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W. R. Chen[a]; Z. L. He[ab]; X. E. Yang[a]; Y. Feng[a]
[a] MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Natural Resource and Environment Science, Zhejiang University, Hangzhou, China [b] Indian River Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, Florida, USA

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W. R. Chen,[1] Z. L. He,[1,2] X. E. Yang,[1] and Y. Feng[1]

[1]MOE Key Lab of Environmental Remediation and Ecosystem Health, College of Natural Resource and Environment Science, Zhejiang University, Hangzhou, China
[2]University of Florida, Institute of Food and Agricultural Sciences, Indian River Research and Education Center, Florida, USA

ABSTRACT

To elucidate physiological mechanisms of zinc (Zn) efficiency in rice (*Oryza sativa* L.), comparative studies on root morphology, ultrastructure, and oxidative enzyme activities were investigated using Zn-efficient rice genotype ('IR8192') and Zn-inefficient rice genotype ('Erjiufeng'). The results showed that moderate Zn-deficient conditions increased root length, root surface, and root tips in both genotypes, but a greater extent occurred in 'IR8192'. Under moderate Zn deficient conditions, many swollen mitochondria were observed in the root tip cells of 'Erjiufeng', whereas most root cells in 'IR8192' remained intact. Disturbances in the ultrastructure of these organelles were accompanied with elevated oxidative stress in both genotypes and the increases were less in 'IR8192' than in 'Erjiufeng'. This may result from the differences that existed in the activities of antioxidative enzymes between these two genotypes. These results suggest that Zn efficiency in 'IR8192' is closely associated with its high root tolerance to Zn-deficiency by maintaining a relatively higher efficient antioxidative system and intact root tip cell and functions.

Keywords: *Oryza sativa*, antioxidative enzyme, root morphology, ultrastructure, zinc efficiency

INTRODUCTION

Zinc (Zn) is essential for many physiological and biochemical processes. Studies of zinc uptake in biology are critical as Zn is essential for all organisms, and

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Address correspondence to Zhenli He, University of Florida, Institute of Food and Agricultural Sciences, Indian River Research and Education Center, 2199 S. Rock Road, Fort Pierce, FL 34945. E-mail: zhe@ufl.edu

human Zn deficiencies rank third in importance after iron (Fe) and vitamin A deficiency (Hambidge, 2000). Zinc deficiency could be partially corrected by application of a Zn compound to soil or plants (Marschner, 1995), but development of Zn efficient rice germplasms is a more promising strategy than fertilizer application, particularly in developing countries (Graham et al., 1992). Genotypic differences in Zn efficiency among large rice germplasm collections were reported by the International Rice Research Institute (IRRI) and its collaborators (Neue et al., 1998; Quijano-Guerta et al., 2002). Zinc efficiency in relation to bicarbonate tolerance in *Oryza sativa* has been reported (Hajiboland et al., 2003; 2005; Yang et al., 1994a; 1994b; 2003). However, the mechanisms of Zn efficiency in *Oryza sativa* were not well understood. Under adverse conditions, roots primarily sense the stress and try to adapt to the adversity through changing its morphology and distribution. Adaptive responses for plants to acquire low mobile nutrients such as phosphorus (P), potassium (K), and Fe have been well documented (Høgh-Jensen et al., 2003; Marschner and Romheld 1996; Schachtman et al., 1998). Compared with the P-inefficient plants, the P-efficient plants have higher root/shoot ratio, longer total root length, more root tips, and the interface area, which closely correlate with phosphorus absorption, was improved in P-efficient plants through decreasing root diameter (Foehes et al., 1998).

Though Zn deficiency can modify a number of physiological processes, the primary site of injury is probably at cell membrane level (Candana and Tarhan, 2003). Cellular organelles, such as the plasma membrane and mitochondria, are known to be critically affected in response to adverse environmental conditions (Ciamporova and Mistrik, 1993). Numerous studies on ultrastructure alteration resistance to the stress of excess heavy metals have been carried out, especially on several metal hyperaccumulation plants (Barbara et al., 2003; Maria et al., 1999; Vani et al., 2001; Ioannis et al., 2004; Chen et al., 2003; Kukkola et al., 2000), but few reports concern the ultrastructure alteration caused by micronutrient deficiency. Study on ultrastructure alteration between the Zn-efficient and –inefficient rice is also helpful to elucidate the mechanisms of Zn efficiency in plants. The damages of ultrastructure (especially the cell membrane) mainly result from the production of reactive oxygen species (ROS) caused by stress conditions (Cakmak, 2000). One of the primary effects of ROS in cells is the peroxidation of membranes (Tewari et al., 2004). To defend from the peroxidation, plants have developed an effective antioxidant defense system comprising of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidases (POD). Superoxide dismutase catalyses dismutation of the superoxide anion ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2), while ascorbate peroxidase (APX), catalase (CAT), and POD detoxify H_2O_2. Enhanced activities of antioxidative enzymes and concentrations of antioxidant molecules have been reported earlier in magnesium (Mg) deficient maize (Tewari et al., 2004) and Zn deficient wheat plants (Hacisalihoglu et al., 2003). However, minimal information is available regarding responses of membrane

ultrastructure together with the anti-oxidative enzymes in rice plants to Zn deficiency.

In previous studies, it was found that Zn inefficient genotype ('Erjiufeng') did not have significantly lower Zn concentrations than the zinc efficient genotype ('IR8192') in all tissues, but it still developed Zn deficient symptoms (Chen et al., 2004), suggesting that total Zn in tissues may be less physiologically available (Ellis et al., 2003). In this case, biochemical tests, such as measurements metal-containing enzyme activities may serve as better measures of physiologically available metals. Metal deficiencies often result in decreases in the activity of metal-requiring enzymes (Lopez-Millan et al., 2005). Superoxide dismutase, as a Zn requiring enzyme, can be a good indicator. The objectives of this study were: (1) to study the morphological plasticity of two rice genotypes differing in zinc efficiency at different Zn activities; (2) to observe differences in ultrastructure alteration in response to Zn deficiency between the Zn-efficient and Zn-inefficient rice plant; and (3) to examine the effects of Zn deficiency on the induction of oxidative stress and antioxidative responses in rice plants under hydroponic conditions.

MATERIALS AND METHODS

Plant Growth and Treatment

One zinc-efficient rice genotype ('IR8192') and one zinc-inefficient rice genotype ('Erjiufeng') were selected for this study. Pre-germinated seeds were precultured in distilled water containing 0.02 mmol/L calcium sulfate ($CaSO_4$) for five days and then grown in nutrient solution prepared as described by Yang et al. (1994a). A chelator-nutrient solution technique (Yang et al., 1994a) was used to control free Zn activities in nutrient solution. Three Zn activities adopted were: pZn^{2+} 9.7, 11.0, and >11.5. Our previous studies indicate that most of the rice genotypes grow normally at pZn^{2+} <9.7, but Zn deficient symptoms occurs at pZn^{2+} 11.0. Therefore, pZn^{2+} 9.7 is defined as sufficient, pZn^{2+} 11.0, moderate deficient, and pZn^{2+} 11.5, as deficient. Nutrient solution composition and preparation were referred to in Yang et al. (1994a). Plant seedlings were transplanted into plastic container, nutrient solution was replaced every three days. All the treatments were randomly placed in the greenhouse, with temperature of 21/29°C (night/ day) and relative humidity of 70%, respectively.

Measurements of Zn Concentration and Root Parameters

Six plants in each replicate were randomly harvested for biomass; three plants in each replicate were used for root parameter measurements after 30 days of treatment. Briefly, plants were rinsed with distilled water twice after harvest

and then blotted dry immediately with tissue paper. Afterwards, plants were divided into shoot and root, respectively. The dry weight of each part was obtained. The dried plant materials were finely ground (60 mesh) and ashed in a muffle furnace at 55°C for 6 hours, then the ash was dissolved in 1:3 hydrochloric acid (HCl) and analyzed with inductively coupled plasma- mass spectroscopy (ICP-MS) (Yang et al., 1994a). Subsamples of plants were rinsed twice with distilled water and scanned to obtain root image using a flat-bed scanner (DT-Scan, Régent Instrument Inc, Nepean, ON).

Electron Microscopy

It is largely recognized that root tips are the primary site of stress-induced injury in plants. Sections were taken from the top part of young roots with a size of about 2 mm. Samples were fixed in 2.5% glutardialdehyde (0.1 mol/L phosphate buffer, pH 7.2) for 90 min at room temperature. After rinsing with appropriate buffer, the sections were postfixed in 1% osmium tetroxide (OsO_4) for 90 min. The fixed material was dehydrated in a graded alcohol series and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were cut with a Reichert-Jung ULTRACUT E ultramicrotome (Reichert-Jung, Depew, NY), with their thickness of 70 nm being controlled by the instrumental setting. The ultrathin sections were stained with lead citrate and uranyl acetate and viewed with TEM (JEM-1230 JEOL, Japan).

Electrolyte Leakage, Hydrogen Peroxide Concentration, and Lipid Peroxidation

The electrolyte leakage was calculated as the ratio of conductivity before boiling to that after boiling. Briefly, roots of four seedlings were immersed in 15 mL distilled water and the initial conductivity was measured. The tubes were then placed in boiling water for 15 min, and cooled to room temperature. Conductivity was again determined.

The oxidant stress induced by Zn-deficiency was studied using the lipid peroxidant, hydrogen peroxide concentration as indicators. Hydrogen peroxide concentrations in the roots were determined as described by Brennan and Frenkel (1977). The hydroperoxide–titanium complex formed by reaction of tissue-H_2O_2 with titanic tetrachloride was precipitated using a concentrated ammonia solution. The pigments in precipitate were removed by repeatedly washing with cold acetone. The precipitate was solubilized in 2N sulfuric acid (H_2SO_4) and absorbance of the solution was read at 415 nm against water blank.

The level of lipid peroxidation products in root tissue was determined in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction

as described by Heath and Packer (1968). Two-hundred mg fresh root tissue was homogenized in 3 mL 10% trichloroacetic acid (TCA); the homogenate was boiled for 30 min at 95°C in a water bath with 0.5% TBA (in 20% TCA) and then cooled quickly in ice bath and centrifuged at 10,000 g for 10 min. Malondialdehyde equivalent was calculated from the difference in absorbance at 532 and 600 nm using extinction coefficient of 155 mM^{-1} cm^{-1}.

ENZYMES ACTIVITIES ASSAY

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to Wang et al. (1983) in terms of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of NBT auto-oxidation under assay condition. Superoxide dismutase activity was expressed as a unit per milligram protein of rice root. Peroxidase (POD; EC 1.11.1.7) activity was determined by the Amako (1994) method. Peroxidase activity was defined as the increase in absorbance recorded at one OD value of A470 per minute under assay condition. Catalase (CAT; EC 1.11.1.6) activity was determined by monitoring the disappearance of H_2O_2 through measuring the decrease in absorbance at 240 nm (an extinction coefficient of 0.036 mM^{-1} cm^{-1}) of a reaction mixture consisted of 25 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and enzyme extract. One unit of CAT activity corresponded to the amount of enzyme that decomposes 1 mol of H_2O_2 per minute under assay conditions. Catalase activity was expressed as a unit per g of fresh weight of root tissues. Determination of ascorbate peroxidase (APX; EC 1.11.1.11) activity of the roots was performed as described by Nakano and Asada (1981). Activity of APX was monitored as a decrease of ascorbate by measuring change in absorbance at 290 nm for 1 min. The 3mL assay mixture consisted of 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 50 mM sodium phosphate buffer (pH = 7). One unit of APX activity was expressed as unit per g of fresh weight of the root tissues. Protein content of the extraction compound was quantified according to Bradford (1976), using bovine serum albumine (BSA) as a standard.

STATISTICAL ANALYSIS

All values reported are means±S.E. of three replicates. The data were analyzed by one-way analysis of variance (ANOVA) procedure and compared for level of significance by Duncan's new multiple range test using SPSS software.

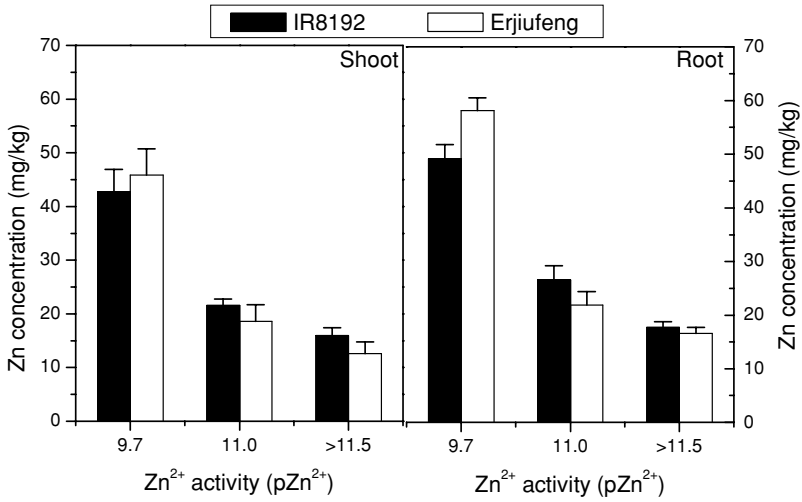

Figure 1. Zinc concentration in the shoots and roots of Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng grown at different Zn^{2+} activities.

RESULTS

Effects on Zn Concentrations

Zinc deficiency greatly decreased shoot and root Zn concentration of both rice cultivars (Figure 1). At sufficient Zn level ($pZn^{2+} = 9.7$), the Zn-inefficient cultivar ('Erjiufeng') can accumulate slightly more Zn in both root and shoot parts than the Zn-efficient cultivar ('IR8192') ($P > 0.05$). However, the reverse was true at moderate and severe Zn deficient condition ($pZn^{2+} = 11.0$ and $pZn^{2+} > 11.5$), respectively, indicating that 'IR8192' can intake and transport Zn more efficiently under Zn deficient stress.

Effects on Shoot and Root Biomass Production

Under the moderate Zn-deficient condition ($pZn^{2+} = 11.0$), the Zn-efficient genotype ('IR8192') grew well and no Zn-deficient symptoms were observed. However, the growth of Zn-inefficient 'Erjiufeng' was obviously inhibited, and Zn-deficient symptoms such as black speckles and yellow-white speckles occurred (picture were not supplied here). For the Zn-efficient genotype ('IR8192'), shoot and root dry matter yields slightly decreased at pZn 11.0, but was not statistically significant. However, both shoot and root dry matter yields of the Zn-inefficient 'Erjiufeng' significantly decreased ($P < 0.05$) grown at pZn 11.0, as compared with those at pZn 9.7 (Table 1).

Table 1
Biomass of Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng at different Zn^{2+} activities

Treatment pZn	Shoot dry weight (g/plant)		Root dry weight (g/plant)	
	IR8192	Erjiufeng	IR8192	Erjiufeng
9.7 (CK)	0.598[ab]	0.745[a]	0.096[ab]	0.126[a]
11.0	0.451[b](75.42%)	0.450[b] (60.40%)	0.094[b] (97.9%)	0.080[b] (63.5.05%)
>11.5	0.196[c] (32.78%)	0.156[c] (20.94%)	0.066[c] (68.75%)	0.046[c] (36.51%)

Note: Data are means of three replications, data in the parentheses represent the percentage of treatment in the CK, a, b, c means significant difference at 0.05 level by Duncan′s new multiple test.

Under severely Zn deficient condition, the biomass of both cultivars decreased greatly, but less extent occurred in 'IR8192'. These results indicate that the extent of decrease in dry matter production due to Zn deficiency was much greater for the Zn-inefficient genotype than for the Zn-efficient genotype.

Effects on Root Morphology

Root Length and Total Tips

Compared to the sufficient Zn condition ($pZn^{2+} = 9.7$), total root length of the Zn-efficient genotype ('IR8192') increased 39% at moderate Zn deficiency ($pZn^{2+} = 11.0$), but minimal change occurred to the Zn-inefficient genotype ('Erjiufeng') (Figure 2). Under severe Zn deficient condition, the root length of 'IR8192' was 68% of the control, and the Zn-inefficient 'Erjiufeng' decreased more, with only 38.7% of the control. These results indicate that Zn efficient genotype develops a larger root system under moderate deficient conditions, which may account for its efficient Zn absorption from low Zn soil.

Similar to root length, total fine root length and root tips of the efficient genotype significantly increased but those of the inefficient genotype hardly changed at moderate Zn deficient level, as compared to Zn sufficient conditions (Figure 3). At the severe Zn deficient level, total fine root length and root tips of both genotypes were much less than the control, but Zn-efficient rice ('IR8192') had significantly longer total fine root length and more root tips than the Zn-inefficient rice ('Erjiufeng'). This result indicates that under moderate Zn deficient conditions, the initiation of new roots (especially the fine roots) is promoted in the efficient genotypes, which may partly account for its increased total root length and enlarged root system.

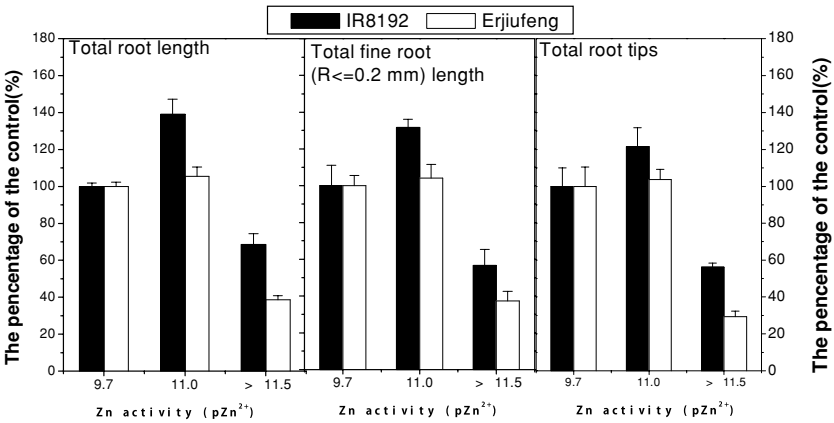

Figure 2. The total root length, total fine root length and total root tips of Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng under different Zn^{2+} activity conditions. The CK is set as 100%. The total root length of IR8192 CK and Erjiufeng CK were 1019.07 cm/plant and 1693.81 cm/plant, respectively; the total fine root (diameter$<=$0.2 mm) length: values of IR8192 CK and Erjiufeng CK were 570.48 cm/plant and 579.20 cm/plant, respectively; the total root tips: values of IR8192 CK and Erjiufeng CK were 570.48 cm/plant and 579.20 cm/plant, respectively.

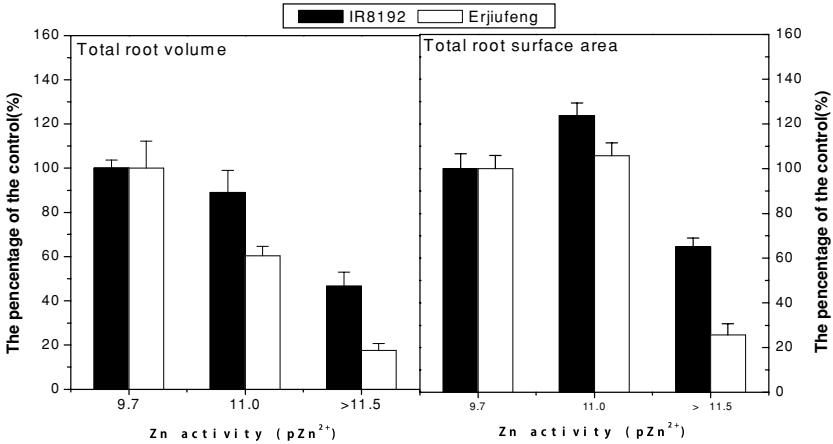

Figure 3. The total root surface areas and total root volume of Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng under different Zn^{2+} activity conditions. The CK is set as 100%. The total root surface areas of IR8192 CK and Erjiufeng CK were 273.18 cm^2/plant and 377.23 cm^2/plant, respectively; the total root volume: values of IR8192 CK and Erjiufeng CK were 3.88 cm^3/plant and 6.92 cm^3/plant, respectively. Data are means of three replications, and bars depict Standard error (SE).

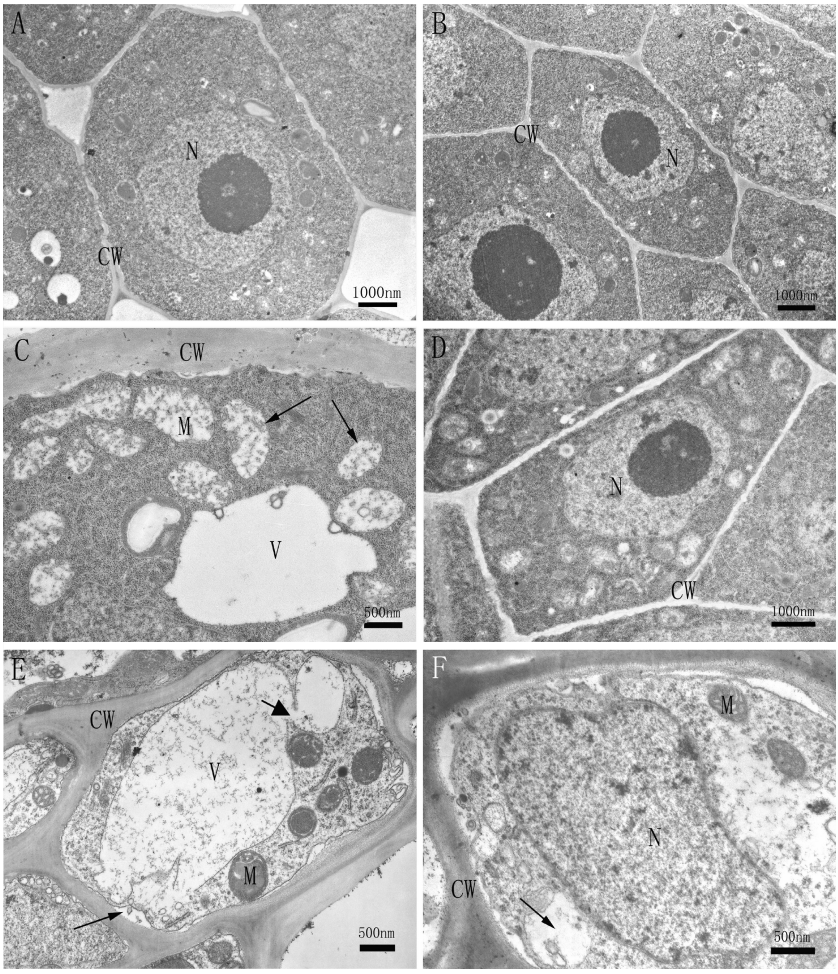

Figure 4. The ultrastructure of the root tip cell in Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng in different Zn^{2+} activity. (A) IR8192 in $pZn^{2+} = 9.7$. nomal cell ultrastructure; (B) IR8192 in $pZn^{2+} = 11.0$, no visible alteration in the cell ultrastructure; (C)IR8192 in $pZn^{2+} > 11.5$, swollen mitochondria (arrow). (D) Erjiufeng in $pZn^{2+} = 9.7$, nomal cell ultrastructure; (E) Erjiufeng in $pZn^{2+} = 11.0$, the plasma membrane was broken (short arrow) and swollen mitochondria (M), plasmolysis emerged (long arrow); (F) Erjiufeng in $pZn^{2+} > 11.5$; the cell ultrastructure were seriously damaged, the vacuolation phenomena (arrow) were observed.

Root Surface Area and Root Volume

Compared with the control, the root surface areas of 'IR8192' increased 23.7% at the moderate Zn deficit level (Figure 4), but that of 'Erjiufeng' decreased

slightly. At the severe Zn deficient level, the root area of 'IR8192' was approximately 2-fold greater than that of 'Erjiufeng', though smaller than that of the control. Total root volume of the Zn-efficient genotypes decreased slightly for 'IR8192', but significantly for the 'Erjiufeng' when grown at the moderate deficient Zn level (Figure 4). Similarly, greater root volume was noted in 'IR8192' than in 'Erjiufeng' grown at a severely Zn deficit level. These results imply that under moderate Zn deficient stress, fine root development of the efficient genotype was enhanced, and the greater surface area helps increase the plant's ability to acquire Zn from soil.

Effects on Root Tip Cell Ultrastructure

Zinc deficient stress primarily leads to the mitochondria's ultrastructural alteration (Figure 4). In the root tips of the control ($pZn^{2+} = 9.7$, Figures 4A and 4D), mitochondria in a condensed state exhibited a typical ultrastructure characterized by numerous cristae and a very dense matrix. Under the moderate Zn deficient condition ($pZn = 11.0$), many swollen mitochondria were observed in the tip cells of the Zn-inefficeint genotype ('Erjiufeng'), with broken vacuole membrane (Figure 4E), whereas the cells in the Zn-efficeint genotype ('IR8192') (Figure 4B) remained intact. Under the severe Zn deficient level ($pZn^{2+} > 11.5$), critical damage of the cells was observed in both genotypes (Figures 4C and 4F). The vacuolation phenomena were popular, plasmolysis were observed other than the swollen mitochondria.

Effects on Electrolyte Leakage, Hydrogen Peroxide Concentration, and Lipid Peroxidation

Electrolyte leakage reflected the plasmalemma damage caused by the stress, and was increased after the rice seedlings were subjected to Zn deficiency (Table 2). However, the increased percentages were much lower in 'IR8192' than in 'Erjiufeng', suggesting that 'IR8192' were less damaged.

The oxidative damage to membranes was examined by measuring the content of thiobarbituric acid reactive substances (TBARS), an indicator of lipid peroxidation. Exposure of seedling to the Zn deficiency caused a general increase in the TBARS content (Table 2). For Zn inefficient genotype 'Erjiufeng', a significant increase in the TBARS level was observed under the moderate Zn deficient condition ($pZn = 11.0$), and attained a peak at the severe Zn deficient condition ($pZn^{2+} > 11.5$), at which the TBARS value was 2.67-fold higher than the control. However, in the Zn efficient genotype 'IR8192', the TBARS content was kept at a fairly low level at the treatment of $pZn = 11.0$, a severely Zn deficient condition also significantly enhanced its TBARS content 2.11-fold higher than the control. The H_2O_2 content in both genotypes had a similar

Table 2
Effects of Zn deficiency on lipid peroxidation (MDA) and hydrogen peroxide (H_2O_2) concentration in roots of Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng grown at different Zn^{2+} activities

	IR8192 (Zn efficient genotype)			Erjiufeng (Zn inefficient genotype)		
	$pZn^{2+} = 9.7$	$pZn^{2+} = 11.0$	$pZn^{2+} > 11.5$	$pZn^{2+} = 9.7$	$pZn^{2+} = 11.0$	$pZn^{2+} > 11.5$
Electrolyte leakage (%)	10.2 ± 0.3^{a}	22.5 ± 0.3^{c}	34.3 ± 0.4^{d}	12.5 ± 0.2^{b}	56.0 ± 0.2^{e}	58.1 ± 0.2^{f}
MDA ($\mu mol \cdot g^{-1}FW$)	3.48 ± 0.54^{c}	4.69 ± 0.72^{c}	7.35 ± 0.95^{b}	3.41 ± 0.25^{c}	7.52 ± 1.51^{b}	9.10 ± 0.86^{a}
H_2O_2 ($\mu mol \cdot g^{-1}FW$)	9.86 ± 1.90^{d}	14.22 ± 3.24^{c}	18.21 ± 2.30^{b}	9.47 ± 1.83^{d}	19.63 ± 0.90^{b}	25.44 ± 2.32^{a}

variation trends as MDA (Table 2). However, in contrast to 'Erjiufeng', lower H_2O_2 concentrations ($P<0.05$) were measured in 'IR8192' under both severe and moderate Zn deficit conditions.

Activities of Antioxidant Enzymes

As a member of ascorbic acid-glutathione cycle, APX plays a crucial role in eliminating poisonous H_2O_2 from plant cells. In the roots of both genotypes, the APX activity was found higher at Zn-deficit treatments than the controls. However, no significant differences were observed between 'IR8192' and 'Erjiufeng'. Activity of catalase, an important H_2O_2 scavenging enzyme, was increased under the Zn deficient stress in both genotypes, but no difference was found between these two genotypes. Peroxidase (POD) activities in both genotypes under moderate Zn deficient condition reached the maximum values, exceeding that of the Zn-sufficient treatment (Control) ($P < 0.01$), which is an evidence of increased antioxidant defense system against the Zn stress conditions (Table 3). However, significant decreases ($P < 0.01$) were observed at severe Zn deficient activity afterwards. These may be explained by the fact that the more peroxidative attacks and damage of the radicals brought by the Zn stress exceeded the maximum ability of antioxidant defense system and diminished the activities of these enzymes. Compared with the Zn inefficient genotype 'Erjiufeng', the Zn efficient genotype 'IR8192' can reach a higher maximum activity in moderate Zn deficit condition (1.72-fold of the control) and keep a fairly higher activity level (85.34% of the maximum under the severe Zn deficit condition $pZn^{2+} = 11.0$), suggesting that 'IR8192' had a higher ability to remove reactive oxygen species.

Superoxide dismutase (SOD) is also a key enzyme in the plant's defense against oxidative damage (Bowler et al., 1992). At the same time, it is a Zn-containing enzyme that can be used to estimate physiological Zn availability. The Zn activity in the solution influenced SOD activity in 'Erjiufeng' more than in 'IR8192' (Table 3). At the moderate zinc deficit activity ($pZn^{2+} = 11.0$), the SOD activity did not change in 'IR8192' ($P > 0.05$), but was greatly decreased in 'Erjiufeng' ($P <0.01$) as compared with the control. The decreases in root SOD activity were by 81.7% and 73.6% for 'IR8192' and 'Erjiufeng', respectively, when the Zn activity (pZn^{2+}) declined from 9.7 (sufficient zinc condition) to >11.5 (severely zinc deficit condition).

DISCUSSION

'IR8192' and 'Erjiufeng' have proven to be Zn-efficient and Zn-inefficient genotypes. 'IR8192' was more tolerant to Zn-deficit than 'Erjiufeng' in terms of maintaining biomass production under Zn-deficit stress, which agreed with

Table 3
Effects of Zn deficiency on the SOD, CAT, POD, and APX activity in roots of Zn efficient genotype IR8192 and Zn inefficient genotype Erjiufeng grown at different Zn^{2+} activities

Antioxidant enzymes	IR8192 (Zn efficient genotype)			Erjiufeng (Zn inefficient genotype)		
	$pZn^{2+} = 9.7$	$pZn^{2+} = 11.0$	$pZn^{2+} > 11.5$	$pZn^{2+} = 9.7$	$pZn^{2+} = 11.0$	$pZn^{2+} > 11.5$
SOD ($U \cdot mg^{-1}$ protein)	73.52 ± 10.46^{a}	72.88 ± 7.52^{a}	60.04 ± 7.52^{abc}	68.19 ± 7.98^{ab}	56.13 ± 8.46^{bc}	50.19 ± 8.40^{c}
CAT ($U \cdot mg^{-1}$ protein)	14.44 ± 2.96^{b}	22.51 ± 5.97^{a}	16.14 ± 5.58^{ab}	13.30 ± 1.77^{b}	22.07 ± 3.24^{a}	15.37 ± 3.33^{ab}
POD ($\triangle A_{470}$ mg^{-1} protein)	4.35 ± 0.98^{cd}	7.53 ± 0.82^{a}	6.42 ± 0.33^{ab}	3.77 ± 0.38^{d}	5.78 ± 0.52^{b}	5.27 ± 0.88^{c}
APX (U mg^{-1} protein)	18.10 ± 2.49^{c}	27.65 ± 2.73^{a}	22.65 ± 2.83^{abc}	17.16 ± 2.10^{c}	25.30 ± 4.24^{ab}	$20.75 \pm 4.48bc$

our previous results (Yang et al., 1994b). In the present study, 'IR8192', as a Zn efficient genotype, was also found to have lower Zn requirement and be more efficient at Zn intake and translocation from roots to shoots when compared to 'Erjiufeng' (Figure 1). As a rule in nutrient efficiency, the acquisition of nutrients by the roots plays the most important role (Gutschick, 1993). Efficiency in acquisition largely depends on root size and morphology. A large surface area (fine roots, long root hairs) is either an inherent property (e.g., grasses vs. legumes) or deficiency-induced (e.g., by P or N, but not K or Mg deficiency) and is of key importance for acquisition particularly of P, and most likely also ammonium, in upland soils (Marschner, 1998). In the present study, the moderate Zn deficiency significantly increased root surface, total root length, total fine root length and total root tips in 'IR8192' (the Zn-efficient genotype), but decreased these root parameters in the 'Erjiufeng' (Figures 2 and 3). A great difference in these root parameters between these two genotypes was noted under severe Zn deficient conditions. Longer and thinner roots and a greater proportion of thinner roots were suggested to be associated with Zn efficiency in wheat (Dong et al., 1995). In calcareous soil, bicarbonate is one of factors inducing Zn deficiency in rice, but Zn-efficient rice genotype had greater tolerance to bicarbonate at low Zn by developing a finer and larger root system (Yang et al., 1994b; Hajiboland et al., 2003, 2005; Yang et al., 2003). The results from this study suggest that Zn efficiency is closely associated with a larger surface area of the 'captor' organ (longer fine root and larger root surface) in lowland rice.

The Zn deficit stress also greatly influenced root tip cells' ultrastructure. The mitochondria was the primary cell organ that was ruined by Zn deficiency. The ultrastructures of both genotype were seriously damaged by the severe Zn deficient stress. The ultrastructure of 'IR8192' maintained intact but that of 'Erjiufeng' was damaged by the moderate Zn deficit stress. These ultrastructure changes are in agreement with changes in morphology between the two genotypes. Mitochondria is the cellular energy factory and 70% of its dry weight is protein. More than 100 enzymes have been found in mitochondria. Zinc is the key component or the requiring active factor for these proteins. Zinc deficiency induced these proteins' degradation and impeded their synthesis, thus resulting in broken inner membrane and swollen mitochondria.

It is widely accepted that oxidative damage to critical cell compounds resulting from attack by ROS is the basis of disturbances in plant growth caused by Zn deficiency (Cakmak, 2000). The ROS damage membrane lipids and proteins as well as nucleic acids (Bolwell and Wojtaszek, 1997) and thus results in a reduction in plant growth and development (Ogawa and Iwabuchi, 2001). Hydrogen peroxide, an important type of ROS, is induced in plants following exposure to a wide variety of abiotic and biotic stress (Karpinski et al., 1999). Thiobarbituric acid reactive substances are an indicator of lipid peroxidation and oxidative damages on membranes. In the present study, 'IR8192' accumulated less H_2O_2 and producing less lipid peroxides, as compared to 'Erjiufeng'. This could be explained in terms of higher degree of protection

against oxidative damage in 'IR8192' by higher efficient ROS scavenging systems.

To get a better insight into the possible role of the antioxidative enzymes in Zn efficiency in rice, we determined the activities of some important antioxidative enzymes—CAT, SOD, POD, and APX. The results revealed that they were increased to corroborate the induction of oxidative stress in Zn-deficient plants. Catalase and APX are the primary H_2O_2 scavenging enzymes in plants, but no difference was found between the activities of these two genotypes. These results indicate CAT and APX may not be the key factors attributed to the high Zn-efficiency in 'IR8192'. Peroxidase is known to respond as a general stress enzyme to different abiotic and/or biotic stress in plants. In the prese study, the POD activity was increased, to a greater extent, in the Zn-efficeint genotype 'IR8192' than in the Zn-inefficeint genotype when grown at the moderate Zn deficient condition (Table 3). This may account for the lower lipid peroxidation in 'IR8192' (Table 2), as POD also catalyses the quenching of organic peroxides besides disproportionation of H_2O_2. These results are in agreement with those of Hacisalihoglu et al. (2003) and Tewari et al. (2004), who have reported increases in the activities of POD in Zn-deficient wheat and Mg-deficient maize, respectively.

SOD, also an essential component of the plants' antioxidative defense mechanism, has been reported to increase in Zn deficient wheat (Hacisalihoglu et al., 2003), Mg deficient plants–beans, menthe, and maize (Tewari et al., 2004). However, a contrast result was obstained in the present study. In both genotypes, SOD activity was decreased under Zn deficient conditions. This may be explained by the facts that SODs are classified into three types based on their metal cofactor: those that contain Mn (Mn SOD), Fe (Fe SOD), or Zn and copper (Cu) (Cu/Zn SOD) (Bowler and Montagu 1992). The Cu/Zn SOD activity is generally lower under Zn-limiting conditions than under standard conditions. However, there is a compensatory mechanism involving the induction of other isoforms, in order to keep an adequate level of SOD in the cell to protect against deleterious effects of superoxide radicals (Lopez-Millan, 2005). So far, this compensatory mechanism has been described between MnSOD and CuZnSOD in leaves of pea and tobacco plants grown under limiting Mn levels (Yu and Rengel, 1999). In this study, the total SOD did not change much in Zn efficient genotype 'IR8192', probably due to the decreases in CuZnSOD associated with an increase in MnSOD and FeSOD activity.

It is concluded that the deleterious effect of ROS caused by Zn-deficiency on root systems was more conspicuous in 'IR8192' than 'Erjiufeng'. The greater tolerance in 'IR8192' to Zn deficiency was due to better synergies between the antioxidative enzymes, that protected the root fine structure and maintained the functions, and lager root surface areas, which improved the ion intake capacity. It is likely is an important mechanism for the high Zn efficiency in 'IR8192'.

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