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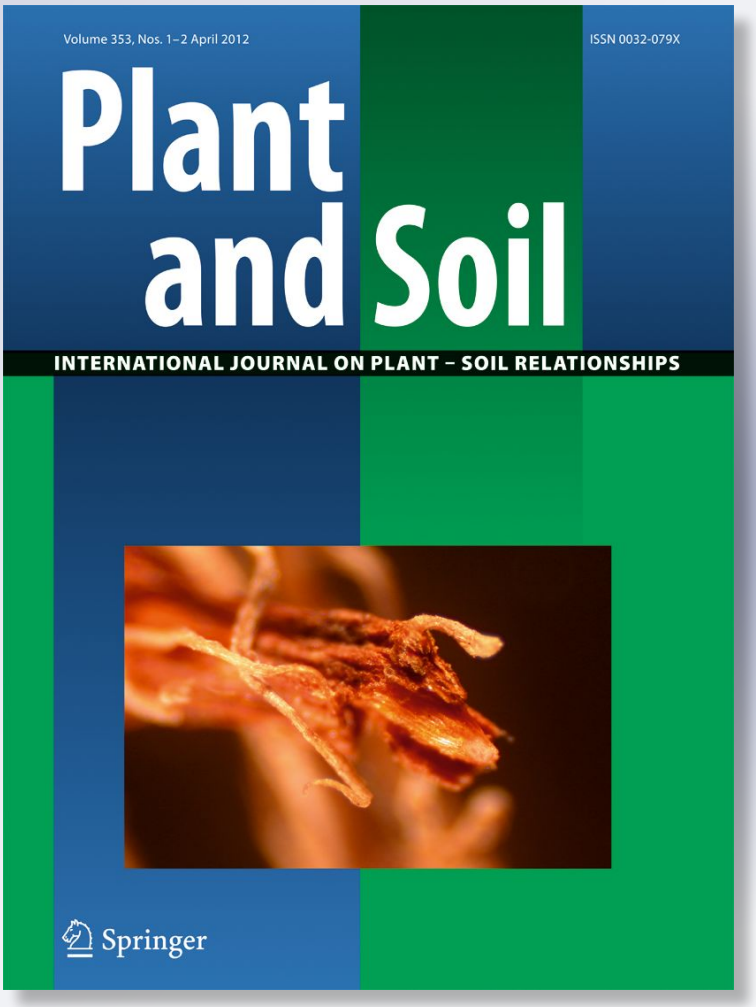

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REGULAR ARTICLE

Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.)

Ping Li · Alin Song · Zhaojun Li · Fenliang Fan · Yongchao Liang

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Abstract
Background and aims This study aimed to investigate the roles of silicon (Si) in ameliorating manganese (Mn) toxicity in two rice (*Oryza sativa* L.) cultivars: i.e. cv. Xinxiangyou 640 (XXY), a Mn-sensitive cultivar and cv. Zhuliangyou 99 (ZLY), a Mn-tolerant cultivar.
Methods Plants were cultured in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM), both with or without Si supply at 1.5 mM Si.
Results Plant growth was severely inhibited by high Mn in cv. XXY, but was enhanced by Si supply. In cv. XXY, Si-enhanced tolerance resulted from a restriction of Mn transport, whereas in cv. ZLY Mn uptake was depressed. In cv. XXY, high Mn significantly increased superoxide dismutase (SOD), catalase and ascorbate peroxidase activities but decreased non-protein thiols and glutathione concentrations, leading to accumulation of H_2O_2 and malondialdehyde. The addition of Si significantly counteracted high Mn-elevated malondialdehyde and H_2O_2 concentrations and enhanced plant growth. In cv. ZLY, high Mn considerably raised SOD activities and glutathione concentrations, thus leading to relatively low oxidative damage.
Conclusions Si-enhanced Mn tolerance was attributed mainly to restricted Mn transport in cv. XXY but to depressed Mn uptake in cv. ZLY. Silicon mainly influenced non-enzymatic antioxidants in these two rice cultivars under high Mn stress.

Keywords Antioxidants · High manganese · Lipid peroxidation · Mn uptake and transport · Rice · Silicon

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbate
CAT	Catalase
DTNB	5,5′-Dithiobis-2,2-nitrobenzoic acid
EDTA	Ethylenediaminetetraacetic acid
GSH	Glutathione (reduced form)
MDA	Malondialdehyde
NBT	Nitro blue tetrazolium
NPT	Non-protein thiols
PVP	Polyvinvinyl pyrrolideone
SOD	Superoxide dismutase

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TBA Thiobarbituric acid
TCA Trichloroacetic acid
XXY Xinxiangyou 640
ZLY Zhuliangyou 99

Introduction

The availability of Mn to plants growing in soil depends not only on total soil Mn content, but also on other soil parameters including the amounts of easily reducible Mn oxides, soil pH, redox potential, soil moisture, and microbial activity (Horst 1988; Foy et al. 1978; Conyers et al. 1997). Mn toxicity is one of the most limiting factors for crop production on acid and insufficiently drained soils with low redox potential (Schlichting and Sparrow 1988; Conyers et al. 1997). It has been suggested that excess Mn induces oxidative stress in barley (Demirevska-Kepova et al. 2004) and in *Cucumis sativus* (Shi et al. 2005b; Dragisic Maksimovic et al. 2007).

Rice is the primary staple food for over two billion people in Asia, Africa, and Latin America (Salekdeh et al. 2002). It is a typical siliciphilous plant species that can accumulate up to 10% SiO_2 in the shoots (Okuda and Takahashi 1965; Epstein 1999) and it is generally recognized that the application of silicon (Si) is beneficial for the growth of rice plants in both glasshouse and field conditions (Liang et al. 1994; Ma and Takahashi 1989, 1990; Ma et al. 1989). Increasing evidence suggests that Si plays an important role in ameliorating both biotic and abiotic stresses in plants (for review see e.g. Epstein 1999; Liang et al. 2007). Although it has been proven that metal toxicities in several plant species can be ameliorated by supply of Si, the mechanisms involved are still poorly understood (Liang et al. 2005; Neumann and Nieden 2001; Rogalla and Römheld 2002; Shi et al. 2005a; Dragisic Maksimovic et al. 2007; Song et al. 2009).

Species-specific differences have been observed in interactions of Si with Mn toxicity. For instance, in pumpkin, a Si accumulating dicot, Si was found to alleviate Mn toxicity through a localized accumulation of Mn with Si in a metabolically inactive form around the base of the trichomes on the leaf surface without decreasing the Mn content of the shoot (Iwasaki and Matsumura 1999). Similarly in cowpea, a non-Si-accumulating plant species, total Mn concentration in shoots was also unaffected by Si supply (Iwasaki et al. 2002). Rogalla and Römheld (2002) showed that Si-mediated tolerance of Mn in cucumber was a consequence of strong binding of Mn to the cell walls and a lowering of Mn concentration within the symplast. Additionally increased binding of Mn to the cell walls was associated with a subsequent lowering in apoplastic Mn concentration, brought about by Mn interactions with soluble Si in the apoplast itself (Iwasaki et al. 2002; Führs et al. 2009). Shi et al. (2005a) reported that Si-mediated alleviation of Mn toxicity in cucumber was regulated significantly by the activities of antioxidant enzymes and non-enzymatic antioxidants. In maize, the addition of Si reduced the density of brown spots per leaf area as well as lipid peroxidation level and enhanced plant growth in Mn-treated plants (Zlatimira et al. 2008). Doncheva et al. (2009) reported that Mn storage in non-photosynthetic tissue by the addition of Si could constitute a Mn tolerance mechanism in maize.

Previous studies have focused mainly on the mechanism of the effect of Si on the uptake or root to shoot translocation of Mn, but little information is available on Si-mediated Mn toxicity relating particularly to reactive oxygen species (ROS) metabolism and oxidative damage in plants. So far, only a few papers have dealt with Si and Mn interactions in terms of ROS metabolism and antioxidant defense systems, and all these studies have been performed in dicotyledonous plant species (Shi et al. 2005a; Dragisic Maksimovic et al. 2007; Führs et al. 2009). By contrast, in rice, a typical monocotyledonous Si-accumulator, information is lacking concerning Si-alleviated Mn toxicity modulated by antioxidant defense systems. In the present work, we hypothesize that Si may ameliorate toxic effects of Mn in rice by regulating antioxidant defense activities and consequently reducing membrane oxidative damage, as has also been proposed to explain why Si alleviates salt or drought stress in plants. To test this hypothesis, we investigated the effect of exogenous Si on Mn uptake and translocation, antioxidant defense enzymes and non-enzymatic antioxidants in two rice cultivars, Xinxiangyou 640 (XXY) and Zhuliangyou 99 (ZLY) differing substantially in their resistance to high Mn stress. The main objectives were to elucidate the possible mechanisms of Si-mediated alleviation of Mn toxicity in rice.

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Materials and methods

Plant material and growth conditions

Firstly, preliminary screening studies were carried out to obtain two rice cultivars differing substantially in Mn tolerance. Seedlings of various cultivars of rice were grown for 1 week in solutions containing different concentrations of Mn to select for metal tolerance by assessing tolerance index (TI). This was calculated on root length according to the formula: TI = Root length (treated)/Root length (control) (Monni et al. 2001). Based on these preliminary screening studies, two rice cultivars differing substantially in Mn tolerance were used in this investigation: i.e. cv. XXY, a manganese-sensitive cultivar and cv. ZLY, a manganese-tolerant cultivar. Rice seeds were surface-sterilized with 10% $NaClO_3$ for 15 min and germinated on moist filter paper in the dark at 28°C for 3 days. The germinated seeds were sown in the plastic containers filled with quartz sand and watered with 1/2-strength Kimura B nutrient solution. The composition of the basic nutrient solution was: 0.37 mM $(NH_4)_2SO_4$, 0.55 mM $MgSO_4{\cdot}7H_2O$, 0.18 mM KNO_3, 0.37 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.21 mM KH_2PO_4, 20 μMNaEDTAFe·$3H_2O$, 6.7 μM $MnCl_2{\cdot}4H_2O$, 9.4 μM H_3BO_3, 0.01 μM $(NH_4)_6Mo_7O_2{\cdot}4H_2O$, 0.15 μM $ZnSO_4{\cdot}7H_2O$, 0.16 μM $CuSO_4{\cdot}5H_2O$. Solution pH was adjusted to 5.6 with dilute HCl or NaOH. After 7 days, the seedlings were transferred to 4 L plastic pots containing full-strength nutrient solution (18 plants per pot). The experiment was started at the onset of the fifth leaf stage when the plants were transferred to solutions with either normal (6.7 μM) or high (2.0 mM) Mn (added as $MnSO_4$), both with or without Si supply at 1.5 mM Si (added as $Na_2SiO_3{\cdot}nH_2O$). The 8 treatments were arranged in a randomized, complete block design with 3 replicates. . The light period was about 12 h. The air temperature was 25–30 and 18–22°C, respectively, during day and night. The nutrient solutions were renewed every 3 days. The plants were harvested 7 days after the onset of Mn treatments and separated into roots and shoots.

Determination of biomass

At harvest, nine rice plants were taken from each replicate of each treatment and divided into shoots and roots. Detached roots and shoots were carefully washed under a continuous stream of deionised water and then oven dried at 70°C to a constant weight after which dry weights of shoots and roots were measured. Three rice plants were taken from each replicate of each treatment for the other estimations described below.

Determination of Si concentration and Mn concentration

Plants of each treatment were harvested and separated into shoots and roots. The shoots were washed thoroughly with distilled water. To remove the ions from the root free space, the roots were washed with 0.5 mM HCl for 30 min followed by thorough rinsing with distilled water. Plant samples were dried as described above.

About 0.1 g of the dried plant samples were used for the high-temperature alkaline fusion method and the alkaline fusion sample was diluted to 50 ml with distilled water. The Si concentration in the solution was determined by the colorimetric molybdenum blue method as described by Dai et al. (2005). The Mn concentration in the plant samples was determined by atomic absorption spectrophotometer (Perkin-Elmer) on the digested dried plant material extracts obtained by oxidative wet digestion using a mixture of nitric, sulfuric and perchloric acids.

Determination of malondialdehyde (MDA) and H_2O_2 concentrations

Lipid peroxidation was expressed as the concentration of MDA that was determined by the thiobarbituic acid (TBA) test. About 0.5 g leaf segments were homogenized in 5.0 ml of 5% (w/v) trichloroacetic acid (TCA), and centrifuged at 12,000 g for 10 min. The supernatant was assayed for MDA concentration spectrophotometrically according to the method of Heath and Packer (1968). All spectrophotometric analyses were performed using an Hitachi 7500 spectrophotometer (Hitachi Ltd, Tokyo, Japan).

Hydrogen peroxide levels were determined according to Velikova et al. (2000). Briefly, leaf tissues (500 mg) were homogenized in ice bath with 5.0 ml 0.1% (w/v) TCA. To 0.5 ml of the supernatant, a reaction mixture was added of 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1.0 ml 1.0 M KI. The absorbance of the supernatant was read at 390 nm and the content of H_2O_2 was calculated from a standard curve.

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Antioxidative enzyme extraction and assays

Fresh leaf segments (about 500 mg) were ground in an ice-cold sodium phosphate buffer (pH 7.8) containing 0.2 mM ethylenediaminetetraacetic acid (EDTA), 2.0 mM reduced ascorbate and 2% poly-vinvinyl pyrrolideone (PVP). The homogenate was centrifuged at 15,000 g for 20 min at 4°C, and the supernatants were used for assay of the activity of enzymes. 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 method described by Sgherri et al. (1994) in terms of the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The solution absorbance was measured at 560 nm and the absorbance of an unirradiated reaction mixture blank was deducted. One unit of SOD represented the amount that inhibited NBT reduction by 50%.

CAT activity was measured following the method of Aebi (1984). The reaction was started by adding H_2O_2 and the activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption.

APX activity was measured according to the method described by Nakano and Asada (1981). The assay solution contained 50 mM sodium phosphate buffer (pH 7.0), 1.0 mM ascorbate, 1.0 mM H_2O_2, 0.1 mM EDTA and 0.1 ml enzyme extract. The reaction was started by adding H_2O_2 and the decrease in absorbance at 290 nm was recorded for 1 min to determine the oxidation rate of ascorbate.

Determination of glutathione (GSH), non-protein thiols (NPT) and ascorbic acid (AsA) concentrations

Glutathione (GSH) concentration was determined by reading the absorbance at 412 nm following the method by Guri (1983). For this, 0.5 g of fresh root segments were homogenized in an ice bath in 5 ml of 5% (w/v) TCA, and homogenate was centrifuged at 12,000 g for 20 min. The supernatant was used for GSH assay using 5,5′-dithiobis-2,2-nitrobenzoic acid (DTNB) as a reagent.

NPT concentrations were determined by reading the absorbance at 412 nm according to Metwally et al. (2003). Plant tissues (0.1 g fresh weight) were homogenized in 0.1 M HCl/1 mM EDTA solution. The supernatant (2.0 ml) was mixed with 0.5 ml of assay buffer containing 0.2 M sodium phosphate (pH 7.8) and 0.5 ml water. The absorbance at 412 nm was measured following the addition of 0.1 ml 6 mM DTNB to a 3-ml sample.

The concentration of ascorbic acid (AsA) was measured according to Law et al. (1983). AsA concentration was measured based on the reduction of Fe^{3+} to Fe^{2+} by AsA and Fe^{2+} was quantified spectrophotometrically at 534 nm for 90 min at 30°C following the method.

Statistical analyses

The data in the tables and figures are expressed as means of 3 replicates ± S.D. Analysis of variance was performed over all the experimental data using SigmaStat version 2.03. Means in the table were subjected to LSD test at $P<0.05$.

Results

Mn toxicity symptoms

Mn toxicity symptoms observed in leaves were more severe in the Mn-sensitive cultivar (XXY) than in the Mn-resistant cultivar (ZLY) under high Mn stress. Mn toxicity symptoms generally appeared as dark-brown spots, necrotic lesions, chlorosis of leaf edges and tips and crinkle leaf. Clearly, Si supply could significantly alleviate Mn toxicity symptoms in both cultivars (Fig. 1).

Plant growth

Treatment with high Mn significantly inhibited the growth of the Mn-sensitive rice (XXY) with shoot and root dry matter depressed by 17.4% and 20.8%, respectively (Table 1). By contrast, under the same treatment the Mn-tolerant cultivar (ZLY) showed a higher shoot dry weight with root dry weight unaffected. The addition of Si with high Mn increased shoot and root dry matter weights by 40.1% and 29.8% respectively in the sensitive rice cultivar, and by 21.1% and 96.7% in the tolerant cultivar.

Si and Mn concentrations

In both cultivars Si concentration in shoots and roots was significantly increased by Si supply (Table 2).

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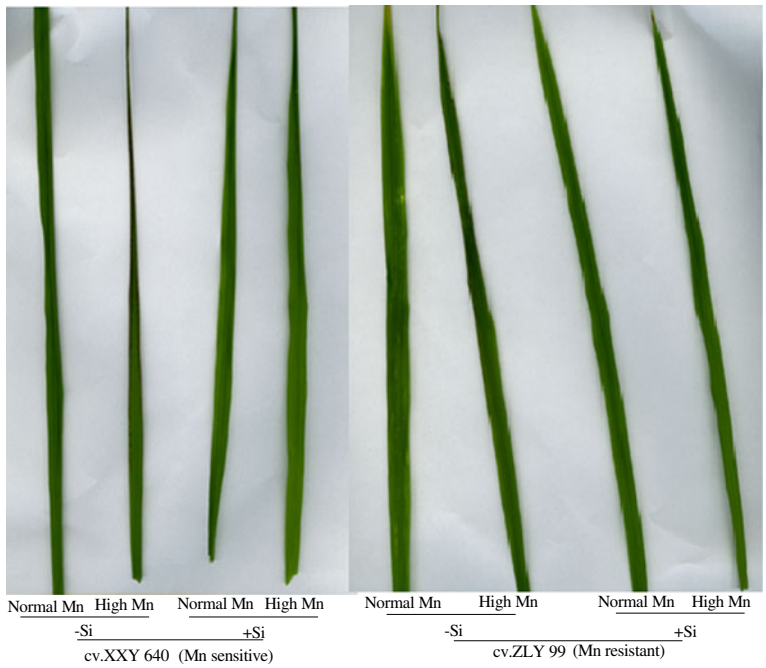

Fig. 1 The toxicity symptoms of two rice cultivars grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply

For example, Si concentration in shoots was at least 9 times greater in the Si treatment than in the non-Si-amended treatments, regardless of Mn level or cultivar tested. Shoot Si concentration was unaffected by high Mn treatment, whereas root Si concentration was increased in both cultivars at the normal Si level. Shoot Si concentration in Si-treated plants was significantly decreased by high Mn application, whereas for the same Mn treatment, root Si concentration was increased in

Table 1 Shoot and root dry weights of rice plants grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply. Data are means ± SD of 3 replicates

Variety	Mn treatment	Si supply	Shoot (g plant^{-1})			Root (g plant^{-1})		
XXY	Normal	−	0.172±0.005			0.072±0.006		
		+	0.192±0.002			0.106±0.008		
	High	−	0.142±0.002			0.057±0.002		
		+	0.199±0.003			0.074±0.001		
ZLY	Normal	−	0.162±0.008			0.040±0.007		
		+	0.284±0.010			0.094±0.006		
	High	−	0.213±0.009			0.061±0.008		
		+	0.258±0.017			0.120±0.006		
			Shoot			Root		
			Df	*P*	$LSD_{0.05}$	*df*	*P*	$LSD_{0.05}$
Source of variation	Cultivar		1	<0.001	0.012	1		0.009
	Mn		1		0.012	1		0.009
	Si		1	<0.001	0.012	1	<0.001	0.009
	Cultivar x Mn		1		0.018	1	<0.001	0.012
	Cultivar x Si		1	<0.05	0.018	1	<0.05	0.012
	Mn x Si		1		0.018	1		0.012
	Cultivar x Mn x Si		1	<0.001	0.025	1		0.018

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Table 2 Si concentrations in shoots and roots of rice plants grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply. Data are means±SD of 3 replicates

Variety	Mn treatment	Si supply	Shoot (mg g^{-1} DW)			Root (mg g^{-1} DW)		
XXY	Normal	−	4.312±0.247			6.237±0.397		
		+	64.930±1.894			10.381±0.465		
	High	−	6.166±0.820			7.633±0.050		
		+	59.961±1.205			11.791±0.388		
ZLY	Normal	−	5.504±0.831			10.755±0.500		
		+	87.416±2.137			17.869±0.730		
	High	−	5.584±0.197			13.296±0.298		
		+	80.787±1.663			18.370±0.140		
			Shoot			Root		
			df	*P*	$LSD_{0.05}$	*df*	*P*	$LSD_{0.05}$
Source of variation	Cultivar		1	<0.001	1.919	1	<0.001	0.615
	Mn		1	<0.05	1.919	1	<0.001	0.615
	Si		1	<0.001	1.919	1	<0.001	0.615
	Cultivar x Mn		1		2.715	1		0.869
	Cultivar x Si		1	<0.001	2.715	1	<0.05	0.869
	Mn x Si		1	<0.05	2.715	1		0.869
	Cultivar x Mn x Si		1		3.839	1		1.229

both cultivars. Irrespective of Si applied, higher shoot and root Si concentrations were found in the Mn-tolerant cultivar (ZLY) than in the Mn-sensitive cultivar (XXY).

At the normal Mn level, the shoot Mn concentration was lower in the Mn-tolerant cultivar (ZLY) than in the Mn-sensitive cultivar (XYY), whereas the root Mn concentration was much higher in the Mn-tolerant cultivar (ZLY) than in the Mn-sensitive cultivar (XYY) (Table 3). In both cultivars shoot and root Mn concentrations were considerably increased by high Mn application in the nutrient solution (Table 3). In the high Mn treatment, greater Mn concentrations in shoots and roots respectively were found in the Mn-tolerant cultivar than in the Mn-sensitive cultivar. The Mn concentration was much higher in shoots than in roots, regardless of the cultivar. At the normal Mn level, the addition of Si did not affect Mn concentration to any extent in either of the cultivars. However, at the high Mn level, for the Mn-sensitive cultivar (XXY), the addition of Si only slightly decreased the shoot Mn concentration in plants, whereas it enhanced root Mn concentration by 83.9%. For the Mn-tolerant cultivar (ZLY), the addition of Si decreased both shoot and root Mn concentrations in the high Mn-treated plants by 63.7% and 58.1%, respectively. In the Mn-sensitive plants treated with high Mn, the root/shoot Mn ratio was 0.88 in the Si-amended plants, as compared to 0.46 in the non-Si-amended plants. In the Mn-tolerant plants treated with high Mn, the root/shoot Mn ratio was 0.71 in the Si-amended plants, as compared to 0.61 in the non-Si-amended plants.

H_2O_2 concentration and MDA concentration

Compared with the normal Mn treatment, high Mn treatment increased H_2O_2 concentration by 33.9% in the Mn-sensitive cultivar (XXY) and by 27.9% in the Mn-tolerant cultivar (ZLY) (Fig. 2a). Likewise, supply with Si decreased H_2O_2 concentration by 37.3% in the Mn-sensitive cultivar (XXY) and by 29% in the Mn-tolerant cultivar (ZLY) (Fig. 2a). High Mn treatment increased MDA concentrations by 112% in the Mn-sensitive cultivar (XXY) and by 7.8% in the Mn-tolerant cultivar (ZLY), compared with the corresponding normal Mn treatment (Fig. 2b). However, addition of Si significantly decreased MDA concentrations in both the sensitive and the tolerant plants exposed to high Mn (Fig. 1b), especially in the sensitive cultivar.

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Table 3 Mn concentrations in shoots and roots of rice plants grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply. Data are means±SD of 3 replicates

Variety	Mn treatment	Si supply	Shoot (μg g^{-1} DW)			Root (μg g^{-1} DW)		
XXY	Normal	−	415.80±44.70			135.93±5.26		
		+	490.51±54.25			187.35±12.86		
	High	−	4515.82±175.58			2069.20±39.76		
		+	4340.81±62.06			3804.40±137.82		
ZLY	Normal	−	335.11±4.70			193.64±12.96		
		+	445.79±60.17			291.83±61.98		
	High	−	5754.97±82.31			3532.94±345.31		
		+	2088.34±185.40			1481.43±81.31		
			Shoot			Root		
			df	*P*	$LSD_{0.05}$	*df*	*P*	$LSD_{0.05}$
Source of variation	Cultivar		1	<0.001	149.918	1		200.263
	Mn		1	<0.001	149.918	1	<0.001	200.263
	Si		1	<0.001	149.918	1		200.263
	Cultivar x Mn		1	<0.05	212.017	1		283.215
	Cultivar x Si		1	<0.001	212.017	1	<0.001	283.215
	Mn x Si		1	<0.001	212.017	1		283.215
	Cultivar x Mn x Si		1	<0.001	299.837	1	<0.001	400.526

Antioxidative enzymes

High Mn treatment significantly increased SOD activity in both cultivars. SOD activity in the Mn-sensitive and the Mn-tolerant plants was 5-fold and 1-fold greater at the high Mn level than at the normal Mn level, respectively (Fig. 3a). Supply with Si did not affect SOD activity in the Mn-sensitive cultivar at the normal Mn level, but considerably decreased it at the high Mn level compared with the Si-untreated plants. However, addition of Si did not alter SOD activity significantly in the leaves of Mn-tolerant cultivar both at the normal Mn level and the high Mn level.

APX activity in the Mn-sensitive plants exposed to high Mn was 2-fold greater than that at the normal Mn level. Supply with Si significantly increased APX activity in the Mn-sensitive cultivar at the normal Mn level, but considerably decreased it at the high Mn level compared with the Si-untreated plants. By contrast, no significant differences were observed between any two treatments in the Mn-tolerant cultivar (Fig. 3b).

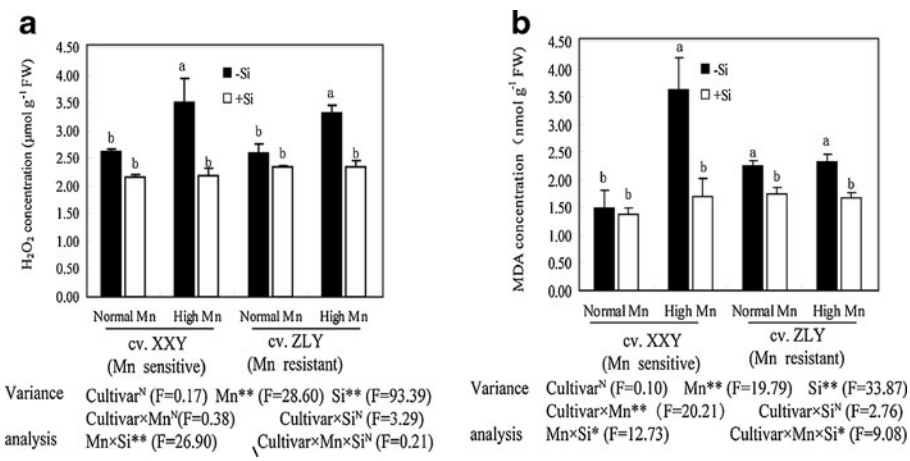

Fig. 2 Concentrations of H_2O_2 (**a**) and MDA (**b**) in the leaves of rice plants grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply. Data are means ± SD of three replicates

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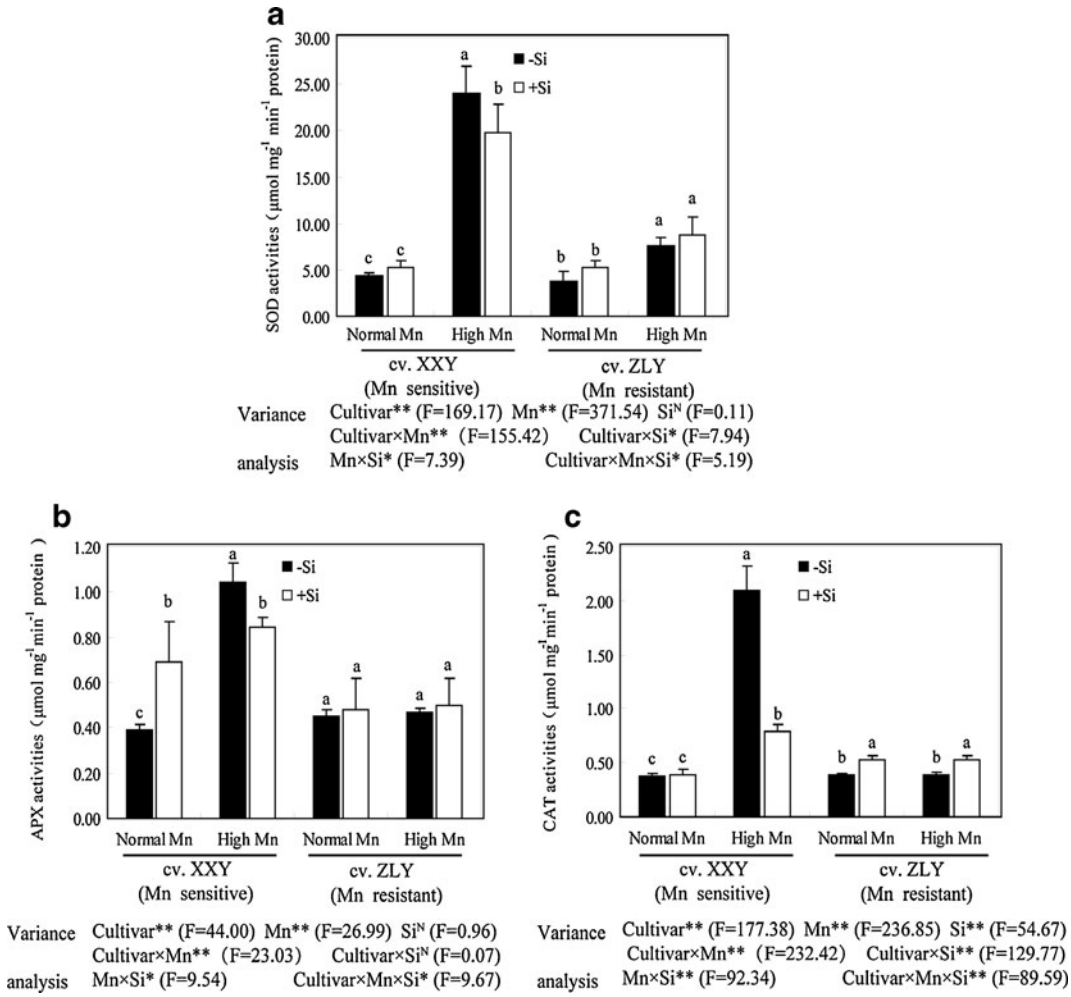

Fig. 3 Activities of SOD (**a**), APX (**b**), and CAT (**c**) in the leaves of rice plants grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply. Data are means ± SD of three replicates

CAT activity in the Mn-sensitive cultivar (XXY) exposed to high Mn was 5-fold higher than that at the normal Mn level. Addition of Si significantly reduced CAT activity in Mn-sensitive cultivar (XXY) at the high Mn level. CAT activity in the Mn-tolerant cultivar (ZLY) was unaffected by application of high Mn (Fig. 3c).

AsA, GSH and NPT concentration

The concentrations of AsA were significantly increased by high Mn treatment in the Mn-sensitive cultivar (XXY) but were not significantly affected in the Mn-tolerant cultivar (ZLY) (Fig. 4a). In the Mn-sensitive cultivar, the concentration of AsA was 99.7% higher at the high Mn level than at the normal Mn level, as compared to 18.3%, in the Mn-tolerant cultivar. Addition of Si significantly increased the concentrations of AsA at the normal Mn level in both cultivars. However, at the high Mn level, addition of Si significantly increased the concentrations of AsA in the Mn-tolerant cultivar but considerably decreased it in the Mn-sensitive cultivar.

The concentrations of GSH were decreased by application of high Mn by 14.8% in the Mn-sensitive cultivar, but were increased by 26.2% in the Mn-tolerant cultivar as compared with the normal Mn treatment (Fig. 4b). Treatment with Si significantly increased the GSH concentration at the high Mn level in the Mn-sensitive cultivar as compared with the Si-untreated plants.

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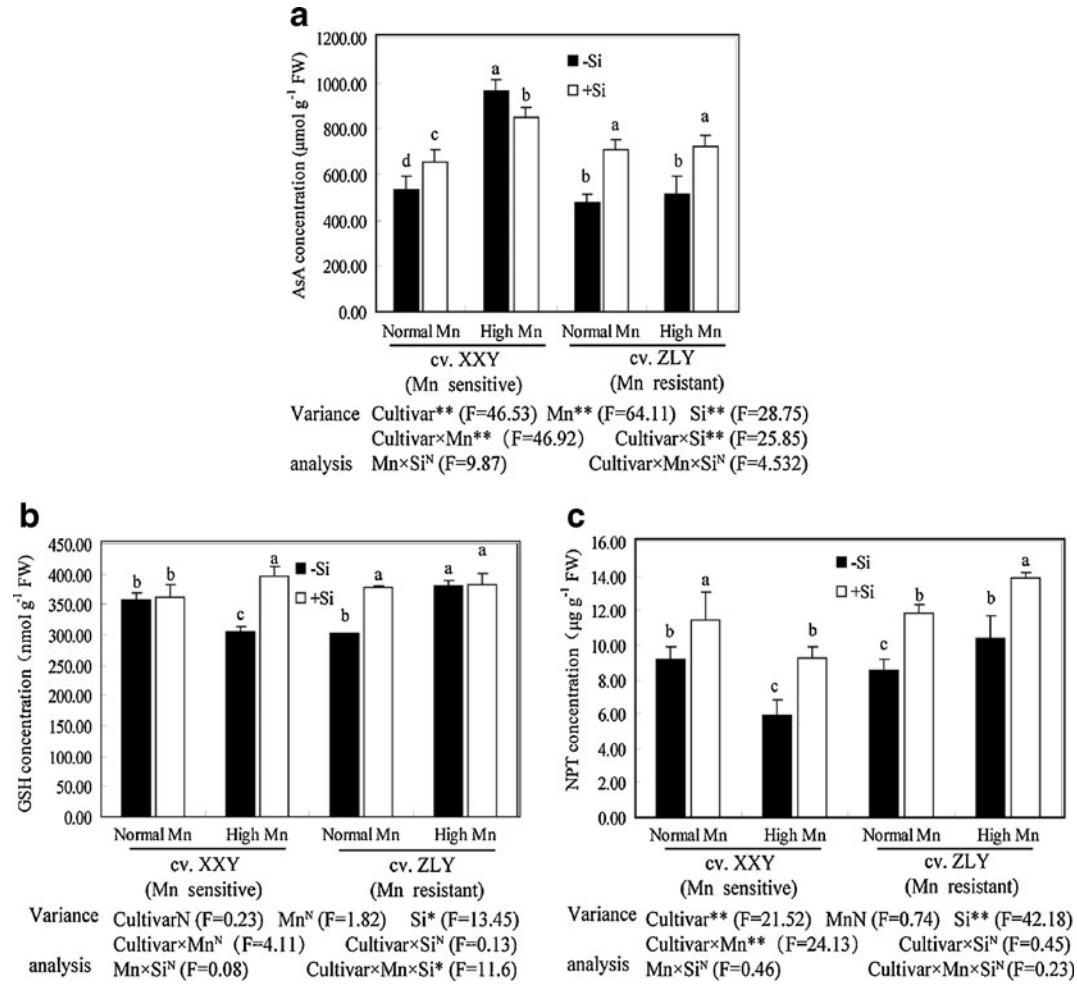

Fig. 4 Concentrations of AsA (**a**), GSH (**b**) and NPT (**c**) in the leaves of rice plants grown in nutrient solution containing normal Mn (6.7 μM) or high Mn (2.0 mM) with or without Si (1.5 mM) supply. Data are means ± SD of three replicates

The concentrations of NPT were decreased by addition of high Mn by 35.9% in the Mn-sensitive cultivar, but were not significantly affected in the Mn-tolerant cultivar (Fig. 4c). Treatment with Si significantly increased NPT concentration at both the normal and the high Mn levels in both rice cultivars.

Discussion

Mn toxicity is one of the most important abiotic stresses in acidic soil (Marschner 1991), and affects physiological and biochemical processes associated with plant growth and development (Paul et al. 2003; Shi et al. 2005b). Plant species and genotypes differ greatly in resistance to excess Mn (Foy et al. 1978). Large genetic differences have been reported between cultivars of various crops: common bean (Gonzalez et al. 1998), cowpea (Horst et al. 1999), rapeseed (Moroni et al. 2003), and maize (Stoyanova et al. 2008; Doncheva et al. 2009). In the present study, treatment with high Mn considerably reduced shoot and root dry matter weights in the Mn-sensitive cultivar (XXY) (Table 1). In this sensitive cultivar the growth of roots was more significantly restricted than that of shoots. This is a phenomenon similarly observed in plants exposed to other metals (e.g. Cu or Al) which characteristically cause damage primarily in the roots (Llugany et al. 2003; Doncheva et al. 2005). In contrast to these findings, however, Doncheva et al. (2009) reported that shoots were more significantly damaged than roots in maize

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under high Mn stress. It seems that in this respect the effects of Mn toxicity in plants may be species dependent. In the present study, high Mn was without effect on the root dry weight of the Mn-tolerant cultivar. Interestingly, the treatment with Si increased dry matter weight of both shoots and roots and enhanced Mn tolerance of Si/Mn-treated plants compared with Mn-treated plants.

At the high Mn level, greater Mn concentrations were found in both shoots and roots of the tolerant cultivar (ZLY) as compared with the sensitive cultivar (XXY), but the dry weight was unaffected by high Mn stress in the tolerant cultivar. This piece of evidence confirms that the cultivar ZLY is resistant to Mn toxicity with an ability to withstand high concentration of Mn in the nutrient solutions. The accumulation of Mn was higher in the roots of cv. ZLY than in the roots of cv. XXY at both the normal and high Mn levels. In this context, it has also been demonstrated that resistant plants accumulated higher concentrations of heavy metals in roots (Straczek et al. 2008; Song et al. 2009) compared with non-resistant plants. Williams and Vlamis (1957) and Vlamis and Williams (1967) clearly demonstrated that, at least in graminaceous species, the uptake of Mn was not reduced by Si application. Horiguchi (1988) reported that in rice, Si treatment altered the distribution of Mn by increasing Mn concentration in roots but decreasing that in shoots, thus reducing the symptoms of Mn toxicity in leaves. In the present study, the addition of Si significantly decreased shoot Mn concentration, but considerably increased root Mn concentration in the Mn-sensitive cultivar (XXY) (Table 3). Similar to the finding of Horiguchi (1988), this phenomenon indicates that Si-enhanced Mn tolerance is due not to restriction of Mn uptake from solutions, but to restriction of root-to-shoot Mn transport. Silicon has been found to enhance the internal ability of roots to inactivate high Mn within the tissues (e.g. Mn binding to the cell wall) and thus inhibit root-to-shoot Mn translocation as has previously been demonstrated (Iwasaki et al. 2002; Wiese et al. 2007), but this needs further investigation. However, for the Mn-tolerant cultivar (ZLY), the addition of Si significantly decreased both shoot and root Mn concentration, indicating that Si-enhanced Mn tolerance is due to restriction of Mn uptake from the nutrient solutions.

Evident genotypical differences were observed in shoot Si concentrations in the present study. Higher shoot and root Si concentrations were found in the Mn-tolerant cultivar (ZLY) than in the Mn-sensitive cultivar (XXY) regardless of Si application, indicating that cv. ZLY is more tolerant to high Mn concentrations than cv. XXY (Table 2). The Si accumulation in the shoots of Si-treated plants was significantly decreased by high Mn treatment, in contrast to the roots in which it was significantly increased, indicating that Mn inhibits Si transport from roots to shoots.

High Mn significantly increased H_2O_2 and MDA concentration in the Mn-sensitive cultivar (XXY), which damaged the rice plants. At the high Mn level, lower H_2O_2 and MDA concentration caused by the addition of Si suggests that Si alleviated this oxidative stress caused by Mn toxicity (Fig. 2). The lower level of lipid peroxidation in the Mn-tolerant cultivar (ZLY) suggests, therefore, that Mn-tolerant plants are better protected from oxidative damage.

High Mn exposure can induce oxidative stress in plants, which can lead to the formation of reactive oxygen species (ROS) including superoxide radicals, hydroxide radicals and hydrogen peroxide (Demirevska-Kepova et al. 2004) that cause oxidative damage to biomolecules such as lipids and proteins, and eventually lead to cell death. Plants possess an antioxidant defense system comprised of enzymatic and non-enzymatic components, which normally maintain ROS balance within the cell. In the present study, significant enhancement of SOD activity, CAT activity and APX activity was observed in the Mn-sensitive plants exposed to high Mn compared to the normal Mn level (Fig. 3), indicating enhanced production of ROS under high Mn stress. Similar results of SOD activity and APX activity have been reported in cucumber under high Mn, although in this plant species CAT activity was decreased by high Mn (Shi et al. 2005a). This discrepancy may be attributed to variability in plant species in evolution to different response mechanisms to cope with high Mn. In the results reported here Si also increased SOD activity, CAT activity and APX activity in the Mn-sensitive plants under high Mn compared with the normal Mn level (Fig. 3), suggesting an important role of Si in removing ROS induced by high Mn. Similar Si effects have also been observed in cucumber under Mn stress (Shi et al. 2005a).

AsA is readily oxidized by catalytic transition metals at an appreciable rate at acid or neutral pH (Buttner 1988; Gallego et al. 1996). In the present

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study, high Mn considerably enhanced AsA concentration in the leaves of Mn-sensitive cultivar compared with the normal Mn level (Fig. 4a), suggesting that rice responded to Mn-induced production of H_2O_2 by expressing higher levels of such scavenging enzymes as in the AsA–APX system. The report that AsA concentration is decreased by high Mn in *Cucumis sativus* (Shi et al. 2005b) may relate to difference between plant species. Owing to its redox active thiol group, GSH has often been considered to play an important role in defense of plants and other organisms against oxidative stress (Rennenberg 1982; May et al. 1998). In the present study, a significant decrease in GSH content is a signal of inhibition of the ascorbate–glutathione cycle in the Mn-sensitive cultivar under high Mn stress. Similar results were obtained in cucumber under high Mn (Shi et al. 2005a). Supply with Si significantly increased AsA concentration and GSH concentration at the high Mn level in the Mn-sensitive cultivar, suggesting that Si supply could increase plant tolerance to oxidative stress under Mn toxicity by increasing the efficiency of the ascorbate–glutathione cycle.

Stoyanova et al. (2008) reported that Mn tolerance in maize plants could be related to an elevated production of non-protein SH compounds. Our study showed that a lower NPT concentration was observed in the Mn-sensitive cultivar at the high Mn level compared to the normal Mn level. Higher NPT concentration was found in the Si/Mn-treated plants than in the Mn-treated plants. These results suggest that stimulation of the non-enzymatic antioxidants system in rice is the key mechanism of Si-mediated alleviation of high Mn stress.

High Mn considerably promoted SOD activity, GSH concentration and NPT concentration, but had no effect on CAT activity, APX activity and AsA concentration in the Mn-tolerant plants, suggesting that SOD is the key enzyme responsible for scavenging ROS in the Mn-tolerant cultivar (ZLY). The addition of Si mainly affected non-enzymatic antioxidants in the Mn-tolerant cultivar (ZLY).

Conclusions

In conclusion, tolerance to Mn toxicity differs between the two rice cultivars used. High Mn severely inhibited the growth of the Mn-sensitive plants (XXY), which could be alleviated by Si supply. Si-enhanced Mn tolerance is the result of restriction of root-to-shoot Mn transport. In the Mn-tolerant cultivar (ZLY), however, Si-enhanced Mn tolerance is the consequence of restriction of Mn uptake from the nutrient solutions. Evidence from this study showed that Si enhanced the enzymatic and non-enzymatic antioxidants in the Mn-sensitive cultivar (XXY) subjected to Mn stress, thus suppressing Mn-induced oxidative damage and enhancing Mn tolerance. For the Mn-tolerant cultivar (ZLY), lower oxidative damage caused by high Mn was associated with its higher free radical scavenging capacity and greater protection mechanism of rice against Mn stress. Supply with Si had a clear ameliorative effect on Mn toxicity in the sensitive cultivar (XXY), while less influence was observed in the Mn-tolerant cultivar (ZLY). The addition of Si mainly regulated non-enzymatic antioxidants in rice cultivars exposed to high Mn stress.

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