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REGULAR ARTICLE

The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice

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Abstract The objective of this study is to elucidate the roles of silicon (Si) in enhancing tolerance to excess zinc (Zn) in two contrasting rice (*Oryza sativa* L.) cultivars: i.e. cv. TY-167 (Zn-resistant) and cv. FYY-326 (Zn-sensitive). Root morphology, antioxidant defense reactions and lipid peroxidation, and histochemical staining were examined in rice plants grown in the nutrient solutions with normal (0.15 μM) and high (2 mM) Zn supply, without or with 1.5 mM Si. Significant inhibitory effects of high Zn treatment on plant growth were observed. Total root length (TRL), total root surface area (TRSA) and total root tip amount (TRTA) of both cultivars were decreased significantly in plants treated with high Zn, whereas these root parameters were significantly increased when Zn-stressed plants were supplied with 1.5 mM Si. Supply of Si also significantly decreased Zn concentration in shoots of both cultivars, indicating lower root-to-shoot translocation of Zn. Moreover, superoxide dismutase (SOD), catalase (CAT), and asorbate peroxidase (APX) activities were increased, whereas malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations were decreased in Si-supplied plants of both Zn-sensitive and Zn-resistant rice cultivars exposed to Zn stress. These alleviative effects of Si, further confirmed by the histochemical staining methods, were more prominent in the Zn-resistant cultivar than in the Zn-sensitive one. Taken together, all these results suggest that Si-mediated alleviation of Zn toxicity is mainly attributed to Si-mediated antioxidant defense capacity and membrane integrity. The possible role of Si in reduction of root-to-shoot translocation of Zn can also be considered.

Keywords Lipid peroxidation · Oxidative stress · Rice · Zn stress

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Introduction

The total zinc (Zn) concentration in soil usually ranges from 10 to 300 mg kg^{-1} with the average of approximately 50 mg kg^{-1} (Mortvedt 2000); however, it can reach thousands of mg kg^{-1} in severely Zn contaminated soils. Over the last decades the Zn concentration in soils has progressively increased in

various regions as a result of human activities. Anthropogenic sources of Zn in soil include sewage sludge, compost, agrochemicals, and mine tailings (Kiekens 1990). The critical soil Zn toxicity level for plants varies as a function of plant genotype and various soil and climatic factors.

Zinc plays an important role in several plant metabolic processes such as enzyme activation, protein synthesis and metabolism of carbohydrate and lipid (Cakmak 2000) and also has an important function in transcription of many genes (Chung et al. 2005). However, the presence of Zn at higher concentrations can cause cytotoxic effects in the presence of hydrogen peroxide (Chung et al. 2005). Many investigators have studied the toxic effect of Zn on various plant species such as *Festuca rubra* (Powell et al. 1986), *Nigella sativa* (EI-Ghamery et al. 2003), *Phaseolus vulgaris* (Cuypers et al. 2001), *Triticum aestivum* (EI-Ghamery et al. 2003), *Vetiveria zizanioides* (Xu et al. 2009), and *Cajanus cajan* (Madhava Rao and Sresty 2000). However, various previous studies were limited to toxic effects of Zn on a shoot level, with less attention to root system, and the data about toxic effects of Zn on hyperaccumulator roots were mostly limited to biomass.

The elevated production of reactive oxygen species (ROS) induced by adverse environmental stresses has been considered to be one of the major factors causing damage of plant cells. Although Zn is not a redox-active metal, i.e. it is not involved as a metal catalyst in the Fenton like reaction, its toxicity can result in oxidative cell damage accompanied by the induction of antioxidative defense mechanisms (Weckx and Clijsters 1997). Indeed, Zn stresses result in the production of superoxide radical (O_2^-), hydrogen peroxide, singlet oxygen and hydroxyl radicals (·OH), which affect various cellular processes mostly the functioning of membrane systems (Weckx and Clijsters 1997). A regulated balance between oxygen radical production and destruction is required if metabolic efficiency and function are to be maintained in both optimal and stress conditions (Foyer et al. 1994). Cells are normally protected against free oxy-radicals by the operation of intricate antioxidant systems, comprising both enzymic systems. Maintaining high activities of the antioxidant enzymes (e.g. superoxide dismutase, catalase and peroxidases) and high contents of the non-enzymatic constituents which act as free radical scavengers (e.g. ascorbate, glutathione and phenolic compounds), is very important for plants to survive under stressful conditions (Bowler et al. 1992; Foyer et al. 1994; Fridovich 1978) as for instance heavy metals stress.

Although silicon (Si) is the second most abundant element in the Earth's crust and its content in plants often reaches values of macronutrients, it is not listed among plant essential elements (Marschner 1995). However, it has been proved to be beneficial for the healthy growth and development of many plant species, particularly graminaceous plants such as rice and sugarcane and some cyperaceous plants (Epstein 1994, 1999; Liang 1999; Liang et al. 2005; Ma et al. 2002). The beneficial effects of Si, mainly in alleviating various biotic (diseases and pests) and abiotic (nutrient deficiency; metal toxicity, including Al; drought and salt stress) stresses in many plant species, are well demonstrated in the literature (for review, see Epstein 1999; Hattori et al. 2005; Liang et al. 1996, 2007; Ma 2004a; Ma and Yamaji 2006; Wallace 1992). It has been shown in many studies so far that Si supply to roots greatly improves the tolerance to metal toxicity as for instance Al toxicity in wheat, rice, maize and soybean (Ma et al. 2004b), Mn toxicity in cowpea (Führs et al. 2009; Iwasaki et al. 2002) and cucumber (Maksimovic Dragisic et al. 2007; Rogalla and Römheld 2002), and Cd toxicity in pakchoi (Song et al. 2009). Si-mediated enhancement of the binding capacity of cell walls has been proposed as a main mechanism of metal detoxification for Zn (Neumann et al. 1997), Mn (Wiese et al. 2007) and Al (Cocker et al. 1998; Hodson and Evans 1995). However, mechanisms underpinning Si-enhanced metal tolerance remain poorly understood and thus need further investigation. Moreover, few reports are available on Si-mediated resistance to Zn toxicity in terms of Zn uptake and transport, ROS-induced oxidative damage and antioxidant defense system. Therefore, further investigation is needed to clarify how Si can mitigate Zn toxicity in plants since Zn is useful to plants at lower concentrations but is extremely toxic at elevated concentrations. The objective of this study was to investigate the impacts of Si on Zn detoxification with special focus on antioxidative enzyme activities, root morphology, plant growth, Zn and Si uptake and accumulation in two rice (*Oryza sativa* L.) cultivars differing greatly in response to Zn excess. Based on our preliminary

studies, cultivar Tyou-167 (Zn-resistant, TY-167) and cultivar Fengyuanyou-326 (Zn-sensitive, FYY-326) were used in the present study.

Materials and methods

Plant material and growth conditions

The hydroponics experiments were carried out in an environmentally-controlled growth chamber of Chinese Academy of Agricultural Sciences. Twenty seeds of rice plants were treated with different Zn levels (0.15 μM (control), 0.04, 0.08, 0.16, 0.8, 2, 4 mM). Seeds of rice plants were selected based on metal tolerance which was assessed as a tolerant index (TI) by calculating root length according to the formula, TI=Root length (treated)/Root length (control) (Monni et al. 2001). In order to evaluate the toxicity of Zn to rice seedlings, a dose-response experiment was performed (Zn treatments: 0.15 μM (control), 0.04, 0.08, 0.16, 0.8, 2, 4 mM). The root and shoot dry weights were determined after 7-day treatment. Compared with the control, the presence of 2 mM Zn in the culture solution significantly inhibited root growth. The inhibition ratio was about 50% in the sensitive cultivar. The toxic symptom was visible at 2 mM Zn after treatment for 1 week. Based on our preliminary screening studies, two rice cultivars differing in Zn tolerance were used in this investigation: i.e. cv. TY-167, a Zn-resistant genotype and cv. FYY-326, a Zn-sensitive genotype. Seeds of two contrasting rice (*Oryza sativa* L.) genotypes, were surface-sterilized with 30% H_2O_2 (10%) for 30 min, followed by rinsing thoroughly with distilled water and germinated on moist filter paper for 48 h in an incubator at 35°C. After burgeoned, seeds were sown in the plastic containers filled with quartz sand and irrigated with 1/2-strength Kimura B nutrient solution. The composition of the basic nutrient solution was: $(NH_4)_2SO_4$ 0.37 mM, $MgSO_4{\cdot}7H_2O$ 0.55 mM, KNO_3 0.18 mM, $Ca(NO_3)_2{\cdot}4H_2O$ 0.37 mM, KH_2PO_4 0.21 mM, $NaEDTAFe{\cdot}H_2O$ 20 μM, $MnCl_2{\cdot}4H_2O$ 6.7 μM, $(NH_4)_6Mo_7O_4{\cdot}4H_2O$ 0.015 μM, $ZnSO_4{\cdot}7H_2O$ 0.15 μM, $CuSO_4{\cdot}5H_2O$ 0.16 μM, and HBO_3 9.4 μM. The solution pH was adjusted to 5.4–5.6 with HCl or NaOH daily. Uniform 7–day–old plants were transferred to 5-L plastic pots (60 plants per pot). Plants were grown under controlled environmental conditions in a growth chamber with light/dark regime of 13/11 h, temperature regime of 27/23°C, photosynthetic photon flux density of 400 μmol m^{-2} s^{-1}, and relative humidity of about 70%.

Plants were exposed to two concentrations of Zn, 0.15 μM (control) and 2.0 mM (high) respectively without or with 1.5 mM Si, added as $K_2SiO_3{\cdot}nH_2O$ to the nutrient solution. Additional K introduced by $K_2SiO_3{\cdot}nH_2O$ was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. In total, there were four treatments with each of them replicated three times. The nutrient solutions were renewed completely every second day and after 7-day treatment plants were harvested for further analyses.

Root morphology

Root morphological parameters were determined by using a root automatism scan apparatus (HP Scanjet 8300 series) equipped with Delta-T scan root analysis system offered by Beijing Channel Scientific Instruments Inc. Delta-T scan root analysis system is a software that recognizes digital root images and analyzes root parameters (length, surface area, and amount) for defined root diameters. Image record was performed at a resolution of 100 dpi and images were saved as TIFF (tagged image file format). Total root length (TRL), total root surface area (TRSA) and total root tip amount (TRTA) were calculated. For each replication, root of one plant was analyzed.

Determination of Zn

After harvest, the roots were washed thoroughly with distilled water. To remove the ions in the root free space, the roots were soaked in 0.5 M HCl for 20 s and rinsed thoroughly with distilled water (Kaya et al. 2009). The pretreated plant tissues were oven-dried for 72 h at 70°C, weighted, and ground to pass a 1.0-mm sieve, and parts of plant tissues were digested in 5 ml of ternary mixture of HNO_3: H_2SO_4: $HClO_4$ in the ratio of 10: 1: 4 (*v*/*v*/*v*). Concentration of Zn was determination by atomic absorption spectrophotometery (Perkin-Elmer, AAnalyst 100).

Determination of Si

About 0.1 g pretreated plant samples was fused using high-temperature alkaline fusion method and the alkaline fusion sample was diluted to 50 ml with distilled water. The Si concentration in the alkaline fusion solution was determined by the colorimetric molybdenum blue method as described by Dai et al. (2005).

Antioxidative enzyme extraction and assays

Root fragments were ground in a potassium phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2% PVP. The homogenate was centrifuged at 15,000×*g* for 20 min at 4°C, and the supernatants were stored at 4°C for analysis of enzyme activities and soluble protein concentration.

The protein contents in the extracts were determined by the method of Bradford (1976), using bovine serum albumin (BSA, Sigma) as a standard.

SOD activity was assayed using the procedure as described by Sgherri et al. (1994). The 3 ml reaction mixture contained 2.4 ml of 50 mM buffered phosphate solution (pH 7.8), 0.2 ml of 195 mM methionine, 0.1 ml of 3 μM EDTA, 0.2 ml of 1.125 mM NBT (nitro blue tetrazolium chloride), 0.1 ml of 60 μM riboflavin, and 40 μM enzyme extract. The reaction mixtures were illuminated for 20 min at a light intensity of 300 μmol $m^{-2}s^{-1}$. One unit SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction. The specific enzyme activity was expressed as the enzyme unit per mg of protein.

CAT activity was measured as the reduction in absorbance at 240 nm due to the reduction of H_2O_2, which was assayed following the method of Aebi (1984). The reaction mixture comprising 1.9 ml 50 mM potassium phosphate buffer (pH 7.0), and 1.0 ml 10 mM H_2O_2 was prepared immediately before use. The reaction was started by adding 0.1 ml of plant extract and H_2O_2 and enzyme activity was measured by monitoring the degradation of H_2O_2 at 240 nm over 1 min. The contents of protein in the extract were measured at the same time. The enzyme activity was expressed as ΔOD_{240} $min^{-1}\times g^{-1}$ protein.

APX activity was measured according to the method of Nakano and Asada (1981). The assay depended on the decrease in absorbance at 290 nm as ascorbates were oxidized. The reaction mixture consisting of 1.1 ml 50 mM potassium phosphate buffer (pH 7.0), 1.5 ml 1 mM AsA, 0.1 ml of plant extract and 0.3 ml 1 mM H_2O_2 was prepared immediately before use. The time interval was 10 min. The reaction was started by adding H_2O_2, and the contents of protein were measured at the same time. The enzyme activity was expressed as ΔOD_{290} $min^{-1}\times mg^{-1}$ protein.

Determination of MDA and H_2O_2 concentrations

The concentration of MDA concentration was determined according to the method of Heath and Packer (1968). Root tissues (500 mg) were homogenised in 3 mL 0.1% TCA (trichloroacetic acid) solution. The homogenate was centrifuged at 2,500 g for 10 min and the supernatant was assayed for MDA concentration. Hydrogen peroxide (H_2O_2) levels were determined by reading the absorbance at 390 nm according to Velikova et al. (2000).

Histochemical analysis

Histochemical detection of loss of plasma membrane integrity in roots was performed by Evans blue (Wang and Yang 2005). The roots were incubated in 5 ml of Evans blue solutions (0.025%, *w*/*v*, in 100 μM $CaCl_2$, pH 5.6) for 30 min. Histochemical detection of lipid peroxidation was conducted with Schiff's reagent (Pompella et al. 1987). The roots were incubated in Schiff's reagent for 20 min. The stained roots were then rinsed with a solution containing 0.5% (*w*/*v*) $K_2S_2O_5$ (prepared in 0.05 M HCl) until the root color became light red. All of the roots stained with the specific reagents indicated above were washed three times with sufficient volume of distilled water, observed under a light microscope (SZX12, Olympus, Japan) and photographed.

Statistical analyses

All the experiment data presented in this paper were means of the data of two independent experiments and statistically examined by three-way analysis of variance. Statistical significance of the means of three replicates was compared at 0.05

probability level using Sigmastat for Windows Version 2.03 (SPSS Inc.).

Results

Root morphology

Data of the total root surface area (TRSA), total root length (TRL) and total root tip amount (TRTA) are presented in Fig. 1. The TRSA, TRL and TRTA in Zn-resistant cultivar under the treatment of Zn were significantly reduced by 29.7, 24.6 and 27.9%, respectively, as compared to the control. In the case of Zn-sensitive cultivar, the TRSA, TRL and TRTA under the treatment of Zn were decreased by 40.6, 31.1 and 31.3%. However, addition of 1.5 mM Si to Zn treatment significantly increased TRSA, TRL and TRTA regardless of cultivars used compared with the corresponding Zn treatments alone. For exam-

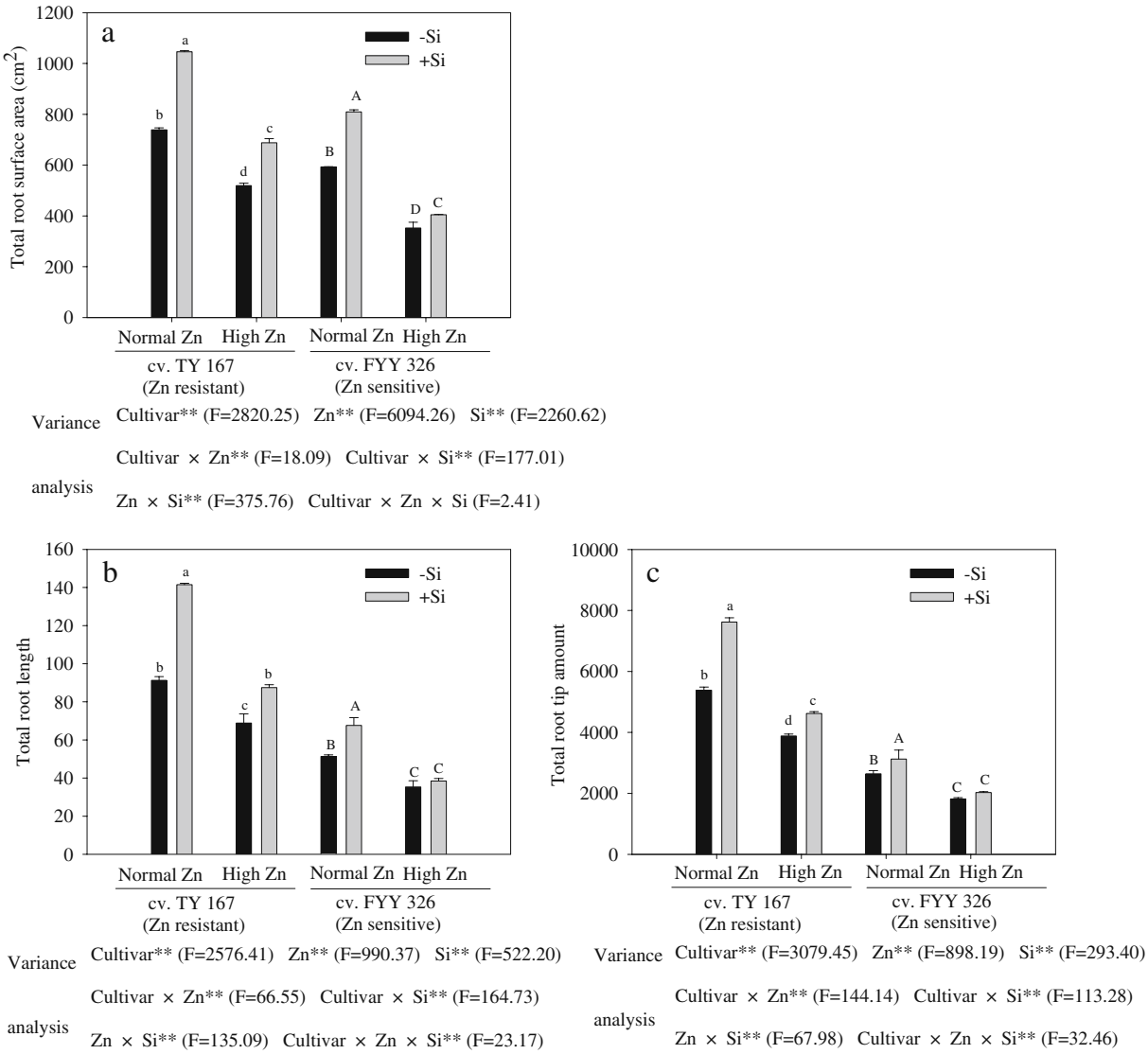


Fig. 1 TRAS (**a**), TRL (**b**) and TRTA (**c**) of two *Oryza sativa* L. cultivars grown hydroponically with either normal (0.15 μM) and high (2 mM) Zn without or with 1.5 mM Si for 7 days. Data are means±S.D. of three replicates. Note: *P*-values indicate significance level based on three-way ANOVA. **P*<0.05,** *P*<0.01. Data followed by different letters within the same cultivar are significantly different (*P*<0.05)

ple, The TRSA, TRL and TRTA increased by 32.5, 27.1 and 19.1% in TY-167 under 1.5 mM Si plus Zn compared with the Zn treatment alone whereas in FYY-326, the TRSA, TRL and TRTA increased by 14.8, 8.8 and 11.6% compared with the corresponding Zn treatment alone. Results from this study showed that the impact of Zn on root morphology of both cultivars also varied with ecotype.

Zn toxicity symptoms and plant growth

Severer leafy symptoms of Zn toxicity were observed in the Zn-sensitive cultivar (FYY-326) than in the Zn-resistant cultivar (TY-167) on Day 7 in the treatment with 2 mM Zn. The symptoms of Zn toxicity were typically manifested as a yellow colour on the lower leaves starting from the tips and spreading toward the bases of the leaves, which became severer as the experiment continued. However, addition of Si could significantly alleviate the symptoms of Zn toxicity (Fig. 2).

Shoot and root growth of both cultivars were negatively affected by a root environment with 2 mM Zn ion concentrations. Compared with the control, high Zn treatment reduced shoot and root biomass by 33.5% and 30.5% in the Zn-resistant cultivar, respectively, and 44.7% and 48.4% in the Zn-sensitive cultivar (Fig. 3). However, supply of 1.5 mM Si to high Zn treatment significantly improved plant growth. For instance, in the Zn-resistant cultivar root biomass was reduced by only 6.1% and shoot biomass was even increased by 1.5% of control treatment, while in the Zn-sensitive one shoot and root biomass was reduced by 19.2% and 36.2%, respectively (Fig. 3). Furthermore, addition of Si 1.5 mM to Zn treatments significantly increased shoot and root biomass regardless of rice cultivars used compared with the corresponding Zn treatments alone. For example, shoot and root dry weights of the Zn-resistant plants treated with Zn plus Si were 52.8% and 34.7% higher than those of plants treated with Zn alone. For the Zn-sensitive cultivar FYY-326, very similar changes were found (Fig. 3) with an exception that the alleviative effect of Si on toxicity to plant growth was more significant in the Zn-resistant cultivar than in the Zn-sensitive cultivar (Fig. 3).

Shoot and root Zn concentration

Zn concentration in both shoots and roots of the two cultivars increased in response to an altered Zn supply in the nutrient solution (from 0.15 μM to 2 mM) (Fig. 4). The Zn concentration in roots of the two cultivars was much higher than that in shoots. The value of Zn concentration in root/shoot was 2.5 and 2.4 in the Zn-resistant and Zn-sensitive cultivar, respectively. However, addition of Si significantly decreased Zn concentrations in shoots of both cultivars under the Zn treatment (Fig. 4a). For example, Zn concentrations in shoots of Zn-resistant cultivar and Zn-sensitive cultivar treated with Zn plus Si were 58.4% and 46.8% lower than those of plants treated with Zn alone. On the contrary, addition of Si considerably increased root Zn concentrations in both cultivars at different degrees, especially in the resistant cultivar (TY-167) (Fig. 4b). Zinc concentrations in roots of Zn-resistant cultivar and

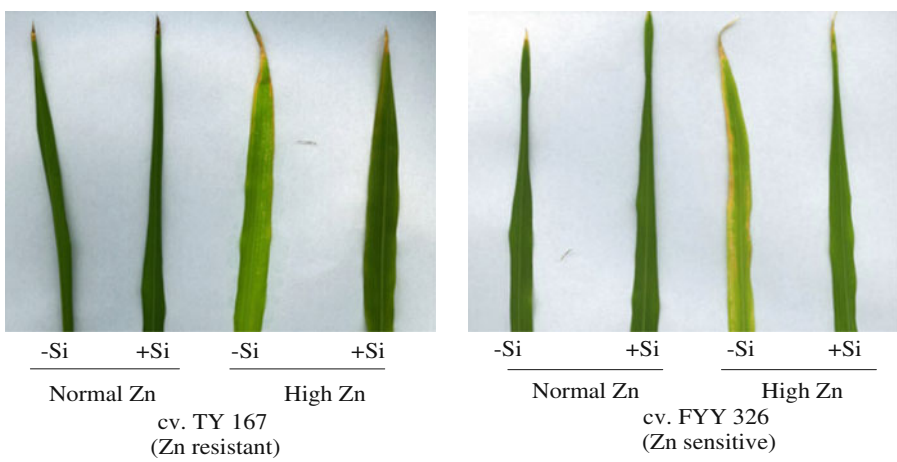
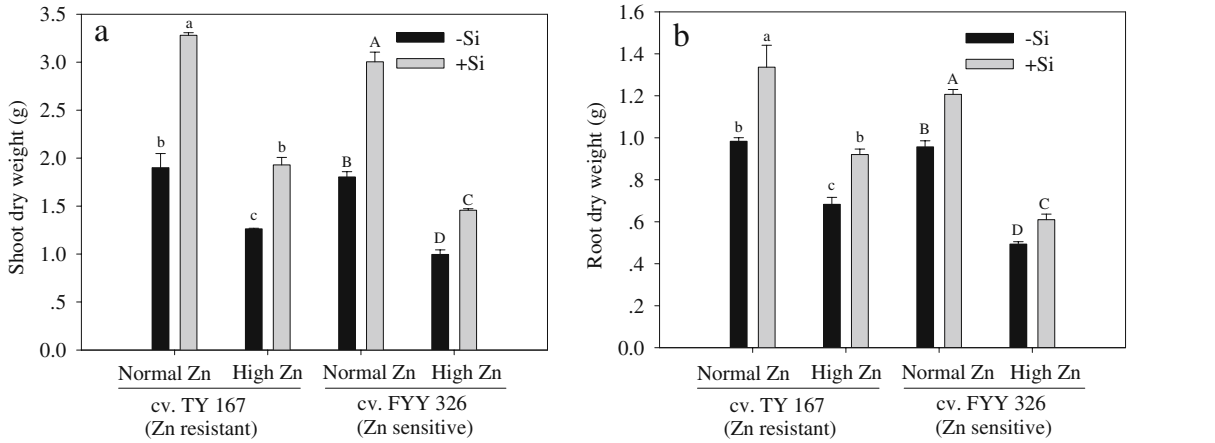


Fig. 2 The toxicity symptoms of two Oryza sativa L. cultivars grown hydroponically with either normal (0.15 μM) and high (2 mM) Zn without or with 1.5 mM Si for 7 days



Variance analysis
Cultivar** (F=84.19) Zn** (F=1279.40) Si** (F=933.24)
Cultivar × Zn** (F=9.13) Cultivar × Si** (F=10.16)
Zn × Si** (F=143.47) Cultivar × Zn × Si (F=0.05)

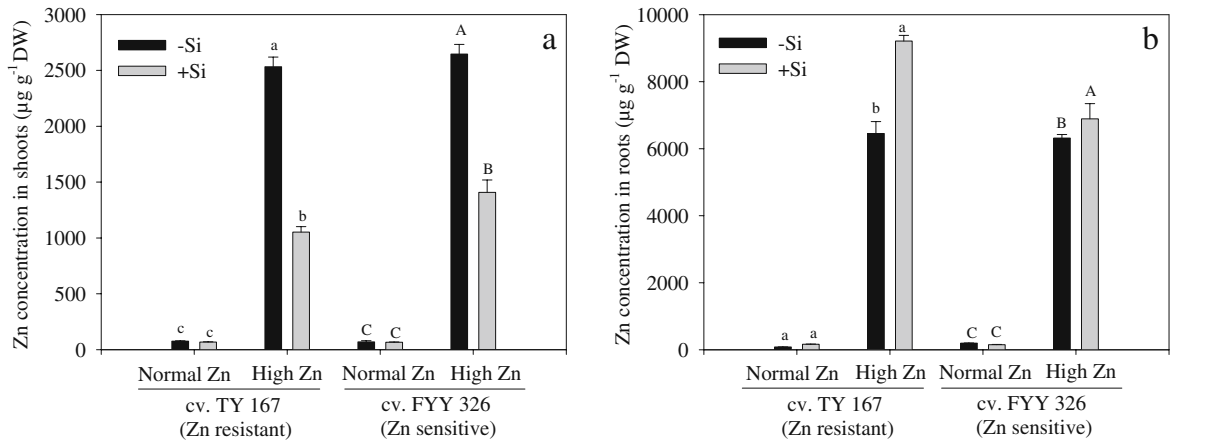
Variance analysis
Cultivar** (F=82.83) Zn** (F=629.34) Si** (F=185.73)
Cultivar × Zn** (F=24.69) Cultivar × Si** (F=9.67)
Zn × Si** (F=13.35) Cultivar × Zn × Si (F=0.08)

Fig. 3 Shoot (**a**) and root (**b**) dry weight of two *Oryza sativa* L. cultivars grown hydroponically with either normal (0.15 μM) and high (2 mM) Zn without or with 1.5 mM Si for 7 days. Data are means±S.D. of three replicates. Note: *P*-values indicate significance level based on three-way ANOVA. **P*<0.05,** *P*<0.01. Data followed by different letters within the same cultivar are significantly different (*P*<0.05)

Zn-sensitive cultivar treated with Zn plus Si were 42.7% and 9.1% higher than those of plants treated with Zn alone. Zn concentration in roots of Zn-resistant and Zn-sensitive cultivar was 8.7 and 4.9 times as high in the Si plus Zn treatment as in shoots, respectively.

Shoot and root Si concentration

As shown in Fig. 5, the concentration of Si in roots and shoots of both cultivars was increased in Si-treated plants. However, Si concentration in roots and espe-



Variance analysis
Cultivar** (F=21.60) Zn** (F=5498.07) Si** (F=756.04)
Cultivar × Zn** (F=23.13) Cultivar × Si* (F=6.16)
Zn × Si** (F=743.75) Cultivar × Zn × Si* (F=5.72)

Variance analysis
Cultivar** (F=44.83) Zn** (F=6647.88) Si** (F=91.22)
Cultivar × Zn** (F=52.70) Cultivar × Si** (F=43.13)
Zn × Si** (F=87.54) Cultivar × Zn × Si** (F=34.34)

Fig. 4 Zn concentration in shoots (**a**) and roots (**b**) of two *Oryza sativa* L. cultivars grown hydroponically in either normal (0.15 μM) or high (2 mM) Zn supplied nutrient solutions without or with 1.5 mM Si for 7 days. Data are means±S.D. of three replicates. Note: *P*-values indicate significance level based on three-way ANOVA. **P*<0.05,** *P*<0.01. Data followed by different letters within the same cultivar are significantly different (*P*<0.05)

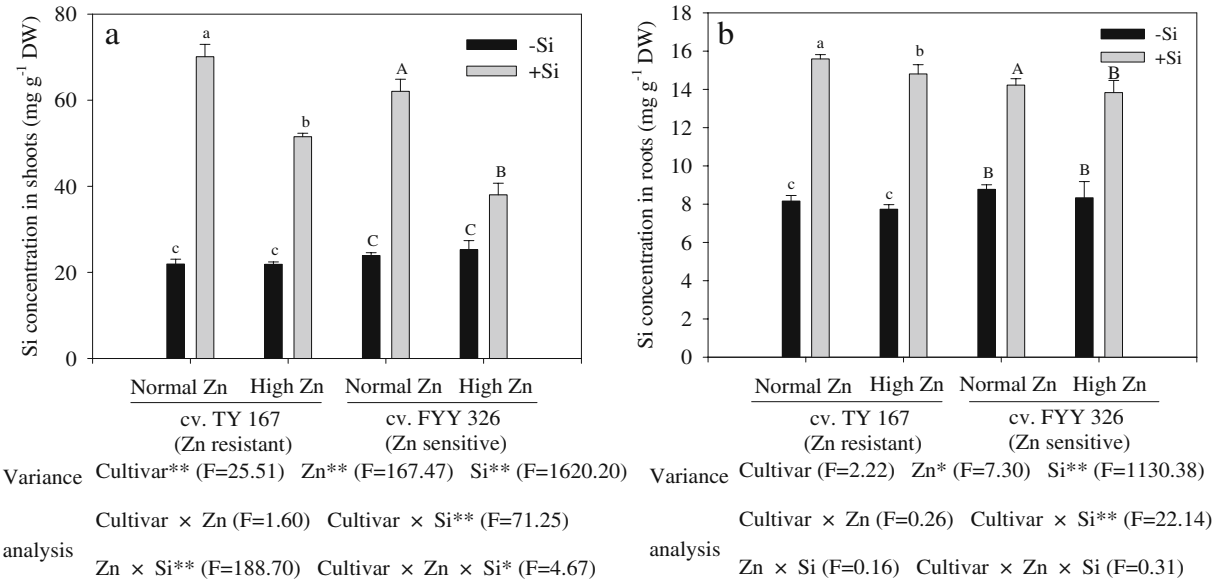


Fig. 5 Si concentration in shoots (**a**) and roots (**b**) of two *Oryza sativa* L. cultivars grown hydroponically in either normal (0.15 μM) or high (2 mM) Zn supplied nutrient solutions without or with 1.5 mM Si for 7 days. Data are means±S.D. of three replicates. Note: *P*-values indicate significance level based on three-way ANOVA. **P*<0.05,** *P*<0.01. Data followed by different letters within the same cultivar are significantly different (*P*<0.05)

cially in shoots of both cultivars grown in the presence of Si was lower in high Zn treatments compared with the normal Zn treatments (Fig. 5). For the Zn-resistant cultivar, Si concentration in shoots and roots decreased by 26.5% and 5.1% in the Zn plus Si treatment compared with the corresponding Si treatment alone, respectively. For the Zn-sensitive cultivar, very similar changes were noted in Si concentration in the Si

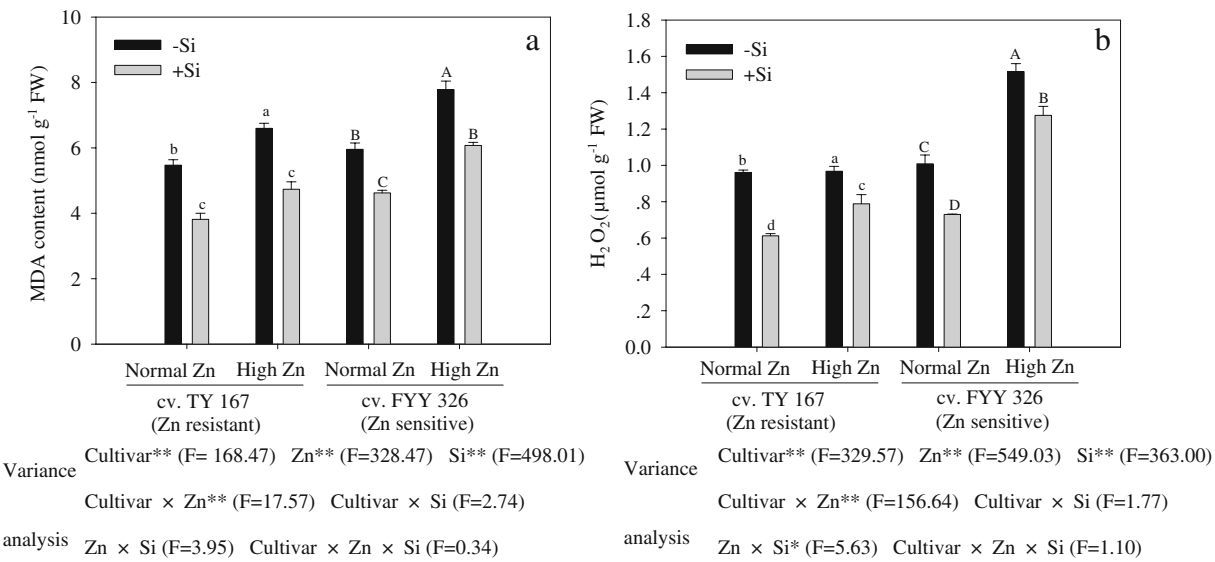


Fig. 6 MDA (**a**) and H_2O_2 (**b**) concentrations of two *Oryza sativa* L. cultivars grown hydroponically with either normal (0.15 μM) and high (2 mM) Zn without or with 1.5 mM Si for 7 days. Data are means±S.D. of three replicates. Note: *P*-values indicate significance level based on three-way ANOVA. **P*<0.05,** *P*<0.01. Data followed by different letters within the same cultivar are significantly different (*P*<0.05)

treatments with or without Zn added, with an exception that shoot Si concentrations were decreased more significantly in the sensitive rice than in the resistant rice.

Lipid peroxidation in roots

Lipid peroxidation in roots of both rice cultivars, measured as MDA concentration, is given in Fig. 6a. Compared with the corresponding control, the concentrations of MDA were significantly increased by treatment with Zn in both rice cultivars (Fig. 6a). However, the MDA concentration under Zn stress was significantly lower in the Si-treated than in non-Si-treated roots. For the Zn-resistant cultivar (TY-167), MDA concentration in the Zn treatment was increased by 20.6%, compared with the control. However, in both cultivars root application of Si decreased MDA concentration. Addition of Si decreased MDA concentration in the Zn-stressed plants by 28.2%, compared with the Zn treatment alone (Fig. 6a). Very similar effects were also observed in the Zn-sensitive cultivar (FYY-326) (Fig. 6a).

H_2O_2 concentration in roots

The concentration of H_2O_2 increased in both rice cultivars exposed to Zn compared with the control (Fig. 6b). However, the concentration of H_2O_2 was significantly lower in the resistant cultivar than in the sensitive one; H_2O_2 concentration in both cultivars was reduced in the presence of 1.5 mM Si to the nutrient solution. Supply of Si decreased H_2O_2 concentrations in the Zn-stressed plants by 23.4%, compared with the corresponding Zn treatment alone. Very similar changes were also observed in the Zn-sensitive cultivar (FYY-326) (Fig. 6b). Si was more effective in decreasing H_2O_2 concentrations in the resistant rice than in the sensitive rice.

Activities of antioxidant enzymes in roots

SOD activities in roots of the two rice cultivars exhibited a fall in relation to increasing concentrations of externally supplied Zn and registered a rise with Si supplied when compared with their corresponding Zn treatment alone. For the Zn-resistant cultivar (TY-167), addition of Zn decreased SOD activities in roots to 93.7% compared with the control. The activity of SOD was increased by 42.9% in the plants treated with Zn plus Si compared with the Zn treatment without Si (Fig. 7a). For the Zn-sensitive cultivar (FYY-326), very similar changes were noted in SOD activity in the Zn treatments with or without Si added, with an exception that significant differences in SOD were found between the Zn treatment alone and the control (Fig. 7a).

For the resistant cultivar (TY-167), CAT activity in the Zn treatment decreased but not significantly with Zn treatment compared with the control. Addition of Si significantly increased CAT activity in Zn-stressed rice roots compared with Zn treatment alone throughout the whole experiment (Fig. 7b). For example, the activity of CAT was 79.4% higher in the Si plus Zn treatment than in the Zn treatment alone (Fig. 7b). For the Zn-sensitive cultivar (FYY-326), very similar results were obtained of CAT activities in the Zn treatment with or without Si, with an exception that significant differences in CAT were found between the Zn treatment alone and the control (Fig. 7b).

For the Zn-resistant cultivar, APX activities in roots was increased by addition of Si by 2.1 times as high in the Zn treatment as in the corresponding Zn alone treatment (Fig. 7c). For the Zn-sensitive cultivar, very similar changes were observed in APX activities in the Zn treatments with or without Si, with an exception that greater increase in APX activity was found in the resistant rice than in the sensitive rice.

Oxidative damage in roots

To confirm the regulatory role of Si in antioxidation, we examined root plasma membrane integrity using histochemical staining with Schiff's reagent and Evans blue (Fig. 8). The Schiff's reagent was applied to determine the degree of peroxidation of membrane lipids and the Evans blue to determine the loss of plasma membrane integrity (Wang and Yang 2005). For the Zn resistant cultivar (TY-167), the roots treated with 2 mM Zn alone were strained extensively. The roots became lighter straining in the treatment with Zn plus Si compared with the Zn treatment alone, indicating that Si had beneficial effects on the protection of cell membrane against Zn-induced oxidative damage. For the Zn-sensitive FYY-326, very similar changes were also observed in the roots (Fig. 8), and the Si beneficial effects on the protection of cell membrane against Zn-induced oxidative

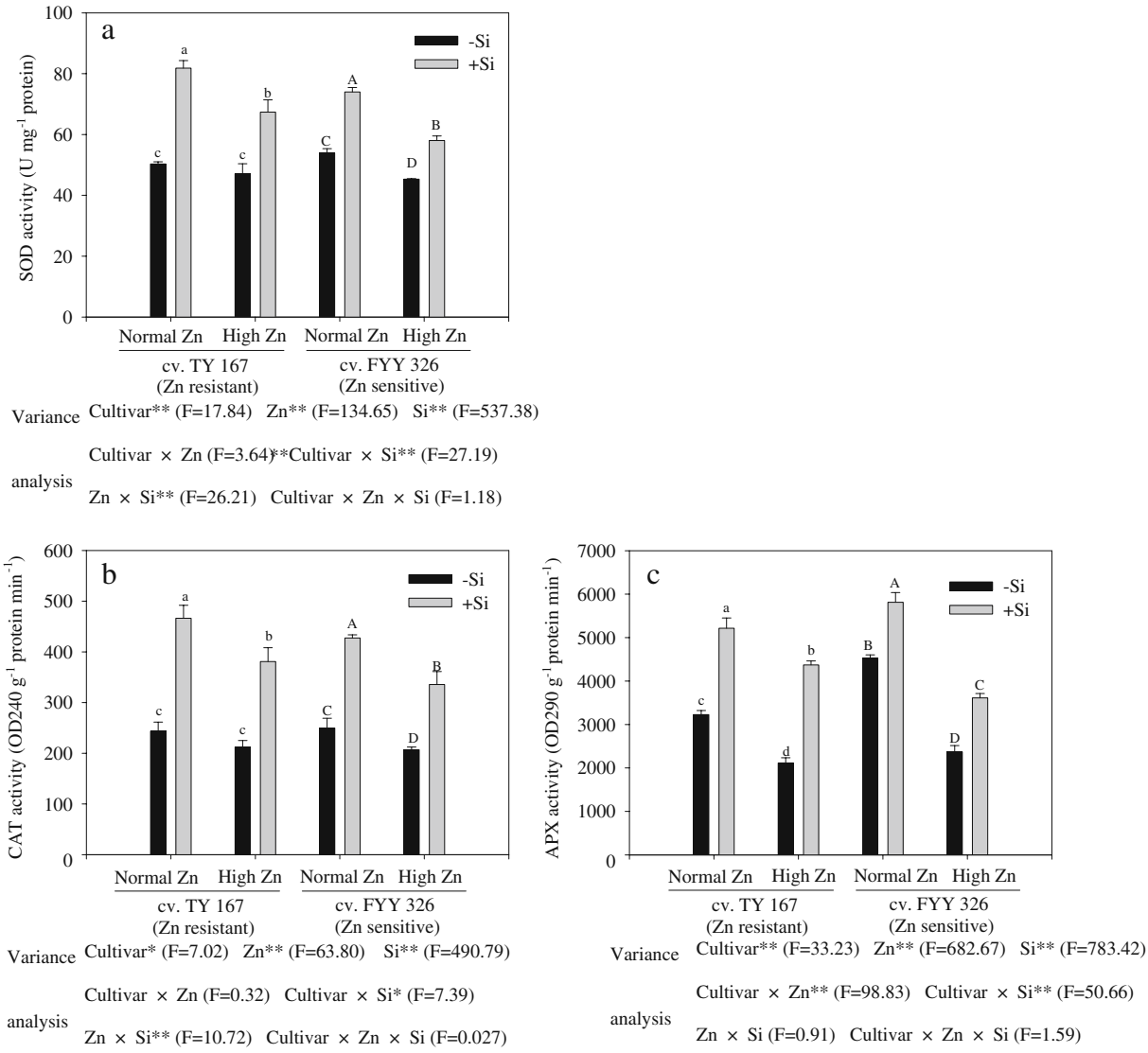


Fig. 7 SOD, CAT and APX activities in roots of Zn-tolerant (TY-167) and Zn-sensitive (FYY-326) Oryza sativa L. grown hydroponically in either normal (0.15 μM) or high (2 mM) Zn supplied nutrient solutions without or with 1.5 mM Si. Data are means±S.D. of three replicates. Note: *P*-values indicate significance level based on three-way ANOVA. **P*<0.05,** *P*<0.01. Data followed by different letters within the same cultivar are significantly different (*P*<0.05)

damage were more significant in the Zn-resistant plant roots than in the Zn-sensitive plant roots.

Discussion

The impact of heavy metals on plant growth, particularly root growth, has been reported in various studies (e.g. Langer et al. 2009; Lin et al. 2005; Qi et al. 2009). Besides the general inhibitory effect of heavy metals on plant biomass production, roots can also respond to heavy metal stress via changes in root growth pattern and morphology. In this study, it was noted that TRSA, TRL and TRTA in both cultivars decreased markedly in the high Zn treatment, whereas these parameters were significantly increased when Si was supplied at either normal (0.15 μM) or high (2 mM) Zn treatments (Fig. 1). This enhancement of root growth might be a result of the higher activities of antioxidative

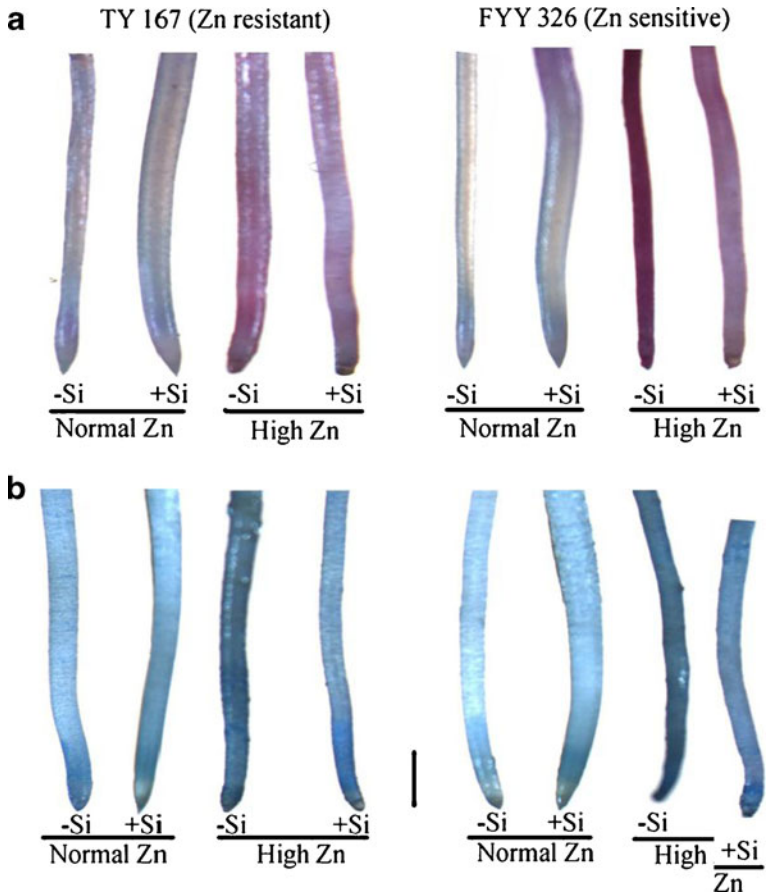


Fig. 8 Lipid peroxidation (**a**) and loss of plasma membrane integrity (**b**) in the root tips of *Oryza sativa* L. cultivars grown hydroponically with either normal (0.15 μM) and high (2 mM) Zn without or with 1.5 mM Si for 7 days. The roots were rinsed with 0.5 M HCl solution and were stained with Schiff's reagent (**a**) or Evans blue (**b**), and immediately photographed under a light microscope. The scale bar in the graph indicates 1.0 cm

enzymes and lower MDA and H_2O_2 concentrations (Song et al. 2009).

Zn uptake as reflected in the concentrations in the roots and shoots of two rice cultivars increased with increasing concentrations of Zn in the nutrient solutions (Fig. 4). The accumulation of Zn in the roots of cv. TY-167 was higher compared to the roots of cv. FYY-326. It was demonstrated that Zn-resistant plants accumulated higher amount of heavy metals in their roots (Straczek et al. 2008). Si could enhance the ability of roots to inactivate the excess Zn within the tissues (e.g. Zn binding to the cell wall) and thus inhibit root-to-shoot Zn translocation. In the present study, supply of Si increased the total root concentration of Zn in both cultivars (Fig. 4). Recently, the root cell wall of *Verbascum thapsus* is proposed to be the major pool of Zn under Zn toxicity that bound more than 60% of total root Zn (Morina et al. 2010). It can be proposed that Si may increase the wall binding potential of Zn as has previously been demonstrated for Mn (Wiese et al. 2007), which can explain the descripancy that the supply of Si did not decrease but even increased the total content of Zn (Fig. 4). High Zn treatment also impaired root and shoot growth of both cultivars (Fig. 3). Interestingly, although the application of Si significantly improved growth of both rice cultivars under Zn excess, the inhibition rates of root and shoot growth were not different in the presence or absence of Si. It was demonstrated that Zn toxicity in plants resulted in inhibited growth of both root and shoot (Ebbs and Kochian 1997; Fontes and Cox 1998). This decrease could be due to their interference with metabolic processes associated with normal development (Lidon and Henriques 1992). Supply of Si partially counteracted the inhibitory effects of high Zn on plant growth (Fig. 3). It has been reported that Si improves tolerance to Zn toxicity by co-precipitation of Zn-Si in the cell walls of *Minuartia verna* (Neumann et al. 1997) or in *Cardaminopsis halleri* by forming Zn-silicate complexes in the cytoplasm (Neumann and Zurnieden 2001). The formation of silicates as part of

the metal tolerance mechanism does not seem only to be restricted to Zn; the alleviation of Al and Mn toxicity by Si has also been observed (Galvez et al. 1987; Hammond et al. 1995; Horiguchi 1988).

In this study, we also provided evidence that Si was able to regulate Zn-induced oxidative stress under normal and high Zn conditions in the roots of rice (Fig. 8). Toxic symptom in the root tips of both cultivars grown with high Zn plus Si was attenuated, as demonstrated by staining with Evens blue (Fig. 8b). This observation was supported by the data of the reduced MDA concentration through staining with Schiff's reagent (Figs. 6a and 8a). Zn-induced cell death with rigid lignification in root cell wall has also been reported in Scots pine (Schützendübel et al. 2001) and rice (Guo et al. 2007). The root cell death induced by Zn stress was significantly alleviated by addition of Si, suggesting that Si played an important role in plant adaptation to Zn-induced root cell death. The Si-mediated reduction of Zn-induced toxicity to the rice roots was also associated with the increase of antioxidant enzyme activities and decrease of H_2O_2 concentration. These results suggest that Si may alleviate the Zn-induced oxidative stress in rice plants.

Environment stresses can increase oxygen-induced cellular damage due to increased ROS generation (Mittler 2002). Therefore, increasing the resistance of Zn stress may depend on the enhancement of the antioxidative defense system, which includes several antioxidative enzymes and antioxidant compounds. In the present study, Zn stress decreased SOD, CAT and APX activities (Fig. 7). For instance, SOD activity modulates the relative amounts of $O_2^{\bullet -}$and H_2O_2, which is highly reactive and may cause severe damage to membranes, protein and DNA (Bowler et al. 1992); CAT activity is directly regulated by the concentration of H_2O_2 (Fornazier and Ferreira 2002); APX plays an important role in removal of H_2O_2 in higher plants (Noctor and Foyer 1998). However, these antioxidative enzymes were all increased by exogenous Si with a consequence of less lipid peroxidation and H_2O_2 concentrations in the stressed plants (Fig. 6). Antioxidative enzymes work in a cooperative or synergistic way to protect against oxidative stress (Bagnyukova et al. 2006; Liang et al. 2003, 2008). Although excess Zn^{2+} generated an oxidative stress, the CAT activity was sufficient to cope with an increased concentration of H_2O_2 following treatment with Zn^{2+}, and the cells presented a positive endogenous protective effect. This was demonstrated previously in the experiments with pigeonpea exposed to high Zn (Madhava Rao and Sresty 2000). After adding Si, greater increases of SOD, CAT and APX activities result in high Si concentration which may be beneficial to plant growth (Song et al. 2009). The higher activities of SOD, CAT and APX of roots of both rice cultivars all found in high Zn treatment in the presence of Si may be due to Si-mediated enhancement of antioxidant defense activities. Among the two cultivars of rice, the resistant cultivar (TY-167) showed higher values of SOD, CAT and APX activities than the sensitive cultivar (FYY-326) in response to high Zn treatment with addition of Si. Similar role of Si in mediating antioxidant defense system has been confirmed in the salt-stressed barley (Liang 1999; Liang et al 2003) and cucumber (Zhu et al. 2004), drought-stressed wheat (Gong et al. 2005), excess Mn-stressed cucumber (Dragišić Maksimović et al. 2007; Shi et al. 2005), excess B-stressed spinach (Gunes et al. 2007a) and wheat (Gunes et al. 2007b) and freezing-stressed wheat (Liang et al. 2008). Therefore, Si-enhanced antioxidant defense capacity appears to be a universal mechanism for Si-enhanced tolerance to various forms of abiotic stress in plants.

In most cases, Si-mediated responses to abiotic stress are also associated with metabolism of MDA and H_2O_2 which are markers for the ROS-induced cell membrane damage (Gong et al. 2005; Liang et al. 2003; Zhu et al. 2004). In our experiments, the concentrations of MDA and H_2O_2 in both cultivars increased significantly in the high Zn treatment, and were significantly decreased when Si was supplied (Fig. 6). Agarie et al. (1998) reported that Si enhanced the stability of lipid component in cell membranes of rice plants exposed to drought and heat stresses, suggesting that Si prevented structural and functional deterioration of cell membranes in abiotic-stressed rice plants. More recently, it has been reported that the stability and functions of plasma membranes in leaves of salt-stressed barley and Cd-stressed pakchoi were mediated by addition of Si (Liang et al. 2005, 2006; Song et al. 2009), Perhaps a constitutively higher antioxidant capacity or increase in the levels of one or more antioxidants could prevent the oxidative damage and improve resistance to oxidative stress (Liang 1999; Liang et al. 2003). In addition, the decrease in

MDA and H_2O_2 concentrations was relatively less in the Zn-sensitive cultivar than in the resistant cultivar in response to high Zn treatment with Si supply.

It is well known that rice is a typical Si-accumulator and Si is a beneficial element for its growth not only under stress condition (e.g. heavy metal toxicity), but also under unstressed conditions. (Epstein 1999; Ma and Takahashi 2002). We found in previous experiments that no tiller and less fertility would occur in rice plants grown in a Si-free nutrient solution (data not published). The present study indicated that Si significantly enhanced TRSA, TRL, TRTA and plant growth, and restricted Zn transport from root to shoot in both rice cultivars at high Zn (Figs. 1 and 4). The rice plants treated with Si presented not only biomass increase (Fig. 3) but also lower Zn toxicity (Fig. 2). This clearly indicates that a Si-mediated mechanism plays an important role in alleviating the metal stress. The lower lipid peroxidation and higher antioxidant defense activity in roots of both cultivars were also observed as a result of Si application (Figs. 6 and 7). The sensitive cultivar (FYY-326) registered lower levels of CAT and APX activities, and higher toxicity of histochemical detection. The pattern of dry matter and lipid peroxidation changes indicated that the cv. FYY-326 seems to be more susceptible to Zn treatment than cv. TY-167. The higher levels of CAT and APX activities in roots may probably lead to greater levels of detoxification mechanisms of H_2O_2 in cv. TY-167 than in cv. FYY-326 in response to high Zn treatment. The rice cultivar TY-167 in addition to better growth showed higher activities of CAT and APX and lower levels of MDA and H_2O_2 concentrations resulting in relatively less oxidative damage. This can further confirm that the TY-167 is less sensitive to Zn treatment than FYY-326. Furthermore, it may be presumed that most of the oxidative stress in these cultivars results from decreased CAT and APX activities. When the resistant and the sensitive cultivars were compared, the levels of Zn were higher in the roots and lower in the shoots of the resistant cultivar, suggesting that roots might act as a kind of heavy metal filters due to better binding properties of the cell walls.

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