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Effects of cadmium exposure on the growth, photosynthesis, and antioxidant defense system in two radish (*Raphanus sativus* L.) cultivars

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Abstract

Radish is representative root vegetable; its edible parts are directly in contact with Cd-contaminated fields. Two radish cultivars (H4 and L19) with different resistance to Cd were selected to compare their growth, photosynthesis, and antioxidant systems. Our results revealed that H4 was more sensitive to Cd pollution than L19; a significant decrease in the biomass of H4 was observed at higher Cd concentrations. With increasing Cd concentrations, the net photosynthetic rate, effective quantum yield of PSII photochemistry, electron transport rate through PSII, and photochemical quenching were all lowered, however, reductions were more obvious in H4 compared to L19. Further, both peroxidase and catalase activities of L19 were remarkably higher than those of H4. Smaller reductions in ascorbic acid and no obvious changes in glutathione were observed in the leaves of H4. These results suggest that the differences in Cd tolerance could be attributed to the distinct photosynthetic parameters and differences in antioxidant system between L19 and H4.

Additional key words: antioxidant enzyme; cadmium stress; chlorophyll fluorescence; gas exchange; hyperaccumulator plant.

Introduction

Cadmium (Cd) has emerged as one of the most serious environmental pollutants due to its widespread occurrence and toxicity. It has been reported that anthropogenic inputs, primarily due to smelting, fossil fuel combustion, and the application of sewage sludge and phosphate fertilizers, have led to the excessive accumulation of Cd in soils (Grant 2011). Although Cd is a nonessential element, it is readily taken by plants and subsequently causes extreme damage to their physiology and biochemistry, including the inhibition of photosynthesis, increased membrane permeability, and adverse effects on protective enzyme activities (Hashem 2014, Pereira de Araújo *et al.* 2017).

Photosynthesis is an integral part of basic plant physiology. It allows energy from sunlight to be converted into a storable form, usually glucose, which plants use to grow and thrive. In general, photosynthesis is negatively impacted by Cd in various ways, including damage of various components in photosynthetic machinery (Parmar *et al*. 2013, Pereira de Araújo *et al.* 2017), inhibition of photochemical reactions (Dias *et al.* 2013, Xue *et al.* 2014, Mesnoua *et al.* 2016), disturbance of enzymatic activities in the Calvin cycle, and disorganization of the inherent reactive oxygen species (ROS) balance of chloroplasts (Pospíšil 2012, 2014). Wang *et al.* (2014) and Daud *et al.* (2013) demonstrated that Cd induced the inhibition of chlorophyll (Chl) biosynthesis, and distorted the chloroplast ultrastructure and Mg^{2+} substitution of both Chl *a* and *b*, thereby leading to a reduction in the Chl content. In recent years, the technique of Chl fluorescence has become a powerful tool for a quick and nonintrusive evaluation of photosynthetic performance *in vivo* and for identification of possible causes of changes in photosynthesis and plant performance (Maxwell and Johnson 2000, Baker 2008). Xue *et al*. (2014) have reported that more excessive excited energy was produced in the young leaves than in the mature leaves, which further damaged the photosynthetic apparatus. Chloroplasts are the most powerful ROS sources in plant tissues (Foyer *et al.* 1994). It has been reported that when ROS are formed in excess, the oxidation of cellular biomolecules by ROS might produce oxidative damage to pigments, proteins, and lipids in thylakoid membranes, as well as the reduction of photosynthesis, which can eventually lead to the plant death (Pospíšil 2012, 2014). Plants have developed ROS-scavenging systems that are

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Abbreviations: AsA – ascorbic acid; CAT – catalase; C_i – intercellular CO_2 concentration; ETC – electron transport chain; ETR – electron transport rate through PSII; g_s – stomatal conductance; GSH – reduced glutathione; MDA – malondialdehyde; POD – peroxidase; q_N – nonphotochemical quenching; q_P – photochemical quenching; ROS – reactive oxygen species; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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composed of enzymatic and nonenzymatic antioxidants, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), reduced glutathione (GSH), and ascorbic acid (AsA) (Namdjoyan *et al.* 2014, Xu *et al.* 2016). The response of plant scavenging systems to Cd is variable, not only among plant species, but also in genotypes within the same species. For *Lolium perenne* L. and the metal-accumulators, *Thlaspi caerulescens* and *Brassica juncea*, POD, CAT, and SOD activities were enhanced after exposure to Cd, whereas CAT activity declined rapidly; SOD and POD activities were enhanced after the Cd exposure in a non-accumulator plant (*Nicotiana tabacum*) (Luo *et al.* 2011, Xu *et al.* 2016). Furthermore, Wu *et al.* (2004) reported that the relatively lesser reductions of GSH and AsA were observed in ZAU 3, which is a Cd-tolerant genotype.

Radish is an important annual or biennial root vegetable crop of the *Brassicaceae* family cultivated worldwide (Wang and He 2005). A risk of Cd contamination in the roots of this plant is becoming of a great concern. Radish leaves, which are important nutritional organs, provide nutrients for plants *via* photosynthesis. Although previous experiments have focused primarily on responses of photosynthetic and antioxidant systems to Cd exposure, there are few reports combining these two systems in order to examine systematically the effects of Cd stress. The objectives of this work were to examine and compare the growth, photosynthesis, and antioxidant systems of the two radish cultivars of a low and high Cd tolerance, which might contribute to a better understanding of the biological mechanisms involved under Cd stress.

Materials and methods

Plant material, growth, and treatments: Two radish (*Raphanus sativus* L.) cultivars, H4 (less Cd-tolerant cultivar) and L19 (highly Cd-tolerant cultivar), were used in this study. The soil was collected from the surface layer (0–15 cm) of the cultivated soil in Huazhong Agricultural University, Wuhan, China. The soil was yellow brown, with the following characteristics: pH 7.12, 52.33 g(available N) kg^{-1}, 7.96 mg(organic C) kg^{-1}, 16.57 mg(available P) kg^{-1}, 193.48 mg(available K) kg^{-1}, 0.06 mg(total Cd) kg^{-1}. The soil was passed through a 1-mm sieve. Five radish plants were planted in a plastic bucket filled with 13-kg soil samples for 30 d. The Cd growing conditions were simulated by applying $CdCl_2{\cdot}2.5H_2O$ to deionized fresh water at three concentrations of 0, 1.0, and 5.0 mg(Cd) kg^{-1}. The same amount of fertilizer was applied to all samples at a rate of 0.20 g(N), 0.07 g(P), and 0.17 g(K). Three replicates were performed for each treatment. Plants were harvested after 30 d (at the seedling stage) and separated into root and leaf samples, which were washed three times with tap water, distilled water, and deionized water. For the physiological tests, the fresh samples were initially required to be stored in liquid nitrogen.

Gas-exchange parameters and Chl fluorescence: The net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of the fully expanded leaves after 30 d following transplantation were recorded using an infra-red gas analyzer (IRGA, *Li-Cor*, *Li*-6400, Lincoln, NE, USA) from 9:00 to 11:00 h.

Chl fluorescence was measured using an integrative fluorescence fluorometer (*Li-6400* leaf chamber fluorometer, *Li-Cor*, USA). Following 1-h adaptation to darkness, the minimal Chl fluorescence (F_0) was determined using an intensity of 0.2 μmol(photon) m^{-2} s^{-1}. A saturation pulse [red and blue light of 7,200 μmol(photon) m^{-2} s^{-1}] was used to obtain the maximal fluorescence (F_m). The leaves were subsequently illuminated with actinic light [1,400 μmol(photon) m^{-2} s^{-1}] for 1 h, The steady-state value of fluorescence (F_s) was recorded and a second saturation pulse of 6,000 μmol(photon) m^{-2} s^{-1} was imposed to determine the maximum fluorescence at the light-saturated stage (F_m'), The actinic light was then removed and the minimal fluorescence at the light-saturated stage (F_0') was determined by illuminating the leaves with far-red light [7 μmol(photon) m^{-2} s^{-1}]. Other fluorescence parameters [quantum yield of PSII photochemistry (Φ_{PSII}), electron transport rate through PSII (ETR), photochemical quenching (q_P), and nonphotochemical quenching (q_N)] were calculated as described by Genty *et al.* (1989) and Maxwell and Johnson (2000).

Malondialdehyde (MDA): The concentration of MDA was determined by the thiobarbituric acid (TBA) reaction as described by Li *et al.* (2000) and Farooq *et al.* (2013). Fresh leaf samples (0.50 g) were homogenized in 5 ml of 10% trichloroacetic acid. The homogenate was centrifuged at 12,000 × *g* for 15 min, and 1.5 ml of the supernatant was mixed with 2.5 ml of 0.5% 2-thiobarbituric acid. The absorbance was measured at 600, 532, and 450 nm (*UV-5200*, *Shanghai Meipuda Instrument Co. Ltd.*, China).

Enzyme activities: The leaf samples were homogenized in 5 ml of potassium phosphate buffer (pH 7.0), and the homogenate was then centrifuged at 12,000 × *g* for 15 min at 4°C, after which the supernatant was used for the following enzyme assays. The content of soluble protein and activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and POD (EC 1.11.1.7) were estimated according to the method described by Li *et al.* (2000).

The reaction mixture contained 0.05 ml of potassium phosphate buffer (pH 7.8), 0.3 ml of 0.13 M methionine, 0.3 ml of 0.75 μM nitroblue tetrazolium (NBT), 0.3 ml of 0.1 μM EDTA, 0.3 ml of 0.02 μM riboflavin, 0.25 ml of distilled water, and 0.05 ml of supernatant. The SOD activity was estimated as the quantity of enzyme required to cause a 50% inhibition in the reduction of NBT at 560 nm (*UV-5200*, *Shanghai Meipuda Instrument Co. Ltd.*, China).

For CAT activity, the reaction mixture contained 1.5 ml of potassium phosphate buffer (pH 7.0), 0.1 ml of 0.1 M H_2O_2, 1.0 ml of distilled water, and 0.2 ml of enzyme extract. The CAT activity was estimated by monitoring the decrease of absorbance at 240 nm as the H_2O_2 was consumed (*UV-5200*, *Shanghai Meipuda Instrument Co. Ltd.*, China). One unit of CAT activity was defined as U g^{-1}(protein) min^{-1}.

For POD activity, the reaction mixture contained 2.9 ml of extraction buffer (pH 5.5), 1.0 ml of 2% H_2O_2, 1.0 ml of guaiacol (0.05 M), and 0.1 ml of supernatant. The POD activity was calculated from the increase in absorbance (470 nm) due to the oxidation of guaiacol (*UV-5200*, *Shanghai Meipuda Instrument Co. Ltd.*, China). The activity was expressed as U g^{-1}(protein) min^{-1}.

AsA and GSH: The leaf samples were homogenized in 5% metaphosphoric acid (w/v) and centrifuged at 12,000 × *g* for 15 min at 4°C. The supernatant was collected for AsA and GSH analysis. The AsA was spectrophotometrically (*UV-5200*, *Shanghai Meipuda Instrument Co. Ltd.*, China) quantified at 525 nm according to Mohamed *et al.* (2012). The assay mixture containing 150 mM NaH_2PO_4, 10% TCA, 44% H_3PO_4, 4% 2,2'-dipyridyl (w/w), and 3% $FeCl_3$ was incubated at 37°C for 60 min. The content was expressed in mg g^{-1}(FM). The GSH was estimated by the method of Anderson (1985). It is based on the absorbance of the supernatant at 412 nm (*UV-5200*, *Shanghai Meipuda Instrument Co. Ltd.*, China). The assay mixture contained 150 mM NaH_2PO_4 and 4 mM DNTP. The content was expressed in mg g^{-1}(FM).

Statistical analysis: The data were analyzed using *SPSS 20.0* software. The differences between cultivars were examined by one-way analysis of variance (*ANOVA*) followed by *Duncan*'s multiple range test. The significant differences between means were tested at a probability $p \leq 0.05$. Figures were created using *Sigma Plot™ v. 10*.

Results

Biomass of the two radish cultivars: The exposure to different Cd concentrations caused variable effects on shoot, root, and total biomass of the two cultivars (Table 1). In L19, the shoot biomass at the 1.0 mg(Cd) kg^{-1} was 37% higher than that of control, which resulted in the aboveground biomass being significantly enhanced compared with the control; however, the aboveground biomass at the 5.0 mg(Cd) kg^{-1} was basically the same as that of control. In contrast to L19, the shoot and total biomass of H4 significantly decreased at 5.0 mg(Cd) kg^{-1}, the reduction was 24% compared to the control. No significant variations between the shoot and root under the 1.0 mg(Cd) kg^{-1} and control treatments were observed. When subjected to Cd stress, the range of root biomass in the same radish cultivar was lesser than that of the aboveground biomass. It indicated that the inhibition of shoot growth by Cd stress is more serious than that for roots and the growth of L19 was promoted by the low Cd concentration.

Gas-exchange parameters of the two radish cultivars: The P_N of both cultivars decreased, while C_i increased with the increasing Cd concentrations (Fig. 1). In comparison with the control, the declines in P_N were 2% in L19 and 22% in H4 at 5.0 mg(Cd) kg^{-1}, respectively. Moreover, the P_N of L19 was 7 and 23% higher than that of H4 at the 1.0 and 5.0 mg(Cd) kg^{-1}; however, there were no significant differences in g_s and C_i between the two cultivars. It indicated that the photosynthetic rate of both radish cultivars was lowered; however, C_i was improved by Cd exposure.

Chl fluorescence parameters of the two radish cultivars: Φ_{PSII}, ETR, and q_P of the two cultivars were reduced, whereas q_N increased with the increase in the Cd concentration (Fig. 2). No significant differences in the Φ_{PSII}, ETR, q_P, and q_N in L19 were observed between the different Cd treatments. For H4 cultivar, Φ_{PSII}, ETR, q_P, and q_N at the 1.0 mg(Cd) kg^{-1} were statistically the same as the control; however, Φ_{PSII}, ETR, and q_P at the 5.0 mg(Cd) kg^{-1} were significantly reduced by 35, 35, and 18%, respectively, compared with control, while q_N significantly increased by 36%.

MDA concentration of the two radish cultivars: Formation of MDA is considered as a measure of lipid peroxidation in plants. The MDA concentration in leaves of the two cultivars increased obviously with the increasing Cd concentrations (Fig. 3*A*). The MDA content in H4 increased by 16 and 49% at 1.0 and 5.0 mg(Cd) kg^{-1}, respectively, while the increases were just 4 and 20% for L19, respectively, in comparison with the control. The MDA concentrations of H4 were 8 and 20% higher than that of L19 under the 1.0 and 5.0 mg(Cd) kg^{-1}, respectively.

Antioxidant systems of the two radish cultivars: With exposure to Cd, CAT activity was not significantly affected in L19 and H4, although CAT activities of L19

Table 1. Effect of Cd concentrations on biomass of the two radish cultivars. Values are means ± SE (*n* = 3). Values followed by *different letters* indicate significant differences followed by *Duncan*'s test ($p \leq 0.05$) for both cultivars at different Cd treatments. DM indicates dry mass.

Cultivar	Cd content [mg kg^{-1}]	Shoot biomass [g(DM) per plant]	Root biomass [g(DM) per plant]	Total biomass [g(DM) per plant]
L19	0	2.09 ± 0.06^{cd}	0.20 ± 0.03^{b}	2.29 ± 0.08^{c}
	1.0	2.87 ± 0.13^{a}	0.28 ± 0.03^{b}	3.14 ± 0.10^{a}
	5.0	2.26 ± 0.15^{bc}	0.32 ± 0.03^{b}	2.52 ± 0.19^{bc}
H4	0	2.49 ± 0.08^{b}	1.03 ± 0.15^{a}	3.39 ± 0.24^{a}
	1.0	2.39 ± 0.06^{abc}	1.24 ± 0.10^{a}	3.44 ± 0.18^{a}
	5.0	1.88 ± 0.09^{d}	1.05 ± 0.04^{a}	2.93 ± 0.13^{ab}

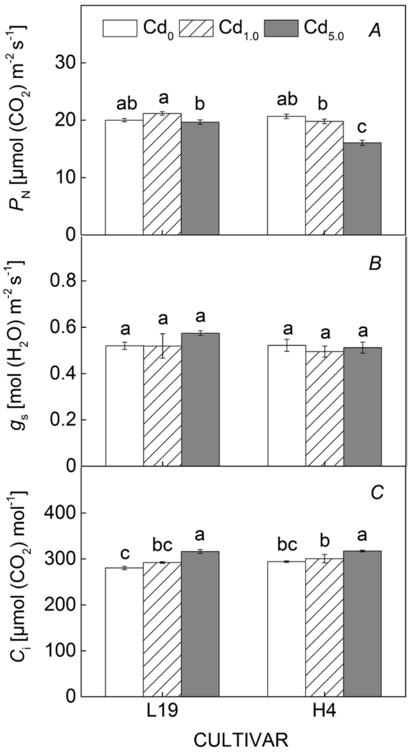

Fig. 1. Effect of Cd concentrations on net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), and intercellular CO_2 concentration (C_i) (*C*) of the two radish cultivars leaves. Values are means ± SE ($n = 3$). The bars with *different letters* between the two cultivars at the different Cd concentrations denote significant differences at $p \leq 0.05$.

were 35, 33, and 7% higher than that of H4 at 0, 1.0, and 5.0 mg(Cd) kg^{-1}, respectively (Fig. 3*B–D*). The POD activity of L19 remarkably increased with the increasing Cd concentrations, whereas POD activity of H4 was initially enhanced at the 1.0 mg(Cd) kg^{-1} and then decreased at 5.0 mg(Cd) kg^{-1}. Further, the POD activities of L19 were 74, 36, and 102% higher than that of H4 at the three Cd treatments. The SOD activities of both cultivars revealed a trend that was similar to the POD activity of H4; however, there were no significant differences between the two cultivars at all the Cd treatments.

AsA concentrations in leaves of both cultivars were significantly reduced with increasing Cd concentrations, while the reduction was sharper in L19 (Fig. 4*A*). Meanwhile, the AsA concentration was obviously higher in L19 than that of H4 at 0 and 1.0 mg(Cd) kg^{-1}, whereas there was no significant difference at 5.0 mg(Cd) kg^{-1} between the two cultivars. No significant differences between treatments were found in GSH concentration of L19, while the GSH concentration of H4 was significantly higher at 1.0 mg(Cd) kg^{-1} compared to the other two treatments (Fig. 4*B*).

Discussion

It has been reported that the photosynthesis and antioxidant defense systems are very sensitive to Cd stress (Xue *et al.* 2014). Plant species and genotypes obviously differ in their photosynthesis, antioxidant enzymatic activities, and antioxidant substances under Cd stress (Mobin and Khan 2007, Zayneb *et al.* 2015).

Generally, photosynthesis can be affected by stomatal and nonstomatal limitations (Farquhar and Sharkey 2003, He *et al.* 2016). In our study, elevated concentrations of Cd were found to decrease the P_N in both radish cultivars, which was accompanied with an increase of C_i and unchanged g_s. The increase of C_i indicates that the decrease of P_N might be primarily attributed to nonstomatal limitations, such as damages to the photosynthetic apparatus and/or inhibitory effects of the Calvin cycle enzymatic activities and PSII electron transport (Parmar *et al.* 2013). Tóth *et al.* (2012) reported that Cd could inhibit the dark phase of the photosynthesis *via* specific binding to enzymes of the Calvin cycle and/or that involved in the carbon concentrating mechanism (CCM) Vitória *et al.* (2006) used radish as experimental material and found that chloroplasts from Cd-exposed samples exhibited alterations in organelle morphology, an increase in the stroma volume, and the deposition of electron-dense material within the double membrane. P_N directly affects photosynthetic products, and photosynthetic products are the main sources for biomass production. In H4, the decrease in shoot biomass might be attributed to the more rapid decline of P_N with increasing Cd concentrations. On the contrary, the shoot biomass of L19 was promoted at the low Cd concentration, along with the mild increase of P_N. Furthermore, Cd concentrations in shoots and roots of H4 were all significantly higher than that of L19 (Xin *et al.* 2017). These facts demonstrate that L19 is more resistant to Cd exposure than H4. Similar results have been reported by Jin *et al.* (2008), who compared Cd-hyperaccumulator (HE) and non-Cd-hyperaccumulator ecotypes (NHE) and reported that the shoot and root biomass of NHE decreased significantly, but those of HE increased significantly with Cd stress.

Chl fluorescence analysis has become a probe for photosynthesis *in vivo*, which can quickly identify the potential causes of changes in photosynthesis and plant performance (Baker 2008). Żurek *et al.* (2014) reported that Chl fluorescence was closely related to the photosynthetic electron transfer chain and further biochemical processes. Roháček (2002) and Lazár (2015) found that q_P reflects activation of the photochemical processes, estimating a fraction of open PSII reaction centers, whereas q_N reflects activation of the nonphotochemical processes, leading mostly to the nonradiative energy dissipation as heat. In our study, Φ_{PSII}, ETR, and q_P in H4 were obviously reduced, whereas q_N increased with the increasing Cd concentrations, which might account for a decrease of biomass and P_N in H4. The decrease of ETR indicated that reoxidation of Q_A was limited by the partial block of electron transport from PSII to PSI (Ekmekçi *et al.* 2008). The reduction of Φ_{PSII} and q_P, as well as the increase of q_N, suggested that less light energy was utilized by photochemical reactions, while less excessive excited energy was produced under Cd treatments (Xue *et al.* 2014). These results are in agreement with Xue *et al.* (2018) and

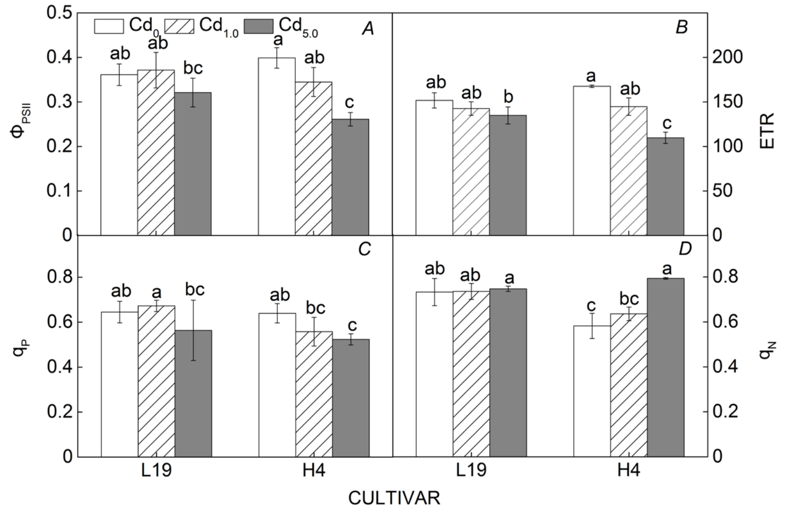

Fig. 2. Effect of Cd concentrations on quantum yield of PSII photochemistry (Φ_{PSII}) (*A*), electron transport rate through PSII (ETR) (*B*), photochemical quenching (q_P) (*C*), and nonphotochemical quenching (q_N) (*D*) of the two radish cultivars leaves. Values are means ± SE ($n = 3$). The bars with *different letters* between the two cultivars at the different Cd levels denote significant differences at $p \leq 0.05$.

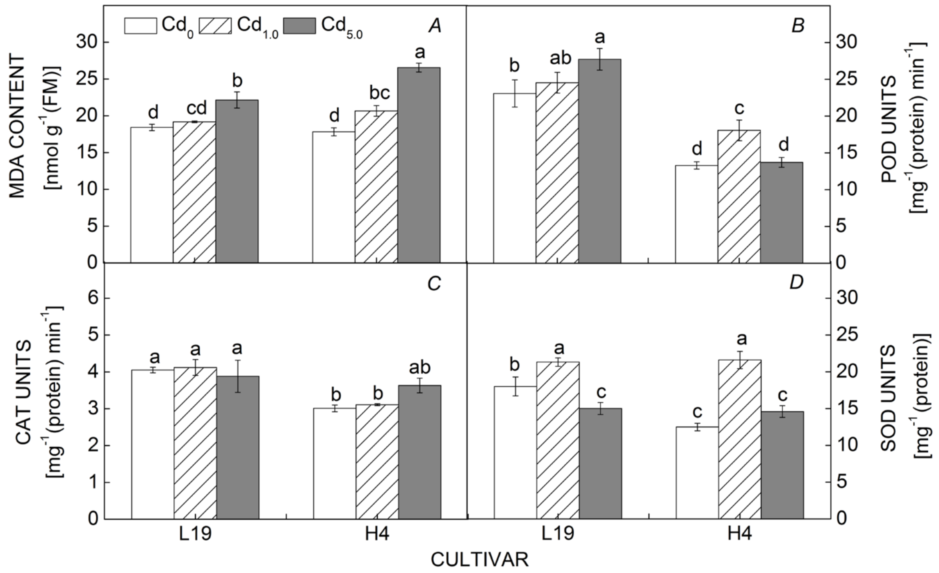

Fig. 3. Effect of Cd concentrations on malondialdehyde (MDA) content (*A*), peroxidase (POD) (*B*), catalase (CAT) (*C*), and superoxide dismutase (SOD) (*D*) activities of the two radish cultivars leaves. Values are means ± SE ($n = 3$). The bars with *different letters* between the two cultivars at the different Cd concentrations denote significant differences at $p \leq 0.05$.

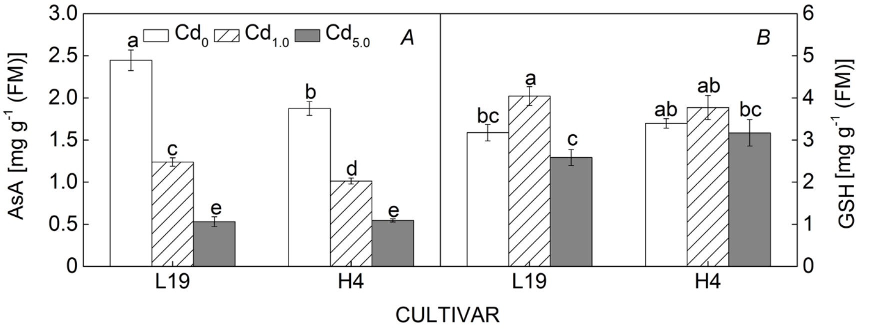

Fig. 4. Effect of Cd concentrations on ascorbic acid (AsA) (*A*) and reduced glutathione (GSH) (*B*) content of the two radish cultivars leaves. Values are means ± SE ($n = 3$). The bars with *different letters* between the two cultivars at the different Cd concentrations denote significant differences at $p \leq 0.05$.

Ekmekçi *et al.* (2008), who suggested that plants would suffer photoinhibition due to reversible inactivation or destruction of the PSII reaction center under Cd exposure. For L19, no significant differences were observed in Φ_{PSII}, ETR, q_P, and q_N, which resulted in no significant changes of P_N compared with the control.

In chloroplasts, electron transport chains (ETCs) of PSI and PSII serve as the primary sources of ROS, which are activated by Cd stress (Foyer *et al.* 1994). Sharma *et al.* (2012) indicated that ETCs in PSI and PSII are the main sources of ROS in chloroplasts. Production of ROS by these sources is enhanced in plants by conditions limiting CO_2 fixation, such as drought, salt, and temperature stresses. MDA is a final product of membrane lipid peroxidation by ROS**.** In the present study, the MDA content in leaves of H4 was higher than that of L19 at any Cd concentration. This result shows that H4 experiences a greater oxidative damage than L19. Our result is in agreement with Shi *et al.* (2010) and Xu *et al.* (2012), who reported that cell membrane could suffer more extensive damage when exposed to high Cd concentrations. Pospíšil *et al.* (2012) reported that an efficient antioxidant defense system plays a critical role in scavenging excessive ROS and maintaining redox balance. POD, CAT, and SOD are universally present antioxidant enzymes in various cellular compartments. Studies have shown that both increases and reductions in enzymatic activities were observed in response to Cd exposure (Mohamed *et al.* 2012, Shu *et al.* 2012, Liu *et al*. 2018). In our study, POD activity of H4 and CAT activities of both cultivars exhibited an increase at low Cd concentrations and a reduction at high Cd concentrations. The increase in POD and CAT activities of both cultivars might be a result of both stimulation by Cd stress, as well as an increase in substrate contents (Tanyolaç *et al.* 2007). The reduction in POD activity of H4 and CAT activities of both cultivars observed at 5.0 mg(Cd) kg^{-1} might be explained by a decrease in enzyme synthesis and/or changes in assembly of its subunits (Verma and Dubey 2003). Further, L19 exhibited higher POD and CAT activity in contrast to H4 at any Cd treatment. This indicates that L19 is more efficient in avoiding oxidative damage from heavy metal.

Low molecular antioxidants such as AsA and GSH are required to protect plant from oxidative stress. In the present study, AsA was reduced significantly by Cd stress. This might be caused due to the following potential mechanisms: (*1*) directly, through the combination with heavy metals, which further prevented their transmembrane transport; (*2*) indirectly, by acting as a reducing agent, protecting thiol-containing proteins and enzymes in cell membranes from oxidation (Rai 1979). In contrast to what was reported for AsA, Cd induced the increase of GSH at low Cd concentration and reduction at high Cd concentration. The increased formation of GSH might comprise 'truly adaptive responses' in response to Cd stress. Moreover, GSH is not only known as general reductant but also serves as substrate participating in control of ROS levels (Xu *et al*. 2016). Hence, the decrease of GSH might be ascribed to its role as an antioxidant or metal chelator involved in Cd tolerance. Compared to L19, the smaller reduction of AsA and unchanged GSH were observed in H4. These indicate that H4 exhibited higher AsA and GSH synthetic capacities which was beneficial for its tolerance to Cd toxicity. Similar results were obtained by Wu *et al.* (2004) and Mohamed *et al.* (2012).

In conclusion, L19 was more tolerant to Cd stress compared to H4. In the present study, a significant increase was found in the biomass of L19 at the 1.0 mg(Cd) kg^{-1}, however, that of H4 decreased significantly at the 5.0 mg(Cd) kg^{-1}. These differences could be a result of distinct photosynthetic and antioxidant system parameters in L19 and H4. In the future, we will continue with in-depth studies toward the elucidation of the relationship between photosynthesis and antioxidant systems in the leaves.

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