Control	3.1±0.4[a)]c[b)]	38.2±0.6c	91.7±0.5c
Fe	25.9±1.2a	573.0±24.1a	95.0±0.1b
Fe+Si	19.2±2.6b	457.3±30.5b	96.0±0.5a

[a)]Means±standard errors ($n = 4$).
[b)]Values followed by the same letter within a column are not significantly different at $P < 0.05$, according to Duncan's new multiple range test.

of Fe^{3+} under all 3 treatments. Fe treatment increased both Fe^{2+} and Fe^{3+} concentrations in iron plaque on the surface of rice roots greatly. While the addition of 1 mmol L^{-1} Si to Fe^{2+} solution decreased the concentrations of both Fe^{2+} and Fe^{3+} in iron plaque by 25.9% and 20.2%, respectively. Moreover, the addition of Si to the Fe^{2+} solution raised the ratio of $Fe^{3+}/(Fe^{3+} + Fe^{2+})$, suggesting that Si addition elevated the oxidative capacity of rice roots (Table II).

Fe and Si concentrations in xylem sap

To explore the mechanism of Si reducing Fe concentration in iron plaque, Fe and Si in xylem sap was examined. Results showed that the volume of rice xylem sap with Fe treatment was much reduced in comparison to that of the control (Table III). Addition of Si to Fe solution increased the volume of xylem sap significantly. For example, the volume of xylem sap of rice cultivars PZTF and TY998 under Fe+Si treatment was 119.3% and 124.8% those of Fe treatment, respectively. Fe treatment increased the concentration of Fe in xylem sap obviously. The addition of Si to the Fe^{2+} solution reduced Fe concentration in xylem sap, but increased Fe transportation from roots to shoots in the two rice cultivars. For example, the amount of Fe in xylem sap was increased by 3.2% and 13.1% in rice cultivars PZTF and TY998, respectively, under Si+Fe treatment in comparison to Fe treatment. Si+Fe treatment increased Si concentration in xylem sap significantly. Rice cultivar TY998 had a larger volume of xylem sap than rice cultivar PZFT (Table III).

TABLE III

Xylem sap flow, Fe and Si concentrations in xylem sap

Rice cultivar	Treatment	Xylem sap velocity	Fe concentration	Si concentration
		μL 5 h^{-1} $plant^{-1}$	μg L^{-1}	mg L^{-1}
PZTF	Control	119.0±3.2[a)]a[b)]	526.3±42.5c	28.7±3.4b
	Fe	109.2±5.5b	713.8±31.2a	22.0±3.1b
	Fe+Si	130.3±10.2a	617.5±34.1b	47.1±4.2a
TY998	Control	218.8±2.5a	371.1±51.7b	37.7±3.5b
	Fe	170.1±6.4b	548.3±25.4a	24.1±6.5c
	Fe+Si	212.2±12.3a	497.1±16.3a	63.5±1.7a

[a)]Means±standard errors ($n = 4$).
[b)]Values followed by the same letter within a column for the same cultivar are not significantly different at $P < 0.05$, according to Duncan's new multiple range test.

Fe and Si uptake in rice seedlings

Fe and Si in rice seedlings were also measured. Results from Table IV indicated that Fe treatment increased Fe uptake significantly in both rice cultivars PZTY and TY998. However, Fe treatment decreased the ratio of shoot Fe to root Fe, suggesting that Fe treatment inhibited Fe transportation from roots to shoots. Addition of 1 mmol L^{-1} Si to Fe^{2+} solution reduced root Fe uptake, but enhanced shoot Fe uptake. The ratio of shoot Fe to root Fe in PZTF under Fe+Si treatment was 145% of that under Fe^{2+} treatment. The corresponding value was 179% in TY998 (Table IV). The results indicated that Si increased Fe transportation from roots to shoots. Interestingly, Si uptake in both roots and shoots did not differ between the control and Fe^{2+} treatment, suggesting that Fe treatment might not influence Si uptake and transportation (Table IV). Addition of 1 mmol L^{-1} Si enhanced both

TABLE IV

Fe and Si uptake in rice seedlings under different treatments

Rice cultivar	Treatment	Fe content		Shoot Fe/ root Fe	Si content		Shoot Si/ root Si
		Root	Shoot		Root	Shoot	
		μg plant^{-1}			mg plant^{-1}		
PZTF	Control	128.2±10.3[a)]a[b)]	235.3±10.5b	2.3±0.3b	0.4±0.04a	7.0± 0.6b	12.8±2.3b
	Fe	139.6±19.9a	278.9± 8.5a	2.0±0.2b	0.3±0.03b	6.4± 0.5b	11.8±5.4b
	Fe+Si	87.9± 5.5b	282.1± 2.7a	2.9±0.2a	0.5±0.05a	16.5±1.8a	27.1±5.7a
TY998	Control	134.0±6.6b	210.6± 3.8c	1.8±0.3b	0.6±0.04b	7.5±0.8b	7.9±0.7b
	Fe	233.0±18.1a	289.1± 5.0b	1.4±0.1b	0.6±0.08b	7.4±0.9b	7.3±0.3b
	Fe+Si	139.0±13.3b	337.6±13.2a	2.5±0.3a	0.8±0.05a	17.3±0.6a	24.0±2.5a

[a)] Means±standard errors ($n = 4$).
[b)] Values followed by the same letter within a column for the same cultivar are not significantly different at $P < 0.05$, according to Duncan's new multiple range test.

root and shoot Si uptake. A larger increase of Si uptake was observed in shoots than in roots. For example, the ratio of shoot Si and root Si was 130% in PZFT and 229% in TY998 under Fe+Si treatment higher than those under Fe treatment.

Ultrastructure of rice roots

To explore the ameliorative mechanism of Si on Fe^{2+} toxicity, variation in apical cell ultrastructure of rice cultivar TY998 roots was observed under different treatments. In the control, the cell nucleus of rice roots grew normally, cell wall was kept intact and normal organelles were within the cytoplasm, though some nucleus membranes seemed slightly wrinkled (Fig. 3a). Exposed to Fe^{2+} toxicity, the epidermal cells were injured severely. Cell wall was deformed and distorted. Moreover, the detachment of cell wall and plasma membrane occurred in some cells (Fig. 3b). Some iron oxides were observed to accumulate in cells and the free space of cells. The organelles of the cytoplasm were dissolved and some cells died (Fig. 3b). Addition of Si to Fe^{2+} solution ameliorated Fe^{2+} toxicity to root cells significantly (Fig. 3c). Despite the deposition of some iron oxides in cells, root cells under Fe+Si treatment grew much better than those under Fe treatment. Furthermore, cell walls of rice roots became thicker under Fe+Si treatment than under Fe treatment (Fig. 3c).

DISCUSSION

Iron toxicity is one of important factors that limit rice growth and yield in waterlogging soils (Liao *et al.*, 1994; Fageria *et al.*, 2008). In this study, Fe treatment of 0.1 mmol L^{-1} Fe^{2+} leaded to severe necrosis and distortion of rice roots (Fig. 1). The cytoplasmic organelles in epidermal cells of rice roots were dissolved and the cell nuclei were scattered throughout the nucleus (Fig. 3b), suggesting that treatment with 0.1 mmol L^{-1} Fe^{2+} could result in Fe toxicity to rice roots. Consistent with our results, Li *et al.* (2001) found that treatment with 0.18 mmol L^{-1} Fe^{2+} induced a damage to rice seedlings, and both root and shoot biomass of rice seedlings were much reduced. Hendry and Brocklebank (1985) also pointed out that excessive Fe^{2+} absorption on the surface of rice roots could trigger the production of reactive oxygen species

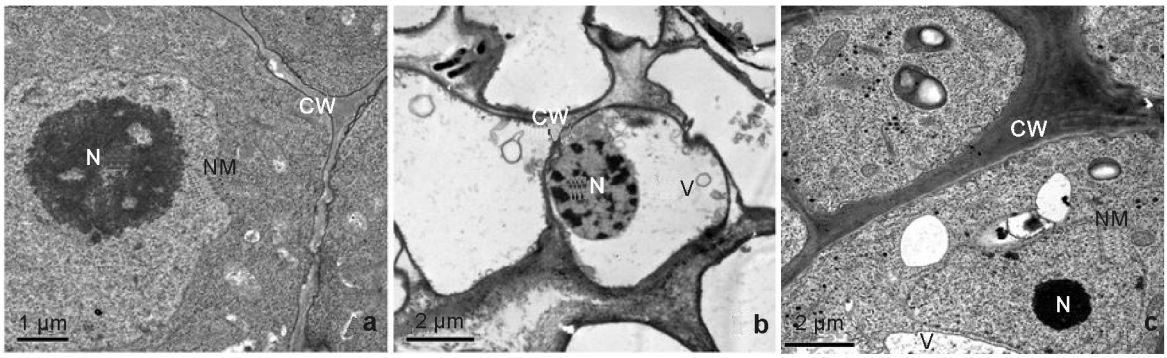

Fig. 3 Transmission electron micrographs of root apical cells of rice cultivar TY998 under treatments of control (a), 0.1 mmol L^{-1} Fe^{2+} (b) and 0.1 mmol L^{-1} Fe^{2+} + 1 mmol L^{-1} Si (c). Rice seedlings were grown for 21 d and another 2-d P starvation, and then transferred to different treatments for 2 d. CW = cell wall; N = nuclear; NM = nucleus membrane; V = vacuole.

(ROS), which induced Fenton reaction causing lipid peroxidation of cell membrane, destroying the integrity of cell, and finally leading to cell death. Due to different experimental conditions, rice varieties, treatment durations, growth conditons and Fe forms, the concentration of Fe toxicity and its toxic effects to rice roots might be different (van Breemen and Moormann, 1978; Zhang *et al.*, 2011).

Si is an essential element for rice seedlings (Tavakkoli *et al.*, 2011). Many studies indicated that Si amendment improved the growth of plants significantly in heavy metal contaminated soils (Ma *et al.*, 2004; Ma and Yamaji, 2006). In this study, the addition of 1 mmol L^{-1} Si to the solution of 0.1 mmol L^{-1} Fe^{2+} solution improved the growth of rice significantly (Fig. 1). Root elongation and root biomass under Fe+Si treatment were increased by 13.5% and 23.1% in comparison to those of Fe^{2+} treatment (Table I). Si addition to Fe^{2+} solution decreased iron precipitation on the surface of rice roots (Fig. 1). Meanwhile, iron deposition into intercellular spaces was reduced greatly (Fig. 3c), suggesting that Si might alter iron distribution. Ma and Yamaji (2006) indicated that Si precipitation was higher in the cortex than in the epidermis and rhizosphere, and Si was mainly located in the epidermal and cortex cells. It is speculated that the ameliorating effects of Si on Fe toxicity might be associated with Si occupying the binding site of Fe, thus reducing Fe precipitation on the surface of rice roots. da Cunha and do Nascimento (2009) reported that Si amendment to a Cd- and Zn-enriched soils could increase maize biomass and the thickness of mesophyll and epidermal cells. In accordance with our results, the addition of Si to Fe^{2+} solution contributed to thickening of epidermal cell walls of rice roots (Fig. 3c). This thickening effect might be caused by the cell's polysaccharide secretions, which would combine with Si and result in the thickening of the cell wall (Harrison *et al.*, 1987). Ma (2010) found the transporters of *Lsi1* and *Lsi2* were required for Si uptake and transport in rice roots. Activation of the above two genes would increase Si uptake and transportation from roots to shoots under Fe+Si treatment. Increased expression of Si transporters might influence Fe uptake and transportation. Further studies on the interaction of Si and Fe are required to elucidate effects of Si on Fe uptake and transportation.

In conclusion, the result of this study indicated that treatment with 0.1 mmol L^{-1} of Fe^{2+} induced the formation of reddish iron plaque and produced the toxicity to rice roots. Addition of 1 mmol L^{-1} Si to 0.1 mmol L^{-1} of Fe^{2+} solution could ameliorate the toxicity of Fe to rice roots obviously. The ameliorating effects of Si on Fe toxicity might be related to thickening cell wall, increasing Fe flow of xylem sap and reducing Fe precipitation on root surface of rice seedlings.

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