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Methyl jasmonate as modulator of Cd toxicity in *Capsicum frutescens* var. fasciculatum seedlings

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ABSTRACT

Methyl jasmonate (MeJA) elicits protective effects as form of plant response to abiotic stress. However, related studies on plant response to metal stress are insufficient. This study aimed to examine the effects of MeJA on growth and physiological responses of *Capsicum frutescens* seedlings exposed to cadmium (Cd) stress. The study was performed in an artificial climate chamber. Results showed that 50 mg L^{-1} Cd significantly impaired the growth of the seedlings by increasing leaf MDA content and decreasing chlorophyll *b*. These effects were significantly mitigated by MeJA at low concentrations (0.1 μmol L^{-1}). The dry weights of different plant parts, chlorophyll content, and leaf catalase and ascorbate peroxidase activities were increased by a low MeJA concentration (0.1 μmol L^{-1}) but were decreased by a high MeJA concentration (1000 μmol L^{-1}). Significant increases in endogenous jasmonic acid were observed at 48 h after the samples were treated with Cd and 0.1 μmol L^{-1} MeJA. These results suggested that low exogenous MeJA concentrations exhibited protective effects on the growth and physiology of *C. frutescens* seedlings under Cd stress.

1. Introduction

Metal pollution has become a serious problem as a result of rapid developments in industrial and agricultural activities (Cheng, 2003). Metals likely cause damages on plant physiology when metal concentrations in the environment exceed the maximum tolerable amount, thereby resulting in photosynthetic output reduction and growth degeneration (Prasad, 2004). Toxic metals such as lead (Pb), cadmium (Cd), and mercury (Hg) are strongly phytotoxic partly because reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) are generated (Prasad, 2004). High levels of ROS usually damage cellular components such as membranes, nucleic acids, and chloroplast pigments, resulting in lipid peroxidation (Tewari et al., 2002). Membrane lipid peroxidation produces lipid radicals and a complex mixture of lipid degradation products, such as malondialdehyde (MDA) (Knight and Voorhees 1990). As a result, plants have evolved multiple mechanisms involving enzymatic antioxidants [such as superoxide dismutases (SOD), peroxidases (POD), and catalase (CAT)] and non-enzymatic scavengers (mainly glutathione, carotenoids, and ascorbate), which help scavenge ROS, delay or inhibit the oxidation of lipids or other molecules, and alleviate their deleterious effects (Prasad, 2004). SOD, CAT, and POD are three groups of important antioxidant enzymes (Vangronsveld and Clijsters, 1994). SOD is the major $O_2^{\bullet-}$ scavenger, and its enzymatic action yields H_2O_2 and O_2. CAT and several classes of peroxidases then scavenge H_2O_2. POD decomposes H_2O_2 by oxidizing co-substrates such as phenolic compounds and/or antioxidants (Blikhina et al., 2003). Ascorbate peroxidase and glutathione reductase, as well as glutathione, are important components of the ascorbate glutathione cycle that removes H_2O_2 from different cellular compartments (Jiménez et al., 1997).

Jasmonates (JAs), including jasmonic acid (JA) and methyl jasmonate (MeJA) are a family of cyclopentanone compounds synthesized from linolenic acid via the octadecanoic pathway; JAs exhibit important functions in the signaling network of plants under various biotic and abiotic stresses (Fujita et al., 2006). JA is involved in NADPH oxidase activation, in which H_2O_2 functions as a second messenger regulating the defense mechanism (Orozco-Cárdenas et al., 2001). Higher levels of JAs destroy chlorophyll, inhibit growth and photosynthetic activity, and intensify senescence processes (Maksymiec and Krupa, 2002; He et al., 2002; Jung, 2004). However, application of certain concentrations of JAs can significantly enhance plant's tolerance. Exogenously applied JAs elicit different physiological responses to stress and increase plant resistance (Walia et al., 2007; Keramat et al., 2009). However, few studies have investigated the mechanism of the toxic effects of MeJA on plants under metal stress. Studies have mainly focused on model plants (*Arabidopsis thaliana*) and herbaceous plants (Maksymiec and Krupa, 2002; Piotrowska et al., 2009; Kováčik et al., 2011). MeJA at 10^{-6} and

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10^{-5} mol L^{-1} elicits protective effects against Cu and Cd ions in *Arabidopsis* plants; by contrast, such protective effects are not observed at 10^{-4} mol L^{-1} (Maksymiec and Krupa, 2002). Lower MeJA concentrations (10^{-7} mol L^{-1} to 10^{-5} mol L^{-1} MeJA) also exhibit stimulatory effects on the photosynthetic pigments of lower aquatic plants, such as *Scenedesmus quadricauda* (Turp.) Bre´b (Kováčik et al., 2011) and *Wolffia arrhiza* (Piotrowska et al., 2009). The application of MeJA and Cd lessens damages caused by Cd by reducing MDA and H_2O_2 content and by increasing antioxidant enzyme activities in soybean plants (*Glycine max* L.) (Keramat et al., 2009).

Abiotic stress such as metal stress stimulates endogenous JA in herbaceous plants such as *A. thaliana*, *Phaseolus coccineus*, and *Oryza sativa* L. (Koeduka et al., 2005; Maksymiec et al., 2005). However, studies on the effect of exogenous MeJA treatment on endogenous JA responses in metal-stressed plants are insufficient; the mechanism by which low exogenous MeJA levels elicit protective effect has not been elucidated yet. The present study aimed to (i) examine the effect of different levels of MeJA on the physiology of toxic metal-induced plant stress and (ii) determine the time-course responses of lipid peroxidation, H_2O_2, and endogenous JA after MeJA and Cd treatment. *Capsicum frutescens* var. fasciculatum, an annual perennial economical crop plant, was selected as a model plant. Cd was selected as a model metal because it is highly prevalent in the farmlands in China and has posed increasing risk of pollution for several years (Niu et al., 2013).

2. Materials and methods

2.1. Experimental setup and sample collection

C. frutescens seeds were surface sterilized with 70% alcohol and then sown on sand in plastic pots [24 cm (open top) × 30 cm (height) × 20 cm (flat bottom)] with half-strength Japanese garden test nutrient solution (Hori 1966). The planted pots were placed on a bench in an artificial climate chamber with daily temperatures of 25 °C, relative humidity of 70%, and light intensity of 800 μmol photons m^{-2} s^{-1}. The seedlings were irrigated with deionized water once every day until the third pair of leaves completely unfolded. Afterward, the pots were randomly divided into nine groups each in triplicate. Six groups were used to determine the effect of MeJA on the responses of the seedlings under Cd treatments: (i) control treatment; (ii) 50 mg L^{-1} Cd; (iii) 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA (MeJA1); (iv) 50 mg L^{-1} Cd + 1 μmol L^{-1} MeJA (MeJA2); (v) 50 mg L^{-1} Cd + 10 μmol L^{-1} MeJA (MeJA3); and (vi) 50 mg L^{-1} Cd + 1000 μmol L^{-1} MeJA (MeJA4). According to the study of Keramat et al. (2009), treatment with MeJA up to 10 μmol L^{-1} showed no effect on MDA and chlorophyll contents in *G. max* L., and we do not intent to investigate the solely effect of MeJA in the present study, hence we do not consider the positive control set (controls with only MeJA at the used concentrations). For the treatments, appropriate amounts of MeJA (sigma) was dissolved in 2 mL of ethanol and diluted to obtain appropriate concentrations with half-strength Japanese garden test nutrient solution containing 50 mg L^{-1} Cd (cadmium chloride, $CdCl_2$). One group without any treatment was used as the control. The seedlings were irrigated with deionized water every day to compensate for water lost by evaporation.

The leaf samples were collected from all of the treatments at the end of the experiment (7 d). To determine the short-term changes in leaf MDA, endogenous JA, and H_2O_2, we collected the leaves of the seedlings from the following treatment groups at 0, 12, 24, 48 h after the treatment: (i) control group; (ii) 50 mg L^{-1} Cd; and (iii) 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA. At each sampling time, two seedlings in each pot were pulled carefully from the sediment and washed with deionized water. The leaf of one seedling was collected, rapidly frozen in liquid nitrogen, and stored at −80 °C. The different parts of another seedling were collected and dried in an oven at 70 °C for 48 h. The dry weights of the different plant parts were recorded.

2.2. Determinations

2.2.1. Chlorophyll concentration

Leaf chlorophylls were determined according to the method described by Wang et al. (2009). In brief, 0.1 g of fresh leaf sample was cut from mature leaf and then finely sliced with stainless steel scissors to increase the surface area of the tissue exposed to the extractant. The sample was then placed in a 15 mL amber glass screw-cap bottle containing 10 mL of a mixed solution of acetone, ethanol, and distilled water (4.5:4.5:1 proportions, respectively) and stored in dark at 4 °C for 2 days. The absorbance readings of the extract were recorded at 645 and 663 nm, and the concentrations (mg L^{-1}) of chlorophyll *a*, chlorophyll *b*, and total chlorophylls in the extract were calculated according to equations of Arnon (1949).

2.2.2. Determination of MDA

MDA in the leaf was determined according to the method of Wang and Jin (2005) with slight modifications. In brief, 0.2 g of fresh plant sample was homogenized in a mortar and pestle with 4 mL of 20% trichloroacetic acid (TCA) (w/v). The homogenate was centrifuged at 9000 × *g* for 5 min. Approximately 1 mL of the supernatant was mixed with an equal volume of 0.6% (w/v) thiobarbituric acid solution containing 10% TCA. The mixture was heated in boiling water for 30 min and then transferred to an ice bath to terminate the reaction. The cooled mixture was centrifuged at 5000 × *g* for 10 min at 25 °C, and the absorbance readings of the supernatant at 450, 532, and 600 nm were recorded. The concentration of MDA (C_{MDA}) was calculated according to the following equation:

$$C_{MDA} = 6\bullet45(A_{532}-A_{600})-0\bullet56A_{450}\bullet$$

2.2.3. Determination of H_2O_2

H_2O_2 reacts with ammonium molybdate to form a stable yellowish complex that exhibits the greatest absorbance at 405 nm. H_2O_2 concentration in the leaves was determined according to the method of Góth (1991) with slight modifications. In brief, 100 mg of leaf sample was homogenized with 3 mL of phosphate buffer (50 mmol L^{-1}, pH 7.4). The homogenate was centrifuged at 9000 × *g* for 10 min. To determine the H_2O_2 content, we mixed 0.5 mL of extracted solution with 50 mmol L^{-1} ammonium molybdate; the absorbance at 405 nm was then recorded. The unit of H_2O_2 concentration was expressed as mmol^{-1} H_2O_2 g^{-1} protein. The H_2O_2 concentration was calculated using an H_2O_2 standard curve. Protein concentration was determined using the Coomassie Brilliant Blue G-250 reagent according to the method of Bradford (1976), in which bovine serum albumin was used as the standard.

2.2.4. Determination of total glutathione (GSH)

Total GSH level was determined according to the method of Beutler et al. (1963). The plant material (0.5 g of fresh weight) was homogenized with mortar and pestle in 5 mL of cold extraction buffer containing 2% 5-sulfosalicylic acid dehydrate, 1 mmol L^{-1} ethylenediaminetetraacetic acid disodium (EDTA–Na_2), and 0.15% ascorbate. The homogenate was centrifuged for 10 min at 9000 × *g* at 4 °C. The reaction mixture containing 1 mL of supernatant, 2 mL of 1% Tris–HCl buffer (pH 8.9), and 50 μL of 10 mmol L^{-1} 5,5′-ditiobis (2-nitrobenzoic acid) (DTNB) was maintained at room temperature for 5 min and the absorbance was obtained at 412 nm. The GSH concentration was calculated using a GSH standard curve.

2.2.5. Determination of endogenous JA concentration

Endogenous JA concentrations were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Rapidbio, USA). Standards or samples are added to the appropriate microtiter plate wells with horseradish peroxidase (HRP)-conjugated JA and incubated, and a competitive inhibition reaction is launched between JA (In standards or samples) and HRP-conjugated JA with the pre-coated antibody specific for JA. All reagents, samples and standards were prepared according to the manufacturer's instructions. The detection limit for the endogenous JA is 80 pmol L^{-1}.

2.2.6. Antioxidative enzyme activity

Approximately 0.3 g of fresh leaf sample was extracted in 4 mL of 50 mmol L^{-1} of ice-cold sodium phosphate buffer (pH 7.4) combined with 1.0 mmol L^{-1} EDTA–Na_2. The homogenate was centrifuged at 9000 × *g* at −4 °C for 10 min, and the supernatant was used for the enzyme assay.

The SOD activity was determined by hydroxylamine assay developed from xanthine oxidase assay according to the method of Elstner and Heupel (1976). In brief, the homogenate was analyzed in a solution containing 0.037 U ml^{-1} xanthine oxidase, 0.375 mmol L^{-1} xanthine, and 0.1 mmol L^{-1} hydroxylamine and enzyme extract. The reaction was initiated using xanthine, incubated at 37 °C for 30 min in a water bath, and terminated by adding 2.5 mmol L^{-1} sulfanilic acid. One unit (U) of SOD activity is defined as the amount that results in a 50% decrease in the rate of nitrite formation from hydroxylamine oxidation induced by $O_2^{\bullet-}$/mg of protein. The $O_2^{\bullet-}$ level was determined at 530 nm after 8 mmol L^{-1} α-naphthylamine (in glacial acetic acid) was added.

CAT activity was determined according to the method of Beer and Sizer (1952) with slight modifications. The reaction mixture (2.5 mL) consisted of 50 mmol L^{-1} phosphate buffer (pH 7.4), 0.1 mmol L^{-1} EDTA, 20 mmol $L^{-1}$$H_2O_2$, and 500 μL of 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 based on its molar extinction coefficient (36 mol L^{-1} cm^{-1}). One unit (U) of CAT activity was defined as a change in absorbance by 0.01 unit/min, and the activity was expressed as U mg^{-1} $protein^{-1}$.

Guaiacol POD activity was determined according to guaiacol method (Fielding and Hall, 1978). Approximately 100 μL of enzyme extract was mixed with 3 mL of 50 mmo L^{-1} phosphate buffer (pH 7.4) containing 0.2% guaiacol (v:v). The reaction was initiated with 1 mL of 0.3% H_2O_2, and guaiacol oxidation was determined based on an increase in the absorbance at 470 nm for 2 min. One unit of POD activity (U) was defined as a change in absorbance by 0.01 unit/min, and the activity was expressed as U mg^{-1} protein^{-1}.

Ascorbate peroxidase (APx) activity was estimated according to the method of Nakano and Asada (1981). Approximately 100 μL of enzyme extract was mixed with 2 mL of 50 mmol L^{-1} potassium phosphate buffer (pH 7.4) containing 0.5 mmol L^{-1} of ascorbate, 1 mmol L^{-1} EDTA–Na_2, and 0.1 mmol $L^{-1}H_2O_2$. Enzyme activity was determined by monitoring the decrease in absorbance at 290 nm for 2 min. One unit (U) of APx activity was defined as the change in absorbance by 0.01 unit/min, and the activity was expressed as U mg^{-1} protein^{-1}.

Glutathione peroxidase (GPx) activity was determined by monitoring the decrease in GSH according to the method of Huang and Wu (1999). In brief, two sets of tubes were prepared. Each tube was filled with 0.2 mL of enzyme extracts, and one tube was heated at 70 °C to inactivate the enzyme. Approximately 0.4 mL of 1.0 mmol L^{-1} GSH and 0.2 mL of 1.5 mmol $L^{-1}H_2O_2$ were added in each tube and incubated at 37 °C for 3 min. Afterward, 2 mL of 1.67% metaphosphoric acid was added. The reaction mixture was centrifuged at 5000 × *g* for 5 min. Two sets of aliquots (2 mL) of the resulting supernatant were then transferred to two test tubes; each test tube contained 2.5 mL of 0.32 mol L^{-1} Na_2HPO_4 and 0.5 mL of 10 mmol L^{-1} DTNB (w:v in 1% trisodium citrate). The test tubes were then stored at room temperature for 5 min. and the absorbances were read at 412 nm. One unit (U) of GPx was defined as 1 mmol of GSH oxidation per min per mg protein, and the activity was expressed as U mg^{-1} protein^{-1}.

2.3. Statistical analyses

The mean and standard deviation (SD) of the three replicates (one sample in each replicate) were calculated. Parametric one-way ANOVA and post-hoc multiple comparison (Tukey's test) was conducted to determine the significant differences in the detected parameters among different levels of Cd treatments. Two-way multivariate ANOVA (MANOVA), in which the treatment and the treatment time were considered as two independent variables, was applied to examine the significant interactive effects and differences between *C. frutescens* seedlings based on different variables (i.e., the contents of H_2O_2, MDA, and endogenous JA in the leaf). Statistical analyses were performed in SPSS version 16.0.

3. Results

3.1. Changes in growth and photosynthetic pigments

The growth and photosynthetic pigments showed significant changes caused by different Cd treatments and increasing levels of exogenous MeJA (Fig. 1). The treatment with 0.1 μmol L^{-1} MeJA significantly enhanced chlorophyll *b* concentrations. The chlorophyll concentrations in the leaf also decreased as the concentration of MeJA treatments increased (Fig. 1a). As the concentration of exogenous MeJA increased, the ratio of chlorophyll *a*/*b* decreased (Fig. 1b), suggesting that MeJA elicited greater inhibitory effects on chlorophyll *a*. The treatment with 50 mg L^{-1} Cd induced significant decreases in the dry weights of the roots, but no significant effects were observed in the other parts of the seedlings. The treatment with a lower concentration (0.1 μmol L^{-1}) of MeJA possibly restored the growth of the seedlings, although this restoration was not significant. As exogenous MeJA increased, the dry weights of different plant parts were gradually reduced particularly under the highest concentrations of MeJA treatment (Fig. 1c). The treatment with exogenous MeJA also resulted in a decrease in the root to shoot (R/S) ratio (Fig. 1d).

3.2. Changes in H_2O_2, MDA, and endogenous JA concentrations

MANOVA, followed by Wilk's lambda test, showed that the treatment time exhibited significant effects on H_2O_2 production in the leaves of *C. frutescens* (Table S1 of supporting information). H_2O_2 is a major component of the ROS and can be induced by abiotic stress. The increase in endogenous H_2O_2 concentrations and the different levels of exogenous MeJA was dose dependent, in which the highest values were recorded at higher levels of exogenous MeJA (10 and 1000 μmol L^{-1}) in the leaves of *C. frutescens* (Fig. 2a). Significant increases in leaf H_2O_2 were only recorded at 50 mg L^{-1} Cd and 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA at 48 h after the seedlings were treated with the Cd (Fig. 2b) compared with the control. However, these increases were no longer observed at 7 d (168 h).

Similar to H_2O_2, the increase in MDA concentration in the leaf of *C. frutescens* as MeJA treatments were increased was dose dependent (Fig. 3a). The treatment time and the interaction of the treatments elicited significant effects on leaf MDA production according to the MANOVA result (Table S1 of supporting information). Leaf MDA content showed no changes in the first 48 h, whereas significant changes were observed at 7 d (168 h) after the treatment (Fig. 3b). At day 7, the treatment with 50 mg L^{-1} Cd significantly induced the production of MDA in the leaves of *C. frutescens* compared with that of the control group. By contrast, the treatment with 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA significantly mitigated the deleterious effect of Cd, and the leaf MDA was reduced to a level comparable to that of the control group (Fig. 3a).

The treatment time and the interaction of the treatments exhibited significant effects on leaf JA concentration according to the MANOVA test result (Table S1 of supporting information). Endogenous JA in the leaves of *C. frutescens* showed no significant changes in the first 24 h after the samples were treated with Cd (Fig. 4b). Significant increases in JA were only observed at 48 h after the samples were treated with 50 mg L^{-1} Cd and 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA. Leaf JA concentrations showed no significant changes as the treatments increased at 7 d (Fig. 4a).

3.3. Changes in GSH and antioxidative enzymes

The changes in the total GSH in the leaves of *C. frutescens* showed a pattern similar to that of H_2O_2 and MDA; in particular, a dose-dependent increasing pattern was observed (Fig. S1 of supporting information).

CAT and APx are the two major H_2O_2 scavengers, which decompose toxic H_2O_2 to non-toxic H_2O. In the present study, the lowest level (0.1 μmol L^{-1}) of MeJA treatment stimulated CAT and APx activities, and the highest values were observed at 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA (Fig. 5a and b). An increase in MeJA treatment significantly decreased enzymatic activities, particularly CAT. Guaiacol POD and glutathione peroxidase (GPx) both detoxify a large family of peroxides, including H_2O_2. In the present study, POD and GPx increased as exogenous MeJA treatments increased. Significant increases in POD and GPx were observed at the highest levels of MeJA treatments (Fig. 5c and d). SOD catalyzes the dismutation of a $O_2^{\bullet-}$ to O_2 and H_2O_2. This process is an important antioxidant defense in almost all of the cells exposed to O_2. In the present study, 50 ppm Cd and exogenous MeJA significantly affected SOD activity in 7 d of treatment (Fig. 5e).

4. Discussion

Most plants are sensitive to low Cd concentrations, which inhibit plant growth as a consequence of alterations in photosynthetic rate as well as in the uptake and distribution of macronutrients and micronutrients (Sandalio et al., 2001; Benavides et al., 2005; Rodríguez-Serrano et al., 2009). These effects were also observed in the present study. In particular, the roots of *C. frutescens* was significantly inhibited by 50 mg L^{-1} Cd after 7 d of treatments, although the dry weights of leaves and the chlorophyll concentrations did not show any significant changes after the seedlings were treated with Cd. Plants, when exposed to higher MeJA concentrations, usually show a substantial

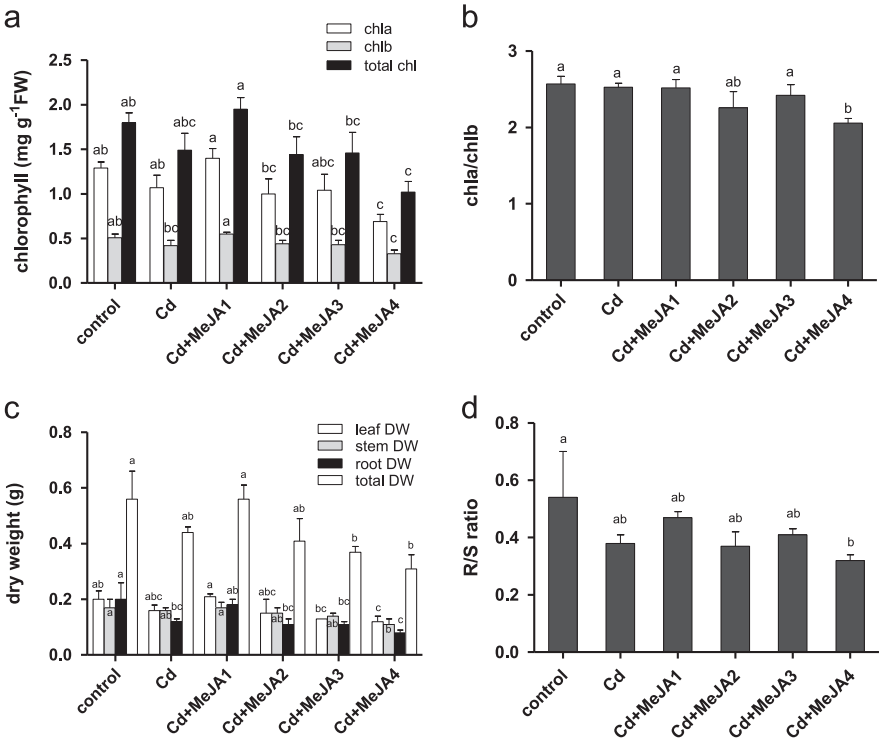

Fig. 1. Changes of (a) chlorophyll a, chlorophyll b and total chlorophylls, (b) ratios of chlorophyll a/b, (c) dry weight of different plant parts and (d) ratios of root/shoot (R/S) in leaf of *C. frutescens* seedlings after 7 days treatment of Cd (50 mg L^{-1}) and different concentrations of MeJA (0.1, 1, 10 and 1000 μmol L^{-1} for MeJA 1, 2, 3 and 4, respectively; values are mean and SD; data with different letters are significantly different at $P \leq 0.05$).

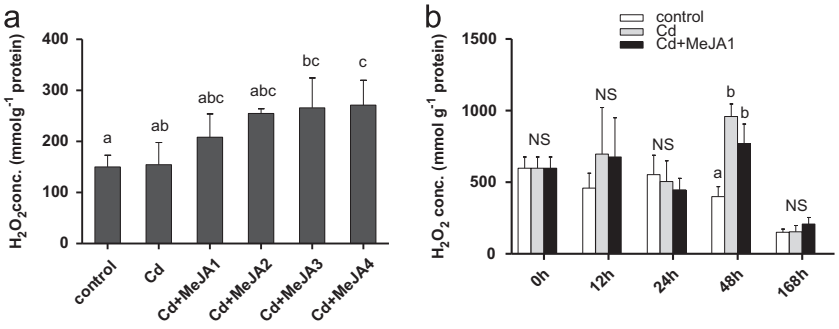

Fig. 2. Changes of H_2O_2 concentrations in leaf of *C. frutescens* seedlings under (a) different treatments of Cd (50 mg L^{-1}) and MeJA (0.1, 1, 10 and 1000 μmol L^{-1} for MeJA 1, 2, 3 and 4, respectively) at day 7, and (b) at different time after the treatments (Values are mean and SD; bars with different letters are significantly different at $P \leq 0.05$; for time series changes of H_2O_2, statistical was made in separate groups of time, NS indicates not significant).

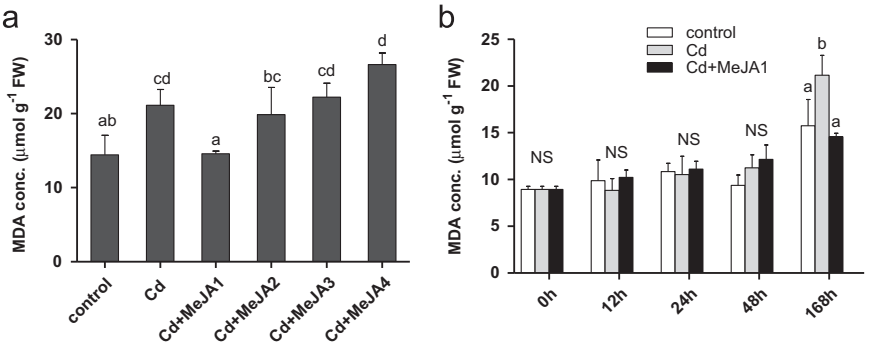

Fig. 3. Changes of MDA concentrations in leaf of *C. frutescens* seedlings (a) under different treatments of Cd (50 mg L^{-1}) and MeJA (0.1, 1, 10 and 1000 μmol L^{-1} for MeJA 1, 2, 3 and 4, respectively) at day 7, and (b) at different time after the treatments (values are mean and SD; bars with different letters are significantly different at $P \leq 0.05$; for time series changes of MDA, statistical was made in separate groups of time, NS indicates not significant).

decrease in photosynthetic activities and chlorophyll concentrations as well as a senescence-like yellowing (Saniewski et al., 1998; Jung, 2004). In a previous study, 100 μmol L^{-1} MeJA reduces the chlorophyll content in the cotyledons of *Cucurbita pepo* (Ananieva et al., 2007) and *W. arrhiza* (Piotrowska et al., 2009). However, lower MeJA concentrations usually stimulate an increase in chlorophyll concentration

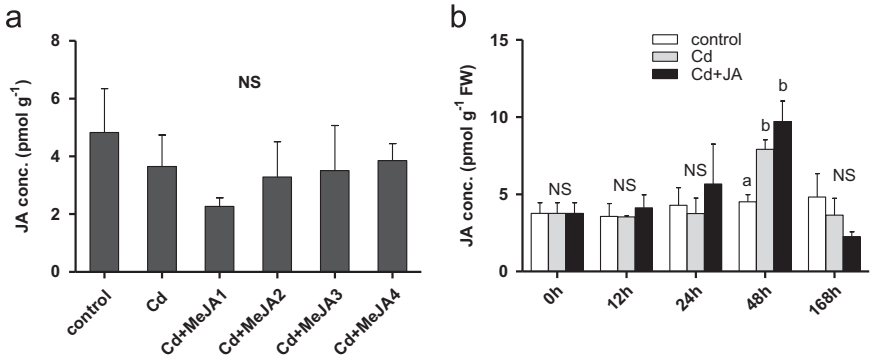

Fig. 4. Changes of JA concentrations in leaf of *C. frutescens* seedlings (a) under different treatments of Cd (50 mg L^{-1}) and MeJA (0.1, 1, 10 and 1000 μmol L^{-1} for MeJA 1, 2, 3 and 4, respectively) at day 7, and (b) at different time after the treatments (values are mean and SD; bars with different letters are significantly different at $P \leq 0.05$; for time series changes of JA, statistical was made in separate groups of time, NS indicates not significant).

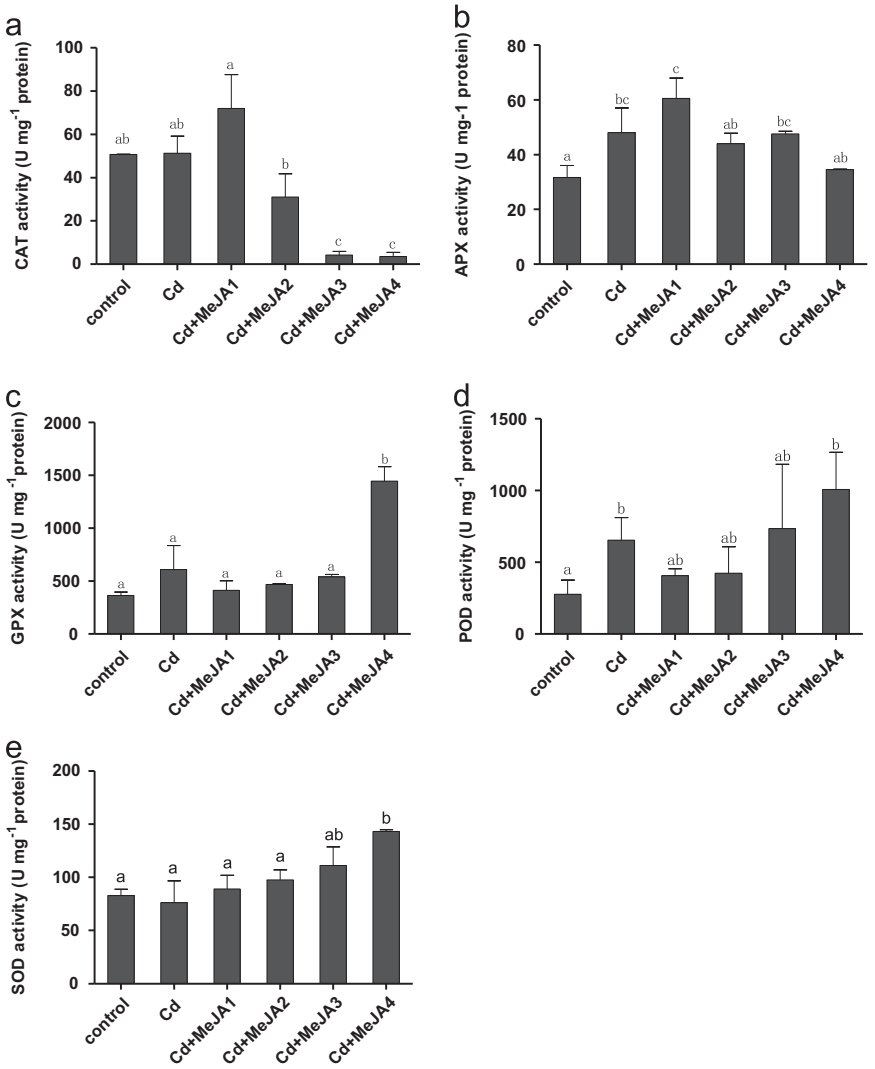

Fig. 5. Changes of activity of (a) CAT, (b) APx, (c) GPx, (d) POD and (e) SOD in leaf of *C. frutescens* seedlings after 7 days treatment of Cd (50 mg L^{-1}) and different concentrations of MeJA (0.1, 1, 10 and 1000 μmol L^{-1} for MeJA 1, 2, 3 and 4, respectively; values are mean and SD; bars with different letters are significantly different at $P \leq 0.05$).

(Keramat et al., 2009; Piotrowska et al., 2009; Kováčik et al., 2011). Other studies also revealed similar findings in *W. arrhiza* exposed to 0.1 μmol L^{-1} JA and lead (Pb) (Piotrowska et al., 2009) as well as in soybean plant treated with 10 μmol L^{-1} MeJA and Cd (Keramat et al., 2009). In the present study, similar effects were observed. In particular, 0.1 μmol L^{-1} MeJA significantly restored the impairment induced by Cd in leaf chlorophyll *b*, whereas the highest levels (1000 μmol L^{-1}) of MeJA significantly decreased the concentrations of the chlorophylls. These results suggested that 0.1 μmol L^{-1} MeJA provided protection during leaf photosynthesis under Cd stress. Leaf chlorophyll *a*/*b* of

C. frutescens also decreased as exogenous MeJA increased, suggesting that high levels of MeJA exhibited greater deleterious effects on chlorophyll *a* than chlorophyll *b*.

Cd does not participate in Fenton-type reactions but can indirectly favor the production of different ROS, such as H_2O_2, $O_2^{\bullet-}$, and $^{\bullet}OH$, resulting in oxidative burst (Olmos et al., 2003). In the present study, although leaf H_2O_2 was not over-produced at 50 mg L^{-1} Cd treatment; significant increases in leaf MDA also indicated the oxidative impairment in the leaves of *C. frutescen* (Figs. 2 and 3). Previous studies suggested that increases in JA and ROS production are common characteristics of senescence, and the toxicity of Cd may accelerate senescence in plants (Rodríguez-Serrano et al., 2009). Exogenous MeJA treatment also results in lipid peroxidation and leaf senescence (Maksymiec, 2007). Hence, the joint influence from MeJA and Cd may result in greater destruction of the lipid membrane and higher yield of MDA products than MeJA or Cd alone. In the present study, exogenous MeJA (0.1–1000 μmol L^{-1}) did not stimulate MDA production compared with Cd treatment (Fig. 3). The MDA content in the leaves treated with 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA was significantly lower than that in the leaves treated with 50 mg L^{-1} Cd alone. Therefore, the lowest MeJA concentration exhibited protective effects on the cell membrane lipid by alleviating lipid peroxidation in *C. frutescens* seedlings under Cd stress.

Plants under toxic metal stress usually exhibit an increase in endogenous stress hormones such as ABA, ethylene, and JA, which usually function as signal molecules involved in regulating antioxidative and growth-related processes (Santner and Estelle, 2009). An increase in endogenous JA is observed in *A. thaliana* treated with Cu and Cd (Maksymiec and Krupa, 2002), pea plants treated with Cd (Rodríguez-Serrano et al., 2009), and rice seedlings treated with Cu (Koeduka et al., 2005). These results suggest that endogenous JA is involved in the cellular response to metal toxicity. Hence, heavy metals may stimulate the octadecenoic pathways leading to JA synthesis (Maksymiec and Krupa, 2002). In the present study, significant increases in endogenous JA were also found at 48 h after the samples were treated with 50 mg L^{-1} Cd and 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA treatments (Fig. 4b). This result suggested that JA may be involved in the cellular responses at the early stage of Cd stress. As a stress hormone, JA is synthesized and becomes accumulated in plants; such processes are considered transient and dynamic. The maximum content is usually reached at 1–24 h after abiotic stress is induced in *A. thaliana*; this content returns rapidly to initial concentration when the stress time is further extended (Maksymiec, 2007). Our previous study also revealed that significant increases in endogenous JA are recorded at 1 d after Pb stress is induced in the mangrove plant *Kandelia obovata* (Yan and Tam, 2013).

The mechanism of Cd toxicity may be a result of a side effect of an increased endogenous JA (Maksymiec and Krupa, 2002). This increase in endogenous JA can contribute to metal toxicity by activating lipoxygenase activity, H_2O_2 production, and lipid peroxidation (Maksymiec, 2007). In the present study, an increase in endogenous JA revealed a close relationship with an increase in H_2O_2, in which JA and H_2O_2 increased at 48 h after the treatments. However, the leaf MDA content did not show any significant increase at 48 h after the samples were treated with 50 mg L^{-1} Cd and 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA treatments compared with the control group. Therefore, the increases in endogenous JA under the appropriate level of exogenous MeJA treatments did not show any deleterious effects on the plant cells.

Evidence has indicated that MeJA can affect the activity and/or pools of stress enzymes, thereby alleviating oxidative stress in plant cells (Li et al., 1998; Wang, 1999; Jung, 2004). Previous reports indicated that the foliage application of 100 μmol L^{-1} MeJA induces CAT activity and mitigates ROS production in maize and strawberry plants subjected to water stress (Li et al., 1998; Wang, 1999). Increases in various antioxidant enzyme activities, including SOD, CAT, and APx in the presence of 10–100 μmol L^{-1} MeJA have also been observed in soybean plant (*G. max* L.) under Cd stress (Keramat et al., 2009). Moreover, JA at 0.1 μmol L^{-1} activated enzymatic (CAT, APx, and NADH peroxidase) and non-enzymatic antioxidant (ascorbate and glutathione) systems in *W. arrhiza* (Piotrowska et al., 2009). The present study also found that low levels (0.1 μmol L^{-1}) of MeJA increased the CAT and APx activities in the leaves of *C. frutescens* and induced a low lipid peroxidation level (indicated by low MDA production), high concentrations of chlorophyll b, and high root biomass yield. These results suggested that MeJA at lower concentrations, particularly at 0.1 μmol L^{-1}, can stimulate the plant to resist damage caused by Cd exposure. High levels of exogenous MeJA also increased SOD, POD, and APx activities, whereas the CAT and APx activities were inhibited. Increases in POD and SOD activities and decreases in CAT activities have also been observed during the senescence of *G. max* L. leaves (Xu et al., 1997). One important function of such high levels of exogenous MeJA is to induce leaf senescence. High doses of exogenous MeJA treatment and leaf senescence possibly share the same process of antioxidative enzyme induction and responses.

5. Conclusions

The present study revealed that the root dry weight and chlorophyll *b* concentrations in *C. frutescens* leaves were significantly inhibited by 50 mg L^{-1} Cd after 7 d of treatments. By contrast, the shoots (leaf and stem) of *C. frutescens* were less susceptible to Cd stress than the roots. Cd at 50 mg L^{-1} also elicited deleterious effects on the leaves of *C. frutescens* as indicated by the overproduction of MDA. Low MeJA concentrations (0.1 μmol L^{-1}) restored the root growth and chlorophyll *b* concentration of the seedling leaves that were suppressed by 50 mg L^{-1} Cd. The MDA content of the leaves treated with 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA was also significantly decreased compared with that treated with 50 mg L^{-1} Cd only. Therefore, low MeJA concentrations may help mitigate the deleterious effect of Cd. Significant increases in endogenous JA were also observed at 48 h after the samples were treated with 50 mg L^{-1} Cd and 50 mg L^{-1} Cd + 0.1 μmol L^{-1} MeJA, suggesting that JA is mainly involved in the cellular responses at the early stage of Cd stress. Low MeJA concentrations (0.1 μmol L^{-1}) also increased the CAT and POD activities in *C. frutescens* leaves. These results suggested that lower MeJA concentrations, particularly at 0.1 μmol L^{-1}, could induce plants to resist damage upon Cd exposure. High levels of exogenous MeJA also increased SOD, POD, and APx activities, while inhibited CAT and APx activities.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2013.08.019.

References

Ananieva, K., Ananiev, E.D., Mishev, K., Georgieva, K., Malbeck, J., Kamínek, M., Staden, J.V., 2007. Methyl jasmonateis a more effective senescence-promoting

factor in *Cucurbita pepo* (zucchini) cotyledons when compared with darkness at the early stage of senescence. J. Plant Physiol. 164, 1179–1187.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1–15.
Beer, B., Sizer, W., 1952. A spectrophotometry method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 1952, 133–139.
Benavides, M.P., Gallego, S.M., Tomaro, M.L., 2005. Cadmium toxicity in plants. Braz. J. Plant Physiol. 17, 21–34.
Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–888.
Blikhina, O., Virolainen, E., Fagerstedt, K.V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 9, 179–194.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Cheng, S., 2003. Heavy metal pollution in China: origin, pattern and control. Environ. Sci. Pollut. Res. 10, 192–198.
Elstner, E.F., Heupel, A., 1976. Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. Anal. Biochem. 70, 616–620.
Fielding, J.L., Hall, J.L., 1978. A biochemical and cytochemical study of peroxidase activity in roots of *Pisum sativum*. II. Distribution of enzymes in relation to root development. J. Exp. Bot. 29, 983–991.
Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr. Opin. Plant. Biol. 9, 436–442.
Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta. 196, 143–152.
He, Y.H., Fukushige, H., Hildebrand, D.F., Gan, S.S., 2002. Evidence supporting a role of jasmonic acid in Arabidopsis leaf senescence. Plant Physiol. 128, 876–884.
Hori, H., 1966. Gravel Culture of Vegetables and Ornamentals. 3. Nurient Solution, Yokendo. Tokyo, Japan, pp. 60–79 (in Japanese).
Huang, A., Wu, Z., 1999. Determination of glutathione peroxidase in rice seedlings. J. Southwest. Agric. Univ. 21, 324–327.
Jiménez, A., Hernández, J.A., del Río, L.A., Sevilla, F., 1997. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea (*Pisum sativum* L.) leaves. Plant Physiol. 114, 275–284.
Jung, S., 2004. Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. Plant Physiol. Biochem. 42, 225–231.
Keramat, B., Kalantari, K.M., Arvin, M.J., 2009. Effects of methyl jasmonate in regulating cadmium induced oxidative stress in soybean plant (*Glycine max* L.). Afr. J. Microbiol. Res. 3 (5), 240–244.
Knight, J.A., Voorhees, R.P., 1990. Peroxidation of linolenic acid-catalysis by transition metal ions. Ann. Clin. Lab. Sci. 20, 347–352.
Koeduka, T., Matsui, K., Hasegawa, M., Akakabe, Y., Kajiwara, T., 2005. Rice fatty acid alpha-dioxygenase is induced by pathogen attack and heavy metal stress: activation through jasmonate signaling. J. Plant Physiol. 162, 912–920.
Kováčik, J., Klejdus, B., Štork, F., Hedbavny, J., Bačkor, M., 2011. Comparison of methyl jasmonate and cadmium effect on selected physiological parameters in *Scenedesmus quadricauda* (chlorophyta, chlorophyceae). J. Phycol. 47 (5), 1044–1049.
Li, L., Staden, J.V., Jager, A.K., 1998. Effect of plant growth regulators on the antioxidant system in seedlings of two maize cultivars subjectd to water stress. J. Plant Growth Regul. 25, 81–87.
Maksymiec, W., 2007. Signaling responses in plants to heavy metal stress. Acta Physiol. Plant. 29, 177–187.
Maksymiec, W., Krupa, Z., 2002. Jasmonate and heavy metals in Arabidopsis plants—a similar physiological response to both stressors? J. Plant Physiol. 159, 509–515.
Maksymiec, W., Wianowska, D., Dawidowicz, A.L., Radkiewicz, S., Mardarowicz, M., Krupa, Z., 2005. The level of jasmonic acid in *Arabidopsis thaliana* and *Phaseolus coccineus* plants under heavy metal stress. J. Plant Physiol. 162, 1338–1346.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Niu, L., Yang, F., Xu, C., Yang, H., Liu, W., 2013. Status of metal accumulation in farmland soils across China: from distribution to risk assessment. Environ. Pollut. 176, 55–62.
Olmos, E., Solano, M.J.R., Piqueras, A., Hellín, E., 2003. Early steps in the oxidative burst induced by cadmium in cultured tobacco cells (BY-2 line). J. Exp. Bot. 54, 291–301.
Orozco-Cárdenas, M.L., Narváez-Vásquez, J., Ryan, C.A., 2001. Hydrogen peroxide behaves as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin and methyl jasmonate. Plant Cell 13, 179–191.
Piotrowska, A., Bajguz, A., Godlewska-Żyłkiewicz, B., Czerpak, R., Kamińska, M., 2009. Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). Environ. Exp. Bot. 66, 507–513.
Prasad, M.N.V., 2004. Heavy Metal Stress in Plants: From Biomolecules to Ecosystems, 2nd Ed. Narosa Publishing House, New Delhi p. 462.
Rodríguez-Serrano, M., Romero-Puertas, M.C., Pazmiño, D.M., Testillano, P.S., Risueño, M.C., Del Río, L.A., Sandalio, L.M., 2009. Cellular response of pea plants to cadmium toxicity: cross talk between reactive oxygen species, nitric oxide, and calcium. Plant Physiol. 150, 229–243.
Sandalio, L.M., Dalurzo, H.C., Gomes, M., Romero-Puertas, M., del Rio, L.A., 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J. Exp. Bot. 52, 2115–2126.
Saniewski, M., Miszczak, A., Kawa-Misczak, L., Wegrzynowicz-Lesiak, E., Miyamoto, K., Ueda, J., 1998. Effects of methyl jasmonate on anthocyanin accumulation, ethylene production, and CO_2 evolution in uncooled and cooled tulip bulbs. J. Plant Growth Regul. 17, 33–37.
Santner, A., Estelle, M., 2009. Recent advances and emerging trends in plant hormone signalling. Nature 459, 1071–1078.
Tewari, R.K., Kumar, P., Sharma, P.N., Bisht, S.S., 2002. Modulation of oxidative stress responsive enzymes by excess cobalt. Plant Sci. 162, 381–388.
Vangronsveld, J., Clijsters, H., 1994. Toxic effects of metals. In: Farago, M.E. (Ed.), Plants and the Chemical Elements. VCH Verlagsgesellschaft, Weinheim, VCH Publishers, New York, pp. 149–177.
Walia, H., Wilson, C., Condamine, P., Liu, X., Ismail, A.M., Close, T.J., 2007. Large-scale expression profiling and physiological characterization of jasmonic acid-mediated adaptation of barley to salinity stress. Plant Cell Environ. 30, 410–421.
Wang, H., Jin, J.Y., 2005. Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. Photosynthetica 43, 591–596.
Wang, L.S., Wang, L., Wang, L., Wang, G., Li, Z.H., Wang, J.J., 2009. Effect of 1-butyl-3-methylimidazolium tetrafluoroborate on the wheat (*Triticum aestivum* L.) seedlings. Environ. Toxicol. 24, 296–303.
Wang, S.Y., 1999. Methyl Jasmonate reduces water stress in strawberry. J. Plant Growth Regul. 18, 127–134.
Xu, S., Shi, L., Hu, B., Zhu, C., Deng, C., Gao, L., 1997. Changes in photosynthetic capacity and related enzyme activities during soybean (*Glycine max*) leaf senescence. J. Northeast Normal Univ. (Nat Sci) 4, 70–73.
Yan, Z., Tam, N.F.Y., 2013. Effects of lead stress on anti-oxidative enzymes and stress-related hormones in seedlings of *Excoecaria agallocha* Linn. Plant Soil 367, 327–338.