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Full Length Research Paper

Effects of methyl jasmonate in regulating cadmium induced oxidative stress in soybean plant (*Glycine max* L.)

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The jasmonates are a group of plant–growth regulators that cause a wide variety of plant responses. Cadmium (Cd) is a toxic heavy metal, which causes toxicity in living organisms, and is considered as an environmental contaminant. In present investigation, the interactive effects of methyl jasmonate (0, 0.01 or 0.1 mM) and cadmium chloride (0 or 500 µM) on malondialdehyde (MDA), hydrogen peroxide (H2O 2) and antioxidant enzymes in soybean (Glycine max L.) leaves were studied. Treatment of methyl jasmonate (MeJA) together with cadmium reduced the amount of H2O2 and MDA when compared with cadmium treated plants. The activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), increased in treated plants with MeJA, and Cd, but guaiacol peroxidase (GPX) activity did not show any noticeable difference. In addition, MeJA treatment increased the amount of dehydro ascorbic acid (DHA) in plants under Cd stress whereas, there was no significant difference in the content of ascorbic acid (ASA). Based on our results, it seems that the application of MeJA together with Cd caused alleviation of Cd damages by the reduction of MDA and H2O2 content and increase in activities of antioxidant enzymes in soybean plants.

Key words: Ascorbate peroxidase, catalase, lipid peroxidation, superoxide dismutase.

INTRODUCTION

Jasmonic acid and its methyl ester, methyl jasmonate, are naturally occurring plant growth regulators, which can affect many physiological and biochemical processes in plants (Wang, 1999; Ueda and Saniewski, 2006; Norastehnia et al., 2007). It has been shown that exogenously applied, jasmonates elicit several different physiological responses to stress and therefore increase plant resistance (Lee et al., 1996; Ding et al., 2001; Walia et al., 2007).

Cadmium is one of the most heavy metals with high toxicity to plants, animals, and human. It is a non-essential heavy metal, having long biological persistence including leaf rolls, chlorosis and reduction of root and stem growth (Smeets et al., 2005; Mishra et al., 2006). many morphological and physiological changes in plants

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When plants are subjected to Cd stress, a variety of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals are generated. These ROS cause oxidative damage in plants (Gallego et al., 1996). Free radicals are toxic to living organisms unless removed rapidly, destroyed or inactivated by various cellular components. In the absence of effective mechanisms that remove or scavenge free radicals, they can seriously damage plant by lipid per- oxidation, protein degradation, breaking of DNA and cell death (Thian and Li, 2006). Plants cope with oxidative stress by using antioxidant enzymes such as SOD, CAT, GPX, APX, GR, and non-enzymatic constituents such as ascorbate and glutathione, which are responsible for scavenging excessively accumulated ROS in plants under stress conditions (Asada, 1999; Shah et al., 2001; Sbartai et al., 2008). There are some evidences indicating that MeJA can affect the antioxidant system in plant cells (Wang, 1999). The role of MeJA in protecting plants from various

stresses has been reported, for example, amelioration of chilling injury and water stress in rice (Lee et al., 1996), tomato (Ding et al., 2001) and strawberry (Wang, 1999). These findings suggest that jasmonates could mediate the defense response to various environmental stresses.

The aim of the present study was to examine the role of MeJA on antioxidant system and oxidative stress resistance in soybean plants subjected to Cd stress.

MATERIALS AND METHODS

Plant growth and treatments

Seeds of *Glycin max* L. Cv.Williams was collected from Karaj Agricultural Center. Seeds were sterilized using 0.1% sodium hypochlorite solution, washed with distilled water and planted in pots filled with perlit. Pots were transferred to growth chamber with day/night temperature of 25/20°C and a 16 h light/8 h dark photoperiod, with a relative humidity of 70%. During the first week of seed sowing, seedlings were irrigated with distilled water. For the rest of experiment, half strength Long-Ashton's nutrient solution was used to irrigate plants every other day. Four weeks after sowing, plants were sprayed with MeJA (0, 0.01 or 0.1 mM) and irrigated by Long-Ashton's solution containing $CdCl_2$ (0 or 500 µM) for 7 days. At the end of experiment, the leaves of the plants were harvested and immediately were frozen in liquid nitrogen and stored at -80°C for the future analysis.

MDA content

For each sample, 0.2 g of the leaf tissue was homogenized in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 $\times$ g for 5 min. For every 1ml of aliquot, 4ml of 20% TCA containing 0.5% thiobarbiturice acid was added. Mixture was heated at 90ºC for 30 min. Samples were cooled on ice for 5 min and then re-centrifuged at 10000 $\times$ g for 10 min and absorbance of the supernatant was recorded at 532 and 600nm. For MDA measurement, the non–specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532nm and an extinction coefficient of 1.55×10^5 / M Cm and was used for determination of MDA concentration (Heat and Packer, 1969).

Hydrogen peroxide content

H_2O_2 content was measured spectrophotometrically after reaction with potassium iodide (KI) according to the method of (Alexieva et al., 2001). Leaf tissues (500 mg) were homogenized in ice bath with 5 ml 0.1% TCA. The homogenate was centrifuged at 12000 g for 15 min. The reaction mixture consisted of 0.5 ml of supernatant, 0.5 ml of 100mM potassium phosphate buffer (pH 7.0) and 2 ml reagent (1 M KI in fresh double-distilled water). The blank probe consisted of 0.1% TCA in the absence of leaf extract. The reaction was carried out for 1 h in darkness and absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2.

Protein and enzyme extraction

One gram of frozen leaves was homogenized in 50 mM phosphate buffer (pH 7.2) containing 1 mM EDTA, 1 mM PMSF and 1% soluble PVP. The homogenate was centrifuged at 14000 g for 20 min at 4°C and the supernatant used for assay of the activity of enzymes. The protein content in the supernatant was measured according to the method of Bradford (1976). Bovine serum albumin was used as standard.

Measurement of antioxidant enzymes activity

The activity of CAT was estimated by monitoring the decrease in absorbance of H_2O_2 within 30 s at 240 nm. The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2 and 100 l enzyme extract. Unit of activity was taken as the amount of enzyme, which decomposes one µmol of H_2O_2 in one minute (Dhindsa et al., 1981).

SOD activity was measured by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm (Giannopolitis and Ries, 1977). The reaction solution (3 ml) contained 50 M NBT, 0.1 mM Na-EDTA, 75 µM of riboflavin, 13 mM methionine, 50 mM phosphate buffer (pH 7.0) and 100µl of enzymes extract. Reaction was carried out in test tubes at 25°C under fluorescent lamp (40) with irradiance of 75 µmol/ms. The reaction was allowed to run for 8 min and stopped by switching the light off. Blanks and controls were run in the same manner but without irradiation and enzyme, respectively. Under the experimental condition, the initial rate of reaction, as measured by the difference in increase of absorbance at 560 nm in the presence and absence of extract, was proportional to the amount of enzyme. One unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photo-reduction by 50%.

The activity of APX was measured according to the method of Nakano and Asada (1981). The 3 ml reaction mixture contained 2.5 ml of 50 mM phosphate buffer (pH 7.0), 200 µl of 3% H_2O_2, 200 µl of 0.5 mM ascorbate and 100 µl of enzymes extract. The change in absorbance was determined within 1 min at 290 nm. One unit of APX was defined as the quantity of enzyme required to oxidize 1µmol of ascorbate per min.

GPX activity was measured using guaiacol as a substrate. Reaction mixture (3ml) contained 25 µl of enzyme extract, 2.77 ml of 50mM phosphate buffer (pH 7.0), 0.1 ml of 1% H_2O_2 and 0.1 ml of 4% guaiacol. The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 3 min. One unit of enzyme activity was defined as the amount that causes a change of 0.01 in absorbance per minute (Zhang et al., 2005).

Measurement of ASA and DHA content

ASA and DHA were extracted in 5% metaphophoric acid. The homogenate was centrifuged at 10000 $\times$ g for 15 min and the resulting supernatant was used for determination of ASA and DHA according to the method of Mc de Pinto et al. (1999).

Statistical analysis

All the experiments were performed in triplicate. Data were analyzed by SPSS, and the means were separated by Duncan test. The significant level was P 0.05.

RESULTS

Malondialdehyde was measured as an indicator of lipid per-oxidation. Cd significantly increased the contents of MDA in leaves of soybean plants in comparison with the control group (Figure1).

Application of MeJA + $CdCl_2$ reduced the amount of MDA about 30%, in compared with Cd-treated plants (Figure1). Thus, it can be deduced that MeJA especially at

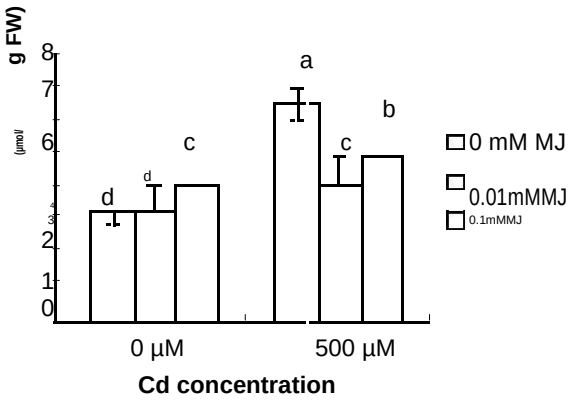

Figure 1. Effect of MeJA and Cd on MDA content in soybean leaves. Data are means ± SE of three replicates. Different letters indicate the significance of difference at P 0.05 levels by Duncan test.

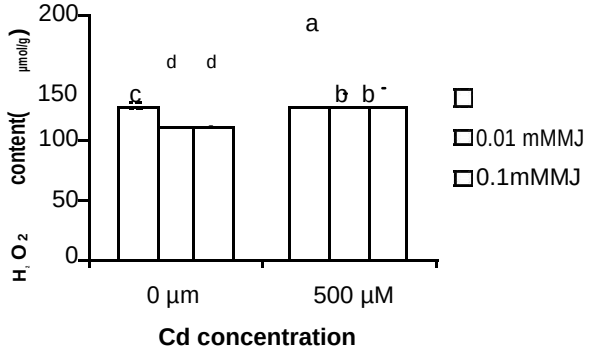

Figure 2. Effect of MeJA and Cd on hydrogen peroxide content in soybean leaves. Data are means ± SE of three replicates. Different letters indicate the significance of difference at P 0.05 levels by Duncan test.

0.01mM concentration, reduces lipid peroxidation in plants subjected to Cd.

Changes in hydrogen peroxide are presented shown in Figure 2. An increase in the hydrogen peroxide content was observed in leaves under Cd treatment. When plants were treated with MeJA and $CdCl_2$, hydrogen peroxide was decreased significantly in comparison with only Cd-treated plants. Significant reduction in H_2O_2 content was detected at both MeJA concentrations (0.01 and 0.1 mM) (Figure 2).

The treatment of plants with 500µM $CdCl_2$ did not have any significant effect on CAT activity in compared with the control plants (Figure 3A).

The study of interactive effects of MeJA and $CdCl_2$ on soybean plants indicated that application of MeJA at 0.01 and 0.1 mM concentrations caused a significant increase in CAT activity compared with plants treated by Cd (Figure 3A).

MeJA + Cd treatment also increased ascorbate peroxidase activity significantly (about 33%) in comparison with

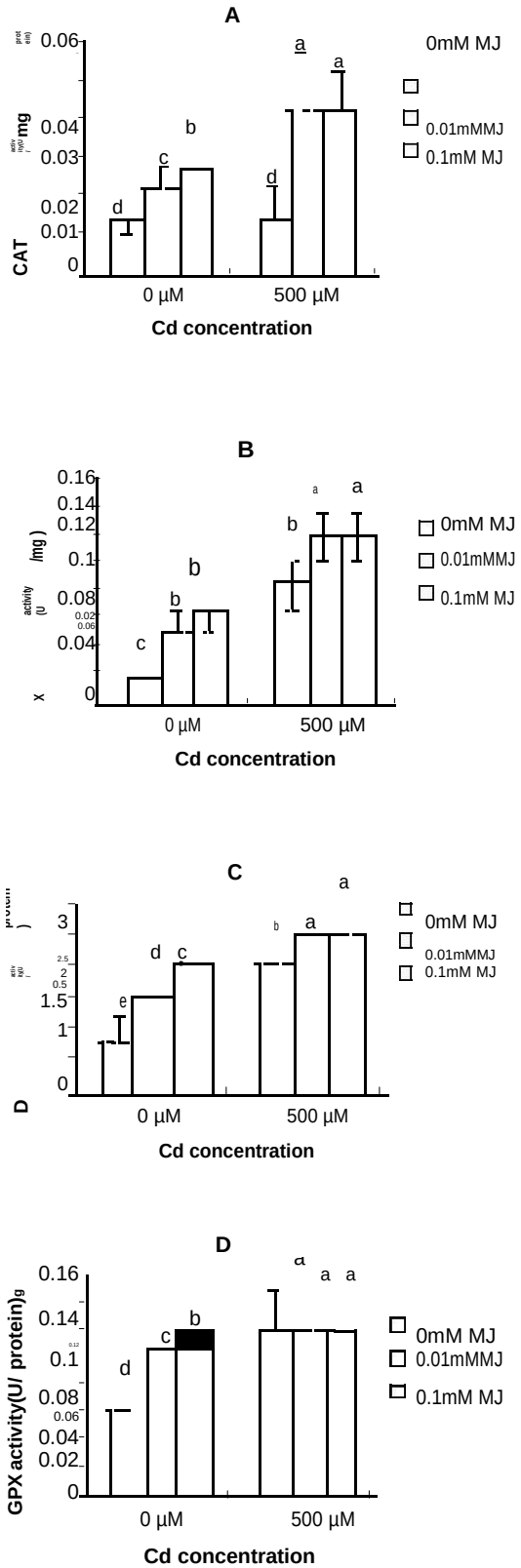

Figure 3. Effect of MeJA and Cd on (A) CAT activity, (B) APX activity, (C) SOD activity and (D) GPX activity in soybean leaves. Data are means ± SE of three replicates. Different letters indicate the significance of difference at P 0.05 levels by Duncan test.

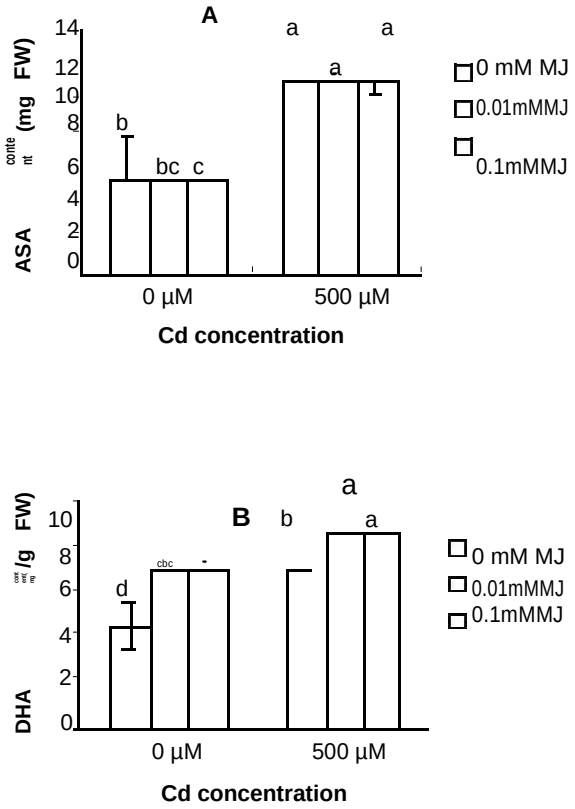

Figure 4. Effect of MeJA and Cd on (A) ASA content and (B) DHA in soybean leaves. Data are means ± SE of three replicates. Different letters indicate the significance of difference at P 0.05 levels by Duncan test.

just Cd-treated plants (Figure 3B). Meanwhile, under stress condition, MeJA treatment increased SOD activity in leaves of soybean plants (Figure 3C).

Unlike the activities of other antioxidant enzymes, when plants were treated with MeJA and $CdCl_2$, GPX activity had no difference with only Cd-treated plants (Figure 3D).

In this study, a significant increase was found in DHA content after application of MeJA on Cd-treated plants (Figure 4B). There was no significant difference in the content of ASA between Cd-treated plants with plants treated by Cd +MeJA (Figure 4A).

DISCUSSION

Cadmium induces oxidative stress in plants. Cd toxicity results in the alteration of oxidant level in plants, including the generation of toxic reactive oxygen species (ROS) like H_2O_2, OH and O_2 and thereby induces oxidative stress (Choudhary and Panda, 2004). MeJA is a plant growth regulator that has a great protective effect against oxidative stress (Wang 1999, Norastehnia and Nojavan-Asghari, 2006, Walia et al., 2007). Data showed that Cd stress induced MDA and H_2O_2 production but, treatment of MeJA caused significant decrease in MDA and H_2O_2 content in plants treated with Cd. MDA is the decomposition product of polyunsaturated fatty acids of biomembrane and increase in MDA content is the indication of oxidative stress in plants (Premachandra et al., 1991). It has been reported that MeJA mitigated the ROS effects in strawberry under water stress and in maize seedlings subjected to paraquat (Wang, 1996; Norastehnia and Nojavan-Asghari, 2006). In strawberry leaves has been shown that MeJA changed the ratio of membranes fatty acids, which is less targeted for free radicals (Wang, 1999). In order to repair the damage initiated by ROS, plants evolve complex antioxidant metabolism. This includes enzymes like SOD, CAT, APX, GPX and non-enzymes like ascorbate and glutathione (Chien et al., 2001; Choudhury and panda, 2004). There is information that shows MeJA affects on the activity and/or pools of stress enzymes and causes the alleviation the oxidative stress (Li et al., 1998; Jung, 2004). This study indicated an increase in the activities of the various antioxidant enzymes in the presence of different concentrations of MeJA. CAT and APX are the most important H_2O_2 scavenging enzymes that can catalyze either the direct decomposition of H_2O_2 or the oxidation of H_2O_2 in plants (Comparot et al; 2002). It has been stated that MeJA induce CAT activity in maize and strawberry plants sub-jected to water stress (Li et al. 1998; Wang, 1999). SOD activity causes an increase in H_2O_2 content that is normally detoxified by CAT in peroxisomes and by APX in cytosol, mitochondria and chloroplasts (Foyer et al., 1997; Asada, 1999). APX reacts with H_2O_2 in the presence of ascorbate to produce water and monodehydroascorbate. Oxidized ascorbate is then regenerated through the ascorbate-glutathione cycle (Comparot et al., 2002). Decrease in H_2O_2 content was observed under MeJA treatment in Cd stressed plants could be related to increase in CAT and APX activity. This increase in enzymes activity may be due to up-regulation of the genes that controlling the synthesis of these enzymes or an increased the activation of constitutive enzyme pools (Li et al., 1998; Norastehnia and Nojavan, 2006). Besides antioxidant enzymes, ascorbic acid is an important non-enzymatic antioxidant, which reacts not only with H_2O_2 but also with O_2^- and OH^- (Reddy et al., 2004). In this research, ASA content increased under Cd treatment. Similar result was reported in *Phaseolus Vulgaris* after heavy metal application (Zengin and Munzuroglu, 2005). Our results showed that MeJA increased DHA content but it had not any significant effect on ASA content in plants subjected to Cd. It has been shown that the levels of low molecular antioxidants such as ascorbate are generally increased in plants under MeJA treatment (Comparot et al., 2002).

Conclusion

According to our results, it could be concluded that MeJA ameliorate oxidative stress Cd-induced by detoxifying

H_2O_2, decrease in lipid peroxidation, increase in some antioxidant enzymes activity and amount of non-enzymatic antioxidants in soybean plants. Increase the activity of antioxidant enzymes could be attributed to the increased tolerance to Cd. Therefore, the protective effect of MeJA against Cd stress can be correlated to the changes in the antioxidant metabolism of soybean leaves.

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