Ecotoxicology and Environmental Safety 74 (2011) 820–825

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Microwave pretreatment can enhance tolerance of wheat seedlings to $CdCl_2$ stress

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ARTICLE INFO

Article history:
Received 25 September 2010
Received in revised form
8 November 2010
Accepted 22 November 2010
Available online 9 December 2010

Keywords:
Microwave
Wheat (*Triticum aestivum* L.)
Cadmium stress
Antioxidative system

ABSTRACT

In order to determine the role of microwave in cadmium stress tolerance of wheat (*Triticum aestivum* L.), seeds were exposed to microwave radiation for 0, 5, 10 and 15 s (wavelength 125 mm, power density 126 mW cm^{-2}, 2450 MHz), and when the seedlings were 7 d old (with one fully expanded leaves), they were treated with 150 μM $CdCl_2$ solution for 10 d. Changes in a number of physiological and biochemical characteristics were measured and used as indicators of the protective capacity of microwave radiation in this experiment. Our results showed that 150 μM $CdCl_2$ treatment reduced plant height, root length, dry weight, AsA and GSH concentration and the activities of SOD, POD, CAT and APX, enhanced the concentration of MDA, H_2O_2 and the production rate of O_2^- when compared with the control. However, seeds with microwave pretreatment 5 or 10 s conferred tolerance to cadmium stress in wheat seedlings by decreasing the concentration of MDA and H_2O_2, the production rate of O_2^- and increasing the activities of SOD, POD, CAT, APX and AsA and GSH concentration. Therefore, antioxidative enzymes and antioxidative compounds may participate in tolerance of wheat seedlings to cadmium stress. The results also showed that the microwave radiation had a positive physiological effect on the growth and development of cadmium stressed seedlings. This is the first investigation reporting the use of microwave pretreatment to enhance cadmium stress tolerance of wheat.

1. Introduction

Cadmium (Cd), a non-essential heavy metal element, is a major environmental pollutant that is toxic to organisms including plants (Harminder et al., 2008). In plants, Cd disturbs various biochemical and physiological processes, leading to cell death and inhibition of growth (Moussa and El-Gamal, 2010; Xu et al., 2009). At cellular level, Cd induces oxidative stress as evidenced by enhanced lipid peroxidation, hydrogen peroxide (H_2O_2) generation and ion leakage (Smeets et al., 2005). Correspondingly plants cope with oxidative stress by using antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), peroxidases (POD) and non-enzymatic constituents such as glutathione (GSH), carotenoids (CAR), ascorbate (AsA) and α-tocopherol, which are responsible for scavenging excessively accumulated reactive oxygen species (ROS) in plants under stress conditions (Shah et al., 2001). Among these defenses, SODs are a group of enzymes that accelerate the conversion of superoxide radicals to H_2O_2 (Carlsson et al., 1995). CAT is one of the main H_2O_2-scavenging enzymes that dismutates H_2O_2 into H_2O and O_2 (Corpas et al., 1999). PODs are enzymes that catalyze the H_2O_2-dependent oxidation of a wide variety of substrates, mainly phenolics (Kawano, 2003). GSH, a disulfide reductant that protects thiols of enzymes, regenerates AsA and reacts with singlet oxygen, H_2O_2 and hydroxyl radicals. GSH plays a central role in protecting plants from ROS (Foyer, 1993). AsA is an important antioxidant, which reacts not only with H_2O_2, but also with O_2^-, OH^- and lipid hydroperoxidases (Ramachandra et al., 2004). Therefore, high activities of antioxidant enzymes and high contents of non-enzymatic constituents are important for plants to tolerate environmental stresses.

Microwaves have various effects on biological system at whole organism, tissue, cell and molecular level (Roux et al., 2006; Hamada, 2007). Microwave irradiation has been shown to have the potential to replace chemical treatment for pest or fungal control in sorghum (More et al., 1992), wheat (Shayesteh and Barthakur, 1996) and walnuts (Wang et al., 2002). Low-intensity microwave radiation enhances germination, plant height, fresh weight and enzymatic activities (Belayavskaya, 2004; Racuciu et al., 2006) and can protect seedlings of *Isatis indigotica* from enhanced UV-B damage (Chen, 2006). Chen et al. (2009a, b) have also found that weak microwave can enhance tolerance of wheat seedlings to salt and osmotic stress. However, little is known if microwave pretreatment can enhance wheat seedlings tolerance to cadmium chloride ($CdCl_2$) stress or whether microwave radiation can cause physiological response of plants to $CdCl_2$ stress. So we hypothesize that microwave pretreatment can increase wheat

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doi:10.1016/j.ecoenv.2010.11.008

seedlings tolerance to $CdCl_2$ stress and a series of experiments were carried out to test this hypothesis.

In this study, wheat (*Triticum aestivum* L.) was the experimental material used to examine the effect of seed pretreatment by microwave radiation on protecting wheat from $CdCl_2$ stress damage. Our objectives were to (1) determine the combined effect of microwave pretreatment and $CdCl_2$ stress on wheat seedlings; (2) evaluate the effect of seed pretreatment by microwave on protecting wheat from $CdCl_2$ stress damage using plant morphological and physiological indices.

2. Materials and methods

2.1. Plant materials and treatment

The uniform seeds of wheat (*T. aestivum* L. cv. Zhengmai No. 004, obtained from Henan Academy of Agricultural Sciences) were surface sterilized for 3 min by immersion in 0.01% $HgCl_2$, washed for 30 min in running water, and then were air dried. Seeds were randomly divided into four batches (1000 seeds per batch) and placed in the center of the microwave board. Seed batches were exposed to microwave radiation for 0 (0 s), 5 (5 s), 10 (10 s) or 15 (15 s) seconds. One batch of seeds was pretreated only once with microwave. Microwave treatment was carried out with a 700-W (power output) experimental prototype microwave oven with variable power at 2450 MHz (wavelength 125 mm, power density 126 mW cm^{-2}, Shunde Electron Industries Ltd., Guangzhou, China). The method and doses of microwave radiation have been described by Chen (2006), who reported that 8 s pretreatment of *I. indigotica* seeds was the best effect among four pretreatments of 3, 8, 13 and 15 s.

After microwave treatment, seeds from each batch were sown in ten Petri dishes (diameter 18 cm, each containing 80 seeds), flushed daily with a half-strength Hoagland's solution, in a growth chamber under a 12 h photoperiod at 400 μmol m^{-2} s^{-1}, 70% relative humidity and 25 °C/18 °C (day/night). When the seedlings were 7 d old (with one fully expanded leaves), they were treated with 150 μM $CdCl_2$ solution (This concentration was chosen based on previous unpublished data) for 10 d. On the 2, 4, 6, 8 and 10 d of $CdCl_2$ stress, leaves and roots were sampled for the various analyses. Seedlings with no $CdCl_2$ stress and no microwave irradiation were regarded as the control (CK).

2.2. Effects test

2.2.1. Malondialdehyde (MDA) determination

MDA concentration was measured according to Predieri et al. (1995). Samples of leaves (0.30 g fresh weight, FW) were homogenized in 50 mM phosphate buffer (pH 7.8), and then centrifuged at 8 000*g* for 15 min at 4 °C. A 1 mL supernatant sample was combined with 2.5 mL thiobarbituric acid (TBA) [5% TBA (w/v) in 20% trichloroacetic acid (w/v)], incubated in boiling water for 20 min and the reaction was terminated in an ice bath for 5 min. The mixture was centrifuged at 10 000*g* for 5 min and the absorbance of supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as μmol MDA g^{-1} FW.

2.2.2. Hydrogen peroxide (H_2O_2) determination

H_2O_2 concentration in the leaves was estimated following the method of Alexieva et al. (2001). Leaves (0.50 g fresh weight, FW) were homogenized with 0.1% trichloroacetic acid (TCA) and centrifuged at 10 000*g* for 10 min at 4 °C. The supernatant was collected and hydrogen peroxide measured spectrophotometrically. The reaction mixture consisted of 1 mL of the extracted supernatant, 1 mL of K phosphate buffer and 2 mL of 1 M KI. The reaction was carried out for 1 h in darkness and absorbance was measured at 390 nm. The concentration of H_2O_2 was calculated using a standard curve plotted with known concentrations of H_2O_2.

2.2.3. The production rate of O_2^- determination

The production rate of O_2^- was measured as described by Elstner and Heupel (1976). Fresh leaves (0.20 g) were homogenized in 1 mL of 50 mM phosphate buffer (pH 7.8), and centrifuged at 10 000*g* for 10 min at 4 °C. Then 0.5 mL of the supernatant was added to 0.5 mL 50 mM phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine hydrochloride. After 1 h reaction at 25 °C, the mixture was added to 1 mL 17 mM sulfanilamide and 1 mL 7 mM α-naphthylamine at 25 °C for 20 min. The specific absorbance at 530 nm was determined. Sodium nitrite was used as a standard solution to calculate the production rate of O_2^-.

2.2.4. Enzyme activity determination

Frozen leaves (0.20 g) were homogenized in a mortar and pestle with 2 mL of 50 mM ice-cold phosphate buffer (pH 7.8) containing 1 mM EDTA. The homogenate was centrifuged at 15 000*g* for 15 min at 4 °C. The supernatant was used for assays of the activities of SOD, POD, CAT and APX. All operations were carried out at 4 °C.

Activity of SOD (EC1.15.1.1) was assayed by measuring its capacity for inhibiting the photo-reduction of nitro blue tetrazolium (NBT), as described by Giannopolitis and Ries (1977). The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 0.75 mM NBT, 0.02 mM riboflavin and 0.1 mL enzyme extract. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 20 W fluorescent lamps (100 μmol m^{-2} s^{-1}). The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. Absorbance of the reaction mixture was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photo-reduction by 50%.

CAT (EC1.11.1.6) activity was measured using the method of Cakmak and Marschner (1992). The reaction solution (3 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 0.1% H_2O_2 and 0.1 mL enzyme extract. The reaction was initiated by adding the enzyme extract. The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (39.4 mM^{-1} cm^{-1}).

Activity of POD (EC1.11.1.7) was determined in terms of oxidation of guaiacol by measuring increase in absorbance at 470 nm (Zhang and Kirham 1994). The reaction mixture (3 mL) contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol (w/v), 1.0 mM H_2O_2 and 0.1 mM EDTA and enzyme extract (0.02 mL). Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}).

APX (EC1.11.1.1) activity was assayed according to the method of Nakano and Asada (1981). The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.1 mL enzyme extract. The reaction was started by the addition of H_2O_2 and ascorbate oxidation was measured at 290 nm for 3 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}).

2.2.5. The determination of antioxidant compounds concentration

Fresh leaves (0.20 g) were homogenized in ice-cold 5% (w/v) trichloroacetic acid and centrifuged at 15 000*g* for 15 min. After centrifuging, to 0.2 mL supernatant was added 2.6 mL phosphate buffer (pH 7.7) and 0.2 mL DTNB (5,5′-dithiobis (2-nitrobenzoic acid)) (2.51 mg mL^{-1}). After 5 min at 30 °C, the absorbance was determined at 412 nm. GSH content was calculated based on a standard curve (Ellman, 1959).

AsA concentration was measured according to Tonamura (1978). Fresh leaves (0.20 g) were homogenized in ice-cold 2 mL 10% metaphosphoric acid. After centrifugation at 15 000*g* for 10 min, to 0.5 mL of supernatant was added 1 mL citric acid-phosphoric acid buffer (pH 2.3) and 1 mL 2,6-dichlorophenol indophenol (30 mg L^{-1}). After 30 s, the absorbance was determined at 524 nm. AsA concentration was expressed as mg g^{-1} FW.

2.2.6. Growth parameter

The green parts in the plant without root were oven dried at 65 °C until constant weight and weighted using electronic scale as biomass (g). Plant height and root length were also measured.

2.3. Statistical analysis

The experiment was a completely random design with three replications. All data obtained were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared by Duncan's multiple range test. Comparisons with $P < 0.05$ were considered significantly different. In all the figures, the spread of values is shown as error bars representing standard errors of the means.

3. Results

3.1. Effect of microwave pretreatment on MDA, H_2O_2 concentration and the production rate of O_2^- of wheat seedlings under $CdCl_2$ stress

Changes of MDA and H_2O_2 concentration and O_2^- production rate in wheat seedlings under continuous $CdCl_2$ stress were presented in Fig. 1. As shown in Fig. 1A, MDA concentration increased in 150 μM $CdCl_2$ treated seedlings (on the initial 8 d) whether they had been pretreated with microwave or not. With prolonged $CdCl_2$ stress treatment, MDA concentration of untreated seedlings (0 s) and 15 s microwave pretreated seedlings gradually increased, whereas MDA concentration of 5 and 10 s microwave