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Full Length Research Paper

Enhanced antioxidative responses of a salt-resistant wheat cultivar facilitate its adaptation to salt stress

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Wheat cultivars capable of accumulating minerals under salt stress are of considerable interest for their potential to improve crop productivity and crop quality. This study addressed the role of antioxidative enzymes in the responses of a salt-resistant wheat cultivar *Cang 6001* to high-salt stress compared to a salt-sensitive wheat cultivar *Shi 4185*. Under NaCl stress, oxidative damage was more severe and the potassium (K), calcium (Ca), zinc (Zn), and iron (Fe) accumulations were lower in *Shi 4185* seedlings than in *Cang 6001* seedlings. Supplementation with antioxidants such as ascorbic acid (AsA) and N-acetyl-L-cysteine (NAC) increased Zn/Fe contents in wheat seedlings, indicating that the increased accumulation of Zn and Fe under salt stress in *Cang 6001* seedlings was at least partially related to the depressed level of reactive oxygen species (ROS). Under salt stress, the superoxide dismutase (SOD) activity was higher in *Cang 6001* than in *Shi 4185*. Semi-quantitative real-time polymerase chain reaction (RT-PCR) analysis indicated that the transcripts of cytoplasmic *Cu/Zn-SOD* and *Mn-SOD* were higher in *Cang 6001* than in *Shi 4185* under salt stress. Our results indicate that during salt stress, elevated SOD activity protected seedlings from ROS damage and may improve micronutrition elements uptake. The possible involvement of SOD activity in Zn/Fe accumulations under salt stress was discussed.

Key words: Anti-oxidative enzymes, wheat, salt, micronutrition elements.

INTRODUCTION

Salinity is a major factor limiting the geographical distribution of plants and is responsible for significant reductions in the yield and quality of many important crops (Boyer, 1982; Jia et al., 2002; Ghoulam et al., 2002; Guo et al., 2009). Increased salinization will result in a 30% reduction in arable land within the next 25 years, and up to a 50% reduction by the middle of 21st century (Wang et al., 2003; Mahajan and Tuteja, 2005). With the goal of increasing the productivity of marginal soils, development and cultivation of salt-tolerant crops has received more attention in recent years.

Salinity has four main constraints on plant growth: (I) salinity inhibits plant growth and development by causing osmotic stress and thereby restricting H_2O uptake, and hence the reduced assimilation, it in turn leads to (II) oxidative stress (secondary, unspecific stress). Furthermore, salt stress also leads to (III) ionic toxicity and (IV) nutrient imbalance (Grattan and Grieve, 1999; Zhu, 2003). Extremely high salt concentrations or long term exposure to salinity condition also induces inhibition of enzyme activities and the over-accumulation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radical, resulting in metabolic disturbance, lipid peroxidation, chlorophyll breakdown and so on (Dong et al., 2001; Tsai et al.,

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Abbreviations: AsA, Ascorbic acid; **NAC,** N-acetyl-L-cysteine; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **RT-PCR,** real-time polymerase chain reaction; **GR,** glutathione reductase; **POX,** peroxidase; **GSH,** glutathione; **AR,** ascorbate; **PCD,** programmed cell death; **CAT,** catalase; **APX,** ascorbate peroxidase.

2004). To avoid the deleterious effects of ROS which played an important role in hindering plant growth and development, plant cells possess efficient antioxidative enzymes, such as superoxide dismutase (SOD EC 1.15.1.1.), catalase (CAT EC 1.11.1.6.) and ascorbate peroxidase (APX EC 1.11.1.11.), among others. These enzymes have been observed to specifically counteract oxidative damage in plants subjected to saline stress (Tsai et al., 2004). In other words, salt stress can trigger enhanced activities of antioxidase, plant varieties with high salt resistance usually showed higher activities of these enzymes (Gossett et al., 1994; Hernandez et al., 1995; Meloni et al., 2003). SOD is thought to be the primary scavenger in the detoxification of active oxygen species in plant, its function is to converts superoxide to H_2O_2 and O_2 (Asada and Kiso, 1973; Fridovich, 1986; Miller et al., 2010). According to the metal binding in the active site, SOD activity can be divided into *Cu/Zn-SOD*, *Mn-SOD* or *Fe-SOD* isoforms. Ascorbate peroxidase (APX) plays an important role in detoxifying H_2O_2, by catalyzing the reduction of H_2O_2 to water (Foyer, 1996). Catalase (CAT) can also reduce H_2O_2 to water but it has lower affinity for H_2O_2 than APX (Miller et al., 2010). Increasing evidence indicates that H_2O_2 functions as a signaling molecule for abiotic stress tolerance in plants; although, H_2O_2 is toxic at high concentrations (Levine et al., 1994; Dong et al., 2001; Akio Uchida et al., 2002; Mercedes et al., 2010). Mercedes (2010) studied a different role for H_2O_2 under salt stress in *Brassica oleracea* roots and found that increased H_2O_2 production occurred in response to salt stress. In the leaves of rice plant, the same phenomenon happened along with the enhanced activities of SOD and APX (Dong et al., 2001). A conceivable explanation for this is, SOD leads to the overproduction of H_2O_2 to eliminate the toxicity of superoxide radicals, then the overproduction of H_2O_2 functions as the signaling of salt stress, which induces the induction of specific APX isoforms (Dong et al., 2001). Akio et al. (2002) also found that pretreating rice seedlings with low levels (<10 mM) of exogenous H_2O_2 can improve its tolerance to salt stress, at the same time, significant increases in CAT, APX, SOD, glutathione reductase (GR) and peroxidase (POX) were observed, it further verified the double action of the H_2O_2 in salt tolerance.

High pH in saline soil reduces mineral dissolubility in most cases. Furthermore, salinity also decreases the water potential and then the water uptake in plants; thereby depresses mineral uptake and causes nutrition disorders in plants. Therefore, the maintenance of stable levels of intracellular mineral ions is critical for plant adaptation to saline stress (Xu et al., 2010a, b). Zn and Fe are essential micronutrients in plants. Although, Fe is abundant in the earth's crust, its availability is always limited, especially in saline soil. Fe deficiency reduces the activity of APX and increases glutathione (GSH) and ascorbate (AR) levels, indicating that Fe deficiency also induces secondary oxidative stress in plants (Ranieri et al., 2001; Zaharieva et al., 2004; Sun et al., 2007). Zn deficiency is one of the most widespread limiting factors in crop production, especially in saline soil. Zinc deficiency reduces the activities of SOD and results in the chlorosis of young leaves (Marschner, 1995).

Cang 6001 is a salt-tolerant winter wheat cultivar that has been cultivated in the coastal salt marshes in Hebei province of China for fourteen years. There is little information about the mechanisms that confer salt tolerance on *Cang 6001*. In order to elucidate the biochemical mechanism of salt tolerance in this wheat cultivar, we chose a salt-sensitive winter wheat cultivar *Shi 4185* whose genetics background is similar to *Cang 6001* as a control, and compared the transcript levels and activities of antioxidative enzymes, as well as the H_2O_2 level, membrane integrity of roots and ion accumulations between the two species. We chose to study salt adaptation during the seedling stage because evaporation and capillary action (especially in late winter and early spring) cause surface soils to accumulate amounts of soluble salts, young seedlings are exposed to much higher salinity than vigorously growing plants, and salt tolerance in young seedlings is critical for the survival of wheat species in saline soil.

MATERIALS AND METHODS

Plant materials and culture conditions

Seeds of the wheat cultivar *Shi 4185* and *Cang 6001* were collected in September, 2008 from Huanghua City, Hebei province of north China and then stored at 4°C until use. Seeds were first treated with 70% ethanol for 30 s, rinsed five times with sterile distilled water, and then left to germinate on double-layer filter paper wetted with various concentrations of NaCl solutions (0, 50, 100, 150, 200 mM). Five-day-old wheat seedlings were transferred to Hoagland's solution supplemented with the same concentration gradients of NaCl as the seeds were treated for 7 days. To examine the effects of ROS on the Zn/Fe accumulations in wheat seedlings, 0.3 mM AsA or N-acetyl-L-cysteine (NAC) were supplemented along with 150 mM NaCl.

Determination of germination, growth and inorganic ions

Germination rate was determined after 48 h of exposure to different concentrations of NaCl stresses. Relative root growth was determined by the method described previously (Errabii et al., 2007). Nine seedlings were analyzed in each set of experiments. After 7 days of stress treatment, the seedlings were harvested and rinsed with deionized water thoroughly especially for the roots and then oven-dried for 72 h. Dried plant tissues were ground to fine homogenized powders and allowed to stand overnight in the digestion tubes with concentrated nitric acid at room temperature and then digested with a programmed temperature accelerated system: first to keep the temperature at 80°C for 1 h and then raised to 120 to 130°C for another 20 h to ensure a complete digestion. Cooled digestion solutions were diluted with millipore-filtered, de-ionized water and briefly centrifuged. The concentrations of K, Na, Ca, Zn and Fe were analyzed by atomic absorption spectrometry (SHIMADZU AA-6300). Statistical analysis was done by Duncan's test ($P<0.05$).

Determination of H_2O_2 level and plasma membrane integrity

For H_2O_2 detection, roots were washed and stained with 25 µM of 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetylester (CM-H_2DCFDA; molecular probe) for 30 min (Xu et al., 2009). Roots were then washed thoroughly and viewed under a Leica laser scanning confocal microscope. All images were scanned under the same conditions (excitation 488 nm laser, emission 525 nm). Evans blue is a membrane-impermeable dye and is generally excluded from living cells. Staining of the cells is a strong indication of a loss of membrane integrity (Baker and Mock, 1994). Roots were stained in 0.25% (v/v) Evans blue solution for 15 min at room temperature, then rinsed three times for 10 min with distilled water. Photos were taken using a Carl Zeiss Imaging System. Six roots were analyzed in each set of experiments.

Enzyme activity

Frozen leaves (0.5 g) were homogenized in a pre-chilled mortar and pestle with 50 mM Na-phosphate buffer (pH 7.8) containing 0.1 mM EDTA and 1% (w/v) of polyvinylpyrrolidone at 4°C. The homogenate was centrifuged at 13,000×g for 30 min at 4°C. The supernatant was collected for the measurement of antioxidant enzyme activities. The activity of SOD was measured as described by Beauchamp and Fridovich (1971). The determination of CAT activity was performed using guaiacol and H_2O_2 as substrates, as described previously (Cakmak and Horst, 1991). The activity of APX was measured as described by Nakano and Asada (1981). Protein concentrations were determined according to the method of Bradford (1976) using bovine serum albumin as a standard.

Semi-quantitative real-time polymerase chain reaction (RT-PCR) Analysis

Total RNA was isolated from wheat seedlings with the TRIZOL reagent (Gibco BRL, USA) (Simms et al., 1993). For semi-quantitative RT-PCR, the concentration of RNA was accurately quantified by spectrophotometric measurements and cDNA was synthesized from DNase-treated total ribonucleic acid (RNA) with a Reverse Transcription System Kit (Promega, USA) using oligo-dT-primers. The genes examined and the primers used to amplify those genes were as follows: Mn-SOD (accession no.AF092524; 5'-ACCAGAAGCACCACGCCACCTAC-3' and 5'-GCTCCCAGACATCAATTCCCAACAAA-3'); cytoplasmic *Cu/Zn-SOD* (accession no. U69632; 5'-CCTCTCTTCCAGG CTCCTGCC-3' and 5'-ATGAACAACAAACGCTCTCCC-3'). Control reactions with the 18S rRNA primers (accession no. AJ272181; 5'-CAAGCCATCGCTCTGGATACATT-3' and 5'-CCTGTTATTGCC TCAAACTTCC-3') were performed to ensure that equal amounts of RNA were used in each set of reactions. Cycle numbers were optimized to ensure that the amplification reaction was tested in the exponential phase.

RESULTS AND DISCUSSION

Salt stress markedly inhibited the germination and root growth of wheat seedlings. As shown in Figure 1, the effects of NaCl on seed germination and root growth varied with the different NaCl concentrations used. In the treatment with 200 mM NaCl, the germination rate decreased by 49.4% and 36% in *Shi 4185* and *Cang 6001* respectively. Moreover, seedlings were more sensitive to salt stress than the seeds at germination stage for both wheat varieties. After 7 days of exposure to 150 mM NaCl, the root growth of *Shi 4185* and *Cang 6001* decreased by 53 and 36.8% respectively relative to the untreated controls; Furthermore, they started to show a significant difference in salt resistance from 150 mM NaCl treatment; on this account, 150 mM was chosen as a sublethal dosage in the following experiments. *Cang 6001* showed a higher germination rate and seedling root growth than *Shi 4185*, indicating that *Cang 6001* was more tolerant to salt stress than *Shi 4185* in the stages of seed germination and seedling root growth.

Salinity stress reduces mineral availability in soil and leads to mineral deficiencies in plants (Xu et al., 2010). Salt stress also decreased the water potential, thereby suppressing water uptake. Decreased water absorption in plants led to the reduction of micronutrients uptake. A case study shows that tomato shoot concentrations and total accumulation of Fe, Cu and Zn decrease as soil salinity increases (Ghazi, 2000). Ionic balance inside the cell is due to an impaired nutrient uptake and transport in the plant due to competition between Na and Cl and other nutrients for transport proteins, also due to plant adaptation to environmental stress. Therefore, we monitored ion accumulation in salt-stressed wheat seedlings. When 150 mM NaCl was applied, both salt-tolerant and salt sensitive wheat genotypes had a much higher Na accumulation in whole plant, however, there is no significant difference in Na contents between the two wheat cultivars (Figure 2). On the contrary, salt treatments reduced the accumulations of Zn and Fe in wheat seedlings (Figure 3). And they both showed significant genotype difference (Figure 3). We also found that salt treatments decreased K and Ca levels in plants and *Shi 4185* exhibited larger reductions than *Cang 6001* (Figure 2). These results suggest that *Cang 6001* had a higher capability on ion accumulation than *Shi 4185* under adverse circumstances. A higher K and Ca contents in the salt-tolerant cultivar may be due to a higher K/Na and Ca/Na selectivity or to higher rates of water uptake, so that ion imbalance can be improved in a certain degree.

To examine *in vivo* level of H_2O_2 in salt-stressed seedlings, we used CM-H_2DCFDA fluorescence probe. As shown in Figure 4A, CM-H_2DCFDA fluorescence intensity increased significantly when seedlings were subjected to NaCl stress and it was higher in *Shi 4185* than in *Cang 6001*. That was to say the salt-tolerant cultivar *Cang 6001* had lower H_2O_2 level than the salt-sensitive cultivar *Shi 4185* had under salt stress just as showed in Figure 4B. Foyer et al. (1994) suggest that the production of ROS under abiotic stresses may serve as a general alarm signal leading to the modification of metabolism and gene expression. However, excess ROS results in cell death and inhibits plant growth. As shown in Figure 4B, Evans blue staining of stressed seedlings indicated that salinity stress reduced plasma membrane integrity markedly, resulting in cell death in wheat roots. Roots of *Cang 6001* showed less staining than those of *Shi 4185* under stress, suggesting that *Cang 6001* had a higher stress tolerance

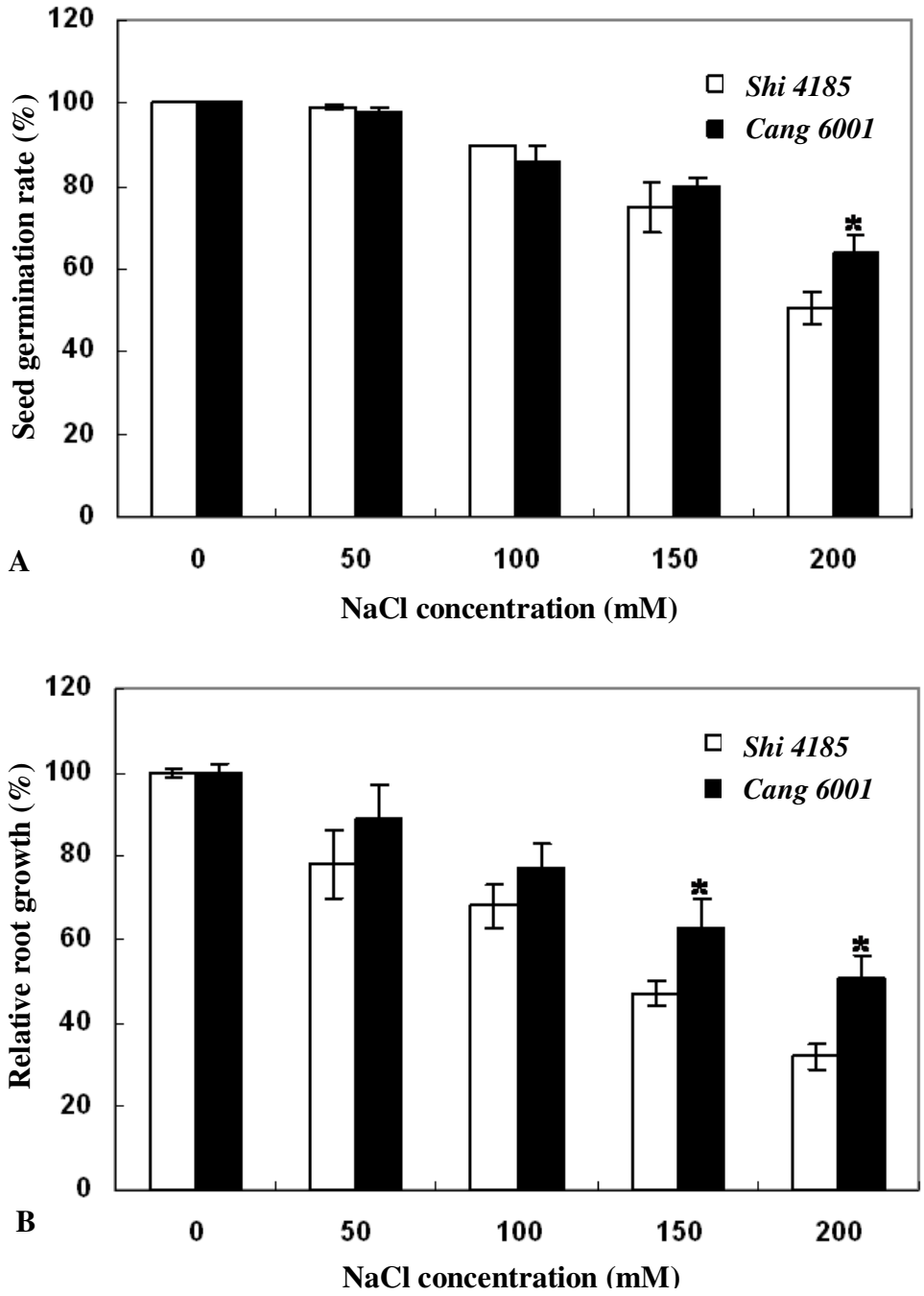

Figure 1. (A) Seed germination rates of *Cang 6001* and *Shi 4185* after 48 h of exposure to different concentrations of NaCl. (B) Relative root growth of wheat cultivars *Cang 6001* and *Shi 4185* after 7d of exposure to different concentrations of NaCl. Each value is the mean of six replicates and *vertical bars* represent ± standard error. Asterisks represent significant difference between the two cultivars ($P<0.05$).

than *Shi 4185*.

The above studies showed that NaCl stress significantly increased H_2O_2 production in salt-stressed seedlings. Meanwhile, salt treatments reduced the accumulations of Zn and Fe in wheat seedlings. It was also apparent that the salt-tolerant cultivar *Cang 6001* had lower H_2O_2 level and higher Zn/Fe levels than the salt-sensitive cultivar *Shi 4185* had under salt stress. To detect the effects of H_2O_2 on Zn/Fe accumulation, salt-stressed wheat seedlings were treated with two antioxidants, AsA and NAC under salt stress. We found that supplementation with either 0.3 mM AsA or 0.3 mM NAC markedly reduced H_2O_2

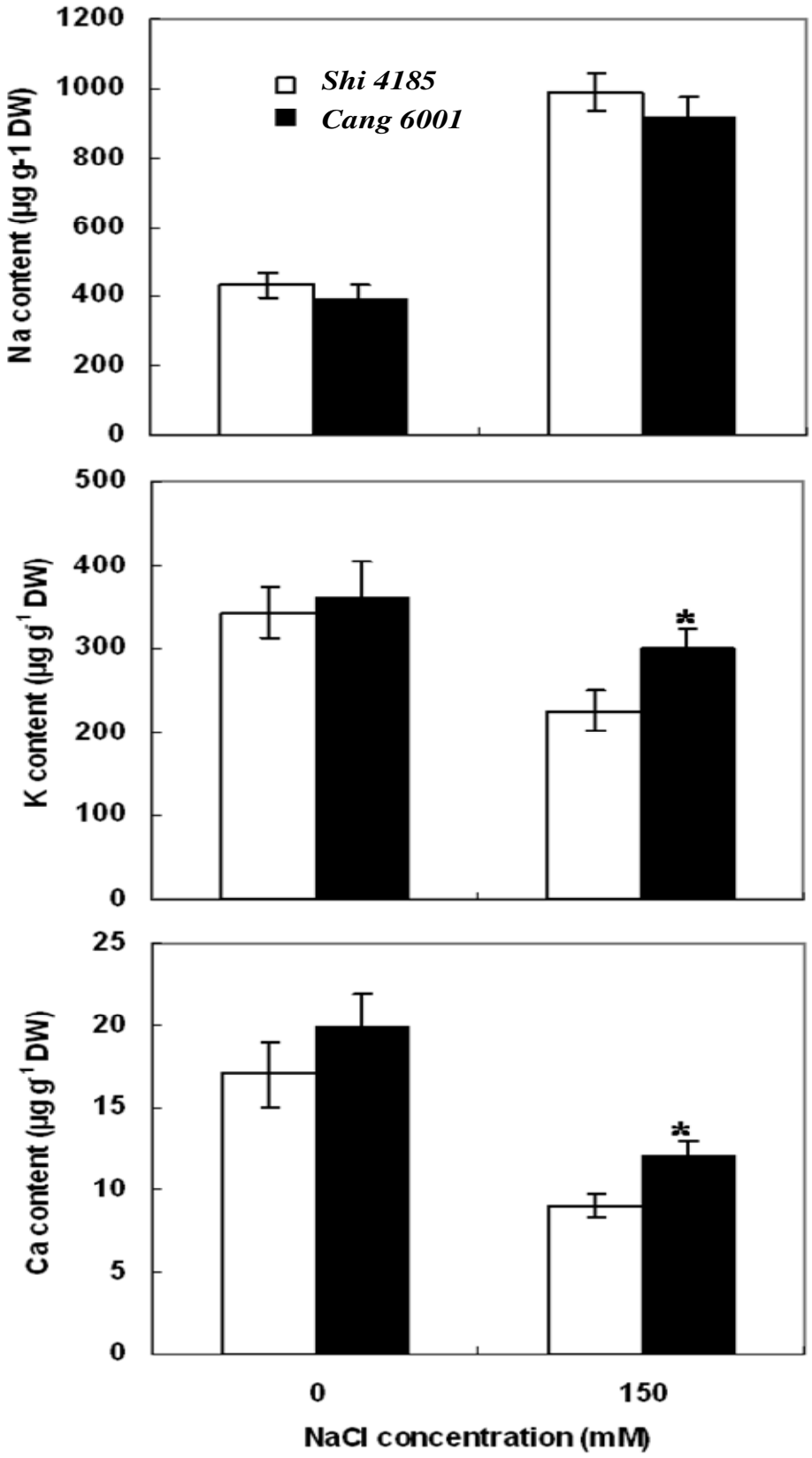

Figure 2. Effects of salt stress on the levels of Na, K and Ca in wheat seedlings in the absence or presence of 150 mM NaCl. Each value is the mean of six replicates and *vertical bars* represent ± standard error. Asterisks represent significant difference between the two cultivars ($P<0.05$).

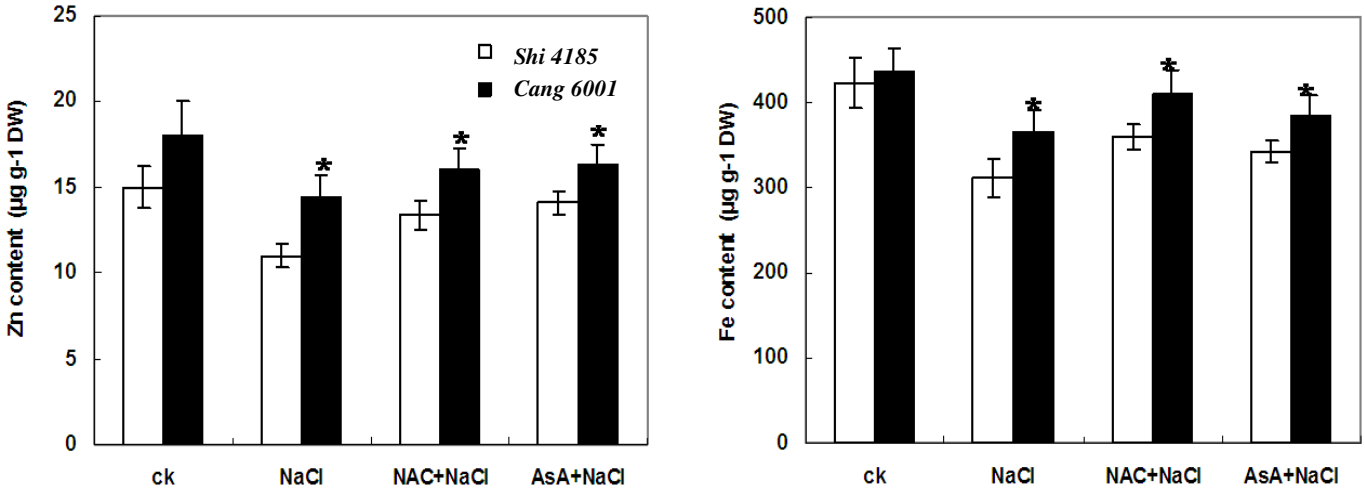

Figure 3. Effects of AsA and NAC on the accumulations of Zn and Fe in the salt-treated wheat seedlings. Wheat seedlings were transferred to Hoagland's solution supplemented with 150 mM NaCl, 0.3mM AsA or 0.3mM NAC. Asterisks represent significant difference between the two cultivars ($P<0.05$).

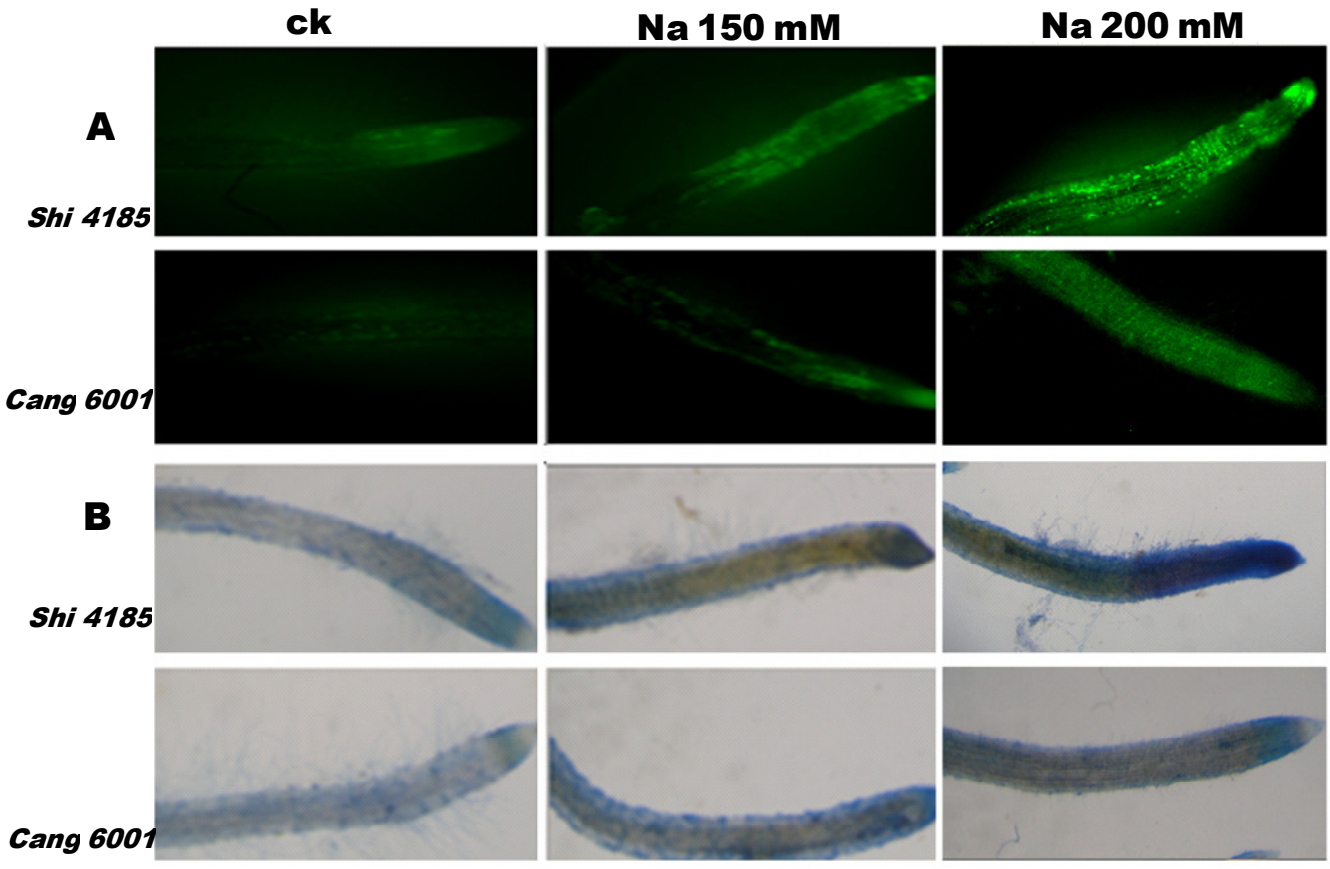

Figure 4. *In situ* fluorescence and histochemical staining showing the effects of NaCl stress on (A) H_2O_2 and (B) plasma membrane integrity in the roots of wheat seedlings. Wheat seedlings were transferred to Hoagland's solution supplemented with two concentrations of NaCl (150, 200 mM). For H_2O_2 detection, roots were excised and then stained with 25 μM of 5-(and-6)-chloromethyl-2',7'- dichlorodihydrofluorescein diacetate, acetylester (CM- H_2DCFDA; Molecular Probe) for 30 min. For plasma membrane integrity detection, roots were excised and then stained in 0.25% (v/v) Evans blue solution for 15 min at room temperature, then rinsed three times for 10 min with distilled water. ck, un-treated control plants.

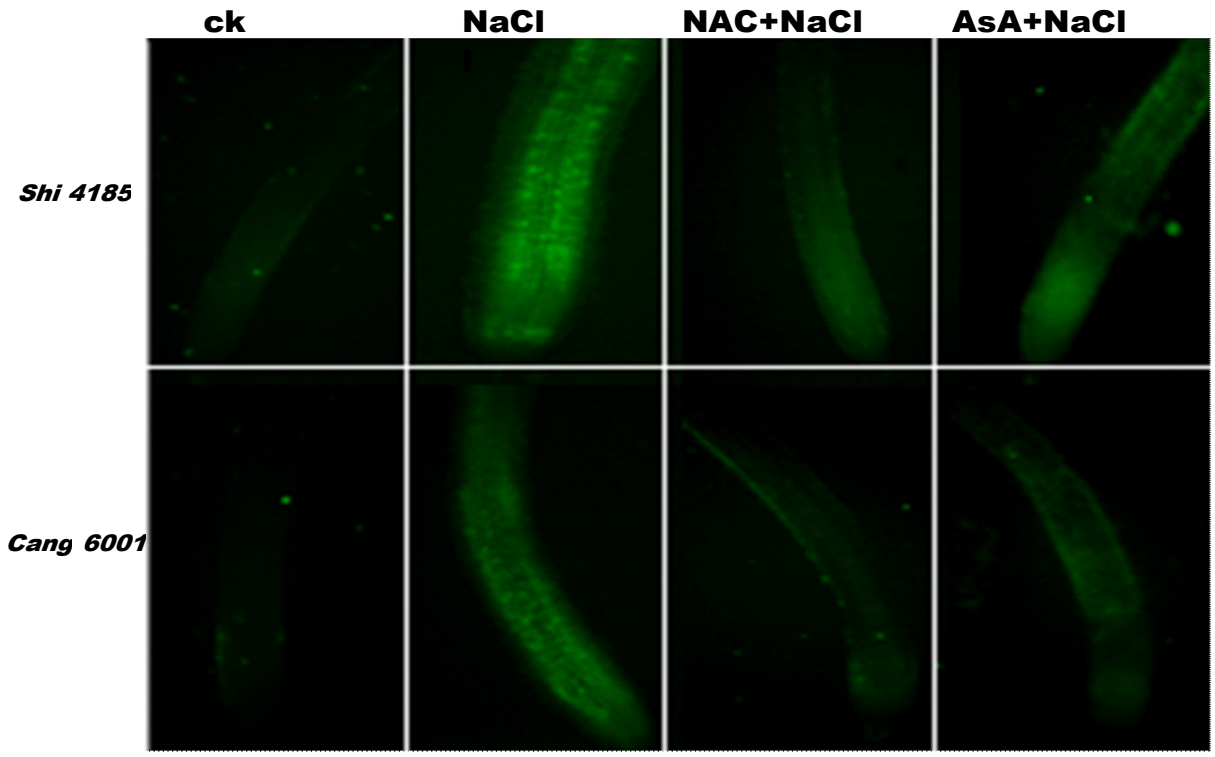

Figure 5. Effects of AsA and NAC on ROS level in the salt-treated wheat seedlings. Wheat seedlings were transferred to Hoagland's solution supplemented with 150 mM NaCl, 0.3 mM AsA or 0.3 mM NAC. ck, un-treated control plants.

productions (Figure 5), while increased Zn and Fe levels in both *Cang 6001* and *Shi 4185* (Figure 3). Our results indicate that the increased accumulation of Zn/Fe under salt stress in *Cang 6001* seedlings is at least partially related to the depressed levels of H_2O_2. ROS burst induced by salt stress results in both necrosis and programmed cell death (PCD) in roots (Katsuhara and Kawasaki, 1996), thus inhibits the root growth and the uptake of micronutrients.

Manchandia et al. (1999) suggest that rapid up-regulation of antioxidant activity provides an initial defense against cellular damage from an oxidative burst. *Cang 6001* accumulated lower H_2O_2 than *Shi 4185* under salt stress. Therefore, we hypothesized that the successful adaptation of *Cang 6001* to salt stress could be due to enhanced antioxidative enzymatic activities. We next monitored the activities of three antioxidative enzymes (Figure 6). The activities of antioxidative enzymes except SOD under salt stress tested were not significantly different between the two wheat cultivars whether with or without NaCl treatment when they were cultured in Hoagland's solution. Salt stress increased the activities of SOD, CAT and APX. The salt-tolerant cultivar *Cang 6001* showed remarkably higher SOD activity than that of salt-sensitive cultivar *Shi 4185*. These results suggest that SOD may play an important role in modulating the different salt tolerance of the two wheat cultivars. For this reason, we next examined the gene expression of *TaSODs*. As shown in Figure 6D, salt treatment induced the expressions of *Mn-SOD* and cytoplasmic *Cu/Zn-SOD*, and *Mn-SOD* and cytoplasmic *Cu/Zn-SOD* transcript levels in *Cang 6001* were higher than in *Shi 4185* under salt stress. These data indicate that SOD activity was closely related to the levels of salt tolerance in the two different wheat cultivars. An enhancement in SOD activity under stress was reported in previous studies, which indicated that SOD played an important role in plant responses to abiotic stress (Khan and Panda, 2008). SOD dismutates superoxide radicals to H_2O_2 and O_2 in the cytosol, mitochondria and chloroplast. Generation of low level superoxide may alleviate cell death in roots (Jabs et al., 1996). Salt treatment increased the activity of SOD in wheat seedlings. Furthermore, *Cang 6001* had higher SOD activity and transcript level than *Shi 4185*, indicating that a high level of SOD activity might protect salt-tolerant wheat from oxidative damage, and thereby reducing cell death in roots of wheat seedlings exposed to salt stress. CAT is one of the key enzymes for converting H_2O_2 to H_2O and O_2. APX is also an essential component of plants' antioxidant defense system (Singh et al., 2006). Bellaire et al. (2000) indicate that NaCl-induced increase in antioxidant activity is an early event during acclimation to high salt conditions. After the seedling has acclimated to

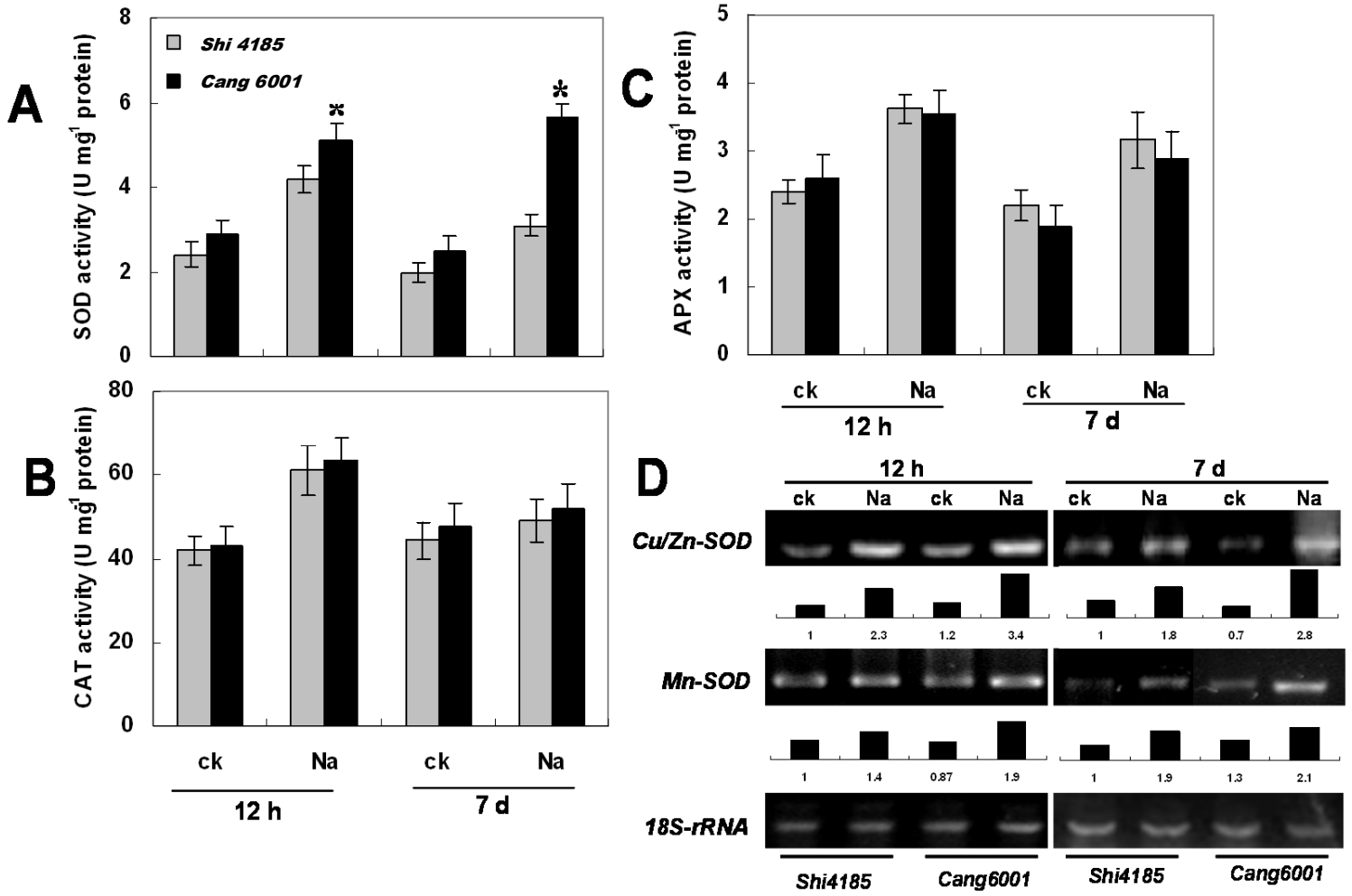

Figure 6. Effects of salt treatment on the activities of (A) SOD, (B) CAT, (C) APX and (D) the transcripts of *Cu/Zn-SOD* and Mn-SOD in wheat seedlings. Wheat seedlings were transferred to Hoagland's solution supplemented with 150 mM NaCl. Asterisks represent significant difference between the two cultivars ($P<0.05$). ck, un-treated control plants; Na, 150 mM NaCl-treated plants.

the oxidative burst, other adaptive mechanisms, such as adjustments in the ion uptake, for example absorption of K, Ca, Zn and Fe, may be induced.

Salt stress induces a rapid increase in ROS production which stimulates the expression and activities of antioxidative enzymes (Khan and Panda, 2008). Salt stress also led to the reduction in Zn/Fe accumulation in plants. Activities of APX, CAT and SOD are significantly decreased by Zn deficiency (Cakmak and Marschner, 1993). Activities of APX and CAT are also inhibited in Fe-deficient plants (Takahiro et al., 2003). Though, salt stress induce a rapid increase in the activities of antioxidative enzymes, high concentration or long-term of salt stress inhibit the antioxidative enzymes activities (Rahnama and Ebrahimzadeh, 2005). The excess or long-term salt stress-induced decrease in antioxidative enzymes activities may be due to salt-induced Zn/Fe deficiency. However, in our study, we did not observe significant reduction in these antioxidative enzymes activities. We considered that (i) the expressions of anti-oxidative enzymes were modulated by both transcriptional and post-transcriptional level (Rodríguez-Serrano et al., 2009); (ii) the expression and activities of antioxidative enzymes were also regulated by both ROS and intracellular Zn/Fe contents (Cakmak and Marschner, 1993). Therefore, salt-affected antioxidative enzymes activities were regulated by both the period and concentration of salt treatment which modulated the ROS production and Zn/Fe accumulation in plants. All of these factors modulated the antioxidative enzymes activities responded to salt stress.

Conclusion

Compared to untreated control plants, the salt-tolerant cultivar was equipped with superior antioxidative defenses that allow it to adapt to the oxidative stress induced

by high salt condition. We found that reducing H_2O_2 production by supplementation with antioxidants such as AsA or NAC increased Zn/Fe accumulation in wheat seedlings, indicating that high rate of cell death was more likely responsible for low nutrient uptake in plants. Also, the transcript level and activity of SOD in the salt-tolerant cultivar increased faster than in the salt-sensitive cultivar, suggesting that SOD played an important role in mediating the different salt tolerance in the two wheat cultivars. Further studies on the roles of SOD in Zn/Fe absorption and accumulation in plants will be helpful for breeding salt-tolerant and Zn/Fe-accumulated plant varieties for agricultural production.

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REFERENCES

Akio U, Andre TJ, Takashi H, Teruhiro T, Tetsuko T (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci. 163: 515-523.

Asada K, Kiso K (1973). Initiation of aerobic oxidation of sulfite by illuminated spinach chloroplasts. Eur. J. Biochem. 33: 253-257.

Baker CJ, Mock NM (1994). An improved method for monitoring cell death in cell suspension and leaf disc assays using Evans blue. Plant Cell Tissue Org. 39: 7-12.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-287.

Bellaire BA, Carmody J, Braud J, Gossett DR, Banks SW, Lucas MC, Fowler TE (2000). Involvement of abscisic-dependent and independent pathways in the up-regulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. Free Radic. Res. 33: 531-545.

Boyer JS (1982). Plant productivity and environment. Sci. 218: 443-448.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Cakmak I, Horst WJ (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean. Physiol. Plantarum. 83: 463-468.

Cakmak I, Marschner H (1993). Effect of zinc nutritional status on activities of superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves. Plant Soil. 155/156: 127-130.

Dong HL, Young SK, Chin BL (2001). The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa L.*). J. Plant Physiol. 158: 737-745.

Errabii T, Gandonou CB, Essalmani H, Abrini J, Idaomar M, Senhaji NS (2007). Effects of NaCl and mannitol induced stress on sugarcane (*Saccharum sp.*) callus cultures. Acta Physiol. Plant. 29: 95-102.

Fridovich I (1986). Biological effects of the superoxide radical. Arch.Biochem. Biophys. 247: 1-11.

Foyer CH, Descourviers P, Kunert KJ (1994). Protection against oxygen radicals: an important defense mechanism in transgenic plants. Plant Cell Environ. 17: 507-523.

Foyer CH (1996). Free radical processes in plants. Biochem. Soc. Trans. 24: 427-434.

Ghazi NA (2000). Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza, 10: 51-54.

Ghoulam C, Foursy A, Fares K (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ. Exp. Bot. 47: 39-50.

Grattan SR, Grieve CM (1999). Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic. 78: 127-157.

Gossett DR, Millhollon EP, Rucas MC, Banks SW, Marney MM (1994). The effects of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cotton cultivars (*Gossypium hirsutum* L.). Plant Cell Rep. 13: 498-503.

Guo R, Shi LX, Yang YF (2009). Germination, growth, osmotic adjustment and ionic balance of wheat in response to saline and alkaline stresses. Soil Sci. Plant Nutr. 55: 667-679.

Hernandez JA, Olmos E, Corpas F, Sevilla F, Del Rio LA (1995). Salt induced oxidative stress in chloroplasts of pea plants. Plant Sci. 105: 151-167.

Jia GX, Zhu ZQ, Chang FQ, Li YX (2002). Transformation of tomato with the *BADH* gene from *Atriplex* improves salt tolerance. Plant Cell Rep. 21: 141-146.

Jabs T, Dietrich RA, Dang JL (1996). Initiation of Runaway Cell Death in an *Arabidopsis* Mutant by Extracellular Superoxide. Science, 273: 1853-1856.

Katsuhara M, Kawasaki T (1996). Salt stress induced nuclear and DNA degradation in meristematic cells of barley roots. Plant Cell Physiol. 37: 169-173.

Khan MH, Panda SK (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol. Plant. 30: 81-89.

Levine A, Tenhaken R, Dixon R, Lamb C (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell, 79: 583-593.

Mahajan S, Tuteja N (2005). Cold, salinity and drought stresses: An overview. Arch. Biochem. Biophys. 444: 139-158.

Manchandia AM, Banks SW, Gossett DR, Bellaire BA, Lucas MC, Millhollon EP (1999). The influence of amanitin on the NaCl-induced upregulation of antioxidant enzyme activity in cotton callus tissue. Free Radic. Res. 30: 429-438.

Marschner H (1995). Mineral nutrition of higher plants. Boston: USA: Academic Press.

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 49: 69-76.

Mercedes H, Nieves FG, Pedro DV, Enrique O (2010). A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in *Brassica oleracea* roots. J. Exp. Bot. 61: 521-535.

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010). Reactive oxygen species homeostasis and signaling during drought and salinity stresses. Plant Cell Environ. 33: 453-46.

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22: 867-880.

Rahnama H, Ebrahimzadeh H (2005). The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol. Plantarum, 49: 93-97.

Ranieri A, Castagna A, Baldan B, Soldatini GF (2001). Iron deficiency differently affects peroxidase isoforms in sunflower. J. Exp. Bot. 52: 25-35.

Rodríguez-Serrano M, Romero-Puertas MC, Pazmiño DM, Testillano PS, Risueño MC, del Río LA, Sandalio LM (2009). Cellular response of pea plants to cadmium toxicity: cross-talk between reactive oxygen species, nitric oxide and calcium. Plant Physiol. 150: 229-243.

Simms D, Cizdziel PE, Chomczynski P (1993). TRI zol™: a new reagent for optimal single-step isolation of RNA. Focus, 15: 99-102.

Singh S, Eapen S, DSouza SF (2006). Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. Chemosphere, 62: 233-246.

Sun BT, Jing Y, Chen KM, Song LL, Chen FJ, Zhang LX (2007).

Protective effect of nitric oxide on iron deficiency-induced oxidative stress in maize (*Zea mays*). J. Plant Physiol. 164: 536-543.
Takahiro I, Rapolu M, Shigeru S (2003). Effect of iron on the expression of ascorbate peroxidase in *Euglena gracilis*. Plant Sci. 165: 1363-1367.
Tsai YC, Hong CY, Liu LF, Kao CH (2004). Relative importance of Na^+ and Cl^- in NaCl-induced antioxidant systems in roots of rice seedlings. Physiol. Plant. 122: 86-94.
Xu J, Yin HX, Li X (2009). Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, *Solanum nigrum* L. Plant Cell Rep. 28: 325-333.
Xu J, Yin HX, Liu XJ, Li X (2010a). Salt affects plant Cd-stress responses by modulating growth and Cd accumulation. Planta, 231:449-459.
Xu J, Yin HX, Li YL, Liu XJ (2010b) Nitric Oxide Is Associated with Long-Term Zinc Tolerance in *Solanum nigrum*. Plant Physiol. 154: 1319-1334.
Wang W, Vinocur B, Altman A (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 18: 1-14.
Zaharieva TB, Gogorcena Y, Abadia J (2004). Dynamics of metabolic responses to iron deficiency in sugar beet roots. Plant Sci. 166: 1045-1050.
Zhu JK (2003). Regulation of ion homeostasis under salt stress. Curr. Opin. Plant. Biol. 6: 441-445.