Plant Physiology and Biochemistry 144 (2019) 254–263

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Measuring the expression and activity of the CAT enzyme to determine Al resistance in soybean

Chao-Qun Zeng[1], Wen-Xia Liu[1], Jing-Yi Hao, Dan-Nan Fan, Li-Mei Chen, Hui-Ni Xu, Kun-Zhi Li*

Faculty of Life Science and Biotechnology, Kunming University of Science and Technology, Chenggong, Kunming, 650500, China

ARTICLE INFO

Keywords:
Soybean
Al stress
Superoxide dismutase
Peroxidase
Catalase

ABSTRACT

To elucidate the mechanism of soybean resistance to Al, physiological and biochemical indices and antioxidant enzyme expression and activities were systematically analyzed in Al-sensitive (*Glycine* max Merr., Yunnan Province of China, SB) and Al-resistant Dambo (*Glycine* max Merr., Kyoto of Japan, RB) black soybean plants. According to the results, the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in RB root tips were significantly lower than those in SB root tips, though the opposite results occurred for soluble protein contents. Moreover, the expression and activities of superoxide dismutase (SOD, $EC_{1.15.1.1.1.1.1.1}$), peroxidase (POD, $EC_{1.11.1.7}$) and catalase (CAT, $EC_{1.11.1.6}$) under 0–400 µM Al for 0–96 h were greater in RB than in SB. However, below 100 µM Al, the activities of those enzymes in SB increased with increasing Al concentration and treatment duration, with SOD activity being lowest and CAT activity exceeding that of POD with increasing Al concentration. Overall, enzyme activity in SB treated with Al at concentrations greater than 200 µM was lower than that in the SB control (CK; not treated with Al) and decreased with treatment duration. Additionally, at Al concentrations lower than 200 µM, enzyme activities in RB were significantly greater than those in RB CK and increased with both Al concentration and treatment duration. Moreover, enzyme activity in RB treated with 400 µM Al was slightly greater than that in RB CK. Thus, CAT activity determines soybean resistance to Al. These results indicate that soybean resistance to Al can be enhanced by regulating the expression and activity of antioxidant enzymes to remove H_2O_2 under Al stress.

1. Introduction

Al toxicity is a major obstacle to the growth and development of crops in acidic soils. Approximately 30% of arable land and more than 50% of potential arable land worldwide is considered acidic (Kochian et al., 2004; Riaz et al., 2018). In China, the Al toxicity of acidic soils reduces crop yields by 35%, and enormous economic losses due to Al toxicity occur worldwide (Jiang et al., 2016). The traditional method for mitigating Al toxicity in acidic soils is the application of lime and organic material, but it is difficult to completely neutralize the topsoil and improve the underlying soil. Furthermore, the cost of treating acidic soils is relatively high. Therefore, the use of modern genetic engineering methods to increase plant resistance to Al is an effective way to improve the productivity of crop species grown in acidic soils (Chen et al., 2011). The tolerance of different plant genotypes to Al toxicity differs significantly and is controlled by genetics. Accordingly, developing crops tolerant to Al is a goal of genetic improvement, which requires an understanding of the physiological and biochemical characteristics and molecular mechanisms of plant tolerance to Al, and many studies have focused on these aspects.

Al stress can cause a metabolic imbalance in reactive oxygen species (ROS) in plants, which can lead to the accumulation of a large amount of ROS such as superoxide free radicals, hydrogen peroxide (H_2O_2) and hydroxyl free radicals, which cause oxidative damage (Apel and Hirt, 2004; Matsumoto, 2000; Yamamoto et al., 2003; Yin et al., 2010). Indeed, Al stress can induce excessive accumulation of ROS in plants, leading to the transformation of various active free radicals to H_2O_2 via the antioxidant damage system and electron transfer. Therefore, the H_2O_2 content is among the main criteria for assessing the degree of oxidative damage in plants. Malondialdehyde (MDA) is a product of membrane lipid peroxidation under oxidative stress, reflecting the degree of cell membrane lipid peroxidation and the strength of the plant response to adverse conditions (Becana et al., 2010; Kanazawa et al., 2010). In addition, soluble proteins are involved in regulating the osmotic potential of plant cells, whereby a high content of soluble proteins can help maintain low osmotic potential and resist stress-induced

---

* Corresponding author.
E-mail address: likzkm@163.com (K.-Z. Li).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.plaphy.2019.09.026
Received 27 April 2019; Received in revised form 17 September 2019; Accepted 17 September 2019

damage (Vassilev and Lidon, 2011). To help combat oxidative stress, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and other enzymes remove excess ROS that have accumulated in plants to maintain active oxygen homeostasis under Al stress (Mittler, 2002). As a free radical scavenger, SOD plays a central role in the cellular protective enzyme system, and its activity is a sign of the ability of plants to resist stress (Takada et al., 2002). CAT is a common and highly active enzyme in organisms that directly catalyzes the decomposition of H_2O_2 to nontoxic water and O_2 (Ali et al., 2005). Many studies have shown that overexpression of CAT can increase plant resistance to abiotic and biotic stresses (Cai et al., 2013; Polidoros et al., 2001) and an important enzyme for alleviating oxidative stress (Gondim et al., 2012; Matsumura et al., 2002; Zhao et al., 2019). POD also catalyzes oxidation using H_2O_2 as an electron acceptor. POD catabolizes H_2O_2 and oxidizes phenols, amines and hydrocarbon oxidation products that have iron porphyrin as an auxiliary group. POD also eliminates toxic H_2O_2, phenols, amines, aldehydes and benzene (Nanda et al., 2010; Saxena et al., 2016). Although antioxidant enzymes that counteract Al toxicity stress induced by an imbalance in the metabolism of ROS play an important role in plants, whether the regulation of these enzymes induces expression and alterations in activity in plants under different Al concentrations and durations of stress remains unclear.

In this study, Al-sensitive small black soybean (SB) and Al-resistant Dambo black soybean (RB) plants, with significantly different tolerance to Al, were used as materials to systematically examine relationships among ROS accumulation, oxidative stress and the antioxidant response in soybean roots treated with different Al concentrations and for various durations. The results showed that the contents of H_2O_2 and MDA in RB were significantly lower than those in SB, though the opposite results were obtained for the soluble protein content. ROS in soybean generated in response to Al stress increased with Al concentration and treatment duration. Overall, the expression and activity of antioxidant enzymes play an important role in eliminating ROS, with CAT activity determining soybean resistance to Al.

2. Materials and methods

2.1. Soybean cultivation

Al-sensitive SB and Al-resistant RB plants were used as experimental materials. Soybean seeds were washed with room-temperature deionized water and placed in a Petri dish containing wet filter paper. The seeds were subsequently incubated at a constant temperature (25 °C) in a dark incubator. Seeds with white sprouts were selected and transplanted onto a thin foam board with needle-sized holes. Floating cultivation was carried out in a black plastic basin filled with deionized water in a growth chamber at 25 °C and under 1000 $\mu mol\ m^{-2}\ s^{-1}$ light for 14 h each day. The culture medium was changed every other day for two weeks.

2.2. Soybean treatment with Al stress

Soybean seedlings displaying identical growth were selected for the experiment, and 3 replicates were established for each treatment. The seedlings were first pretreated overnight with 0.5 mm $CaCl_2$ (pH 4.3) under continuous illumination and then treated with $AlCl_3$ solution (pH 4.3) at concentrations of 0, 50, 100, 200, and 400 μM for 0, 6, 12, 24, 48 and 96 h. Any residual $AlCl_3$ on the root surface was rinsed with sterile water at the different processing time points, and the root surface was blotted with absorbent paper. Root tips of 0–10 mm were collected and stored at −80 °C for ensuing assays.

2.3. Determination of physiological and biochemical indexes of soybean root tips

One gram of soybean root sample was ground in 2 mL of 50 mM (containing 0.2 mM EDTA) phosphate-buffered saline (PBS; pH 7.8) and 0.04 g of polyvinylpyrrolidone (PVP), after which the homogenate was transferred to a tube and centrifuged at 12,000 rpm for 20 min. The supernatant was used as the enzyme solution. The H_2O_2 content was determined via the xylenol orange method (Wang et al., 2017), the MDA content via the thiobarbituric acid (TBA) method (Wang et al., 2017), and the soluble protein content via Coomassie brilliant blue staining (Vassilev and Lidon, 2011). The activity of SOD was determined by the nitro blue tetrazolium (NBT) method; 1 enzyme unit (U) was equal to the amount used to inhibit 50% of the photochemical reduction of NBT (Wang et al., 2017). POD activity was determined by the guaiacol method, whereby an absorbance increase of 0.01 OD_{470} nm $minute^{-1}$ was considered 1 U of POD activity (Wang et al., 2017). CAT activity was determined by UV spectrophotometry, and a decrease in absorbance of 0.01 at OD_{240} nm $minute^{-1}$ was considered 1 U of CAT activity (Wang et al., 2017).

2.4. Extraction of total RNA and synthesis of plant cDNA

In this study, RNA was extracted from young roots using TRIzol reagent (Invitrogen) (Dhar et al., 2014). Using the mRNA from total RNA as a template, oligo (dT) primers were used to synthesize first-strand cDNA via a reverse transcriptase-catalyzed reaction. The target gene was amplified by RT-PCR using cDNA as the template and specific primers.

2.5. SOD, POD and CAT expression analysis

Specific primers for SOD, POD and CAT were designed (Table 1). The same amount of cDNA was used as the template to amplify all genes. The effect of Al treatments on SOD, POD, and CAT expression was determined according to the brightness of the amplified bands. The result of internal reference gene amplification was used to determine whether the template quantity of each reaction system was consistent.

2.6. Data analysis

Determinations of physiological and biochemical indices were repeated three times. SPSS 20.0 was used to analyze statistical significance, and Excel 2010 was used to edit the data.

3. Results

3.1. Effects of different Al stress concentrations and treatment durations on the H_2O_2 content in SB and RB root tips

The content of H_2O_2 can reflect ROS accumulation in the cells of plants after stress, and changes are often used to measure the degree of plant stress (Zhang et al., 2016). The H_2O_2 content in RB and SB roots stressed with different Al concentrations for different treatment durations is shown in Fig. 1. In the absence of Al stress, the two genotypes showed the same low H_2O_2 content. The H_2O_2 content in SB increased slowly with increasing Al stress duration under 50 μM treatment (Fig. 1 a); in RB, the H_2O_2 content decreased gradually to the control (CK)

Table 1
The primer sequences used for gene expression.

Gene (accession No.)	Forward (5′-3′)/reverse (5′-3′)	Product Size (bp)
CAT (AB_109090)	CTGTTGGAGGAAGAAGCC CAGCCTGAGACAAGTAAGAT	721
POD (NM_001251254)	TCACTGAGTCCTTCTGTTG CTCTGCTCCTTCTACCATC	427
SOD (JQ_043347)	TTCTCAACCCTTACAACTTCC ATCTTCCACCAGCATTTCC	478

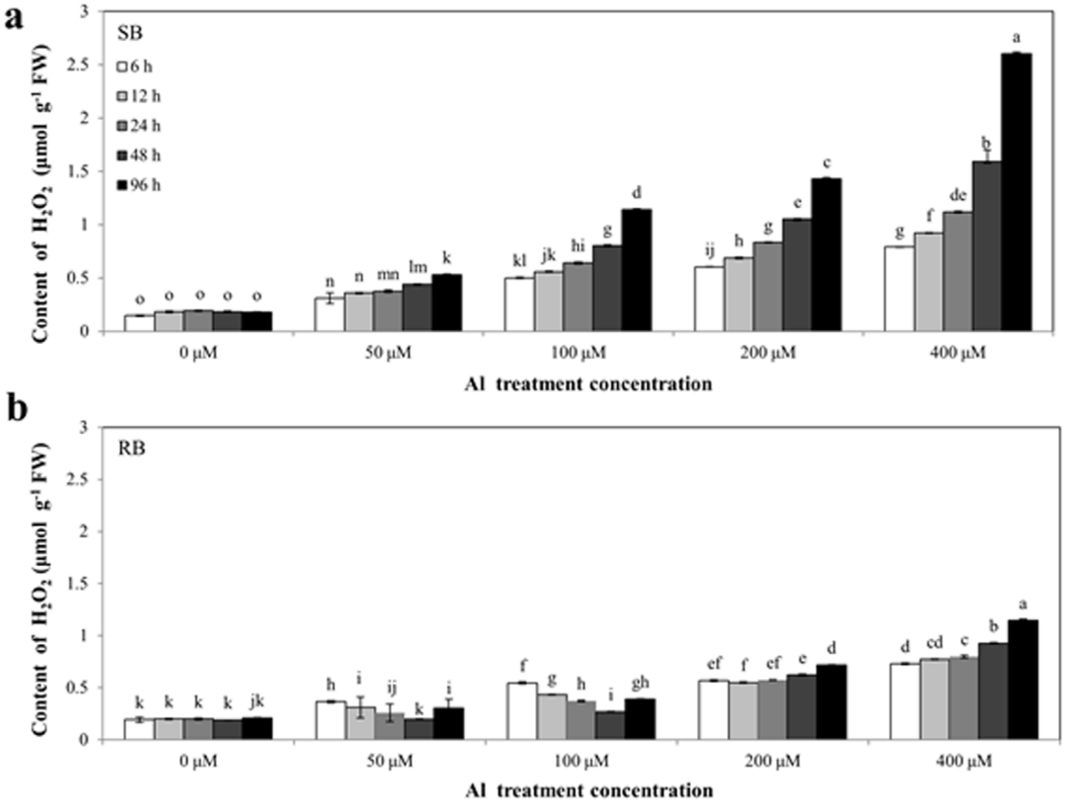

Fig. 1. Effects of different Al concentrations on the H_2O_2 content in the root tips of SB and RB. The different lowercase letters indicate differences at the 0.05 level.

level within 6–48 h but then increased slightly (Fig. 1 b). When treated with 100 μM Al, the content of H_2O_2 in SB roots increased linearly with increasing stress duration, though the content of H_2O_2 in RB was the same as that after treatment with 50 μM Al. Under 200 μM Al, the content of H_2O_2 in SB increased with increasing stress duration, though the concentration in RB increased only slightly. Under treatment with a high concentration of Al (400 μM), the content of H_2O_2 in SB increased rapidly with increasing stress duration, whereas the content increased slowly in RB. Overall, there was a large difference in the content of H_2O_2 between SB and RB; at 96 h, the content of H_2O_2 in SB was 3 times that in RB, and the level of oxidative stress in SB was significantly greater than that in RB.

3.2. Effects of Al stress concentration and duration on MDA contents in root tips of SB and RB

The MDA content reflects the degree of plasma membrane oxidation after stress, and changes in MDA content can be employed to measure the degree of stress (Hao et al., 2006). MDA contents in RB and SB roots of plants subjected to different Al stress concentrations for different durations are presented in Fig. 2. The MDA contents in untreated RB and SB plants were lowest, with the content in the former being lower than that in the latter. With increasing Al concentration, the MDA content in SB increased, whereas the MDA content in RB root tips was significantly lower than that in SB root tips. When RB was treated with a low Al concentration (up to 100 μM), the MDA content in root tips decreased gradually within 48 h and then remained at a relatively low level. Under treatment with 200 μM Al, the MDA content in RB root tips first decreased but then increased, and the MDA content in RB increased slowly when treated with a high concentration (400 μM) of Al.

3.3. Changes in soluble protein contents in root tips of SB and RB under different Al concentrations and treatment durations

Tolerance to abiotic stress can be reflected by the soluble protein content of plants (Chen and Lidon, 2011), and the soluble protein contents in the roots of RB and SB plants under different Al concentrations and stress durations are illustrated in Fig. 3. Without Al treatment, the soluble protein content in the two soybean genotypes was similar, at 2.2 mg g^{-1} fresh weight (FW). Moreover, the plants exhibited the same trend after different stress durations at concentrations of Al lower than 100 μM; the soluble protein contents reached the highest level in SB after Al treatment for 24 h and in RB after Al treatment for 48 h, after which they remained at a certain level. The soluble protein content in the roots of SB and RB plants treated with 100 μM Al was significantly greater than that of plants treated with 50 μM Al (Fig. 3 a b).

In SB exposed to greater than 200 μM Al, the soluble protein level was significantly lower than that in CK, though the levels in response to 400 μM Al were significantly lower than those in response to 200 μM Al. However, RB treated with 200 μM Al presented the highest level of soluble protein, which was three times greater than that presented by CK. The protein content in RB in response to the 400 μM Al treatment was lower than that in response to the other treatments but greater than the CK levels. Overall, the protein content in RB root tips was significantly greater than that in SB root tips under different Al concentrations, but a significant decrease in the RB protein content under the high-Al treatment (400 μM) revealed inadaptability.

3.4. Changes in expression and activity of SOD in root tips of SB and RB under different Al concentrations and treatment durations

Fig. 4 shows SOD expression and activity in the roots of SB and RB plants under different Al concentrations and treatment durations. Without Al treatment (CK), SOD expression and activity in the root tips

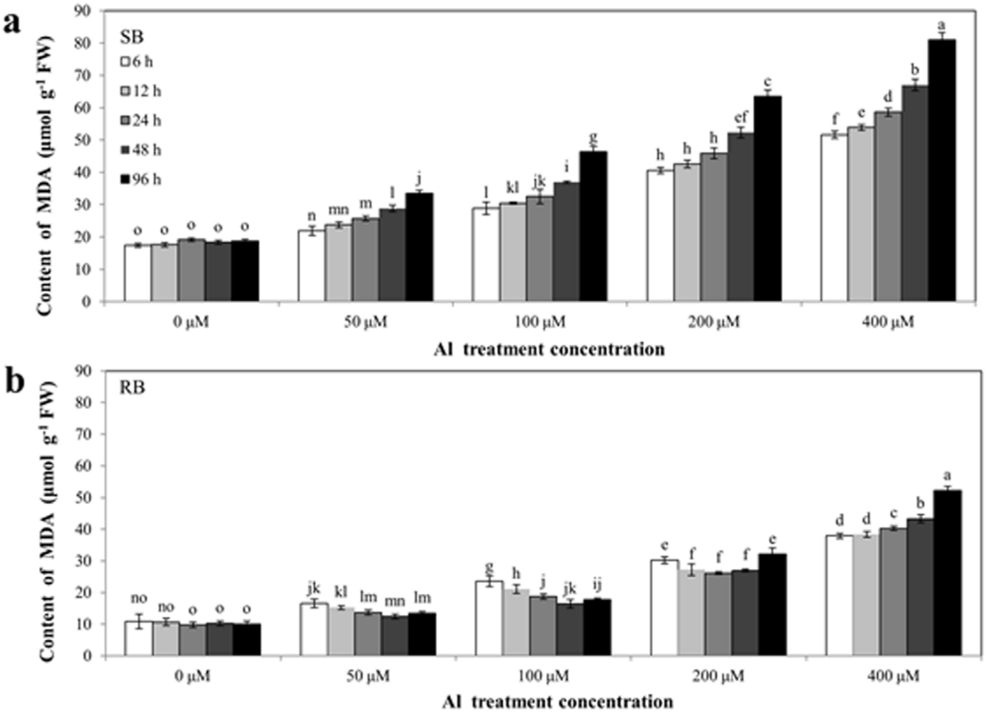

Fig. 2. Effects of different Al concentrations and treatment durations on the MDA content in the root tips of SB and RB. The different lowercase letters indicate differences at the 0.05 level.

of SB plants were lower than those in the root tips of RB plants. SOD activity in SB and RB treated with concentrations below 100 μM Al for different treatment durations was significantly greater than that in CK, and SOD activity in response to 100 μM Al was significantly greater than that in response to 50 μM Al. In SB, SOD activity first increased but then decreased; it was highest at 24 h, after which it decreased slowly and was maintained at a relatively high level. In RB, SOD activity was highest at 48 h and then maintained at a relatively high level. SOD activity in SB treated with 200 μM Al or greater was significantly lower than that in CK; activity in response to 400 μM Al, which was only one-sixtieth of that of CK, was significantly lower than that in response to 200 μM Al. In RB, activity of SOD was significantly greater under treatment with 200 μM Al than with 50 μM Al, first increasing but then decreasing with treatment duration. SOD activity in RB treated with

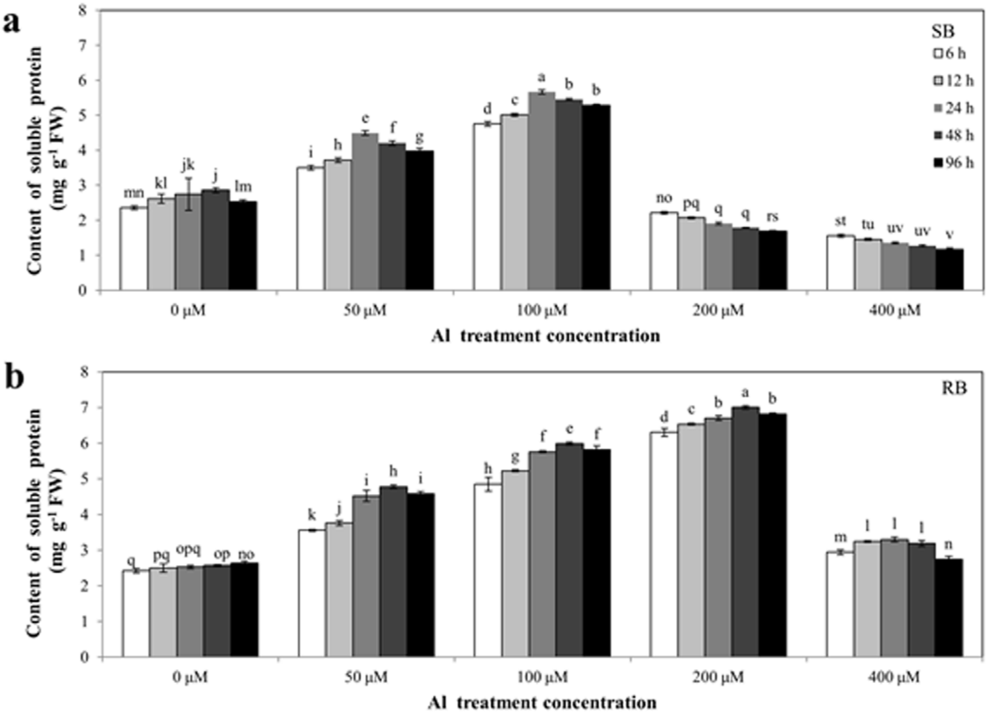

Fig. 3. Effects of different Al concentrations and treatment durations on the contents of soluble proteins in the roots of SB and RB. The different lowercase letters indicate differences at the 0.05 level.

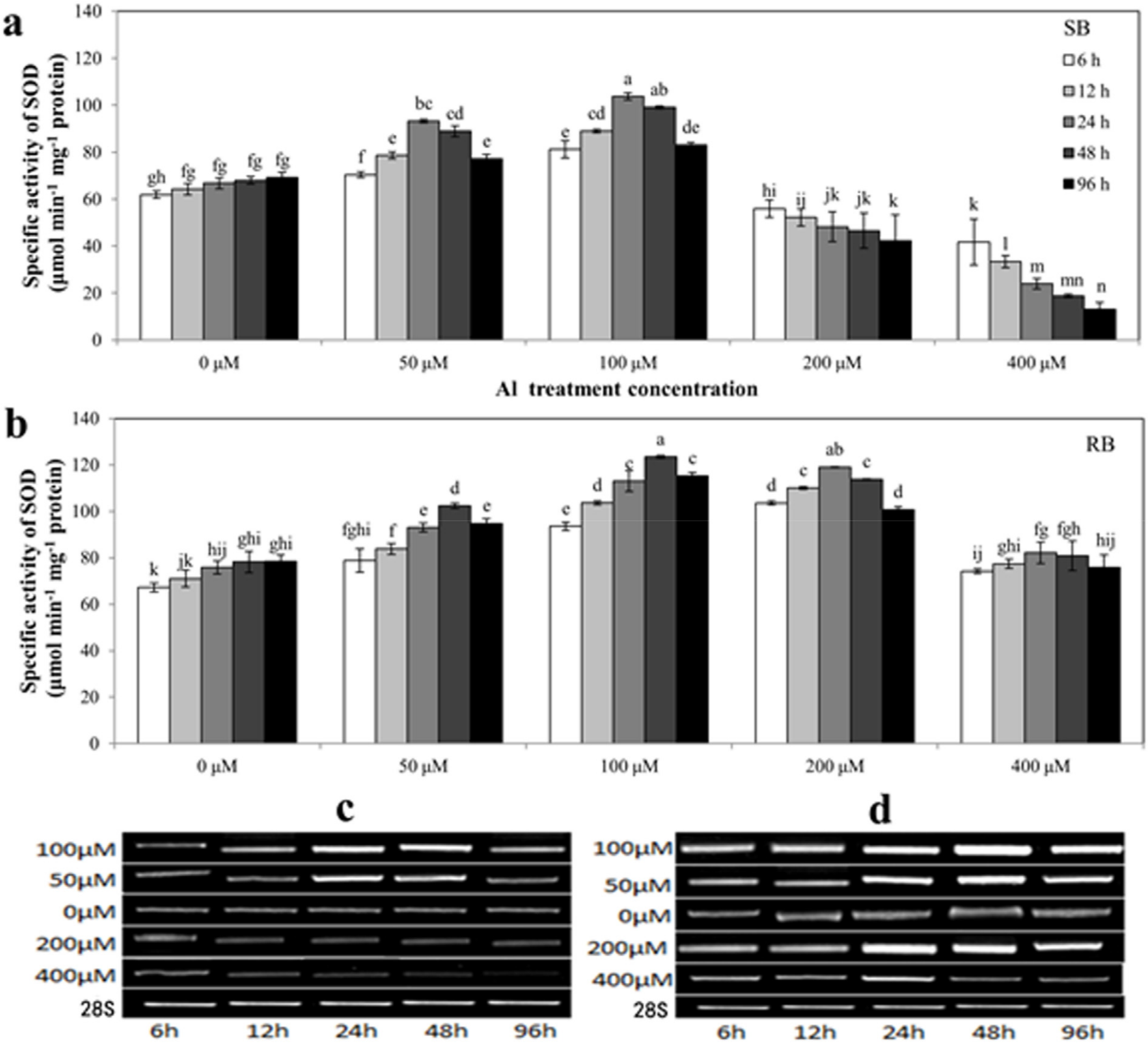

Fig. 4. Changes in the specific activity of SOD (a b) and its expression (c d) in the root tips of SB and RB under Al stress. The different lowercase letters indicate differences at the 0.05 level.

400 μM Al was slightly greater than that of CK within 48 h, but it then decreased after 96 h. Compared with SB, RB presented greater enzyme activity in response to Al stress. Moreover, analysis of gene expression revealed that the SOD level in SB and RB roots tended to be consistent with the level of enzyme activity under different Al concentrations at the same time point, with a positive correlation between them (Fig. 4 c d).

3.5. Changes in expression and activity of POD in root tips of SB and RB under different Al concentrations and treatment durations

POD expression and activity in the root tips of RB and SB plants treated with different Al concentrations and treatment durations are depicted in Fig. 5. In general, activity in root tips of SB plants not subjected to Al stress (CK) was slightly greater than that in root tips of RB plants under the same treatment (Fig. 5 a b), and the POD expression level was also slightly greater than that in RB (Fig. 5 c d), indicating that enzyme activity was proportional to the level of gene expression. In SB and RB treated at concentrations lower than 100 μM Al, POD activity was significantly greater than that in CK, reaching a maximum at 48 h and then maintaining a relatively high level, though that in response to 100 μM Al was significantly greater than that in response to 50 μM Al. Under 200 μM Al stress, POD activity in SB root tips reached a maximum at 12 h but remained below that detected in response to 50 μM Al, after which activity decreased with treatment duration to a level below that of CK at 24 h. In RB, POD activity increased with increasing stress duration, reaching a maximum at 24 h, which was greater than that detected in response to 100 μM Al, after which activity was maintained at a high level—approximately 3.5 times that of CK. Under 400 μM Al stress, POD activity in SB decreased sharply and was significantly lower than that in CK, at only 11% of CK activity at 96 h. In contrast, POD activity in RB was greater than that in CK within 48 h, which was approximately 1.25 times greater than that of CK. Subsequently, POD activity in RB was lower than that in CK, and it was greater in RB than in SB in response to Al stress.

The gene expression analysis shown in Fig. 5 c d indicated that POD gene expression in RB was greater than that in SB under the same stress duration with different Al concentrations. POD gene expression in SB under 200 μM and 400 μM Al stress was inhibited, was lower than the expression level in CK and was barely detected under 400 μM Al stress for 96 h; under 200 Al stress, RB exhibited the highest POD expression, which was much greater than that in CK. POD gene expression in RB under 400 μM Al stress decreased but was greater than that in CK, except at the 96 h time point. POD gene expression in SB and RB was

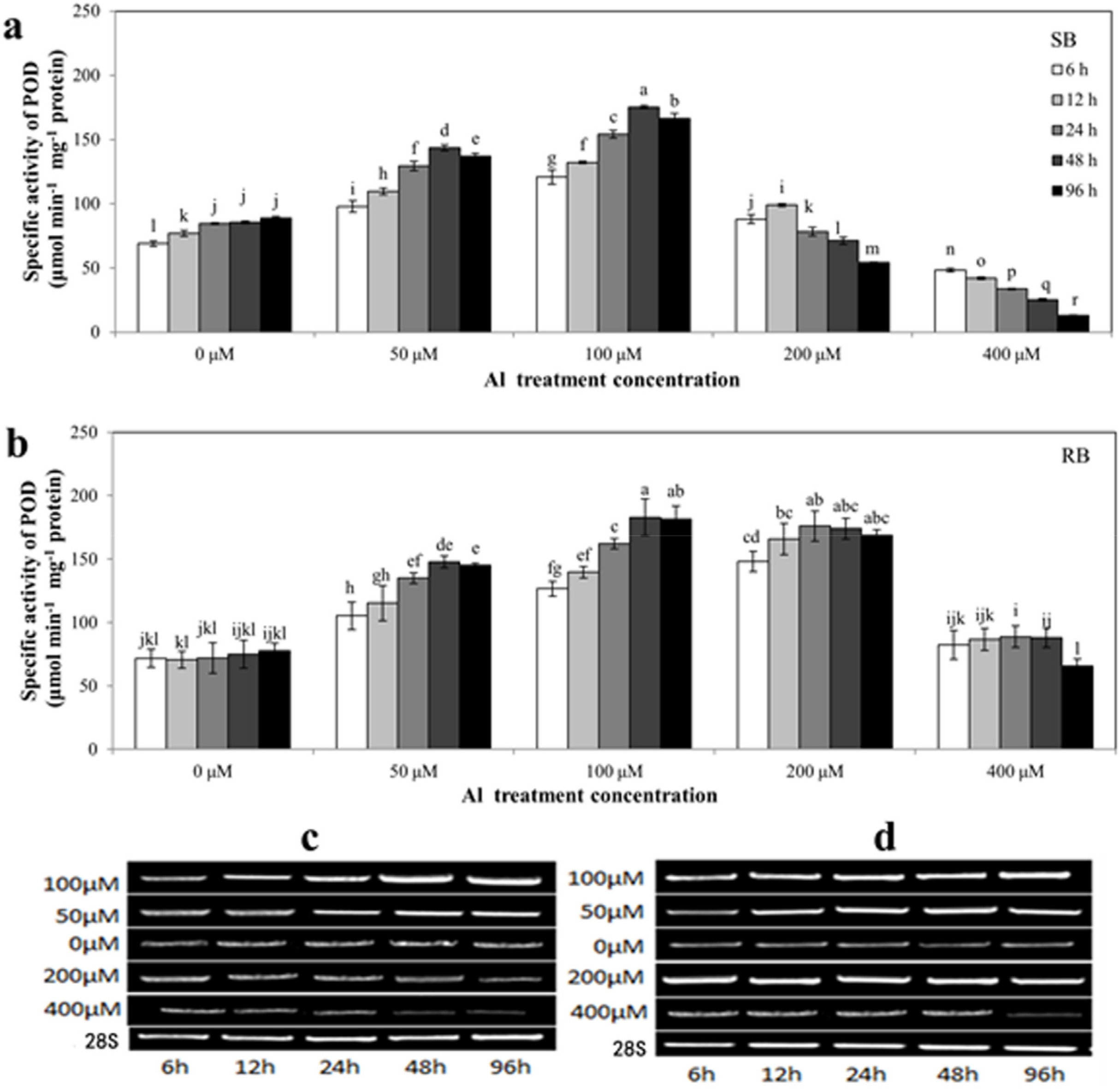

Fig. 5. Changes in the specific activity of POD (a b) and its expression (c d) in the root tips of SB and RB under Al stress. The different lowercase letters indicate differences at the 0.05 level.

highest at 48 h after 100 μM Al treatment. In addition, POD gene expression in RB treated with 200 μM Al was relatively high. Overall, the gene expression levels were consistent with the corresponding changes in enzyme activity.

3.6. Changes in expression and activity of CAT in root tips of SB and RB under different Al concentrations and treatment durations

The results of CAT activity and CAT expression in the root tips of SB and RB under different Al concentrations and treatment durations are presented in Fig. 6. There was no significant difference in CAT activity or expression between the root tips of SB and RB in the absence of Al (CK), both of which were at relatively low levels.

Under Al stress below 100 μM, CAT activity in SB and RB was relatively high, exhibiting two peaks within 96 h. The first activity peak in SB and RB appeared at 12 h after 50 μM Al treatment, which was 3 and 3.4 times that of CK, respectively. CAT activity then declined to 2 times (SB) and 2.7 times (RB) that of the CK level at 24 h and subsequently reached the second peak at 48 h, which was 3.7 times (SB) and 4 times (RB) that of CK; CAT activity then decreased from the second peak to 2.4 times (SB) and 3.5 times (RB) that of CK at 96 h. Under 100 μM Al, the change in enzyme activity was the same as that under the 50 μM treatment. The first peak was 4.4 times (SB) and 4.9 times (RB) that of the CK activity at 12 h; afterward, the activity began to decrease to 3.4 (SB) and 3.7 times (RB) that of the CK level at 24 h, after which it recovered with a second peak, which was 6 times (SB) and 7 times (RB) that of the CK activity at 48 h. CAT enzyme activity subsequently decreased to 3.5 times (SB) and 6 times (RB) that of CK at 96 h. The activity of CAT in SB root tips was slightly greater than that in CK root tips at 6 h after treatment with 200 μM Al and then decreased to less than one-third of the CK activity at 96 h, though the activity of CAT in RB treated with 200 μM Al still tended to increase. The first peak of CAT activity was 5.6 times that of CK at 12 h after treatment, and the second peak of enzyme activity at 48 h was 6.3 times that of CK. Under high concentrations of 400 μM Al stress, the change in CAT activity in SB root tips was similar to that under 200 μM Al (which was lower than the CK activity), whereas the activity in RB peaked at 12 h and 48 h and was 2.4 times that of the CK level, after which it decreased and remained at approximately 2 times that of CK.

Fig. 6 c d illustrates that expression of the CAT gene in the SB and

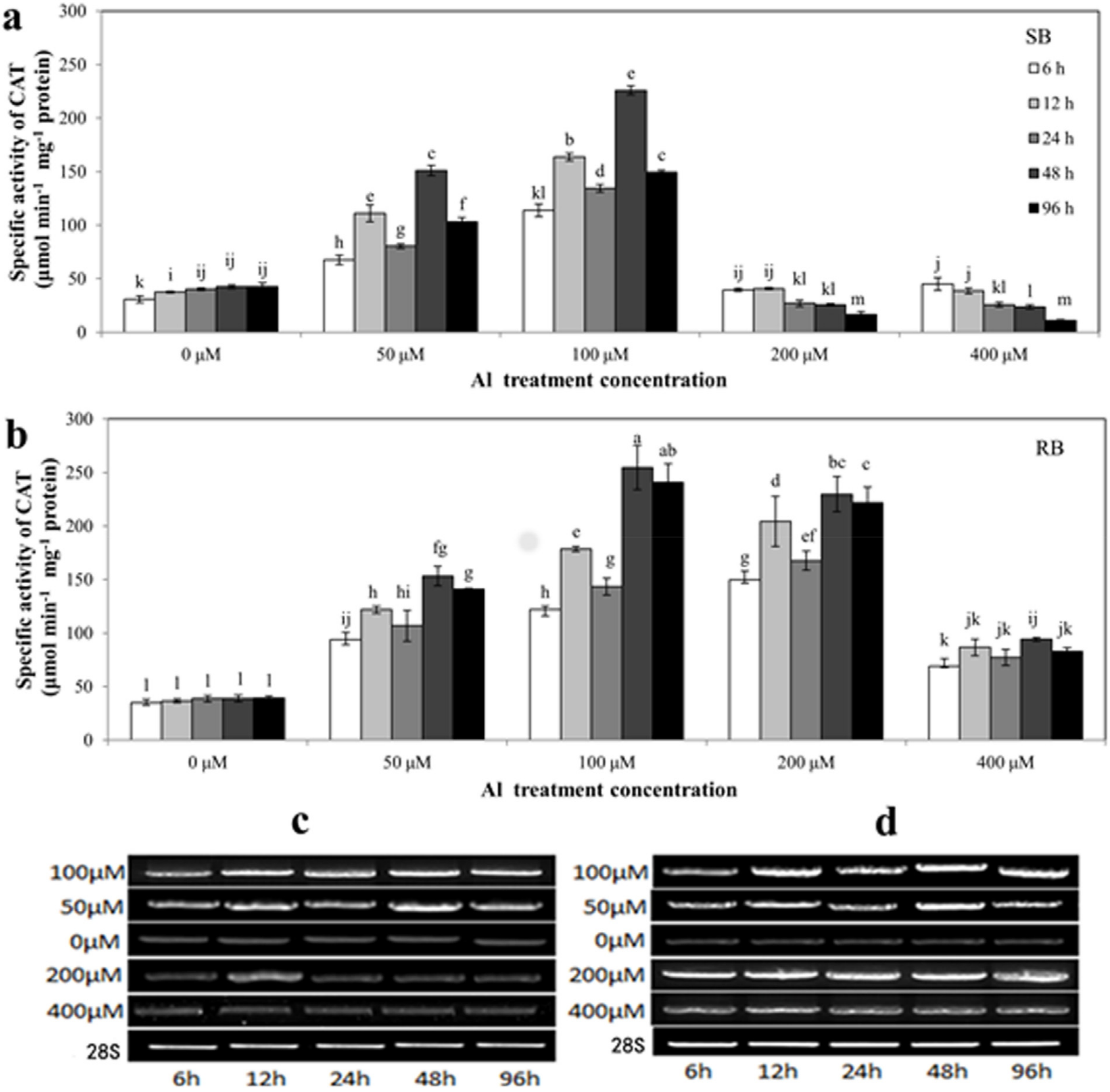

Fig. 6. Changes in the specific activity of CAT (a b) and its expression (c d) in the root tips of SB and RB under Al stress. The different lowercase letters indicate differences at the 0.05 level.

RB plants in the CK treatment was relatively low, with both genotypes exhibiting high expression levels under 50 μM and 100 μM Al. With an increase in Al concentration to 200 μM and 400 μM, the expression level of the CAT gene in SB decreased and was lower than that in CK; however, the expression level was still greater in RB than in CK, the latter of which was much greater than CAT gene expression in SB. Expression of the CAT gene in RB was upregulated to varying degrees at different concentrations; in SB, CAT gene expression was upregulated only under 50 μM and 100 μM Al. This difference in gene expression between the two soybean genotypes was obvious: RB responded to the marked amount of H_2O_2 produced under Al stress by significantly increasing expression of the CAT gene to provide CAT activity.

4. Discussion

Many studies have reported that plants can produce and accumulate ROS such as superoxide anion radicals (O^{2-}), hydroxyl radicals (·OH), hydrogen radicals, methyl radicals and H_2O_2 under Al stress (Swanson and Gilroy, 2010; Yamamoto et al., 2003). These ROS can damage cells by oxidizing cell membranes, lipids, proteins and nucleic acids. Additionally, plants can induce the expression and activity of antioxidant enzymes to eliminate ROS in cells to avoid the oxidative stress damage caused by Al stress (Yuan et al., 2013; Zhang et al., 2016). However, there are few reports on the regularity of expression and activity of antioxidant enzymes in different genotypes of plants under different Al concentrations for different stress durations. In this study, the expression levels and activities of antioxidant enzymes in the root tips of SB and RB plants treated with different Al concentrations and stress durations were analyzed. SOD activity was lowest in SB and RB roots, whereas POD and CAT activities were high when the Al concentration was lower than 100 μM. In general, greater concentrations (200 μM and 400 μM) of Al inhibited the expression and activity of the genes of three antioxidant enzymes in SB, though activities in RB were still greater than those in CK. These results demonstrate that RB has a stronger anti-Al toxicity capacity than SB.

The concentration and duration of Al stress significantly increased the production of ROS in soybean root tips (Fig. 1). Such accumulation of intracellular ROS promoted an increase in MDA content, but the

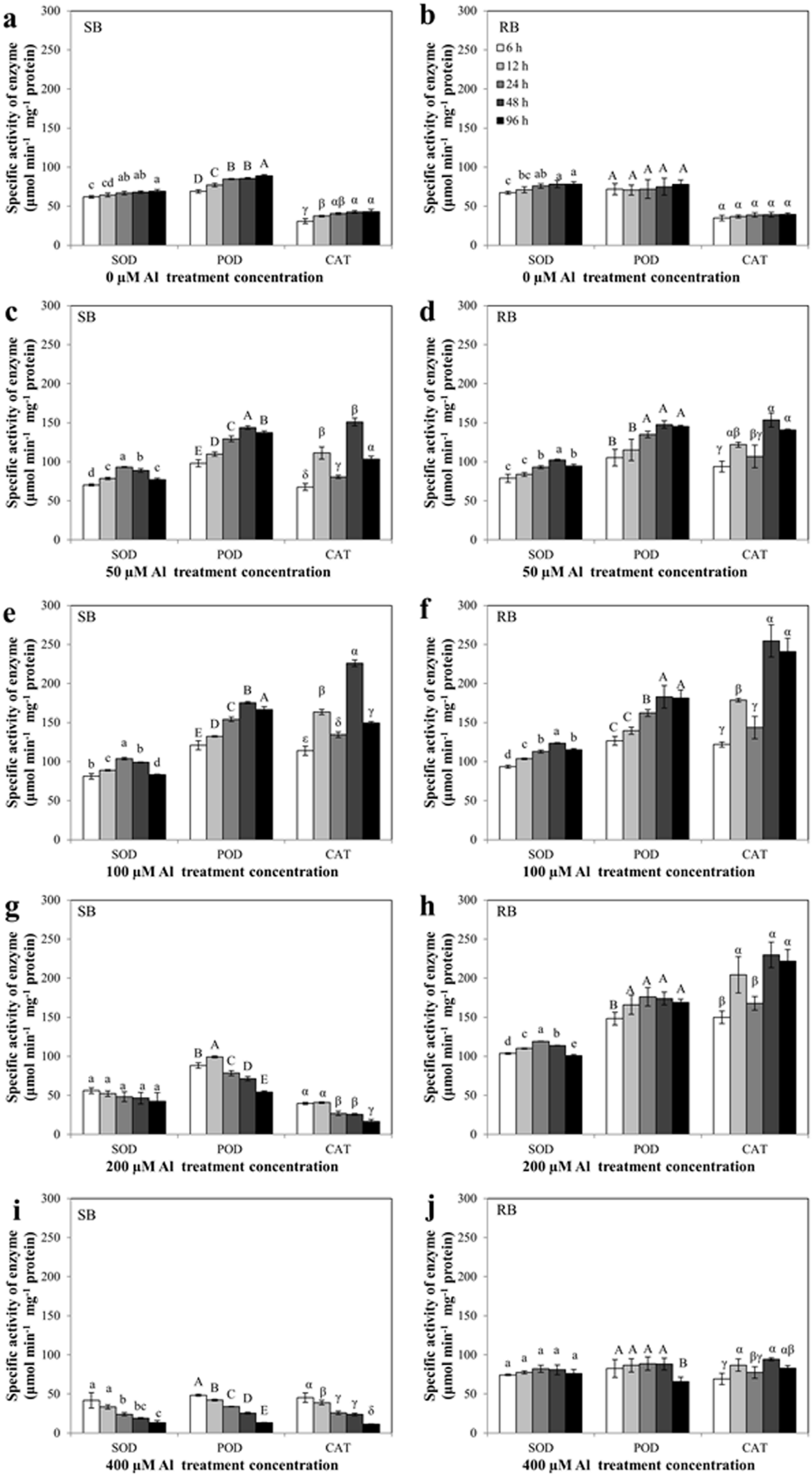

Fig. 7. Spatiotemporal expression of three enzymes in the root tips of soybean plants under Al stress. The different lowercase, uppercase and Greek letters indicate differences in SOD, POD and CAT enzyme activity, respectively, at the 0.05 level.

accumulation of ROS and production of MDA differed between the two genotypes (Figs. 1 and 2). Specifically, the contents of ROS and MDA in SB were significantly greater than those in RB under the same Al treatment concentration and duration. The results also showed that ROS accumulation in soybean root tips was affected by genotype, and changes in H_2O_2 and MDA contents and plasma membrane permeability were consistent with those reported by Liu et al. (2017). The increase in soluble protein content under stress is an important means of maintaining normal osmotic pressure in plants (Šimková et al., 2016). We found that soluble protein contents in SB treated with less than 100 μM Al and RB treated with less than 200 μM Al were significantly greater than those in CK and increased with increasing Al treatment concentration (Fig. 3). The soluble protein content in SB treated with Al at concentrations greater than 100 μM was lower than that in CK and decreased with increasing Al concentration and treatment duration, though the soluble protein content in RB treated with a high concentration of Al (400 μM) was still greater than that in CK. A recent study speculated that a low concentration of Al is able to induce expression of genes related to protein synthesis in plant cells and increase protein synthesis (Aremu et al., 2011). However, the decrease in protein synthase activity and the increase in protein catabolic activity in plants under Al stress results in a reduction in the soluble protein content, which can damage plants (Kidd et al., 2001).

The plant antioxidant system plays an important role in eliminating oxidative stress caused by the accumulation of ROS. Under normal physiological conditions, ROS in plants are in a dynamic balance of continuous production and removal. This balance in plants becomes disrupted when plants are exposed to high-Al stress (Zhang et al., 2016). SOD, POD and CAT are important enzymes involved in metabolizing ROS in plants. SOD, which exists mainly in the cytoplasm, chloroplasts and mitochondria, represents the first line of defense against oxidative stress in plants. When the content of ROS increases, the synthesis and expression of SOD, which catalyzes the conversion of O^{2-} and plasma free radical ions to H_2O_2, increase. POD and CAT remove the excess H_2O_2 produced by SOD in cells to maintain normal cellular levels of H_2O_2 (Nanda et al., 2010).

The results of this study indicate that the gene expression and activity of antioxidant enzymes in different Al-tolerant genotypes are related to the concentration and duration of Al treatment. The expression levels and activities of the SOD, POD and CAT genes in RB were greater than those in SB under to Al stress, especially in response to concentrations greater than 200 μM (Fig. 7). The order of the three antioxidant enzyme activities in SB and RB under 50 μM Al treatment was POD > CAT > SOD; that under 100 μM Al stress was CAT > POD > SOD. When the Al concentration increased to 200 μM, expression and activity of the three enzymes in SB decreased rapidly below the CK level, with that for CAT decreasing the most (Fig. 7g); however, in RB, enzyme activity was still greater than that in CK, and the order was CAT > POD > SOD (Fig. 7h). When the Al concentration was increased to 400 μM, the gene expression of the three enzymes in SB was severely inhibited, and the activity continued to decline rapidly (Fig. 7 i), though enzyme activity was still greater in RB than in CK; the order was still CAT > POD > SOD (Fig. 7 j). The ability of these enzymes to scavenge the ROS caused by Al stress in SB was low, resulting in Al stress injury, whereas RB exhibited high activity for removing the ROS caused by Al stress and for alleviating the associated damage. Moreover, the results indicated relatively stable SOD activity in the soybean genotypes under different Al concentrations, though POD and CAT activity changed significantly. POD, with relatively high activity, was the main enzyme that removed H_2O_2 under low concentrations of Al stress. In the case of high concentrations of Al stress, CAT enzyme activity was relatively high, and CAT was the main enzyme for removing H_2O_2. The asynchronous results for the three antioxidant enzymes in response to Al stress showed that CAT plays an important role in resistance to Al stress. Thus, the highest level of Al tolerance in soybean will be reached when expression and activity of CAT reaches the highest level under Al stress. If the concentration of Al stress continues to increase, then CAT activity will decline, and the plant will become undergo damage. Therefore, CAT enzyme activity is closely related to Al tolerance in soybean. Moreover, CAT is widely found in plants and animals, and its activity is affected by many biotic and abiotic factors, such as temperature, high salinity, heavy metals, drought and various pathogenic microorganisms (Hu et al., 2016). Studies have shown that plant CAT activity is closely related to high salt tolerance and that CAT activity can be used as an important physiological indicator to identify plant drought tolerance (Arias-Moreno et al., 2017; Miller et al., 2010). Heavy metal stress can reduce the activity of CAT in tobacco and can increase that in both *Monstera deliciosa* and *Cassia angustifolia* Vahl, suggesting different responses to heavy metal stress among plant species (Nanda and Agrawal, 2016).

Studies have shown that the induction and regulation of antioxidant enzymes in response to Al stress differ for various plants. Al stress causes an increase in SOD activity in the roots of rice, soybean, maize and onion (Chen et al., 2011; Mittler, 2002; Wu et al., 2013); CAT activity in maize roots was not affected by Al stress but was inhibited in wheat and rice roots (Gondim et al., 2012; Liu et al., 2018; Wang et al., 2015). Regardless, the mechanism by which Al stress induces or inhibits antioxidant enzyme activity needs to be further studied.

In summary, compared with CK root tips, both SB and RB root tips treated with Al at concentrations lower than 100 μM presented relatively low levels of H_2O_2 and MDA and relatively high soluble protein contents. Plants can reduce damage due to Al stress by activating expression and activity of antioxidant enzymes to scavenge ROS. Additionally, protein synthesis-related enzymes are activated to enhance protein synthesis. High concentrations of Al (200 μM and 400 μM) may inhibit expression of antioxidant enzymes in SB and RB, reducing activities and the ability to resist Al stress, resulting in plant damage. The activity of CAT is closely related to the Al tolerance of soybean. High expression and activity of CAT in soybean under high-Al stress confers strong resistance to Al toxicity, which determines the Al tolerance of the plants. The molecular mechanism by which Al stress affects the expression and activity of antioxidant enzymes needs further investigation.

Contributions

LKZ and ZCQ contributed to the conception of the study. ZCQ and LWX contributed significantly to the analysis and manuscript preparation. ZCQ, HJY and FDN performed the data analyses and wrote the manuscript. ZCQ, CLM and XHN assisted with the analysis via constructive discussions.

Acknowledgment

This work was supported in part by the National Natural Science Foundation of China (No. 31560351; No. 31760349).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.09.026.

References

Ali, M.B., Hahn, E.J., Paek, K.Y., 2005. Effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in Phalaenopsis. J. Plant Biochem Physiol. 43, 213–223.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. J. Annu Rev Plant Biol. 55, 373–399.

Aremu, D.A., Ezomo, O.F., Meshitsuka, S., 2011. Gene expression in primary cultured astrocytes affected by aluminum: alteration of chaperons involved in protein folding. J. Environ Health Prev Med. 16, 16–24.

Arias-Moreno, D.M., Jimenez-Bremont, J.F., Marurilopez, I., Delgado-Sánchez, P., 2017. Effects of catalase on chloroplast arrangement in Opuntia streptacantha chlorenchyma cells under salt stress. J. Sci Rep-UK 7, 8656.

Becana, M., Dalton, D.A., Moran, J.F., Iturbeormaetxe, I., Matamoros, M.A., Rubio, M.C., 2010. Reactive oxygen species and antioxidants in legume nodules. J. Physiol Plant. 109, 372–381.

Cai, Y.Z., Zhu, J.B., Hao, X.Y., Wang, A.Y., Xu, D.X., 2013. Drought resistance of KatG transgenic cotton. J. Acta Agriculturae Boreali-occidentalis Sinica. 22, 56–61.

Chen, Q., Zhang, X.D., Wang, S.S., Wang, Q.F., Chen, L.M., 2011. Transcriptional and physiological changes of alfalfa in response to aluminium stress. J. J Agric Sci . 149, 737–751.

Dhar, N., Rana, S., Razdan, S., Bhat, W.W., Hussain, A., Dhar, R.S., Vaishnavi, S., Hamid, A., Vishwakarma, R., Lattoo, S.K., 2014. Cloning and functional characterization of three branch point oxidosqualene cyclases from Withania somnifera (L.) dunal. J. J Biol Chem. 289, 17249–17267.

Gondim, F.A., Gomes-Filho, E., Costa, J.H., Mendes Alencar, N.L., Prisco, J.T., 2012. Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize. J. Plant Physiol Biochem. 56, 62–71.

Hao, F., Wang, X., Chen, J., 2006. Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. J. Plant Sci. 170, 151–158.

Hu, L.F., Yang, Y.G., Jiang, L.W., Liu, S.Q., 2016. The catalase gene family in cucumber: genome- wide identification and organization. J. Genet Mol Biol. 39, 408–415.

Jiang, Q., Gao, Z.X., Lv, C.Y., Hu, H.J., Han, C., 2016. Research progress on mechanism of plant resistance to aluminum toxicity. 2016. J. Nanfang Nongye Xuebao. 10, 211–213 (chinese).

Kanazawa, S., Sano, S., Koshiba, T., Ushimaru, T., 2010. Changes in antioxidative enzymes in cucumber cotyledons during natural senescence: comparison with those during dark-induced senescence. J. Physiol Plant. 109, 211–216.

Kidd, P.S., Llugany, M., Poschenrieder, C., Gunsé, B., Barceló, J., 2001. The role of root exudates in aluminum resistance and silicon-induced amelioration of aluminum toxicity in three varieties of maize (Zea mays L.). J. J Exp Bot 52, 1339–1352.

Kochian, L.V., Hoekenga, O.A., Pineros, M.A., 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. J. Annu Rev Plant Biol. 55, 459–493.

Liu, N., Song, F., Zhu, X., You, J., Yang, Z., Li, X., 2017. Salicylic acid alleviates aluminum toxicity in soybean roots through modulation of reactive oxygen species metabolism. J. Front Chem. 5, 96.

Liu, W., Xu, F., Lv, T., Zhou, W.W., Chen, Y., Jin, C.W., Lu, L.L., Lin, X.Y., 2018. Spatial responses of antioxidative system to aluminum stress in roots of wheat (Triticum aestivum L.) plants. J. Sci Total Environ. 627, 462–469.

Matsumoto, H., 2000. Cell biology of aluminum toxicity and tolerance in higher plants. J. Int Rev Cytol. 200, 1.

Matsumura, T., Tabayashi, N., Kamagata, Y., Souma, C., Saruyama, H., 2002. Wheat catalase expressed in transgenic rice can improve tolerance against low temperature stress. J. Physiol Plantarum. 116, 17–327.

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. J. Plant Cell Environ. 33, 453–467.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. J. Trends Plant Sci. 7, 405–410.

Nanda, A.K., Andrio, E., Marino, D., Pauly, N., Dunand, C., 2010. Reactive oxygen species during plant-microorganism early interactions. J. J Integr Plant Biol. 52, 195–204.

Nanda, R., Agrawal, V., 2016. Elucidation of zinc and copper induced oxidative stress, DNA damage and activation of defence system during seed germination in Cassia angustifolia Vahl. J. Environ Exp Bot. 125, 31–41.

Polidoros, A.N., Mylona, P.V., Scandalios, J.G., 2001. Transgenic tobacco plants expressing the maize Cat2 gene have altered catalase levels that affect plant- pathogen interactions and resistance to oxidative stress. J. Transgenic Res. 10, 555–569.

Riaz, M., Yan, L., Wu, X.W., Hussain, S., Aziz, O., Wang, Y.H., Imran, M., Jiang, C.C., 2018. Boron alleviates the aluminum toxicity in trifoliate orange by regulating antioxidant defense system and reducing root cell injury. J. J Environ Manage. 208, 149–158.

Saxena, I., Srikanth, S., Chen, Z., 2016. Cross talk between H_2O_2 and interacting signal molecules under plant stress response. J. Front Plant Sci. 7, 570.

Šimková, L., Fialová, I., Vaculíková, M., Luxová, M., 2016. The effect of silicon on the activity and isozymes pattern of antioxidative enzymes of young maize roots under zinc stress. J. Silicon. 375, 1–4.

Swanson, S., Gilroy, S., 2010. ROS in plant development. J. Plant Physiol. 138, 384–392.

Takada, Y., Hachiya, M., Park, S.H., Osawa, Y., Ozawa, T., Akashi, M., 2002. Role of reactive oxygen species in cells overexpressing manganese superoxide dismutase mechanism for induction of radioresistance. J. Mol Cancer Res. 1, 137–146.

Vassilev, A., Lidon, F., 2011. Cd-induced membrane damages and changes in soluble protein and free amino acid contents in young barley plants. Emirates J Food Agr 23, 130–136.

Wang, L., Fan, X.W., Pan, J.L., Huang, Z.B., Li, Y.Z., 2015. Physiological characterization of maize tolerance to low dose of aluminum, highlighted by promoted leaf growth. J. Planta. 242, 1391–1403.

Wang, N., Zhang, W., Qin, M., Li, S., Qiao, M., Liu, Z., Xiang, F., 2017. Drought tolerance conferred in soybean (Glycine max. L) by GmMYB84, a novel R2R3-MYB transcription factor. J. Plant Cell Physiol. 58, 1764–1776.

Wu, K., Xiao, S., Chen, Q., Wang, Q.F., Zhang, Y.N., Li, K.Z., Yu, Y.X., Chen, L.M., 2013. Changes in the activity and transcription of antioxidant enzymes in response to Al stress in black soybeans. J. Plant Mol Biol Report. 31, 141–150.

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumoto, H., 2003. Oxidative stress triggered by aluminum in plant roots. J. Plant & Soil. 255, 239–243.

Yin, L., Wang, S., Eltayeb, A., Uddin, M.I., Yamamoto, Y., Tsuji, W., Takeuchi, Y., Tanaka, K., 2010. Overexpression of dehydroascorbate reductase, but not monodehydroascorbate reductase, confers tolerance to aluminum stress in transgenic tobacco. J. Planta. 231, 609–621.

Yuan, H.M., Liu, W.C., Jin, Y., Lu, Y.T., 2013. Role of ROS and auxin in plant response to metal-mediated stress. J. Plant Signal Behav. 8, 234–245.

Zhang, M., Smith, J., Andrew, C., Harberd, N.P., Jiang, C., 2016. The regulatory roles of ethylene and reactive oxygen species (ROS) in plant salt stress responses. J. Plant Mol Biol. 91, 651–659.

Zhao, M.X., Wen, J.L., Wang, L., Wang, X.P., Chen, T.S., 2019. Intracellular catalase activity instead of glutathione level dominates the resistance of cells to reactive oxygen species. J. Cell Stress Chaperones. 24, 609.