Changes In Antioxidant Systems And Sucrose Metabolism In Maize Varieties Exposed To Cd

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Research Article

Keywords: cadmium stress, antioxidant systems, sucrose metabolism, Maize, phytohormone, seed germination

Posted Date: September 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-830110/v1

Abstract

While it is generally accepted that different maize varieties respond in various ways to cadmium (Cd) stress, the physiological mechanisms that determine how they respond are not well-defined. We do know, however, that antioxidant systems and sucrose metabolism help plants to cope with abiotic stresses, including stress from Cd. Seed is sensitive to Cd stress during germination stage. In this study, we investigated how the antioxidant systems, sucrose metabolism, abscisic acid (ABA) and gibberellin (GA_3) concentration in two maize varieties with low (FY9) or high (SY33) sensitivities to Cd changed when Cd was added at 20 mg L^{-1} over different germination stages (3, 6, and 9 days). As Cd accumulated, the germination rate decreased, and growth was inhibited. The $O_2^{\bullet-}$, malondialdehyde (MDA), and proline (Pro) concentrations, and the superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and lipoxygenase (LOX) activities increased compared to the CK (without Cd). The expression levels of three genes (ZmOPR2, ZmOPR5 and ZmPP2C6) that respond to oxidative stress increased differently in two varieties under Cd stress. The activity of the antioxidant system including the transcript levels of oxidative stress response genes were higher in Cd-tolerant variety, FY9, than in sensitive variety, SY33. And then, we also examined sucrose metabolism levels that were increased compared to the CK. However, it was more active in the Cd-sensitive variety, SY33. Therefore, these results also suggest that antioxidant systems are first respond to Cd stress in maize plants, and sucrose metabolism is cooperation and complement that are exposed to Cd.

Introduction

Between 4,000 and 13,000 t of cadmium (Cd), a heavy metal, are released into the environment each year from human industrial activities (ATSDR. 2005). In the environment, Cd causes problems for humans because of its high toxicity and high solubility in water (Guo et al. 2014). Cd can be absorbed easily by crop roots and transported throughout the entire plant, even to the seeds, and, once in the food chain, poses a threat to human health (Satohnagasawa et al. 2012). When Cd accumulates in plants, photosynthesis is inhibited, biomass and yields decrease, and plants might die (Skorzynskapolit et al. 2010; Zhang et al. 2013).

In plant, the mechanisms to overcome the Cd stress are complex. The Cd absorbed from the environment are first deposited in cell wall or chelated by organic acids and phytochelatins (Keltjens et al., 1998; Radhouane et al., 2006). It may be a mechanism by which plants avoid Cd stress. Other free Cd in the cell induced the damage to plants. In plant cells, Cd induces the production of reactive oxygen species (ROS), including singlet oxygen (O_2), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), which can impair redox homeostasis, leading to peroxidation of membrane fatty acids and enzyme inactivation (Sharma and Dietz 2009; Gill and Tuteja 2010). Meanwhile many metabolism systems activated to respond to Cd stress are also very important defense mechanisms. Through evolution and artificial selection, plants have developed multifarious strategies to cope with accumulation of ROS by Cd stress. For example, plants use their antioxidant systems to regulate their response to Cd stress (Mittler et

al. 2004). Antioxidant enzymes such as SOD, POD, and CAT scavenge and decompose ROS to maintain redox in a steady state to save cells (Moller et al. 2007). The activities and transcript levels of antioxidative enzymes were affected on the response of plants to oxidative stress. Free L-proline (Pro) is a non-enzyme antioxidant that accumulates in plants stressed by heavy metals (Sharma and Dietz 2006). It can reduce the toxicity of ROS by scavenging O_2 and OH^- to protecting the cellulose backbone (Kaul et al. 2008). Seeds are very sensitive to environmental factors during germination, the first phase in the life cycle (Jisha et al. 2013). Germination begins when dry seeds absorb water. The reserves in seeds are mobilized to drive different metabolic processes (Bewley 1997). Stress from heavy metals can inhibit or delay seed germination and impair the establishment of seedlings (Ahmad et al. 2012). Previous studies reported that the redox balances and the mobilization of reserves could be disturbed if crop seeds experienced Cd stress during germination (Kuriakose and Prasad 2008; Junyu et al. 2008). Seed germination is also regulated by plant hormones, such as abscisic acid (ABA) and gibberellic acid (GA). These two hormones play antagonistic roles in regulating seed germination (Kucera et al. 2005; Holdsworth et al. 2008). ABA maintains dormancy and inhibits germination, while GA regulates dormancy release and promotes germination. These two hormones are also involved in the signal transduction pathways of abiotic stresses, and the levels of ABA and GA may change in plants that are subjected to Cd or other heavy metals (Guo et al. 2019). Sucrose metabolism also plays an important role in a plant's growth, development, and response to stress. The metabolites such as sucrose, glucose, fructose, and other soluble sugars, that are produced during sucrose metabolism are directly or indirectly involved in protecting plants from abiotic stresses, for example, as signal molecules that regulate gene expression and osmotic protectants that protect biomolecules and membranes (Ruan 2014). Studies have shown that the metabolite concentrations in plants may change under Cd stress (Verma and Dubey 2001; Sfaxibousbih et al. 2010).

Different accessions of the same crop respond differently to Cd stress. Researchers have noticed that the amounts of Cd that accumulate, and the responses to Cd, vary considerably among different varieties of several crops (Liu et al. 2007; Guo et al. 2019). Maize (*Zea mays* L.) is an important grain crop that is grown worldwide. Cd impairs the growth of maize, leading to decreased yields (Zhao et al. 2018). The effects of Cd on different maize varieties have been studied by various researchers; for example, Zhao et al. (2018) analyzed the genetic structure of Cd accumulation in 269 maize accessions.

Seed was more sensitive to Cd stress in germination than seedling stage. The sucrose and substrate of sucrose metabolism are mainly derived from photosynthesis in seedling stage, while those were derived from the decomposition products of stored substances in grains during germination stage. In previous research, we also found that sucrose metabolism that protects the plant from Cd stress was more active in SY33 than in FY9 in seedling stage (Li et al. 2020). However, the underlying physiological mechanisms that help to mitigate and alleviate Cd toxicity during germination of different varieties of maize are not completely understood. The responses to Cd toxicity in plant are complex processes involving multiple systems. Therefore, in this study we investigated how Cd affected two varieties of maize, a Cd-tolerant

variety (FY-9) and a Cd-sensitive variety (SY33), during the germination phase and evaluated how antioxidant systems and sucrose metabolism interacted to alleviate Cd toxicity.

Materials And Methods

Plant material, growth conditions, and Cd treatments.

Seeds of SY33 and FY9 were obtained from the Shenyang Academy of Agricultural Sciences and the Dongya Seed Company, Shenyang, Liaoning Province, China, respectively. The seeds were sterilized with 1% sodium hypochlorite solution (v/v) for 10 min, rinsed with sterile water at least three times, and then soaked in distilled water for 12 h. One part of soaked seeds was used for germination analysis. The same number seeds were transferred into a petri-dished lined with three filter papers and covered with gauze that were moistened with distilled water or 20 mg L^{-1} $CdCl_2$ and placed in an incubator at 28°C in the dark to germinate for 9 days. Germination rates were then calculated, respectively.

The other part of soaked seeds was used for sampled for other assays. The soaked seeds were transferred in to petri-dishes lined with three filter papers and covered with gauze that was moistened with distilled water or $CdCl_2$ at 20 mg L^{-1}, and germinated for 3 days, then were transferred into plastic containers and cultivated in a 1/4 Hoagland solution (pH 6.0) with 20 mg L^{-1} $CdCl_2$ treatment at 28°C and a 16/8 h light/dark photoperiod for 3 or 6 days. A control was also set up, without cadmium. Tissues were collected and stored directly in liquid nitrogen or dried at 80°C until the weight was constant before storage. Each treatment was repeated at least four times, with three seedlings in each replicate.

Calculation of the relative growth rate.

The seed was considered germinated when the radicle or coleoptile was at least 2 mm. The germination rates (%) were calculated as the number of seeds out of the total number of seeds that germinated in 3, 6, and 9 days in either water or Cd solution (20 mg L^{-1}). The length of the plumules and radicles or shoots and roots of between 10 and 15 of the Cd-treated plants for each time period were measured with a ruler. The biomass was determined by measuring the dry weights of the plumules and radicles or shoots and roots.

Cd estimation.

The dry plant tissues were ground to powder, and then 0.2 g of the dry powder was digested with HNO_3 and $HClO_4$ (v/v, 83/17) for 24 h. The Cd concentration was measured using an atomic absorption spectrophotometer.

Determination of the $O_2^{\bullet-}$, MDA, and Pro concentrations and the relative electrolyte leakage rates.

To determine the concentration of $O_2^{\bullet-}$, a portion of the sample (2.0 g) was mixed with 3 mL 65 nmol L^{-1} of a phosphate buffer (pH 7.8) and centrifuged for 10 min at 10,000 rpm. The supernatant (2.0 mL) was

mixed with a phosphate buffer (1.5 mL) and hydroxylamine hydrochloride (0.5 mL) at 25°C for 20 min. Then 2.0 mL of the reaction mixture was mixed with 17 mmol L^{-1} sulfanilic acid (2.0 mL) and 27 mmol L^{-1} of α-naphthylamine (2.0 mL) at 30°C for 30 min. The absorbance was measured at 530 nm using a UV-T6 spectrophotometer.

The concentrations of MDA were measured using the thiobarbituric acid chromogenic method as described by Aravind and Prasad (2003). The free L-proline concentrations and relative electrolyte leakage rates were measured as described by Bates et al. (1973)

Determination of the plant hormones.

Fresh tissue (1 g) was ground to powder and mixed with 10 mL of 80% methanol and 0.2 g of Crosslinked Polyvinylpyrrolidone at 4°C for 12 h and centrifuged at 15,000 rpm for 10 min. The supernatant was extracted by ethyl acetate three times and then dissolved in methanol and stored at −20°C. The levels of GA_3 and ABA were measured using a slightly modified version of the method described by Jia et al. (2020).

Determination of sugar concentrations.

For the sugar assays, the dried samples were powdered and homogenized in 80% ethanol, boiled at 70°C for 30 min, and centrifuged at 8000 g at 4°C for 10 min. The total soluble sugars in the supernatants were measured as described by McCready et al. (1950). The fructose, glucose, and sucrose concentrations were measured by high performance liquid chromatography (HPLC, Waters 600 HPLC) using the method of Sanchez-Linares et al. (2012).

Enzyme activity assay.

The activity of LOX was determined as described by Surrey (1964). 1 g fresh tissue was powdered and homogenized with 50 mM Hepes (pH 7.0), 5 mM cysteine, and 10 mM EDTA. The mixture was centrifuged at 10,000 g at 4°C for 20 min, and the supernatant was examined for enzymes.

To determine the activities of the antioxidant enzymes, samples of fresh plant tissue (500 mg) were homogenized in 5 mL of 100 mM pre-cooled phosphate buffer (pH 7.5) containing 1 mM EDTA. The homogenate was centrifuged at 12,000 g for 15 min at 4°C and the activities of antioxidant enzymes of the supernatant were determined. The SOD activity was examined spectrophotometrically at 560 nm, as described by Tewari et al. (2008). The POD activity was measured as the guaiacol oxidation at 470 nm by H_20_2, as described by Lacan and Baccou. (1998). The CAT activity was determined by measuring the decrease in H_20_2 at 240 nm, as described by Ishibashi et al. (2008).

To measure the activities of the sucrose metabolism enzymes, fresh plant tissues (1 g) were ground to powder with quartz sand and homogenized with a 50 mM phosphate buffer (pH 7.5) at 4°C. After centrifugation at 12,000 g for 20 min at 4°C, the supernatant was divided into two portions, one of which

was analyzed for the activities of SPS, SS, and NI, as described by Saher et al. (2005), and the other was stored at − 80°C. Meanwhile, the precipitate was resuspended in the same extraction buffer with 1 M KCl and agitated continuously for 60 min at 4°C. The homogenate was centrifuged at 12,000 g for 20 min, and then the supernatant was mixed with the stored supernatant solution. The AI activities of the mixture were determined using the method of Saher et al. (2005).

RNA extraction and analysis.

RNA was isolated from tissues using an RNA pure Plant Kit (Qiagen). Total RNA (about 2 μg) was reverse transcribed to synthetic cDNA using a MMLV reverse transcriptase (Promega). Quantitative reverse polymerase chain reaction (qRT-PCR) assays were performed with a real-time PCR detection system (ABI 7500) using SuperReal PreMix Plus Kit (Qiagen). The reaction conditions were 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 30 s. The ΔΔCT method was used to analyze the transcript levels of the relevant genes (Livak et al. 2001). The primers were used to amplificated *SOD* gene were 5'-CGGTGCACCAGAAGACGAAG-3' and 5'-GCCAGTCTTCCACCAGCATT-3'. The *CAT* gene primers were 5'-TCCCAACTACCTGATGCTGC-3' and 5'-GTTGGGCTTGCGTATGGTTG-3'. The *POD* gene primers were 5'-TGGAACACAAGCACGAACCC-3' and 5'-CCTTCCACAGCGTCTCGTT-3'. The *ZmTubulin1* (ID: Zm00001d013367) gene was used as an internal control. The primers of *ZmTubulin1* were 5'-GTGTCCTGTCCACCCACTCTCT-3' and 5'-GGAACTCGTTCACATCAACGTTC-3'.

Statistical analysis.

All data are shown as means and the standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out using SPSS version 26. The significance level was $P < 0.05$. Each experiment had four replicates.

Results

The effects of Cd accumulation on germination rate and biomass of the seeds of two maize varieties

We examined how Cd affected the germination of two maize varieties, FY9 and SY33, selected from 16 varieties that are widely grown in the northeast of China because of their response to Cd stress. The germination rates of the FY9 and SY33 seeds that were treated with 20 mg L^{-1} Cd were 15% and 36% lower than the germination rates of the control seeds, respectively (Fig. 1A). The growth of the plumules and radicles of the seeds treated with Cd for 3 days was also inhibited. The shoots and roots of seedlings treated with Cd for 6 and 9 days showed less growth than the control seedlings, with the seedlings treated with 20 mg L^{-1} Cd showing noticeably less growth. The lengths of the shoots and roots of SY33 were noticeably less than those of FY9 (Table S1). The biomass accumulation was also affected by the Cd treatments, and followed a similar pattern as the lengths of the shoots and roots (Table 1).

We investigated the relationship between the Cd accumulation and growth inhibition by examining the Cd concentrations in the shoots and roots. The Cd concentrations in shoots and roots increased as the

duration of the Cd treatment increased (Fig. 1B and C). The Cd concentrations in the shoots and roots of SY33 were higher and lower than those in FY9, respectively. These data suggest that the Cd stresses impaired the germination and growth of maize, and that SY33 was more sensitive to Cd stress than FY9 during the germination stage.

The effect of Cd on redox homeostasis in the seeds of two maize varieties during germination

The Cd stresses induced oxidative stress in the plants. The concentrations of the main indexes of oxidative damage, LOX, $O_2^{\bullet-}$, and MDA, increased noticeably as the number of Cd treatment days increased (Fig. 2A-F). With 20 mg L^{-1} Cd treatment for 9 days, the LOX concentrations were 23.69% and 35.45% higher in the leaves, and 34.06% and 42.85% higher in the roots, of FY9 and SY33 than in CK, respectively; the $O_2^{\bullet-}$ concentrations were 26.40% and 36.86% higher in the shoots, and 31.53% and 54.10% higher in the roots, of FY9 and SY33 than in CK, while the MDA concentrations were 42.51% and 53.08% higher in the leaves, and 41.13% and 54.98% higher in the roots, of FY9 and SY33 than in CK, respectively. ROS accumulated to higher levels in the Cd-treated SY33 than in Cd-treated FY9. The relative conductivities also increased in the Cd-treated plants, with a greater increase in SY33 than in FY9 (Fig. 2G and H).

We also examined how the oxidative stress changed in the Cd-treated FY9 and SY33. The activities of antioxidative enzymes significantly increased in the leaves and roots as the Cd treatment days increased (Fig. 3A-F). With the 20 mg L^{-1} Cd treatment, the SOD activities in the shoots of FY9 and SY33 were 33.71% and 22.07% higher, while those in the roots of FY9 and SY33 were 15.97% and 9.46% higher, than in Ck, respectively. The activities of POD were 4.98% and 2.83% higher in the shoots, and 3.19% and 1.84% higher in the roots, of FY9 and SY33 than in CK, respectively. The activities of CAT were 3.66% and 2.63% higher in the shoots, and 2.68% and 1.84% higher in the roots, of FY9 and SY33 than in CK, respectively. The activities of the antioxidative enzymes were generally higher in FY9 than in SY33. Proline, a non-enzyme antioxidant, accumulated in the shoots and roots of the Cd-treated plants, with more accumulation in FY9 than in SY33 (Fig. 3G and H). These results show that more antioxidants accumulated in the Cd-tolerant variety, FY9, than in the Cd-sensitive variety, SY33, with oxidative stress having less effect on FY9 than on SY33.

The effects of Cd on endogenous hormones in the seeds of two maize varieties during germination

ABA and GAs are phytohormones that regulate seed germination and responses to abiotic stress. We examined the concentrations of ABA and GA_3 in the Cd-treated plants to determine how these phytohormones changed during seed germination. The ABA concentrations were 29.6% and 36.7% higher in the shoots of FY9 and SY33 treated with 20 mg/L Cd stress for 6 days than in CK, respectively, but did not change noticeably in the roots (Fig. 4A and B). The ABA shoot concentrations were higher in SY33 than in FY9. The GA_3 concentrations in the shoots and roots of the two Cd-treated maize varieties were noticeably lower than in CK (Fig. 4C and D). The data suggest that the accumulation of ABA was greater

than that of GA_3 in the Cd-treated plants, and that these hormones accumulated to higher levels in SY33 than in FY9.

The effects of Cd on sucrose metabolism in the seeds of two maize varieties during germination

Sucrose metabolism is inhibited in Cd-stressed plants. In this study, we used the sucrose metabolite concentrations to indicate the sucrose metabolism. The total soluble sugar concentrations increased considerably in the shoots and roots of FY9 and SY33 under the Cd treatment, with a greater accumulation of total soluble sugars in SY33 than in FY9, especially in the shoots (Fig. 5A and B). The fructose concentrations followed a similar pattern as the total soluble sugars, with more fructose accumulation in SY33 than in FY9 (Fig. 5C and D). The concentrations of sucrose generally increased as the Cd concentrations increased and reached a maximum in the plants treated with 20 mg L^{-1} of Cd for 6 days (Fig. 5E and F). The concentrations of glucose did not change noticeably in the Cd-treated plants (Fig. 5G and H).

The activities of many of the enzymes that help to regulate sucrose metabolism are affected by Cd stress. The activities of SPS decreased in the shoots and roots as the duration of the Cd treatment increased (Fig. S1A and B). The activities of SS in the sucrose synthesis direction also decreased, and showed a greater decrease in SY33 than in FY9 (Fig. S1C and D). However, the activities of sucrose hydrolysis enzymes, including AI, NI, and SS (in the hydrolysis direction), increased significantly in the Cd-treated shoots, particularly in SY33 (Fig. 6A-F). These results suggest that, when treated with Cd, the production and accumulation of soluble sugars in germinating maize seeds increased, especially in SY33, the Cd-sensitive variety.

The transcript levels of antioxidant enzymes in response to Cd stress in two varieties during germinations

Transcript levels of antioxidant enzymes are the important indicators of plants response to Cd stress. We measured the expression of *SOD* (Zm00001d031908), *CAT* (Zm00001d054044) and *POD* (Zm00001d040702) under 20 mg L^{-1} $CdCl_2$ stress for 0, 3, 6 and 9 days. The transcript level of *SOD* increased gradually as Cd treatment time increased in FY9, but that decreased gradually on 6th day Cd treatment in SY33 (Fig. 7A). The transcript levels of CAT significant increased on 3th and 6th under Cd treatment in FY9 and SY33 and decreased lightly on 9th (Fig. 7B). The transcript level of POD significant increased as Cd treatment time increased in FY9, but that was down-regulated on 3th to 6th in SY33 (Fig. 7C). These results suggest that the transcript levels of antioxidant enzymes were different to response to Cd stress in different maize varieties.

Discussion

Many researchers have reported that heavy metal toxicity inhibits seed germination (Sfaxibousbih et al. 2010). While the seed coat is the main barrier that protects the embryo from Cd contamination, the Cd ion can still penetrate into the germinating seeds by imbibition, and delay or inhibit seed germination (Wierzbicka and Obidzinska 1998; Kuriakose and Prasad 2008). When the radicle breaks through the seed

coat, most of the Cd is absorbed from the soil by the root system. The Cd that accumulates in the germinating seeds and seedlings impairs many metabolism processes in plant cells, with effects on the germination rate and biomass accumulation (Sfaxibousbih et al. 2010; Zhang et al. 2013). We found that the germination rate, biomass, and growth, including the lengths of the shoots and roots, of the two different maize varieties decreased when Cd was added, which is consistent with the previous results (Sfaxibousbih et al. 2010; Xu et al. 2014; Guo et al. 2019). Cd had more effect on SY33 than on FY9, and SY33 was more sensitive to Cd than FY9, that is consistent with our previous research[27].

When Cd ions accumulate in cells, the cellular metabolic balance is altered and the generation of ROS is stimulated, causing oxidative stress and inhibiting plant growth and development (Gill and Tuteja, 2010). $O_2^{\bullet-}$, one of the main products of ROS, is generated by xanthine oxidase and NADPH-dependent oxidase that are induced by heavy metals such as Cd (Rio et al. 2006; Rodriguezserrano et al. 2006). LOX induces lipid peroxidation, which then produces MDA in the plants (Liang et al. 2003; Montillet et al. 2004). The LOX activities and the $O_2^{\bullet-}$ and MDA concentrations are good indicators of the damage caused by Cd-induced oxidative stress. The results showed that the LOX activity was enhanced, and the $O_2^{\bullet-}$ and MDA concentrations increased, when exposed to Cd, especially in SY33. Also, the relative conductivity, which represents the degree of damage to cell membranes, was higher in SY33 than in FY9. These results indicate that the Cd caused more damage to SY33 than FY9. In response, plants produce antioxidants to help them cope with the Cd-induced oxidative stress (Liang et al. 2003; Guo et al. 2017). SOD, POD, and CAT are representative enzymatic antioxidants that scavenge ROS induced by biotic and abiotic stressors. SOD scavenges $O_2^{\bullet-}$ by catalyzing the detoxification of $O_2^{\bullet-}$ to O_2, while CAT and POD mainly decompose and remove H_2O_2 (Mittler 2002; Xu et al. 2014). The activities of SOD, POD, and CAT increased as the duration of the Cd treatment increased, and were higher in FY9 than SY33. The expression of *SOD*, *POD* and *CAT* were examined in two varieties. The results showed that transcript levels of *SOD*, *POD* and *CAT* increased under Cd treatment and were obviously higher in FY9 than that in SY33. The changes of transcript level of antioxidant enzymes were similar to the changes of their activities. It may mean that more antioxidant enzymes were transcribed, translated and accumulated in FY9 than that in SY33 under Cd treatment. Therefore, the antioxidant ability is higher in FY9 than SY33. Pro, a representative non-enzymatic antioxidant and osmotic protectant, scavenges OH^- and $^1O_2$, and protects the cellular backbone (Kaul et al. 2008). The concentration of Pro increased in the Cd-treated plants and showed more increase in FY9 than in SY33. While more Cd accumulated in FY9 than in SY33, the antioxidants were more active in FY9 than in SY33, indicating that less ROS accumulated, and the germination rate and growth were better, in FY9 than in SY33. These factors may help to explain why FY9 was tolerant to, and SY33 was sensitive to, Cd stress.

Sucrose metabolism provides energy and material for plant growth, and its products accumulate in plants as dry matter. Meanwhile, various researchers have reported that sucrose metabolism in plants changes when exposed to Cd stress (Verma and Dubey 2001; Junyu et al. 2008; Sfaxibousbih et al. 2010). The sugars help to maintain the cellular osmotic balance and protect cells from Cd stress. We found that the activities of the sucrose metabolism enzymes were modulated by the Cd. The activities of

the sucrose enzymes in the synthesis direction decreased, while the activities of the sucrose hydrolysis enzymes increased. The concentrations of sucrose, glucose, and fructose increased, and the concentrations of total soluble sugars increased noticeably under Cd treatment. It means that more sugars were used to maintain osmotic balance under Cd stress, while less sugars were provided for plant growth when Cd was added, especially in the Cd-sensitive SY33. Oxidative stress induced by Cd damages the cellular membrane and breaks the osmotic balance in cells (Sharma and Dietz 2009; Gill and Tuteja 2010). The results showed that oxidative stress was more serious in SY33 than in FY9, so more soluble sugars accumulated in SY33 to counteract the Cd stress. Together, the above results indicate that the antioxidant system actively protects the cells from damage by ROS, while sucrose metabolism passively maintains the basic environment for physiological activities in Cd-stressed plants. These two systems may be cooperative ones complementary to each other to help plants cope with Cd stress.

Plant hormones regulate the growth and development of plants and help plants respond to abiotic stresses. ABA and GA cooperate to regulate seed germination (Duval et al. 2012). The ABA and GAs concentrations changed when Cd was added (Guo et al. 2019). The GA_3 concentrations first increased and then decreased before Cd was added, but then decreased further as Cd was added and the duration of the treatment increased. GA_3 is needed to activate germination and so its concentrations increased in the early stages of germination and then decreased as the germination progressed. The GA_3 concentrations continued to decrease when the seedlings were treated with Cd. ABA regulates the transcription of resistant genes that respond to Cd stress[36]. The concentrations of ABA in the leaves of FY9 and SY33 obviously increased under the Cd treatment; in their studies, Chaca et al. (2014) and Guo et al. (2019) also observed increases in ABA in soybean and wheat.

In conclusion, we found that, as Cd accumulated in germinating seeds, the transcript levels and activities of antioxidases and Pro concentrations increased, and ROS was scavenged, such that the oxidative damage in FY9 and SY33 decreased. Meanwhile, sucrose metabolism was also induced to maintain the osmotic balance in the damaged cells and to protect the plants from Cd stress. The antioxidant system was more active in the Cd-tolerant variety, FY9, while sucrose metabolism was more important in the Cd-sensitive variety, SY33. These findings suggest that multiple systems involved in the response to Cd stress, among them the antioxidant system and sucrose metabolism provide active and passive responses, respectively in maize during the germination stage.

Declarations

Ethical Approval and Consent to Participate

Not applicable

Consent to Publish

All the authors have consent to publish the content of, this manuscript.

Availability of data and materials

The data and materials obtained in this study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was financially supported by National Key R& D Program of China (No. 2017YFD0300601), the scientific research projects of Liaoning Provincial Department of Education, China (No. LSNZD201608) and National Nature Science Foundation of China (No. 31601233).

Authors Contributions

JJF, CL, YSZ, XLM Planned the experiments. YDC, TFL, MYG performed the experiments. JJF, CL, YDC, Analyzed the data and wrote the manuscript.

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Tables

Table 1 The biomass (dry weight) of the two maize varieties.

Treatment time (d)	Treatment	Variety	Biomass (DW)	
			Shoot	Root
3	CK	FY9	0.0024±0.0003e	0.0022±0.00011f
		SY33	0.0023±0.00019e	0.0021±0.00031f
	Cd	FY9	0.0015±0.00014e	0.0015±0.0002f
		SY33	0.0011±0.00006e	0.001±0.00003f
6	CK	FY9	0.053±0.0013b	0.021±0.0013b
		SY33	0.051±0.00089b	0.019±0.00084b
	Cd	FY9	0.028±0.00092c	0.012±0.00091d
		SY33	0.02±0.0006d	0.008±0.00059e
9	CK	FY9	0.104±0.00066a	0.031±0.0012a
		SY33	0.101±0.0021a	0.03±0.0011a
	Cd	FY9	0.051±0.0047b	0.02±0.00066b
		SY33	0.022±0.0011d	0.014±0.00057c

The data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at $P<0.05$. The experiments were repeated four times.

Figures

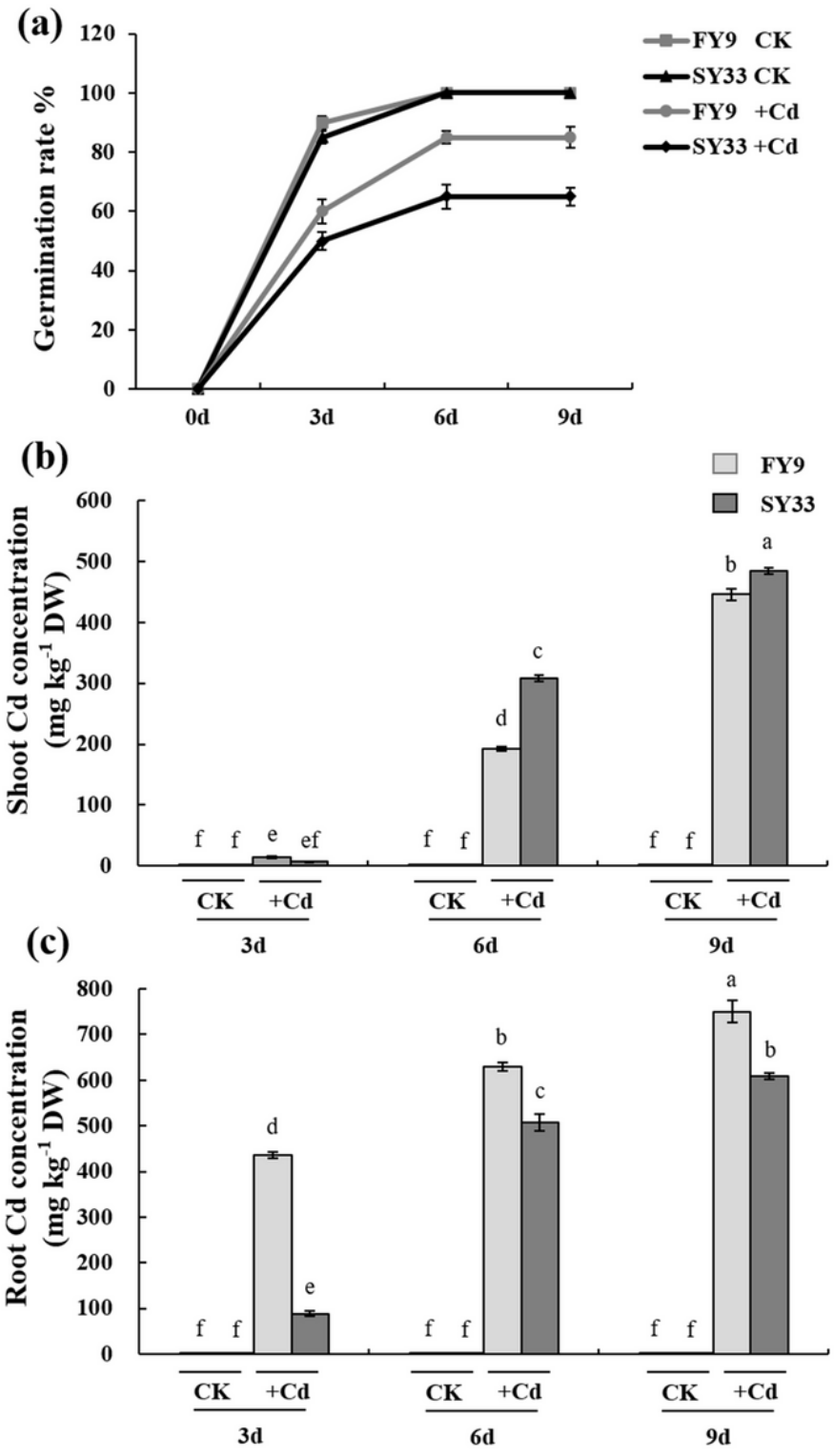

Figure 1

a Germination rate of the two maize varieties at 20 mg L−1 Cd concentrations, respectively, b Cd concentration in the shoots of two maize varieties at 20 mg L−1 Cd concentrations, respectively, c Cd concentration in the roots of two maize varieties at 20 mg L−1 Cd concentration, respectively. The data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at $P<0.05$. The experiments were repeated four times.

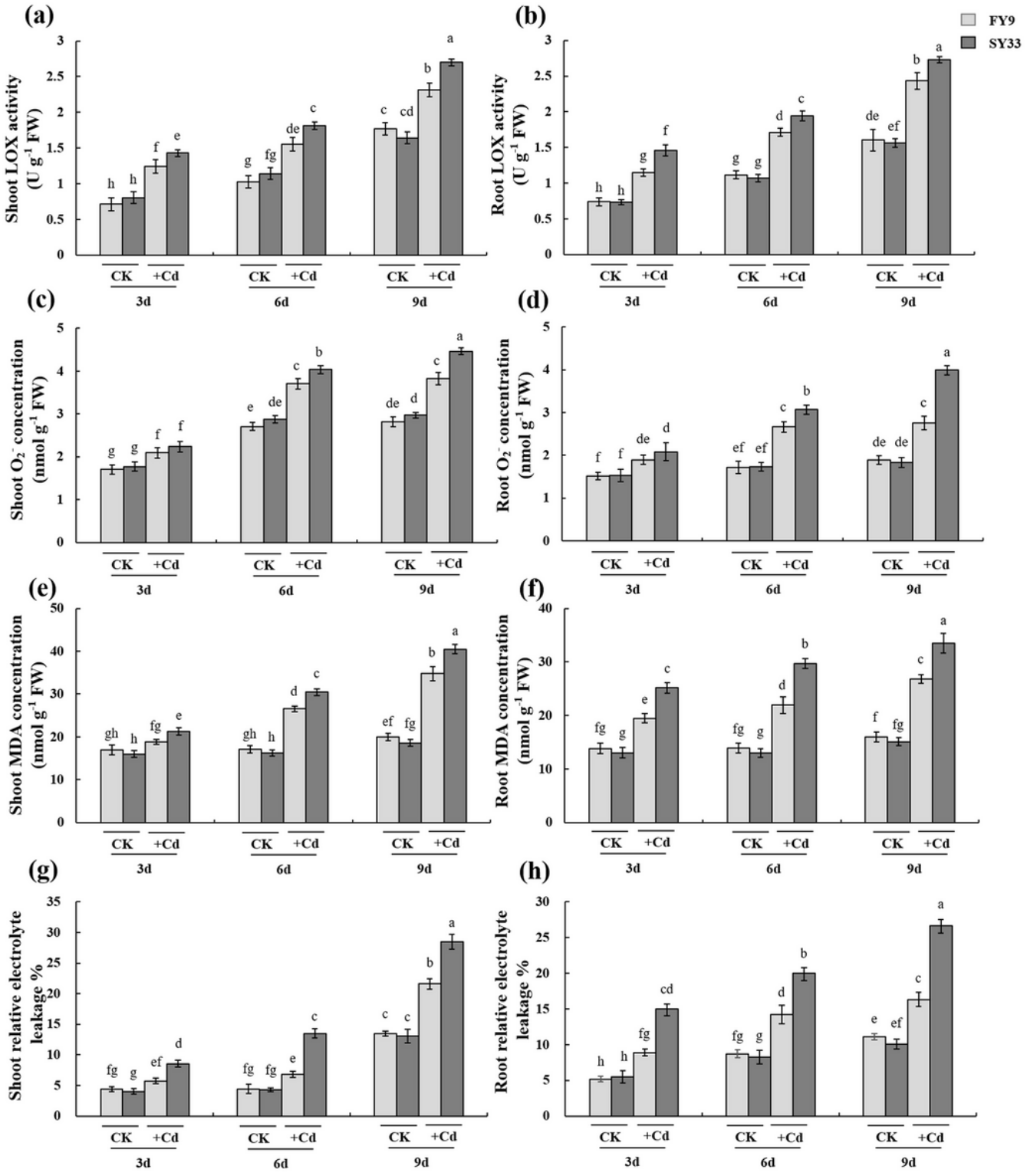

Figure 2

a LOX activity in shoots at 20 mg L−1 Cd concentrations, b LOX activity in roots at 20 mg L−1 Cd concentrations, c O2•- concentration in shoots at 20 mg L−1 Cd concentrations, d O2•- concentration in roots at 20 mg L−1 Cd concentrations, e MDA concentrations in shoots at 20 mg L−1 Cd concentrations, f MDA concentrations in roots at 20 mg L−1 Cd concentrations, g relative electrolyte leakage in shoots at 20 mg L−1 Cd concentrations, h relative electrolyte leakage in roots at 20 mg L−1 Cd concentrations. The

data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at P<0.05. The experiments were repeated four times.

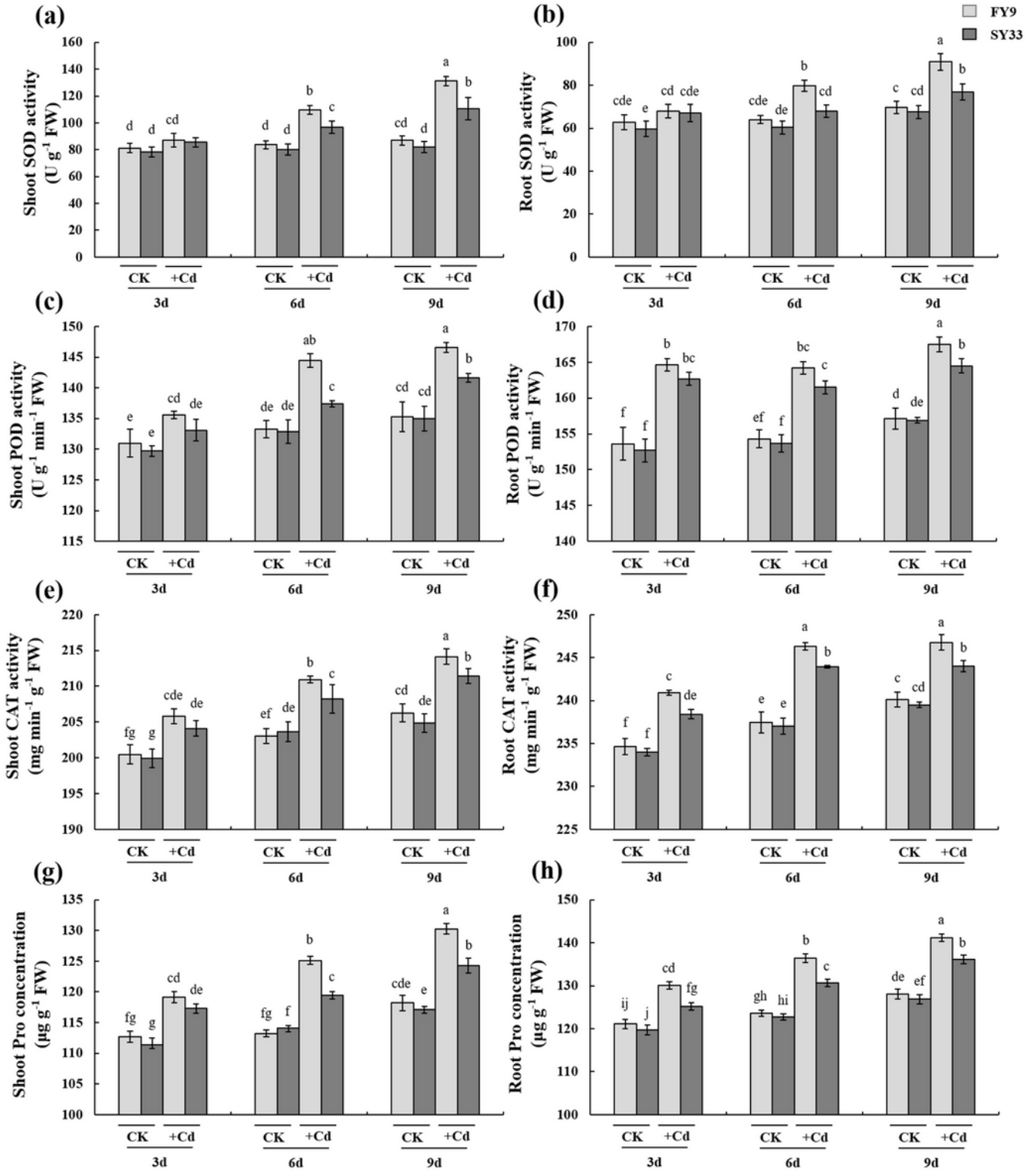

Figure 3

a SOD activity in shoots at 20 mg L−1 Cd concentrations, b SOD activity in roots at 20 mg L−1 Cd concentrations, c POD activity in shoots at 20 mg L−1 Cd concentrations, d POD activity in roots at 20 mg L−1 Cd concentrations, e CAT activity in shoots at 20 mg L−1 Cd concentrations, f CAT activity in roots at

20 mg L−1 Cd concentrations, g Pro concentrations in shoots at 20 mg L−1 Cd concentrations, h Pro concentrations in roots at 20 mg L−1 Cd concentrations. The data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at P<0.05. The experiments were repeated four times.

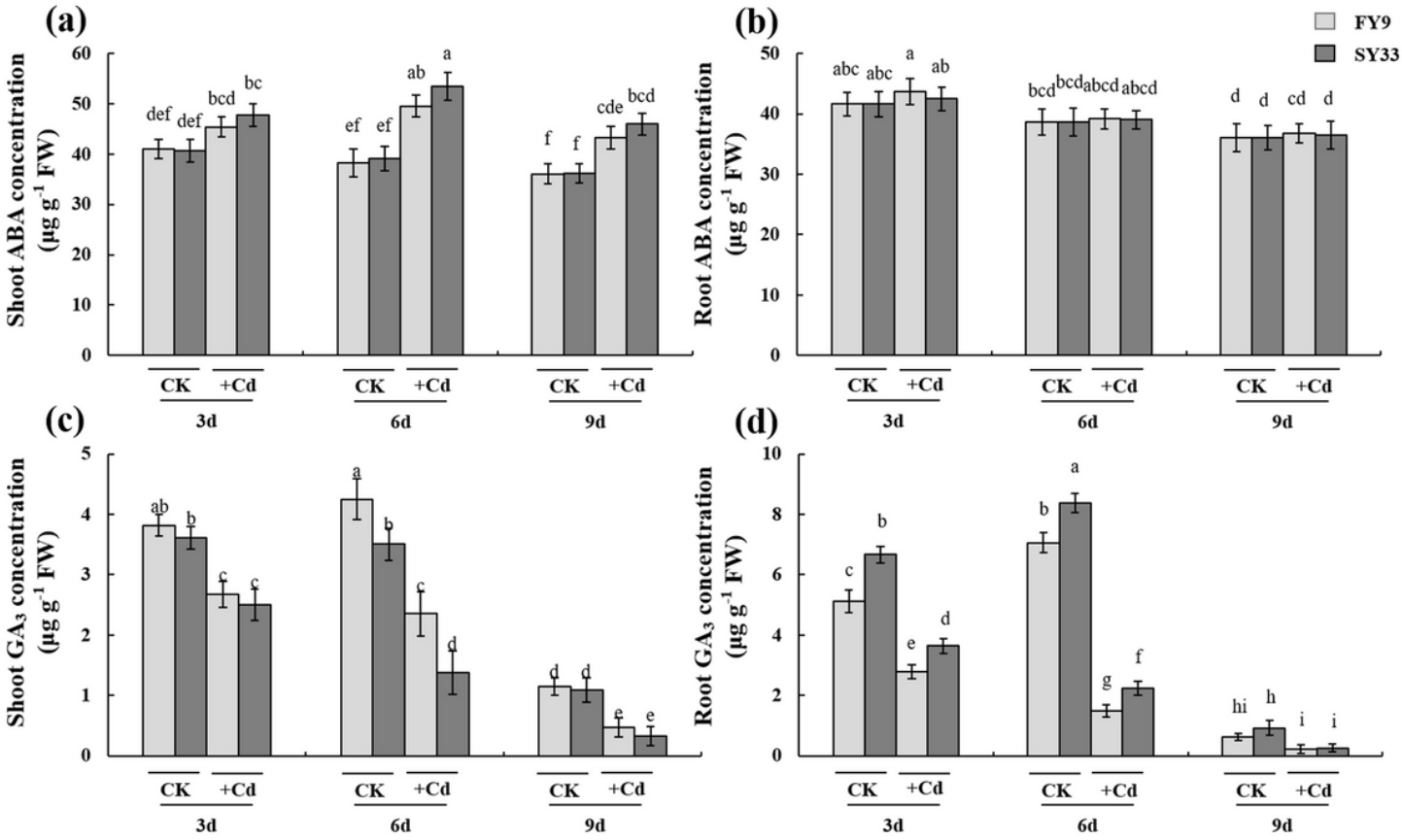

Figure 4

a ABA concentration in shoots at 20 mg L−1 Cd concentrations, b ABA concentration in roots at 20 mg L−1 Cd concentrations, c GA3 concentrations in shoots at 20 mg L−1 Cd concentrations, d GA3 concentrations in roots at 20 mg L−1 Cd concentrations. The data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at P<0.05. The experiments were repeated four times.

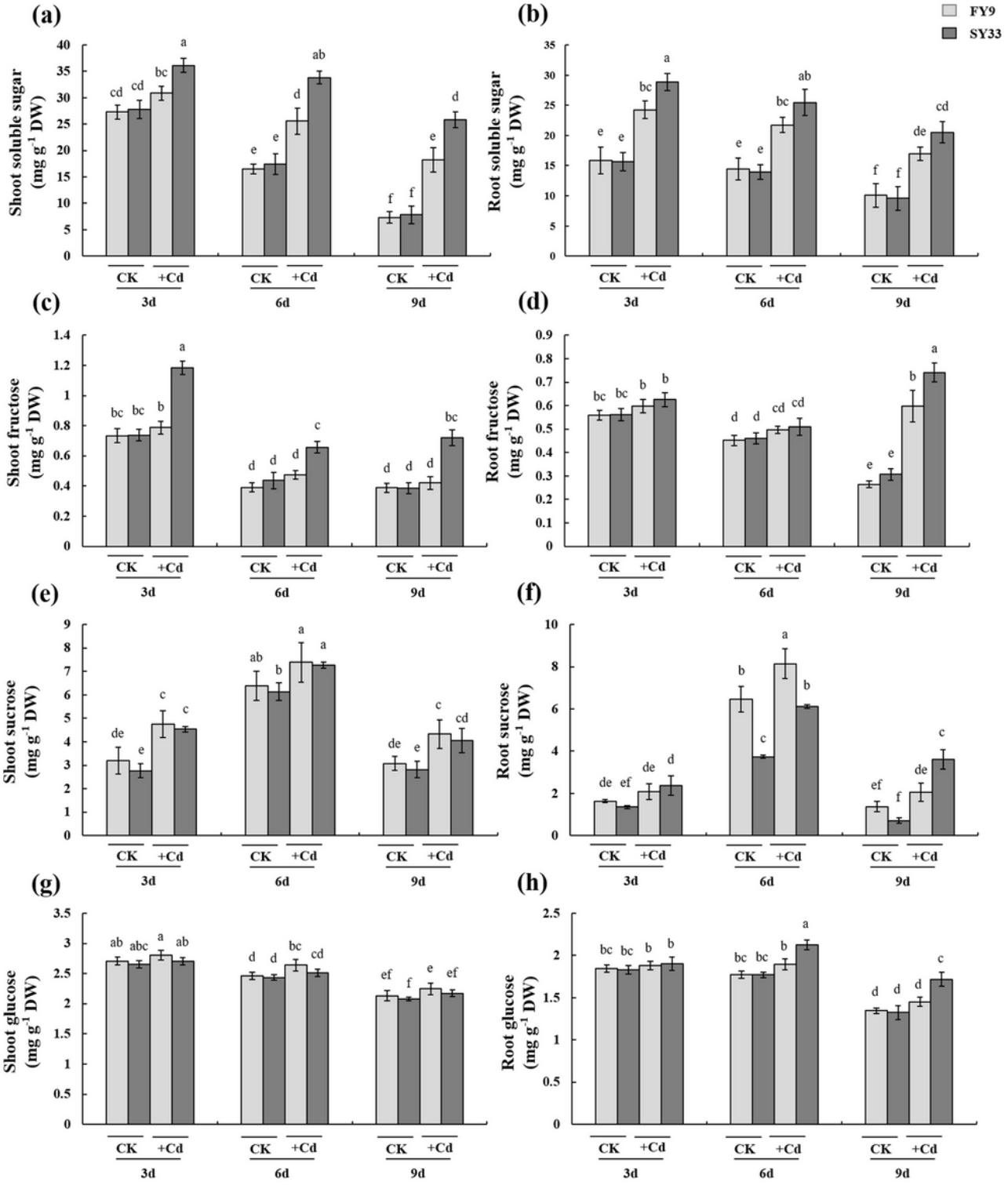

Figure 5

a Total soluble sugar concentrations in the shoots at 20 mg L−1 Cd concentrations, b total soluble sugar concentrations in roots at 20 mg L−1 Cd concentrations, c fructose concentrations in the shoots at 20 mg L−1 Cd concentrations, d fructose concentrations in roots at 20 mg L−1 Cd concentrations, e sucrose concentrations in the shoots at 20 mg L−1 Cd concentrations, f sucrose concentrations in roots at 20 mg L−1 Cd concentrations, g glucose concentrations in the shoots at 20 mg L−1 Cd concentrations, h

glucose concentrations in roots at 20 mg L−1 Cd concentrations. The data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at P<0.05. The experiments were repeated four times.

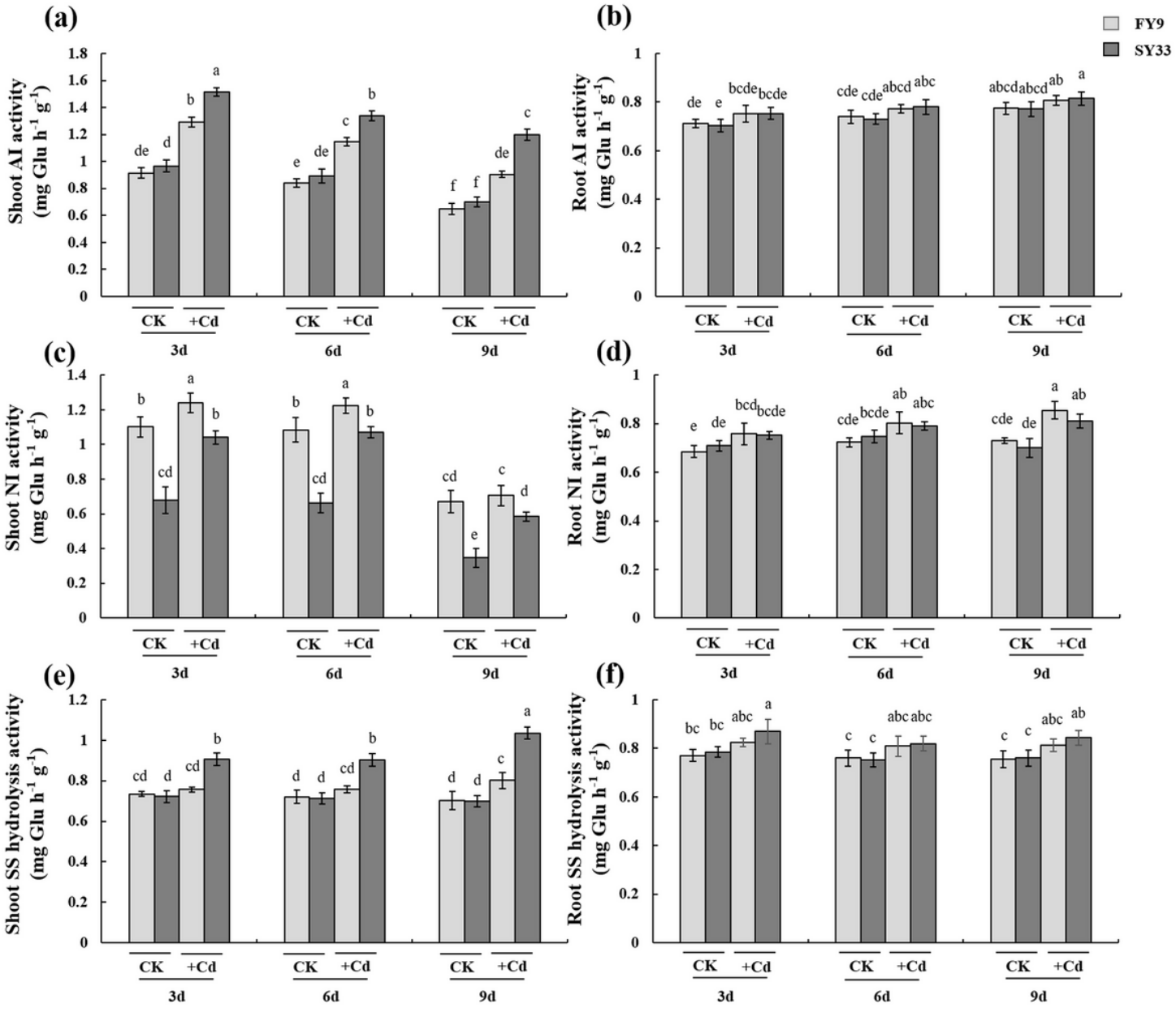

Figure 6

a AI activity in shoots at 20 mg L−1 Cd treatment, b AI activity in roots at 20 mg L−1 Cd treatment, c NI activity in shoots at 20 mg L−1 Cd treatment, d NI activity in roots at 20 mg L−1 Cd treatment, e SS hydrolysis activity in shoots at 20 mg L−1 Cd treatment, f SS hydrolysis activity in roots at 20 mg L−1 Cd treatment. The data shown are the means ± SD (n=4). The different lowercase letters indicate significant differences by Duncan analyze at P<0.05. The experiments were repeated four times.

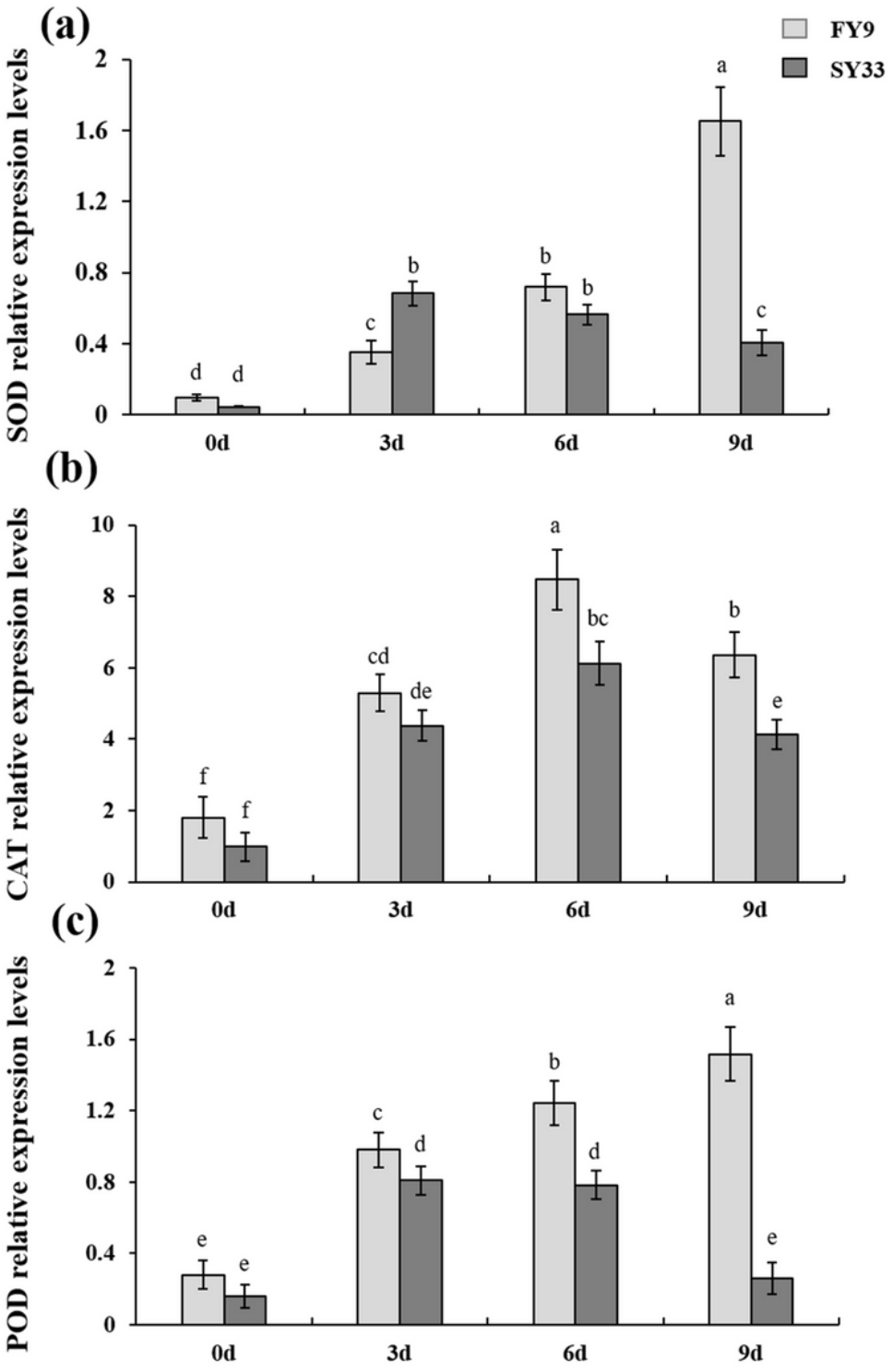

Figure 7

a Expression profiles of SOD genes in FY9 and SY33 at different Cd treatment time, b expression profiles of CAT genes in FY9 and SY33 at different Cd treatment time, c expression profiles of POD genes in FY9 and SY33 at different Cd treatment time. Data are presented as the treatment means ± SD (n=3). Different letters indicate significant difference when $P < 0.05$ (Duncan's test).

Supplementary Files

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