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A Cu/Zn superoxide dismutase from *Jatropha curcas* enhances salt tolerance of *Arabidopsis thaliana*

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ABSTRACT. Superoxide dismutases (SODs) are involved in protecting plants against diverse biotic and abiotic stresses. In the present study, a novel *Cu/Zn-SOD* gene (*JcCu/Zn-SOD*) was cloned from *Jatropha curcas* L. Quantitative reverse transcription-polymerase chain reaction analysis revealed that *JcCu/Zn-SOD* is constitutively expressed in different tissues of *J. curcas* and induced under NaCl treatment. To characterize the function of this gene with respect to salt tolerance, the construct *p35S:JcCu/Zn-SOD* was developed and transformed into *Arabidopsis* using *Agrobacterium*-mediated transformation. Compared with wild-type, transgenic plants over-expressing *JcCu/Zn-SOD* showed enhanced tolerance to salt stress during germination, seedling establishment, and growth in terms of longer root, larger rosette area, and a larger number of leaves in addition to higher SOD activity levels

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under NaCl stress. In addition, over-expression of *JcCu/Zn-SOD* resulted in lower monodialdehyde content in transgenic *Arabidopsis* compared to wild-type plants under the same NaCl stress. Therefore, *JcCu/Zn-SOD* can increase a plant salt stress tolerance potentially by reducing oxidant injury.

Key words: Abiotic stress tolerance; Anti-oxidative enzymes; Cu/Zn-SOD; NaCl stress; *Jatropha curcas*

INTRODUCTION

Soil salinity, particularly NaCl content, is principally regarded as an inherent problem in irrigated agriculture, and limits plant growth and crop yield worldwide (Zhu, 2001). Increased salinization of arable land has devastating global effects, with 30% land loss predicted to occur within the next 25 years and up to 50% by the year 2050 (Wang et al., 2003). Under salt stress, both the osmotic and ionic balances are severely damaged, resulting in the accumulation of reactive oxygen species (ROS) (Patade et al., 2012), which is toxic to cells at high concentration by causing oxidative damage to membrane lipids, proteins, and nucleic acids (Price et al., 1989; Moran et al., 1994). To protect against oxygen-mediated injuries, plants have developed different strategies, either by suppressing ROS generation or scavenging ROS that they have already produced (Apel and Hirt, 2004).

Superoxide dismutases (SOD; EC: 1. 15. 1. 1), a family of metalloenzymes, is one of the most important enzymes in the plant defense system against oxidative stress, and it occurs ubiquitously in every cell of all plant types (Mittler, 2002). As the first-line defense against oxidative damage, SOD catalyzes the conversion or dismutation of toxic superoxide anion radicals to molecular oxygen and hydrogen peroxide. Therefore, increased SOD expression can protect plants against physical stresses (salinity, chilling, drought, and high light intensity). It has been reported that SOD over-expression in some transgenic plants enhanced their tolerances to stresses (Lee et al., 2007; Tseng et al., 2007). Thus, SOD is considered to be a key enzyme in the regulation of intracellular ROS levels and in the maintenance of normal physiological conditions under oxidative stress (Mittler, 2002).

Jatropha curcas L., commonly known as physic nut, is a NaCl-resistant tropical shrub or small tree that belongs to the Euphorbiaceae family. The lifespan of *J. curcas* is up to 50 years, and it can grow in mining areas where soils are contaminated by heavy metals (Openshaw, 2000). Recently, this plant has attracted considerable attention because its seeds are an ideal raw material for biodiesel production (Mohibbe Azam et al., 2005; Fairless, 2007; Achten et al., 2008). Moreover, *J. curcas* can be used as a source of food, soap, cosmetics, pesticides, and anticancer medicine (Makkar and Becker, 2009). Previous studies showed that SODs may be the most important factors for *J. curcas* against salt, drought, and heavy metal stress (Gao et al., 2009, 2010). However, in contrast to other plants, the antioxidant response to oxidative and environmental stress has not yet been well-characterized in *J. curcas* at the molecular level.

In the present study, a *JcCu/Zn-SOD* gene was cloned and sequenced from *J. curcas*. We detected the expression of *JcCu/Zn-SOD* in different tissues of *J. curcas*. To characterize the role of *JcCu/Zn-SOD in vivo*, the gene was introduced into *Arabidopsis* and over-expressed. Furthermore, differences between transgenic and wild-type (WT) *Arabidopsis* plants were compared under NaCl stress.

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MATERIAL AND METHODS

Plant materials and treatments

Mature seeds, flowers, leaves, stems, roots, and silique of *J. curcas* were collected in the summer from Panzhihua city of Sichuan Province, China, and instantly frozen in liquid nitrogen. Mature seeds were immersed in 70% ethanol for 5 min and then 0.1% mercuric choride for 10 min. Seeds were rinsed with distilled water soaked for 24-36 h at room temperature and sown in trays filled with sand for germination and growth.

Arabidopsis thaliana seeds were surface-sterilized and sown on plates containing 20 mL MS medium (Murashige and Skoog, 1962). Seeds were stratified in the dark at 4°C for 2 days and then transferred to a tissue culture box at 22°C (16-h light/8-h dark).

Cloning and sequencing of the *JcCu/Zn-SOD* gene

Total RNA was extracted from plant tissues using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer instructions. First-strand cDNA was synthesized using Superscript II (Invitrogen). A specific fragment of *JcCu/Zn-SOD* was amplified using 2 degenerate primers, *JcSOD1* and *JcSOD2* (Table 1), which were designed based on the conserved regions of the corresponding genes from other plants. The polymerase chain reaction (PCR) was performed as follows: 94°C for 3 min; 32 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s; and a final extension step of 72°C for 2 min. The PCR fragment was purified using an Agarose Gel DNA Purification Kit (Takara, Shiga, Japan), and ligated into the pMD18-T vector (Takara). Sequence analysis was performed by Invitrogen in Shanghai. The 5'- and 3'-ends of *JcCu/Zn-SOD* were obtained using a BD SMART RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) with the specific primers *JcSOD3*, *JcSOD4*, *JcSOD5*, and *JcSOD6* (Table 1).

Table 1. Primer sequences used in the experiments.

Primers	Sequences (5'-3')
JcSOD1	AT(T/C)TC(A/C)A(T/G)CCG(A/T)ACAA(A/C)AAGACACA
JcSOD2	AT(T/C)G(C/T)CAAC(A/G)ACC(T/C)AC(A/G)AGC(T/C)A
JcSOD3	ACTAAGTTCATGCCCACCCTTTCA
JcSOD4	GTGCTCTTCCGACTACTGCATTTGG
JcSOD5	CCGTCATGCGGGTGACCTGGGAAC
JcSOD6	TAGTTGCTAACGCTGATGGGGTGGC
JcSOD7	TCAAACTTCCTCGTCAATCGC
JcSOD8	CTGTTGTGGGACCATCGTCTT
Actin-F	ATGAGCTTCGAGTTGCACCA
Actin-R	AGCATCAGTGAGATCACGAC

Real-time PCR

Total RNA was extracted from different tissues or leaves by 200 mM NaCl treatment. Real-time PCR was performed using 1 µg total RNA for reverse transcription. The RNAs were quantified using the Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA, USA). Expression levels of genes were determined using the iCyclerTM IQ Real-Time PCR Detection System (Bio-Rad) according to the QuantiTect SYBR Green PCR Kit instruction manual and

were analyzed using the iCyclerTM Real-Time Detection System software (version 3.0). A *JcCu/Zn-SOD* fragment (168 bp) was amplified using the gene-specific primers *JcSOD7* and *JcSOD8* (Table 1). *J. curcas* actin, amplified using the primers *Actin-F* and *Actin-R* (Table 1), giving a product of 180 bp, was used as a reference to normalize *JcCu/Zn-SOD* cDNA levels. *JcCu/Zn-SOD* cDNA levels were normalized with those of actin in the same samples and the final relative cDNA amounts of *JcCu/Zn-SOD* were the means of 3 replicates.

Plasmid construction and *Arabidopsis* transformation

Full-length *JcCu/Zn-SOD* was cut from *pMD18-JcCu/Zn-SOD* using *Bam*HI and *Sac*I restriction sites, and subcloned into the cognate sites of pBI121. The *JcCu/Zn-SOD* gene was located between the CaMV35S promoter and the NOS 3'poly (A) signal to generate 35S: *JcCu/Zn-SOD*. The construct was transformed into *Agrobacterium* (GV3101). *A. thaliana* was transformed by *Agrobacterium* using the floral-dip procedure (Clough and Bent, 1998). Transformants were selected for their ability to grow on 1/2 MS medium containing 50 μg/mL kanamycin and by PCR. T3 generation plants were used in all experiments unless other indicated.

Salt tolerance assay

For the germination test, WT and transgenic *Arabidopsis* seeds were surface-sterilized, seeded on plates containing MS medium with 150 mM NaCl or without NaCl. The plates were incubated at 4°C for 2 days and then the temperature was increased to 22°C. The germination rates were scored every day. For the plant growth assay, half of the 7-day-old seedlings grown on MS medium were transferred onto plates containing 150 mM NaCl. The seedlings were allowed to grow on this medium for 3 weeks. Finally, the root length, rosette area, number of leaves, and morphology of individual plants were observed and calculated.

Total SOD activity

After 3 weeks of growth in the presence or absence of 150 mM NaCl, the WT and *JcCu/Zn-SOD* transgenic *Arabidopsis* plants were analyzed for SOD activity as previously described (Alonso et al., 2001). Leaves were homogenized in 50 mM potassium-phosphate buffer, pH 7.5, and then centrifuged at 15,000 *g* for 30 min at 4°C. The supernatant was added to a reaction mixture containing 50 mM phosphate buffer, pH 7.0, 13 mM methionine, 75 mM nitro blue tetrazolium, 100 mM EDTA. The reaction was started by adding 2 μM riboflavin and placing the tubes under 15 W fluorescent lamps for 15 min. The control set of reaction mixtures was kept in the dark. Activity was calculated by determining absorbance at 560 nm.

Determination of lipid peroxidation

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) using thiobarbituric acid. Next, 0.2 g leaf was excised into 5 mL 10% trichloroacetic acid containing 0.5% thiobarbituric acid and 0.5% Triton X100 and homogenized using a cold pestle and mortar. The mixture was placed in boiling water bath for 30 min and then quickly cooled in an ice bath. After centrifugation at 13,000 *g* for 10 min, the absorbance of the supernatant was

determined at 532 and 600 nm by atomic emission spectrophotometry (Cao et al., 2009).

Statistical analyses

The SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Analysis of variance was performed followed by the Duncan multiple comparison tests. Statistically significant differences ($P < 0.05$) are reported in the figures.

RESULTS

Cloning of the *JcCu/Zn-SOD* gene

Several *Cu/Zn-SODs* have been reported in many plants, and sequence analysis revealed numerous conserved regions (Abreu and Cabelli, 2010). These sequences were used to design degenerate primers to clone *Cu/Zn-SOD* from *J. curcas*. A 265-bp fragment was amplified from leaf cDNA. A cDNA, referred to as *JcCu/Zn-SOD*, was obtained by random amplification of cDNA ends-PCR and consisted of 901 bp, including 25-bp 5'-untranslated and 145-bp 3'-untranslated regions. The cloned *JcCu/Zn-SOD* contained an open reading frame encoding a polypeptide of 243 amino acids with a calculated molecular mass of 24.85 kDa and an isoelectric point of 6.57. Sequence alignment of the deduced amino acids (Figure 1) was found to share approximately 70% identical amino acids with its homologs in *Populus trichocarpa*, *Fragaria vesca*, *Ricinus communis*, and *Theobroma cacao*. Phylogenetic analysis indicated that *JcCu/Zn-SOD* is most closely related to *Cu/Zn-SOD* from *Ricinus communis* (Figure 2). The sequence of JcCu/Zn-SOD was deposited in the NCBI GenBank under accession No. KF268341.

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JcCu/Zn-SOD   MQAAAAVAA   TILAASPSSHPLLYPFPNPIL......SHSSPLH S H V LKLP.RQ LPLSLTT AAPKKPLA V        78
TcCu/Zn-SOD   MQ.AAAIAA   AILTVTT.......SQHTLLAPFPINPSTPAVLQ S R V LKLP.CQ LSIA... TVPKKPLI F        73
FvCu/Zn-SOD   MQ..AALAA   SVMLSTY.......SSHTLFAQIP....TPPTLH S H V LKLP.VK FSLA... SAAPKPLA V        68
PtCu/Zn-SOD   .........   AILTATP.......PTRYPLL......SPLPPNH S H V LKLP.RQ FSFSL.. AKKQQPPF V        60
RcCu/Zn-SOD   .........   TIIAASPSYHALLSPASSPNL......T.....H L H N VLKLSRQ VALSLSS A..KKSLT V        63

JcCu/Zn-SOD         T N      T E      N V      P      Y      I   A    NNK   A   EI        V    163
TcCu/Zn-SOD         D K      T D      N I      P      F      M   A    NNM   A   KV        V    158
FvCu/Zn-SOD         T A      T E      N I      P      F      I   P    NNL   A   EV        I    153
PtCu/Zn-SOD         T S      S E      S I      P      F      M   P    KKL   A   EI        V    145
RcCu/Zn-SOD         N N      T G      N I      L      Y      M   A    KGL   S   DI        V    148

JcCu/Zn-SOD      V      N    S    VV                    LT          LLAMCKAAWLSQCKEMGSEVDT..      243
TcCu/Zn-SOD      V      K    S    II                    LT          VVGLTPI.................      223
FvCu/Zn-SOD      E      S    T    VI                    LS          VVGLTPV.................      218
PtCu/Zn-SOD      V      S    S    VI                    ST          VVGLTPI.................      210
RcCu/Zn-SOD      V      S    S    VI                    LT          VVGLTPV.................      213
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Figure 1. Comparison of the amino acid sequence of Cu/Zn-SOD from several plant species using the MEGA 5.0 software. The GenBank accession No. and name of these sequences are as follows: JcCu/Zn-SOD, *J. curcas* Cu/Zn-SOD (KF268341); TcCu/Zn-SOD, *Theobroma cacao* Cu/Zn-SOD (EOY33683.1); FvCu/Zn-SOD, *Fragaria vesca* Cu/Zn-SOD (XP_004287550.1); PtCu/Zn-SOD, *Populus trichocarpa* Cu/Zn-SOD (EEF10665.1); RcCu/Zn-SOD, *Ricinus communis* Cu/Zn-SOD (EEF38668.1). Identical amino acids are highlighted in black and similar residues are in gray.

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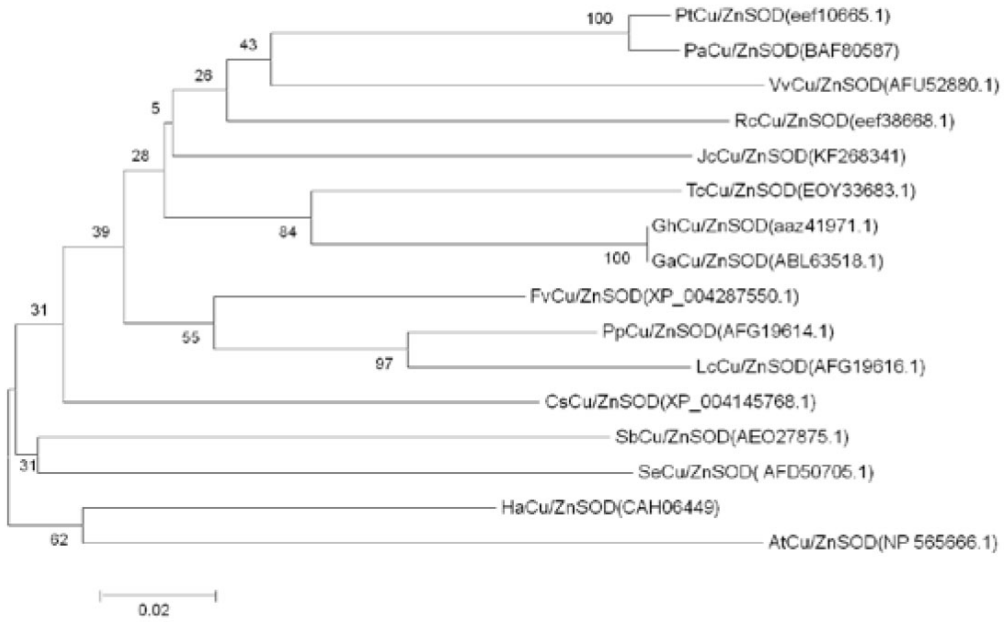

Figure 2. Phylogenetic tree for the amino acid sequences of Cu/Zn-SODs. The tree was constructed using the neighbor-joining method. Plant sources and GenBank accession No. are indicated. Jc = *Jatropha curcas*; Pt = *Populus trichocarpa*; Pa = *Populus alba*; Vv = *Vitis vinifera*; Rc = *Ricinus communis*; Tc = *Theobroma cacao*; Gh = *Gossypium hirsutum*; Ga = *Gossypium arboreum*; Fv = *Fragaria vesca*; Pp = *Prunus persica*; Lc = *Litchi chinensis*; Cs = *Cucumis sativus*; Sb = *Scutellaria baicalensis*; Se = *Salicornia europaea*; Ha = *Helianthus annuus*; At = *Arabidopsis thaliana*. The scale bar represents 2 residue differences per 100 amino acids.

Expression profile of *JcCu/Zn-SOD*

Quantitative real-time PCR analysis indicated that the *JcCu/Zn-SOD* gene was expressed in all *J. curcas* tissues examined (root, stem, leaf, flower, and silique). Moreover, the expression of *JcCu/Zn-SOD* was significantly higher in the leaf than in other tissues (Figure 3A).

Previous studies showed that *Cu/Zn-SOD* levels were increased under NaCl stress in *A. thaliana* (Attia et al., 2011). In this study, to determine whether the expression of *JcCu/Zn-SOD* is regulated by NaCl, the mRNA accumulation profile was determined under 200 mM NaCl treatment. As indicated in Figure 3B, *JcCu/Zn-SOD* expression began increasing within 6 h, peaked at 18 h, and subsequently decreased. The results showed that *JcCu/Zn-SOD* expression was closely related to NaCl stress.

Over-expression of *JcCu/Zn-SOD* in *Arabidopsis* plants

The induction of *JcCu/Zn-SOD* expression by 200 mM NaCl prompted us to analyze its function in NaCl stress resistance. Accordingly, we developed *JcCu/Zn-SOD* homozygous transgenic lines in *A. thaliana*. Among the 36 putative T0 transgenic lines, only 8 lines segregated for *JcCu/Zn-SOD* in 3:1 ratio in the T1 generation. Subsequently, *JcCu/Zn-SOD* levels

were detected in transgenic plants by PCR and semi-qPCR. Three transgenic lines (S3, S12, and S26) that expressed relatively higher *JcCu/Zn-SOD* levels were used for further analysis (Figure 4A and B).

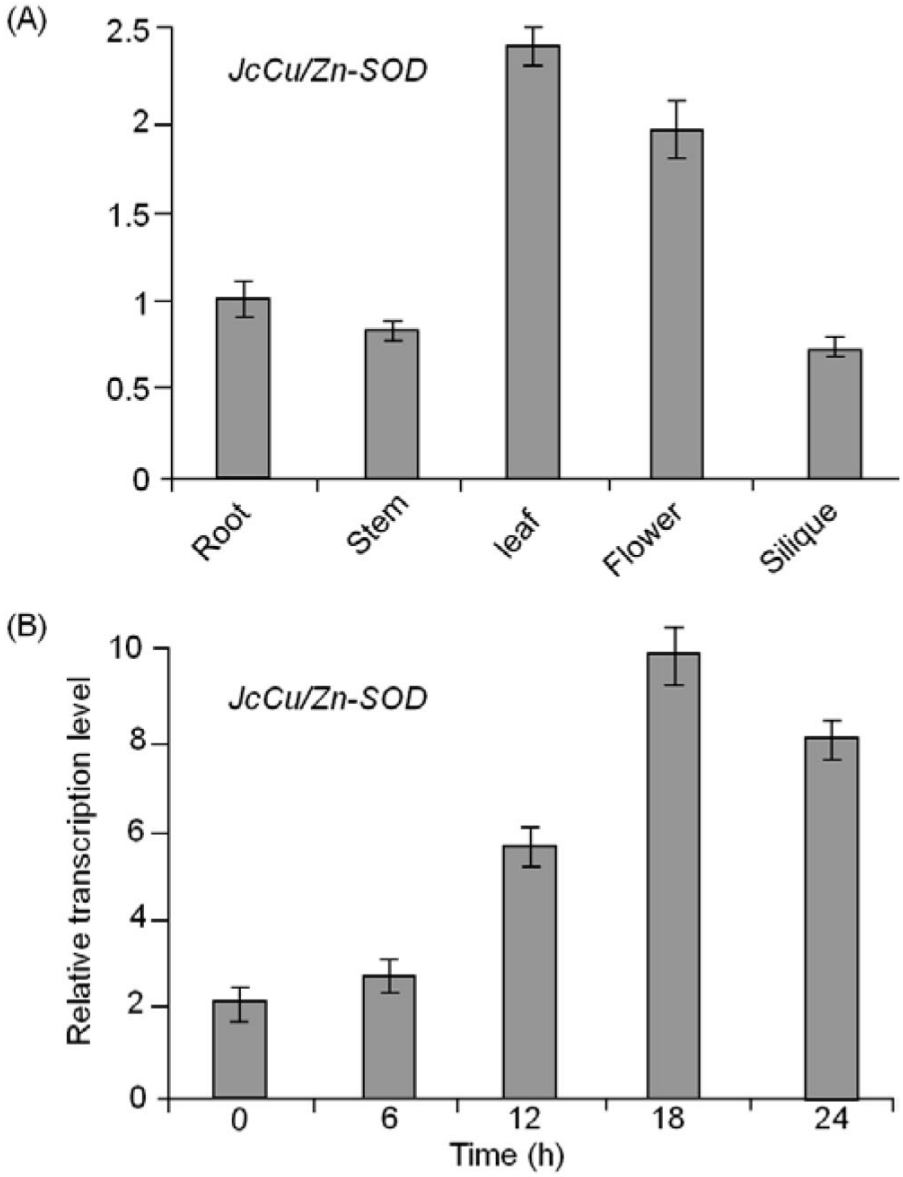

Figure 3. Expression of *JcCu/Zn-SOD*. **A**. q-PCR analysis of *JcCu/Zn-SOD* in different tissues of *Jatropha curcas*. Total RNA was extracted from the root, stem, leaf, and silique of wild-type plants. **B.** q-PCR analysis of *JcCu/Zn-SOD* expression in *J. curcas* exposed to NaCl. Total RNAs were isolated from 4-week-old leaves of *J. curcas* treated at 200 mM NaCl and duration from 0-24 h. Transcripts of actin were used as a control. Error bars indicate the standard deviation of the mean (N = 3). Three replicates were analyzed.

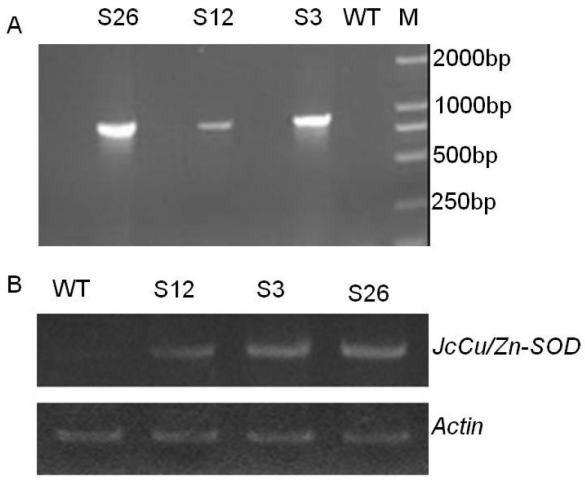

Figure 4. Detection of the transgene from kanamycin-resistant lines. **A.** Confirmation of *JcCu/Zn-SOD* transgenic *Arabidopsis* plants by PCR. WT, wild-type plants; S3, S12, and S26, independently transformed plant lines; *M*, DNA marker (DL2000). **B.** Semi-quantitative RT-PCR analysis of *JcCu/Zn-SOD* in transgenic lines. Actin was used as a reference to show that equal amounts of RNA were used in the analysis. WT, wild-type plants; S3, S12, and S26, independently transformed plant lines.

JcCu/Zn-SOD enhances the resistance of plants to NaCl stress

In *Arabidopsis*, salt sensitivity was most evident during seed germination and subsequent seedling growth (Gao et al., 2003). In our study, no difference between transgenic lines and WT in germination percentage was observed when seeds were maintained on MS medium without NaCl (Figure 5A and B) while a significant difference between those two groups of *Arabidopsis* plants was found in the rate of germination following supplementation with 150 mM NaCl. Three days after spotting, transgenic lines began germinating, whereas the WT germinated on the 5th day. Eleven days later, the germination rate of WT was 45%, while the germination rates of S3, S12, and S26 transgenic lines were 91, 83, and 96%, respectively (Figure 5B and D).

In the post-germination phase, the seeds continuously growing on medium plates without NaCl supplementation showed morphology and growth characteristics of transgenic seedlings similar to those of WT (Figure 6A). In contrast, under 150 mM NaCl treatment, WT seedlings showed retarded growth, whereas the growth of transgenic seedlings (S3, S12, and S26) was slightly restrained. Moreover, primary root length and lateral root number of the WT lines were dramatically reduced compared to transgenic lines (Figure 6B).

After 3 weeks of plant growth, both the number of leaves and area of rosette were measured. No significant difference was observed in number of leaves in the transgenic plants compared to controls under normal growth conditions. However, under 150 mM NaCl stress, the number of leaves on transgenic plants S3, S12, and S26 were 2, 2, and 4, respectively, which was more than their corresponding controls (Figure 7A). It was also observed that the rosette area of the transgenic lines was similar compared with WT on normal MS medium. However, it was more pronounced in transgenics than in WT plants under NaCl stress conditions. In transgenic plants, the rosette area of S3, S12, and S26 was 57, 37, and 67%, respec-

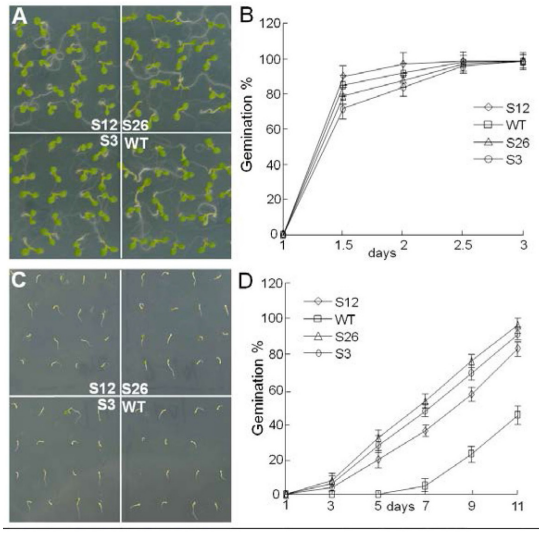

Figure 5. Effect of salt (NaCl) stress on germination of *JcCu/Zn-SOD* transgenic *Arabidopsis* seeds. Seeds were germinated in MS medium supplemented in the presence or absence of NaCl. Photos were taken on the 11th day without NaCl (**A**) or 150 mM NaCl stress (**C**). The germination rate was determined for a period as indicated under normal condition (**B**) or NaCl-treated (**D**). N = 50 ± 5. The data are reported as the mean value of 3 individual experiments.WT, non-transgenic *Arabidopsis*; S3, S12, and S26, independent *JcCu/Zn-SOD* transgenic lines.

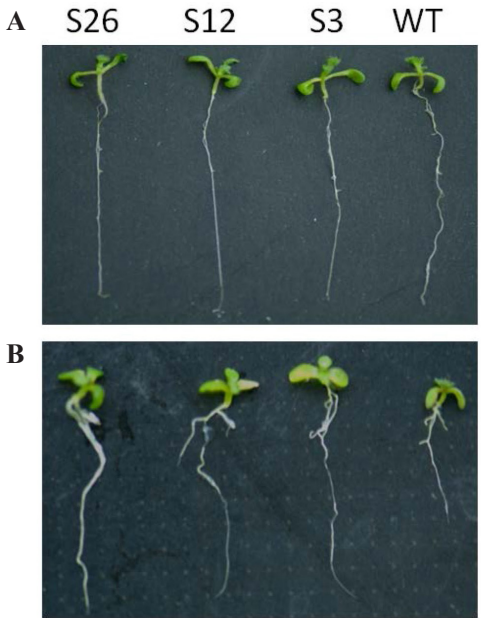

Figure 6. Growth of wild-type and transgenic *Arabidopsis* seedlings (S3, S12, and S26) on MS medium supplemented or not with NaCl. Seven-day-old seedlings were transformed onto MS agar plates supplemented with different concentrations of NaCl and allowed to grow for 2 weeks. **A.** Normal condition; **B.** 150 mM NaCl.

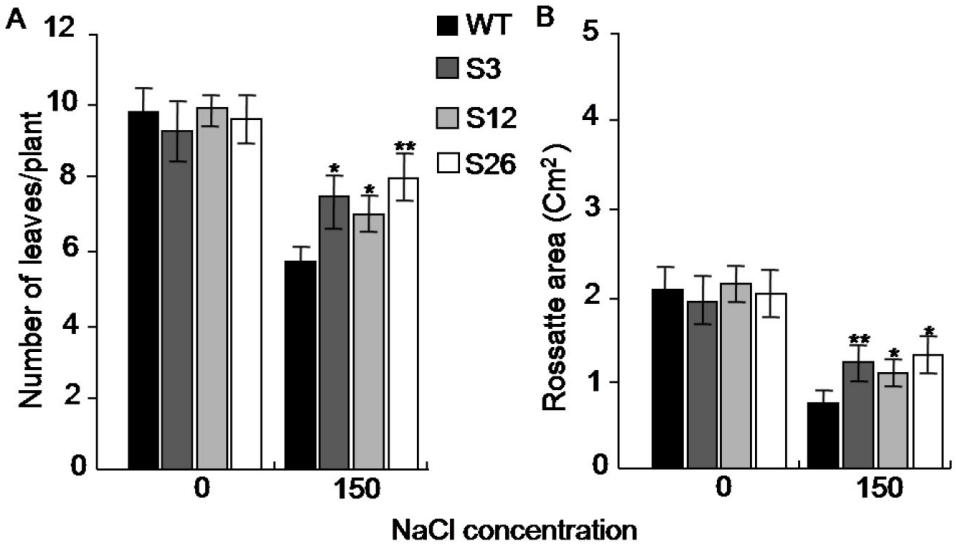

Figure 7. Over-expression of *JcCu/Zn-SOD* gene in *Arabidopsis* improves number of leaves and rosette area. Wild-type and transgenic plants transferred to MS agar plates supplemented with 150 mM NaCl or without NaCl. After 3 weeks of growth on these plates, the number of leaves (**A**) and rosette area (**B**) were recorded. Data are reported as mean values ± standard deviation of 3 replications. The single and double asterisks represent significant difference determined by the Student *t*-test at $P < 0.05$ and $P < 0.01$, respectively.

tively; values that were higher than in WT (Figure 7B).

These results suggested that over-expression of *JcCu/Zn-SOD* enhanced tolerance to salt stress in transgenic plants.

Higher SOD activity in transgenic *Arabidopsis* plants under NaCl stress

It was hypothesized that the increased tolerance to salt stress during *Arabidopsis* growth resulted from higher SOD levels. As expected, total SOD activity in transgenic plants was significantly higher than in WT after 3 weeks of growth under 150 mM NaCl treatment. On MS medium without salt stress, the SOD activities of S3, S12, and S26 were 4.1, 3.9, and 4.5 U/mg protein, respectively, and that of WT was 3.5. Under 150 mM NaCl stress, activity in the WT was only 5.4, while the 3 transgenic lines examined, S3, S12, and S26, showed SOD levels of 10.2, 9.8, and 11.6, respectively (Figure 8A).

Transgenic plants suffered less MDA than WT

Salt stress causes rapid and excessive accumulation of ROS in plant cells, which further causes a lipid peroxidation chain reaction. MDA is the principal by-product of lipid oxidation. In our study, there was no significant difference in MDA content between WT and transgenic lines without NaCl treatment. However, under 150 mM NaCl treatment, MDA content in transgenic over-expressing lines was lower than that in WT. Lines S3, S12, and S26 exhibited approximately 22, 15, and 28%, respectively, lower MDA concentration than the non-transformed control (Figure 8B).

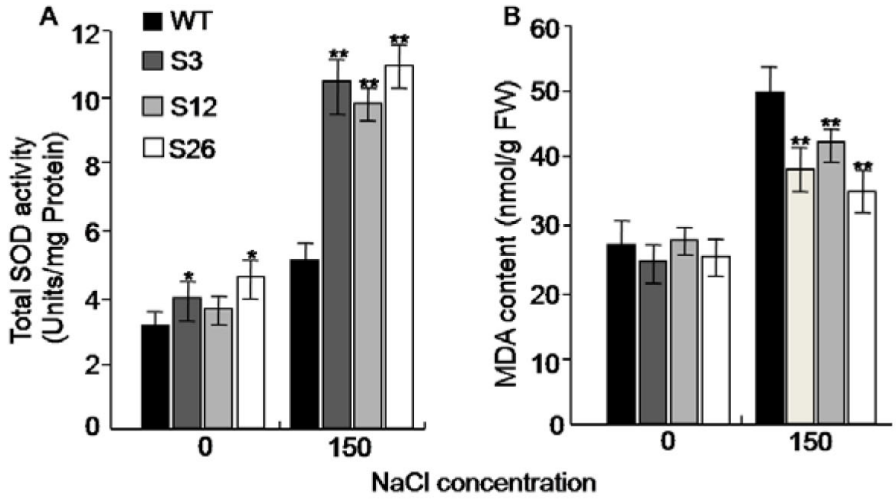

Figure 8. Changes in SOD activity (**A**) and MDA content (**B**) in wild-type and transgenic lines under salt stress. Plants were treated for 3 weeks with 150 mM NaCl or were untreated and sampled for measurement. Data are reported as mean values ± standard deviation of 3 replications. The single and double asterisks represent significant difference determined by the Student *t*-test at $P < 0.05$ and $P < 0.01$, respectively.

DISCUSSION

It has been reported that increased SOD activity is positively correlated with the tolerance to different stresses such as low/high temperature, drought, salinity, high light intensity, and ozone (Ueda et al., 2013). In the present study, we isolated a novel *Cu/Zn-SOD* from *J. curcas*. Sequence alignment of *JcCu/Zn-SOD* with other plants revealed that the gene shares highly identity with other reported Cu/Zn-SOD proteins (Figure 1), suggesting that *JcCu/Zn-SOD* likely has the same function as other reported homologous proteins. Quantitative real-time PCR analysis showed that the *JcCu/Zn-SOD* gene was expressed in all *J. curcas* tissues examined and increased under NaCl stress (Figure 3A and B). These findings generally agree with those other studies reporting increased *Cu/Zn-SOD* expression in plants exposed to salt stress (Hernandez et al., 1999; Wang et al., 2010). However, no significant changes in *Cu/Zn-SOD* mRNA levels were observed in other experiments under NaCl treatment (Menezes-Benavente et al., 2004; Jithesh et al., 2006). Differences between *Cu/Zn-SOD* may allow them to function in diverse responses.

The seeds of *Arabidopsis* plant over-expressing the *JcCu/Zn-SOD* gene exhibited a higher germination rate and better survival ability than those of WT under NaCl stress (Figure 5-7). These results agreed with those of previous studies (Badawi et al., 2004; Gill et al., 2010; Diaz-Vivancos et al., 2013). Saline induced an increase in the ROS level, leading to seed deterioration (Xi et al., 2010). Under salt stress, ROS production may exceed the removal ability of native antioxidant enzymes, thus harming the germinating seeds and resulting in slower germination in WT seeds. In contrast, in transgenic lines, over-expression of *JcCu/Zn-SOD* may help the plant to maintain safe ROS levels by scavenging over-produced ROS. Moreover, without salt stress, similar germination response and early seedling establishment were ob-

served in transgenic and WT seeds, suggesting that over-expression of the *JcCu/ZnSOD* gene to a moderate level did not affect the growth of transgenic plants.

Although *JcCu/Zn-SOD* over-expression in transgenic lines was driven by the high activity of cauliflower mosaic virus 35S promoter, SOD activity was only slightly higher in transgenic lines than in the WT without salt treatment. In contrast, the activity was much higher in transgenic lines compared to in WT under NaCl stress (Figure 8A). These results suggest that post-transcriptional or post-translational regulation occurs for the *JcCu/Zn-SOD* gene, which has been reported in *Arabidopsis* (Sunkar et al., 2006; Xi et al., 2010).

Salt stress causes extensive lipid peroxidation. Therefore, the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of salt-induced oxidative damage (Gossett et al., 1996). Our results showed that transgenics had significantly lower MDA contents compared to WT under salt stress (Figure 8B), and over-expression of *JcCu/Zn-SOD* can eliminate salt-induced ROS by decomposing it into water and oxygen, thus helping the plant to overcome the stress induced by NaCl. Several studies have reported upregulation of the antioxidative system decreases MDA content to protect plant against stress (Gill and Tuteja, 2010).

In conclusion, our results demonstrated that the over-expression of *JcCu/Zn-SOD* enhances the salt resistance during the plant life without negatively affecting development. Transgenic *Arabidopsis* plants exhibited higher levels of SOD activity WT and may reduce MDA content under NaCl stress. Importantly, over-expression of *JcCu/Zn-SOD* in crops may be useful for promoting their resistance in high soil salinity areas.

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