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Methyl jasmonate alleviates cadmium toxicity in *Solanum nigrum* by regulating metal uptake and antioxidative capacity

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Abstract

The growth of the Cd-hyperaccumulator *Solanum nigrum* L. and its physiological responses to a short-term (7 d) Cd stress and to exogenous methyl jasmonate (MeJA) were investigated. Compared with the leaves of *S. nigrum*, the roots were more liable to Cd and showed a significantly decreased dry mass and increased malondialdehyde content. Cd accumulation in the shoots and roots of *S. nigrum* were proportional to the Cd concentration in the hydroponic solution. The application of a low concentration of MeJA (0.01 μM) significantly reduced the translocation/accumulation of Cd in both the shoots and roots compared with a 40 mg dm^{-3} Cd treatment only. Moreover, 40 mg dm^{-3} Cd significantly decreased the activity of leaf superoxide dismutase, but 0.01 μM MeJA restored it. MeJA also enhanced the activity of catalase in the leaves but showed no significant effect on peroxidase activity. The content of both endogenous jasmonic acid (JA) and MeJA in the leaves of *S. nigrum* increased with the increase of exogenous MeJA concentration.

Additional key words: catalase, Cd translocation, glutathione, jasmonic acid, peroxidase, superoxide dismutase.

Introduction

The increasing environmental cadmium pollution caused by natural and anthropogenic activities is a significant global problem (Nada *et al.* 2007). Cd is a nonessential element that strongly inhibits plant growth and development and causes plant death even at rather low concentrations (Andresen and Küpper 2013). The overproduction of reactive oxygen species (ROS), such as super oxide radical ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2) is an indirect consequence of Cd toxicity (Andresen and Küpper 2013). These ROS damage membranes of cellular organelles, nucleic acids and chloroplast pigments, cause lipid peroxidation and malondialdehyde (MDA) formation (Gill and Tuteja 2010). In response, plants have evolved enzymatic antioxidants, *e.g.*, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), and non-enzymatic scavengers, *e.g.*, glutathione, carotenoids, and ascorbate (Gallego *et al.* 2012).

Phytoremediation is a technology that uses metal-accumulating plants to extract metals from contaminated soil, groundwater, or surface water (Raskin *et al.* 1997, Liu *et al.* 2012). Understanding the detoxification strategies that these plants adopt against metal stress is vital for their application in phytoremediation (Dixit *et al.* 2001, Xu *et al.* 2009). *Solanum nigrum* is a promising Cd hyperaccumulator (Wei and Zhou 2004). The effects of Cd treatments on growth, physiology, Cd accumulation, and distribution of *S. nigrum* seedlings have been studied in recent years (Sun *et al.* 2007, 2008, Pinto *et al.* 2009, Deng *et al.* 2010). Strategies, such as the application of N-acetyl-L-cysteine (Deng *et al.* 2010), silicon (Liu *et al.* 2013), ethylenediaminetetraacetic acid (Sun *et al.* 2009), and citric acid (Gao *et al.* 2012), that help *S. nigrum* increase its Cd tolerance and metal-uptake efficiency have also been investigated.

Jasmonates (JAs) including jasmonic acid (JA) and methyl jasmonate (MeJA) are a family of cyclopentanone compounds synthesized from linolenic acid through the

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Abbreviations: CAT - catalase; GSH - glutathione; H_2O_2 - hydrogen peroxide; JA - jasmonic acid; MDA - malondialdehyde; MeJA - methyl jasmonate; $O_2^{\cdot-}$ - superoxide radical; $^{\cdot}OH$ - hydroxyl radical; POD - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
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octadecanoic pathway. These compounds play important roles in the signalling network of plants under various biotic and abiotic stresses (Fujita *et al.* 2006). The exogenous application of high JAs concentrations can inhibit growth and photosynthesis and speed up senescence (Jung 2004). However, low concentrations of exogenous JAs can increase plant resistance to abiotic stresses (Keramat *et al.* 2009). A few studies have indicated that 10^{-7} to 10^{-5} M MeJA exert a stimulatory effect on photosynthetic pigments (Piotrowska *et al.* 2009, Kováčik *et al.* 2011) and alleviate Cd damage by reducing metal uptake (Piotrowska *et al.* 2009). Consequently, the activities of antioxidant enzymes increase, and the content of malondialdehyde (MDA) and H_2O_2 decrease (Keramat *et al.* 2009).

The changes induced by excessive metals occur relatively rapidly in plants, and their stress responses to short- and long-term stresses generally differ (Maksymiec *et al.* 2005). Available studies on the effect of Cd stress on *S. nigrum* have been carried out based on relatively long-period (months) greenhouse experiments (Sun *et al.* 2007, 2008, Pinto *et al.* 2009, Deng *et al.* 2010), whereas studies on the responses of *S. nigrum* to short-term (days) metal stress are still lacking. The physiological and biochemical mechanisms underlying metal accumulation in hyperaccumulator plants under short-term Cd stress are still not fully understood. The protective role of exogenous MeJA on *S. nigrum* response to Cd has also not yet been elucidated. Accordingly, the present study was aimed to investigate whether MeJA is involved in the induction of defence responses in the typical Cd-hyper-accumulatory plant, *S. nigrum*, to a short-term Cd stress, and to test the hypothesis that the protective ability of MeJA against Cd toxicity is mediated by its effect on the activities of antioxidative enzymes and on the uptake and translocation of Cd.

Materials and methods

Experimental setup and sample collection: *Solanum nigrum* L. seeds were surface sterilized with 70 % (v/v) ethyl alcohol and then sown into sand in plastic pots with a half-strength Japanese garden test nutrient solution (Hori 1966). The planted pots were placed on a bench in a growth chamber with a daily temperature of 25 °C, a relative humidity of 70 %, and an irradiance of 800 μmol(photon) m^{-2} s^{-1} during a 14-h photoperiod. The seedlings were irrigated with tap water daily until the third pair of leaves completely unfolded. Then the pots were randomly divided into nine groups, each in triplicate. Five groups were treated with different concentrations of $CdCl_2$ (control, 10, 25, 40, and 55 mg dm^{-3}). Other four groups were treated with different concentrations of MeJA (0.01, 0,1, 10, and 1 000 μM) together with 40 mg dm^{-3} Cd. Tap water irrigation of the seedlings was performed every day to compensate for the water loss through evaporation. Leaf, stem, and root samples of all the treatments were collected at the end of the experiment (day 7). The leaf and root samples of one seedling were separated, rapidly frozen in liquid nitrogen, and then stored at -80 °C. The tissues of another seedling were collected for growth and metal analyses. The different plant parts were first dried in a hot air oven at 120 °C for 2 h and dried at 70 °C for 48 h.

Cd in plant tissues was determined according to the method of Wong *et al.* (1993). About 0.25 g of oven-dried plant samples were initially charred on a hot plate for about 1 h and then incinerated in a muffle furnace at 500 °C for 6 h. The ash was digested and diluted to 10 cm^3 with 1 % (m/m) HNO_3. Cd in the digested solutions was analyzed with an atomic absorption spectrometer (*Analyst 800*, *PerkinElmer*, Waltham, MA, USA). The precision of the method was determined in terms of the recovery of spiked Cd standards (as $CdCl_2$) in homogenized plant tissue samples at 10 mg dm^{-3}. The average recoveries Cd was 91.5 ± 9.9 % (n = 3), and the limit of detection 0.05 mg(Cd) kg^{-1}(d.m.).

MDA in the leaves was determined according to the method of Kosugi and Kikugawa (1985) with modifications. Fresh samples (0.2 g) were homogenized using a mortar and pestle with 4 cm^3 of 20 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 9 000 *g* for 5 min. Then, 1 cm^3 of the supernatant was mixed with an equal volume of 0.6 % (m/v) thiobarbituric acid solution comprising 10 % TCA. The mixture was heated in boiling water for 30 min and then transferred to an ice bath to arrest the reaction. The cooled mixture was centrifuged at 5 000 *g* and 25 °C for 10 min, and the absorbances of the supernatant were read at 450, 532, and 600 nm. The MDA content was calculated as $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$.

Endogenous JA and MeJA content was determined using an enzyme-linked immunosorbent assay (ELISA) kit (*Rapidbio*, CA, USA) according to the manufacturer's instructions. Approximately 100 mg of tissue was rinsed once with 1× phosphate-buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1.46 mM KH_2PO_4, then homogenized in 1 cm^3 of 1× PBS and stored at -20 °C overnight. Two freeze-thaw cycles were performed to break the cell membranes and then the homogenates were centrifuged at 9 000 *g* and 4 °C for 5 min. The supernatant was used for the hormone assay. Standards or samples were added to appropriate microtiter plate wells with horseradish peroxidase (HRP)-conjugated JA and MeJA and then incubated. Subsequently, a competitive inhibition reaction was launched between JA/MeJA (in standards or samples) and HRP-conjugated JA/MeJA with the pre-coated antibody specific for JA/MeJA. The detection

limits for the endogenous JA and MeJA were both 80 pM.

Total GSH content was determined according to the method of Beutler *et al*. (1963). Fresh tissue (0.5 g) was homogenized with a mortar and pestle in 5 cm^3 of a cold extraction buffer containing 2 % (m/v) 5-sulfosalicylic acid dihydrate, 1 mM ethylenediaminetetraacetic acid disodium (Na_2EDTA), and 0.15 % (m/v) ascorbate. The homogenate was centrifuged at 9 000 *g* and 4 °C for 10 min. The reaction mixture containing 1 cm^3 of the supernatant, 2 cm^3 of a 1 % (m/v) Tris-HCl buffer (pH 8.9), and 0.05 cm^3 of 10 mM 5,5'-ditiobis-(2-nitrobenzoic acid) (DTNB) was maintained at room temperature for 5 min and the absorbance was read at 412 nm. The GSH content was calculated using a GSH standard curve.

Proline was extracted and determined according to Bates *et al.* (1973). Fresh leaf or root tissue (about 0.1 g) was homogenized with 3 cm^3 of 3 % (m/v) sulfosalicylic acid, and the homogenate was centrifuged at 1 700 *g* for 10 min. After transferring 0.5 cm^3 of the supernatant to a test tube, 0.5 cm^3 of acetic acid and 1 cm^3 of acid ninhydrin were added, and the mixture was boiled for 1 h. The reaction was terminated in an ice bath. The reaction mixture was extracted with 1 cm^3 of toluene and thoroughly mixed by vortexing. The absorbance of the upper toluene phase was determined at the wavelength of 520 nm using toluene as blank and L-proline as standard.

Antioxidative enzymes: About 0.3 g of fresh samples (leaf or root) was extracted in 4 cm^3 of a 50 mM ice-cold sodium phosphate buffer (pH 7.4) mixed with 1 mM Na_2EDTA. The homogenate was centrifuged at 9 000 *g* at -4°C for 10 min, and the supernatant was used for the enzyme assay.

SOD activity was determined by the hydroxylamine assay developed from the xanthine oxidase assay according to the method of Elstner and Heupel (1976). The homogenate was tested in a solution containing 0.037 U cm^{-3} xanthine oxidase, 0.375 mM xanthine, 0.1 mM hydroxylamine, and the enzyme extract. The reaction was initiated using xanthine and then incubated at 37 °C for 30 min in a water bath. The reaction was terminated with 2.5 mM sulfanilic acid. One unit of SOD activity was defined as the amount of the enzyme that caused a 50 % decrease in the rate of nitrite formation from the oxidation of hydroxylamine by $O_2^{\cdot-}$ per mg of protein. $O_2^{\cdot-}$ was measured at 530 nm after adding 8 mM α-naphthylamine in glacial acetic acid.

CAT activity was measured according to the method of Beer and Sizer (1952) with minor modifications. The reaction mixture (2.5 cm^3) consisted of a 50 mM phosphate buffer (pH 7.4), 0.1 mM EDTA, 20 mM H_2O_2, and 0.5 cm^3 of the enzyme extract. The reaction was initiated by adding the extract. The decrease in H_2O_2 was monitored at 240 nm for 2 min and quantified by the coefficient of absorbance of 39.4 mM cm^{-1}. One unit of CAT activity was defined as the amount of the enzyme that decomposed 1 mmol of H_2O_2 per min.

POD activity was determined according to the method of Fielding and Hall (1978) with some modifications. The enzyme extract (0.1 cm^3) was mixed with 3 cm^3 of a 50 mM phosphate buffer (pH 7.4) containing 0.2 % (v/v) guaiacol. The reaction was initiated with 1 cm^3 of 0.3 % (v/v) H_2O_2, and guaiacol oxidation was measured by the increase in absorbance at 470 nm for 2 min. The POD activity was calculated using a coefficient of absorbance of 26.6 mM cm^{-1}. One unit of POD activity was defined as the amount of the enzyme catalyzing the formation of 1 μmol of tetraguaiacol per min.

Protein content was determined according to the method described by Bradford (1976) using bovine serum albumin as standard.

Statistics: The mean and standard deviation (SD) of three replicates were calculated. Parametric one-way *ANOVA* followed by post-hoc multiple comparisons (the Tukey's test) were used to test significance of differences among treatments. All statistical analyses were performed with *SPSS version 16.0*.

Results

Cd did not significantly affect the leaf and stem dry masses of *S. nigrum* according to one-way *ANOVA*, whereas the root dry mass showed a dose-dependent decrease (Fig. 1*B*). The root/shooot ratio also decreased with the increased Cd concentration (Fig. 1*D*). The visual appearance of the seedlings under the low concentrations of exogenous MeJA (0.01 to 10 μM) was comparable to that of the control, whereas 1 000 μM MeJa induced some impairement symptoms, such as yellow leaves, wilting leaves, and blackening roots (Fig. 1*A*). The treatments with exogenous MeJA did not show any effect on the dry mass of the seedlings grown under 40 mg dm^{-3} Cd, but 0.01 μM MeJA restored seedling growth that had been depressed by 40 mg dm^{-3} Cd (Fig. 1*C*,*E*).

The Cd content in the shoots and roots significantly increased with the increased Cd concentration after 7 d of growth (Fig. 2*A*,*C*). The Cd accumulation was proportional to the Cd concentration in the substrate ($y = 9.426 x - 13.3$, $r^2 = 0.97$, $P = 0.0004$ for shoots, and $y = 132.2 x - 573.7$, $r^2 = 0.99$, $P = 0.0019$ for roots). Interestingly, the MeJA treatment at a low concentration (0.01 μM) significantly inhibited Cd uptake from the substrate, whereas 1 000 μM MeJA showed no significant effect (Fig. 2*B*,*D*).

The MDA content of the roots increased significantly at Cd concentrations 40 and 55 mg dm^{-3}, but the changes in the leaves were not significant (Fig. 3*A*). Low concentrations of exogenous MeJA had no significant

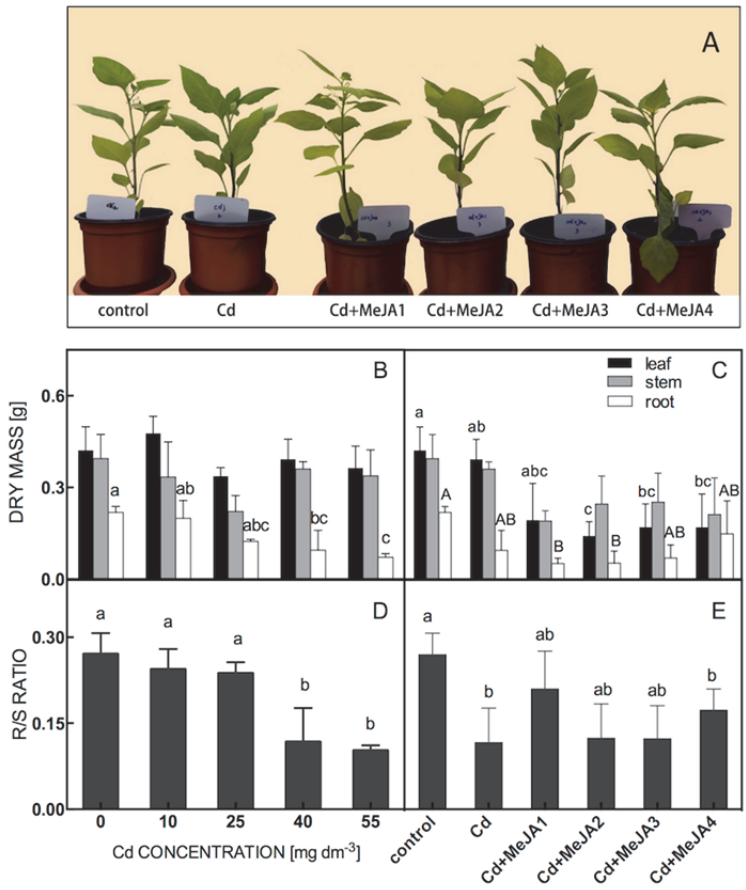

Fig. 1. The effect of different concentrations of Cd on *S. nigrum* appearance (*A*), dry masses of different parts (*B*), and root to shoot (R/S) ratio (*D*) at day 7 after the treatment. The effect of 40 mg dm^{-3} Cd and 0.1, 1, 10, or 1000 µM (MeJA1, 2, 3, and 4, respectively) on the plant dry mass (*C*) and R/S ratio (*E*). Values are means ± SD, $n = 3$, data with different lowercase and capital letters are significantly different at $P \leq 0.05$.

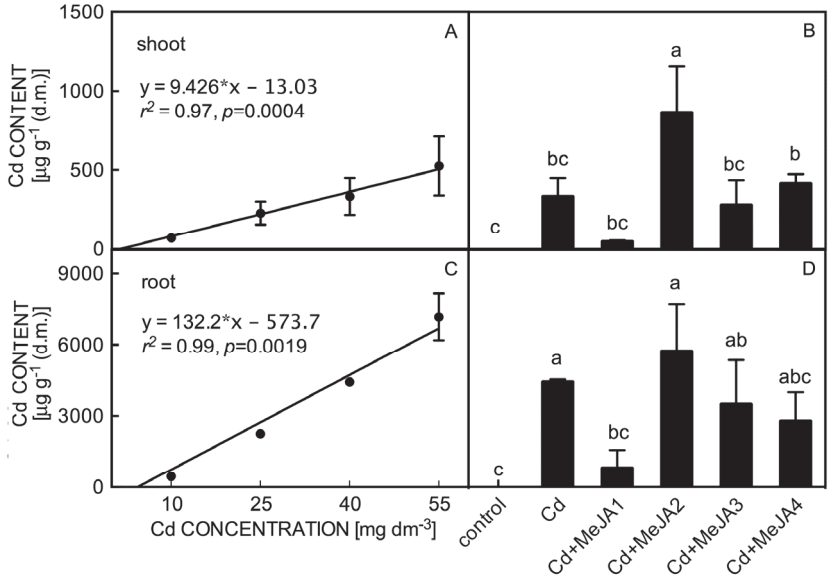

Fig. 2. The accumulation of Cd in shoots and roots of *S. nigrum* under different concentrations of Cd (*A*, *C*), and under 40 mg dm^{-3} Cd with 0.1, 1, 10, and 1000 µM MeJa (MeJA1, 2, 3, and 4, respectively) (*B*, *D*). Means ± SD, $n = 3$, for each plant part, data with different letters are significantly different at $P \leq 0.05$.

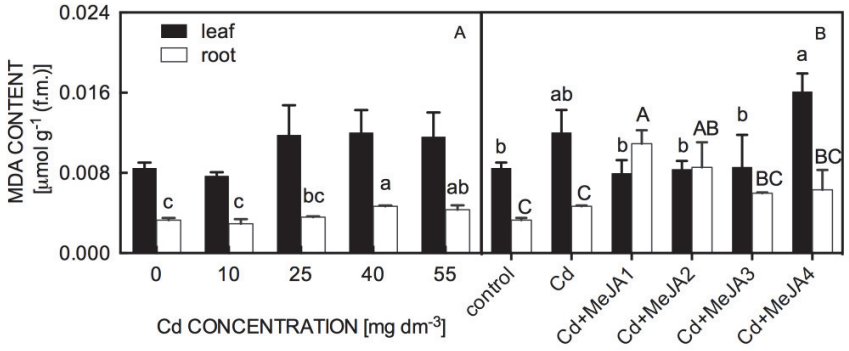

Fig. 3. The content of MDA in roots and leaves of *S. nigrum* under different concentrations of Cd (*A*), and under 40 mg dm^{-3} Cd with 0.1, 1, 10, and 1000 μM MeJa (MeJA1, 2, 3, and 4, respectively) (*B*). Means ± SD, $n = 3$, for each plant part, data with different lowercase or capital letters are significantly different at $P \leq 0.05$.

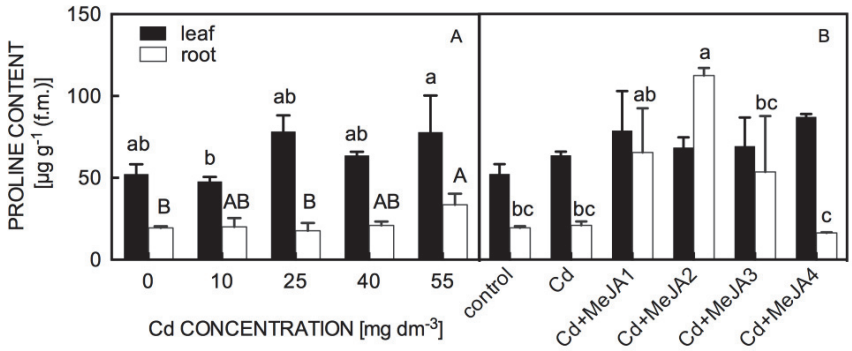

Fig. 4. The content of proline in roots and leaves of *S. nigrum* under different concentrations of Cd (*A*), and under 40 mg dm^{-3} Cd with 0.1, 1, 10, and 1000 μM MeJa (MeJA1, 2, 3, and 4, respectively) (*B*). Means ± SD, $n = 3$, for each plant part, data with different lowercase or capital letters are significantly different at $P \leq 0.05$.

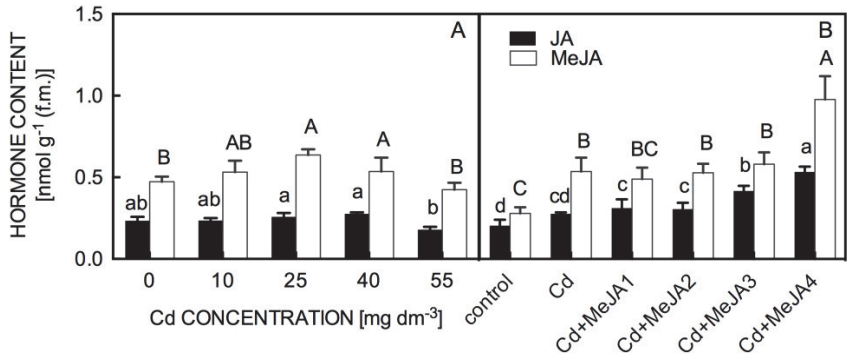

Fig. 5. The content of JA and MeJA in leaves of *S. nigrum* under different concentrations of Cd (*A*), and under 40 mg dm^{-3} Cd with 0.1, 1, 10, and 1000 μM (MeJA 1, 2, 3, and 4, respectively) (*B*). Means ± SD, $n = 3$, for each plant part, data with different lowercase or capital letters are significantly different at $P \leq 0.05$.

effect on the MDA content in the leaves and roots. Moreover, a significant increase in MDA was found in the leaves treated with 1 000 μM MeJa (Fig. 3*B*).

The treatment with Cd in different concentrations did not show any stimulatory effect on proline accumulation in the leaves. Only the highest Cd concentration increased the proline content in the roots (Fig. 4*A*). The treatment with 0.01 μM MeJA significantly increased the leaf proline content compared with the control and 40 mg dm^{-3} Cd treatments (Fig. 4*B*). A similar effect was also observed in the roots, whereas a significant increase in the proline content was observed under a treatment with 0.1 μM MeJA.

On day 7, the treatments with different concentrations

of Cd did not show any significant effect on a leaf endogenous JA content compared to the control, whereas 25 and 40 mg dm^{-3} Cd induced a significant accumulation of endogenous MeJA in the leaves (Fig. 5*A*). Leaf JA and MeJA concentrations under the combined treatments of exogenous MeJA and Cd showed an increasing pattern with the increase of exogenous MeJA concentration (Fig. 5*B*).

Dose-dependent increasing patterns were observed in the leaf and root GSH content under different Cd treatments (Fig. 6*A*). Low concentrations of exogenous MeJA further increased the GSH content in the leaves (0.01 μM MeJA) and roots (0.01 and 0.1 μM MeJA), but this stimulatory effect was not observed under 1 000 μM MeJA (Fig. 6*B*)

Cd did not affect the CAT activity in the leaves, but

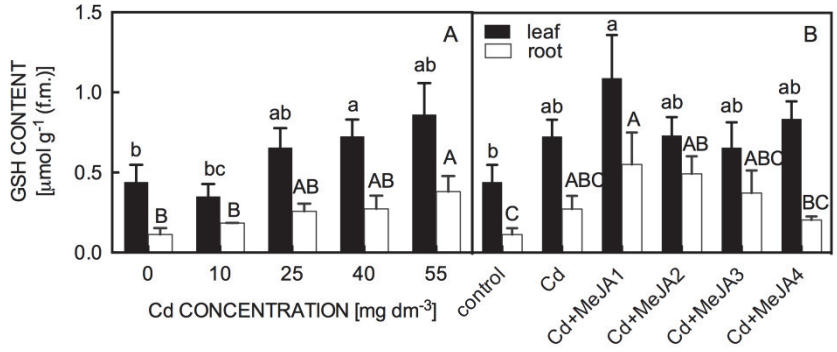

Fig. 6. The content of GSH in roots and leaves of *S. nigrum* under different concentrations of Cd (*A*), and under 40 mg dm^{-3} Cd with 0.1, 1, 10, and 1000 μM MeJa (MeJA1, 2, 3, and 4, respectively) (*B*). Means ± SD, $n = 3$, for each plant part, data with different lowercase or capital letters are significantly different at $P \leq 0.05$.

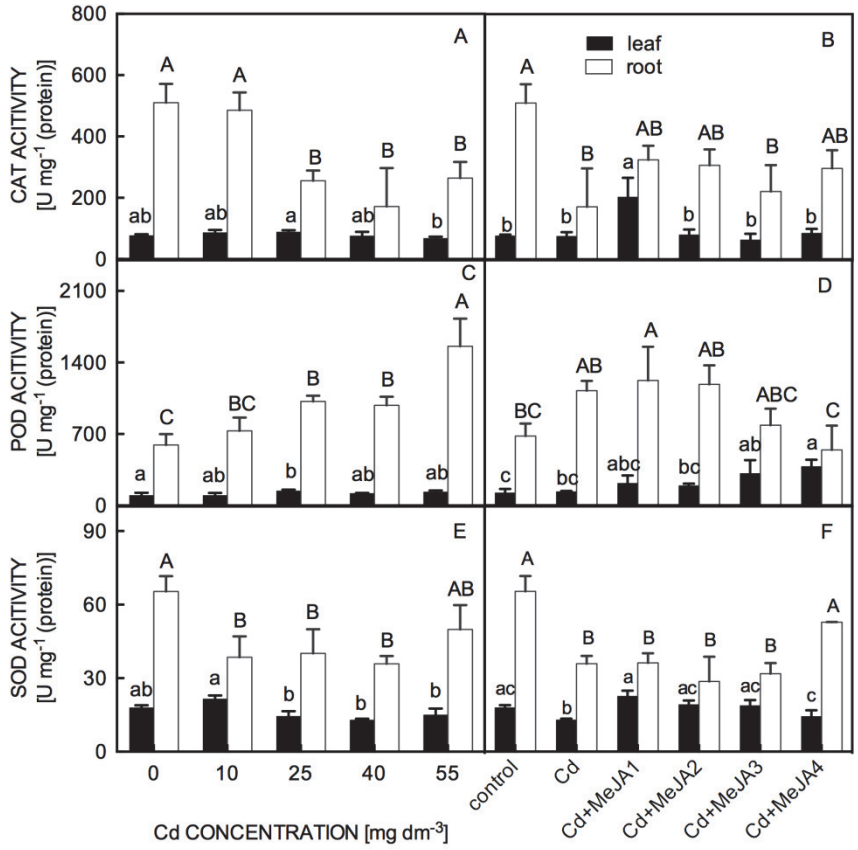

Fig. 7. Activities of CAT (*A*, *B*), POD (*C*, *D*), and SOD (*E*, *F*) in roots and leaves of *S. nigrum* under different concentrations of Cd (*A,C,E*), and under 40 mg dm^{-3} Cd with 0.1, 1, 10, and 1000 μM MeJa (MeJA 1, 2, 3, and 4, respectively) (*B,D,F*). Means ± SD, $n = 3$, for each plant part and enzyme, data with different lowercase or capital letters are significantly different at $P \leq 0.05$.

the CAT activity in the roots was significantly depressed by concentrations ≥ 25 mg dm^{-3} (Fig. 7*A*). This depression was significantly mitigated by 0.01 μM MeJA, but not by any other MeJA concentration (Fig. 7*B*). A similar effect was also observed in the roots, *i.e.*, 0.01 μM MeJA promoted the CAT activity.

The POD activity in the leaves was promoted by 25 mg dm^{-3} Cd, but no change was observed under other Cd treatments (Fig. 7*C*). In contrast, the POD activity in the roots significantly increased at Cd concentrations ≥ 25 mg dm^{-3}. The treatment with MeJA increased the leaf POD activity, and an increasing trend was generally dose dependent (Fig. 7*D*). MeJA of 0.01 μM also significantly increased the root POD activity, but this effect was not observed at higher doses.

The SOD activity showed no significant change in the leaves under various Cd treatments, but the SOD activity in the roots significantly decreased and significant decreases were observed at 10 and 40 mg dm^{-3} Cd (Fig. 7*E*). The decrease in the leaf SOD activity under 40 mg dm^{-3} Cd was mitigated by 0.01 μM MeJA, but such an effect was not observed in the roots (Fig. 7*F*).

Discussion

In the present study, Cd inhibited the root dry mass of *S. nigrum*, and thus the root/shoot ratio decreased with the increased Cd concentration. This observation was expected considering that the root is the first organ affected by Cd present in soil (Andresen and Küpper 2013). By contrast, the dry mass of the leaves and stems of *S. nigrum* were not affected by Cd similarly as reported in previous studies (Sun *et al.* 2007, 2008). Nevertheless, a long-term exposure to Cd may also inhibit the growth of the shoot of *S. nigrum* (Sun *et al.* 2007, Pinto *et al.* 2009). Plants subjected to high MeJA concentrations usually shows a substantial decrease in photosynthetic rate and chlorophyll content, however, MeJA increase chlorophyll content and growth when the concentration is low (Keramat *et al.* 2009, Piotrowska *et al.* 2009, Kováčik *et al.* 2011). In the present study, the treatment with a 0.1 μM MeJA restored the R/S of the seedlings depressed by 40 mg dm^{-3} Cd suggesting that a low MeJA concentration mitigated the inhibitory effect of Cd on root growth.

The translocation/accumulation of Cd in both the shoots and roots of *S. nigrum* under 0.01 μM MeJA were significantly reduced compared with the Cd treatment only. These results are similar to a previous finding that the application of 0.1 μM JA significantly inhibits Pb accumulation in *Wolffia arrhiza* (Piotrowska *et al*. 2009). The addition of another stress hormone ABA to a hydroponic solution also reduces both xylem sap Cd concentration and shoot Cd accumulation (Salt *et al.* 1995, Zhao *et al.* 2006). Also the application of phytohormone 2,4-epibrassinolide decreases nickel uptake in *Brassica juncea* (Kanwar *et al.* 2012). The xylem loading by heavy metals including Cd is mainly influenced by plant transpiration (Lux *et al.* 2011) and the reduced uptake of metals under ABA and brassinosteroid treatments is interpreted as the result of reduction in transpiration rate (Salt *et al.* 1995). Similarly to ABA, the application of MeJA also affects plant transpiration by promoting stomatal closure (Hossain *et al.* 2011). The reduced uptake of Cd in the shoots and roots of *S. nigrum* in the present study might also be a result of stomatal closure and a decreased transpiration. To verify this, future works are necessary to follow the changes of transpiration rate of seedlings under combined metal and MeJA treatments. A high dose of MeJA can initiate oxidative stress (Mur *et al.* 2006), which induces damage to the physiological barriers and results in a free flow of Cd to the tissue. This phenomenon can explain why the accumulation of Cd in the shoots and roots of *S. nigrum* at a high concentration of MeJA were not reduced compared with the Cd treatment only.

The generation and accumulation of ROS lead to the destruction of membrane lipids and results in a significant MDA accumulation (Gill and Tuteja 2010). In the present study, a significant increase in MDA was observed at 40 and 55 mg dm^{-3} Cd in the roots but not in the leaves. The present results are in accordance with those obtained by Sun *et al.* (2007) and Pinto *et al.* (2009) and indicate that the leaves of *S. nigrum* had a higher ROS detoxifying ability than the roots. Indeed, organic acids in *S. nigrum* have been suggested to play important roles in binding incoming Cd and alleviating its deleterious effect (Sun *et al.* 2007).

Proline plays an important protective role against heavy metal stress, and the free proline accumulation capacity is reportedly a good indication of self-protection of plants growing under stressful conditions (Chen *et al.* 2004). The Cd-induced proline accumulation is an important mechanism (Schat *et al.* 1997). In the present study, a significant increase in proline was observed only in the roots of *S. nigrum* under the highest concentration of Cd (55 mg dm^{-3}), but Sun *et al.* (2007) found that the proline content significantly increases under Cd stress in the leaves and roots of *S. nigrum*. The treatment with 0.01 or 0.1 μM MeJA induced a significant increase in proline content in both the leaves and roots suggesting that MeJA exerted a protective effect on *S. nigrum* under the Cd stress by enhancing the proline synthesis. Similar to proline, the GSH content in the leaves and roots of *S. nigrum* were also positively regulated by low concentrations of MeJA. Previous studies also showed that exogenous JA treatments increase mRNA levels and the capacity for the synthesis of the GSH in *Arabidopsis* under Cd stress (Xiang and Oliver 1998), which indicates that jasmonates (both JA and MeJA) at certain concentrations stimulate the GSH synthesis and may thus help to increase resistance to the metal.

In the present study, the POD activity significantly

increased in the roots of *S. nigrum* with the elevated Cd concentration, whereas the increase in the leaves was not significant. Similar results were found by Pinto *et al.* (2009). A significant increase in the root POD activity was accompanied by a significant decrease in the root CAT activity and an increase in the root MDA content indicating that the POD activity was not adequate to eliminate ROS induced by the Cd stress. The SOD activities in the leaves and roots decreased with the increased Cd stress, which may be due to a direct inhibition by Cd. Similar results were obtained in cotyledons of *Brassica napus* seedlings under 200 to 500 μM Cd stress (Ali *et al*. 2014). The results of the present study diverge from those obtained by Sun *et al.* (2007), who found that POD and CAT activities significantly increase after five weeks of Cd treatment. Pinto *et al.* (2009) also found that CAT activity increases both in the leaves and roots of *S. nigrum* after 90 d of Cd treatment. Guo *et al*. (2014) also found that leaf POD and SOD activities in seedlings of *Agropyron cristatum* increase with the increase of Cd concentration and treatment duration. MeJA can affect the activity and/or pools of antioxidative enzymes, thereby causing the alleviation of oxidative stress (Keramat *et al.* 2009, Piotrowska *et al.* 2009). An increased activity of various antioxidant enzymes including SOD, CAT, and APX in the presence of 10 to 100 μM MeJA has been observed in soybean under Cd stress (Keramat *et al.* 2009). JA at 0.1 μM was found to activate the enzymatic (CAT, APX, and POD) and non-enzymatic antioxidants (ascorbate and glutathione) of *Wolffia arrhiza* (Piotrowska *et al.* 2009). Similarly, the present study also found that 0.01 μM MeJA significantly increased the CAT and SOD activities in the leaves and the POD activity in the roots of *S. nigrum.*

Endogenous JA is suggested to be involved in a cellular response to metal toxicity (Maksymiec 2005). Increases in endogenous JA were also observed in *Arabidopsis thaliana* and *Phaseolus coccineus* treated with Cu and Cd (Maksymiec *et al*. 2005), pea plants treated with Cd (Koeduka *et al.* 2005), and mangrove seedlings, such as *Kandelia obovata*, *Acanthus ilicifolius*, and *Excoecaria agallocha*, treated with Pb (Yan and Tam 2013a,b). In the present study, endogenous JA and MeJA both significantly increased under the Cd treatments. All genes of JA biosynthesis enzymes are JA-inducible, and the JA biosynthesis is regulated by a positive feedback (Wasternack 2007). In the present study, the increases of endogenous JA and MeJA under the exogenous MeJA treatment might be attributed to the *de novo* biosynthesis or the intake of exogenous JA *per se*, which need further investigation.

Conclusions

The present study revealed that the root dry mass and R/S of *S. nigrum* significantly decreased with the increased Cd concentration, whereas the shoots (leaves and stems) were less affected by Cd than the roots probably due to rather a low translocation of Cd to the shoots. The treatment with 0.1 μM MeJA restored the R/S ratios of the seedlings under 40 mg dm^{-3} Cd, and decreased the translocation and accumulation of Cd in both the shoots and roots. The treatment with low concentrations of MeJA also enhanced the content of proline and GSH, and the activities of POD and SOD compared with those under 40 mg dm^{-3} Cd alone.

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