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Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress

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ABSTRACT

Jasmonic acid (JA) is regarded as endogenous regulator that plays an important role in regulating stress responses, plant growth and development. To investigate the physiological mechanisms of salt stress mitigated by exogenous JA, foliar application of 2 mM JA was done to wheat seedlings for 3 days and then they were subjected to 150 mM NaCl. Our results showed that 150 mM NaCl treatment significantly decreased plant height, root length, shoot dry weight, root dry weight, the concentration of glutathione (GSH), chlorophyll *b* (Chl *b*) and carotenoid (Car), the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), enhanced the concentration of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and the rate of superoxide radical ($O_2^{\bullet -}$) generation in the wheat seedlings when compared with the control. However, treatments with exogenous JA for 3 days significantly enhanced salt stress tolerance in wheat seedlings by decreasing the concentration of MDA and H_2O_2, the production rate of $O_2^{\bullet -}$ and increasing the transcript levels and activities of SOD, POD, CAT and APX and the contents of GSH, Chl *b* and Car, which, in turn, enhanced the growth of salt stressed seedlings. These results suggested that JA could effectively protect wheat seedlings from salt stress damage by enhancing activities of antioxidant enzymes and the concentration of antioxidative compounds to quench the excessive reactive oxygen species caused by salt stress and presented a practical implication for wheat cultivation in salt-affected soils.

1. Introduction

Salinity is one of the major environmental factors that severely limit growth and yield of crop plants worldwide because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil (Wu et al., 2012; Li et al., 2011). Salt stress can induce several morphological, physiological, and metabolic responses and cause osmotic stress and reactive oxygen species (ROS) stress, leading to gradual peroxidation of lipid and antioxidant enzyme inactivation (Garg and Manchanda, 2009). ROS interact with a wide range of molecules causing a series of responses, including lipid peroxidation, membrane destruction, protein denaturation and DNA mutation. To mitigate and repair the damage initiated by various ROS, plants have developed a wide range of non-enzymatic and enzymatic defense mechanisms to detoxify free radicals and therefore help protect them from destructive oxidative stress (Parida et al., 2004; Li et al., 2011). The antioxidant defense system includes non-enzymatic antioxidants such as glutathione (GSH), ascorbic acid (AsA), α-tocopherol, proline and carotenoids, whereas antioxidant enzymes include superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidases (POD, EC 1.11.1.7). SOD is a major scavenger of toxic $O_2^{\bullet -}$ radicals and its enzymatic action results in the formation of H_2O_2 that is subsequently converted to H_2O by enzyme peroxidase (Triantaphylides and Havaux, 2009). POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants, whereas CAT breaks down H_2O_2 into H_2O and O_2 (Mittler, 2002; Li et al., 2011). A large body of evidence has demonstrated that the antioxidant systems play important roles in protecting plants against oxidative damage induced by salt stress. Furthermore, salt tolerance in most crop plants is often correlated well with a more efficient oxidative system (Mittler, 2002; Xie et al., 2008). Therefore, enhancing the activities of antioxidant enzymes and contents of non-enzymatic compounds in plants are necessary for improving plant's tolerance to salt stress (Li et al., 2011; Bidabadi et al., 2013).

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), are naturally occurring plant growth regulators, regulating the morphological, physiological and biochemical processes in

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbate; Car, carotenoid; CAT, catalase; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; GR, glutathione reductase; GSH, glutathione; H_2O_2, hydrogen peroxide; JA, jasmonic acid; MDA, malondialdehyde; MeJA, methyl jasmonate; NBT, nitroblue tetrazolium; $O_2^{\bullet -}$, superoxide radical; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid

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plants (Seo et al., 2005; Mahmood et al., 2012). In recent years, plant growth regulators were considered a new way to improve plant stress tolerance, alleviate stress damage, and ensure the quality and yield of plants (crop) (Anjum et al., 2011; Mahmood et al., 2012). Many studies showed that JA plays an important role in abiotic stress tolerance, and considerable interests have focused on JA due to its ability to induce a protective effect on plants under stress (Cao et al., 2009; Takeuchi et al., 2011). Liu et al. (2012) reported that exogenous 1 and 2.5 mM JA could offset the effects of UV-B radiation by increasing the activity of SOD and the content of proline and anthocyanin in wheat seedlings. Studying the role of methyl jasmonate (MeJA) in alleviating NaCl-induced salt stress on pepper, Rezai et al. (2013) reported that exogenous 500 mg L^{-1} MeJA could improve the resistance of pepper seedlings to salinity stress by increasing the concentration of chlorophyll and root length, shoot and root fresh weight. However, it is not known if exogenous JA treatment also induces salt stress tolerance in wheat seedlings. So we put forward a hypothesis that exogenous JA treatment can confer salt tolerance to wheat seedlings by regulating the growth, the transcript levels and activities of SOD, POD, CAT and APX, the concentration of photosynthetic pigments, AsA, GSH and membrane lipid peroxidation.

The present study was carried out in an attempt to evaluate the interactive effect of exogenous JA and salt stress, ascertain the role of JA in the resistance to salt stress at physiological and molecular level using wheat seedlings. This work provides new knowledge on the roles of exogenously applied JA in modifying responses of wheat seedlings under salt stress.

2. Materials and methods

2.1. Plant materials, growth and treatment conditions

Uniform seeds of wheat (*Triticum aestivum* L. cv. Wenmai No. 6, obtained from Henan Academy of Agricultural Sciences) were surface sterilized using 0.01 percent $HgCl_2$ for 1 min, followed by washing several times with flowing water. The seeds were grown in Petri dishes (diameter 16 cm), flushed daily with Hoagland solution, in a growth chamber under a 12 h photoperiod at 600 μmol m^{-2} s^{-1} provided by fluorescent lamps, 65 percent relative humidity and 25 °C/18 °C (day/night) temperature. One-week-old seedlings (with one fully expanded leaves) were used as experimental materials for treatment with jasmonic acid (JA) and NaCl. Treatments were (1) seedlings were sprayed with distilled water containing 0.02 percent (v/v) ethanol (the control, CK), (2) seedlings were treated with 150 mM NaCl containing 0.02 percent ethanol (v/v) (NaCl), (3) seedlings were sprayed with 2 mM JA alone containing 0.02 percent (v/v) ethanol (JA), and (4) seedlings were sprayed with 2 mM JA containing 0.02 percent ethanol (v/v) before additional 150 mM NaCl (JA+NaCl). Because JA (Sigma-Aldrich) has low solubility in water, it was dissolved in 0.02 percent (v/v) ethanol and diluted with distilled water. For JA treatment, 2 mM JA containing 0.02 percent (v/v) ethanol was sprayed two times at 2-day intervals and control plants received the same amount of distilled water containing 0.02 percent (v/v) ethanol. Three days after commencing the JA treatments, 150 mM NaCl was added to the Hoagland solution for seedlings with or without JA treatment. 2 mM JA was chosen according to Liu et al. (2012), who reported that exogenous 1 and 2.5 mM JA could offset the effects of UV-B radiation by increasing the antioxidant defense of wheat seedlings. Five petri plates of 80 seedlings each were regarded as a group of treatments and all experiments were independently repeated at least three times. After 4 days of salt stress, leaves were collected and immediately frozen in liquid nitrogen and stored at −70 °C until use. In our preliminary experiment, we have found that the effect of exogenous JA on a few parameters in wheat seedlings under salt stress was significant on 3–6 days. So we selected the data for 4th day of exposure to salt stress. Then, the shoots and roots of another wheat seedlings were separately incubated at 105 °C for 10 min and were incubated at 65 °C in a hot-air oven until constant weight and weighed using electronic scale as dry weight, respectively. Plant height and root length were also measured.

2.2. Determination of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) concentration and superoxide radical ($O_2^{\bullet -}$) production rate

The concentration of MDA which is a product of lipid peroxidation was assessed by the thiobarbituric acid (TBA) as described by Predieri et al. (1995). Samples of leaves (0.2 g fresh weight, FW) were homogenized with a mortar and pestle in 50 mM phosphate buffer (pH 7.8), and then centrifuged at 8000*g* for 15 min. One mL supernatant was combined with 2.5 mL thiobarbituric acid (TBA) incubated in boiling water for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 10,000*g* for 5 min and the absorbance of supernatant was monitored at 532 and 600 nm. The value for non-specific absorbance at 600 nm was subtracted. MDA concentration was calculated by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as μmol MDA g^{-1} FW.

H_2O_2 concentration was estimated according to Shi et al. (2005). Leaf samples (0.5 g fresh weight, FW) were homogenized in an ice bath with 3 mL of three percent (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000*g* for 15 min. Afterwards, 1 mL of the supernatant was added to 1 mL of 100 mM potassium phosphate buffer (pH 7.0) and 2 mL of 1 M KI. The absorbance was measured at 390 nm and the content of H_2O_2 was calculated based on a standard curve.

The production rate of $O_2^{\bullet -}$ was measured by the modified method of Elstner and Heupel (1976). Fresh leaves (0.1 g) were homogenized in 1 mL of 50 mM phosphate buffer (pH 7.8) and were then centrifuged at 10,000*g* for 10 min. The supernatants (0.5 mL) were mixed with 0.5 mL of 65 mM phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine chlorhydrate and then were incubated at 25 °C. After 1 h, the mixture was added to 1 mL of 17 mM sulfanilamide and 1 mL 7 mM α-naphthylamine at 25 °C for 20 min. The absorbance was measured at 530 nm and the production rate of $O_2^{\bullet -}$ was calculated from a standard curve of $NaNO_2$.

2.3. Antioxidant enzyme activities determination

Fresh leaves (0.50 g) were homogenized with a mortar and pestle under chilled conditions in the presence of 3 mL phosphate buffer (50 mM, pH 7.8) containing 1 mM EDTA. The homogenate was centrifuged at 15,000*g* for 15 min at 4 °C, and the resulting supernatant was used for the enzyme assay.

SOD activity was determined using the method of Giannopolitis and Ries (1977) that spectrophotometrically measures inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. The 3 mL reaction mixture consisted of 0.1 mL enzyme extract, 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 0.75 mM NBT and 0.02 mM riboflavin. The reaction was initiated by placing the tubes under two 40 W fluorescent lamps and terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. One unit of SOD activity was defined as the amount of enzyme required to cause a 50 percent inhibition of the reduction of NBT.

CAT activity was assayed spectrophotometrically at 240 nm in a reaction mixture containing 0.1 mL of enzyme extract, 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA and 0.1 percent H_2O_2. The decomposition of H_2O_2 was measured by following the decline in absorbance at 240 nm for 3 min and quantified by its molar extinction coefficient (39.4 mM^{-1} cm^{-1}). One unit of CAT activity was defined as a change of 0.01 absorbance min^{-1} caused by the enzyme extract (Qiu et al., 2011).

Analysis of POD capacity was based on oxidation of guaiacol using H_2O_2 according to the method of Zhang and Kirham (1994). The enzyme extract (20 μL) was added to the reaction mixture containing 20 μL guaiacol solution and 10 μL H_2O_2 solution in 3 mL of phosphate buffer solution (pH 7.0). The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 5 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}).

The method of Nakano and Asada (1981) was used for the assay of APX activity. The reaction mixture (3 mL) contained 0.1 mL of enzyme extract, 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.5 mM ascorbate and 0.1 mM H_2O_2. APX was assayed as a decrease in absorbance at 290 nm of ascorbate and enzyme activity was quantified using the molar extinction coefficient for 2.8 mM^{-1} cm^{-1}.

2.4. Determination of non-enzymatic antioxidants and photosynthetic pigments concentration

The method described by Tonamura (1978) was applied to quantify AsA concentration. Fresh leaves (0.1 g) were homogenized in ice-cold 2 mL ten percent metaphosphoric acid. After centrifugation at 15,000*g* for 10 min, to 0.5 mL of supernatant, 1 mL of citric acid–phosphoric acid buffer (pH 2.3) and 1 mL of 2, 6-dichlorophenol indophenol (30 mg L^{-1}) were added. After 30 s, the absorbance was determined at 524 nm and the content of AsA was calculated from a standard curve of ascorbate.

Fresh leaves (0.1 g) were ground with mortar and pestle in ice-cold five percent (w/v) trichloroacetic acid, and centrifuged at 15,000*g* for 15 min. The supernatants were used for assays of GSH content. Reduced GSH content was measured according to the method of Ellman (1959). Supernatant (0.2 mL) was added to 2.6 mL NaH_2PO_4 (pH 7.7) and 0.2 mL 5, 5-dithiobis (2-nitrobenzoic) (DTNB, 2.51 mg mL^{-1}, pH 6.8). After 5 min at 30 °C, the absorbance was determined at 412 nm and reduced GSH content was calculated based on a standard curve.

The photosynthetic pigments were extracted from seedling leaves with 10 mL of 80 percent acetone for 24 h in dark at room temperature, and the extracted solution was analyzed spectrophotometrically (Liu et al., 2012). The content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid (Car) was estimated according to the