J Plant Growth Regul (2009) 28:381–385
DOI 10.1007/s00344-009-9106-7

Weak Microwave Can Enhance Tolerance of Wheat Seedlings to Salt Stress

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Received: 3 June 2008 / Accepted: 24 April 2009 / Published online: 15 July 2009

Abstract The aim of our investigation was to determine the effect of microwave pretreatment of wheat seeds on the tolerance of seedlings to salt stress. Selected parameters (for example, plant growth and biochemical parameters related to oxidative status) were measured. The results showed that microwave pretreatments for 5, 10, 15, or 20 s resulted in an increase in root length and shoot height in seedlings, with 10- and 15-s treatments giving the greatest effect. Salt stress, produced by treatment with 200 mM NaCl, reduced the length and fresh weight of shoots and roots, enhanced the leaf concentrations of malondialdehyde (MDA) and oxidized glutathione (GSSG), indicators of oxidative stress, while decreasing the activities of nitric oxide synthase (NOS), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and glutathione reductase (GR). Furthermore, the salt treatment reduced the concentration of nitric oxide (NO) and glutathione (GSH) in the shoots. However, treatments of seeds with microwave radiation followed by salt stress restored all of these parameters close to those in non-salt-treated seedlings. The results indicate that application of a suitable dose of microwave radiation to seeds can enhance tolerance to salt stress in wheat seedlings.

Keywords Wheat · Microwave pretreatment · Salt stress

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Introduction

Agricultural production is of paramount importance in an era in which the Earth's population is over six billion and growing. To ensure that food supplies keep pace with population growth, a complete understanding of the processes involved in crop growth and development is required to inform agronomic practices. Salinity is one of the major abiotic stress factors for plant growth. Some studies estimate that 20–50% of all irrigated croplands is affected by high salt concentration, resulting in considerable economic losses (Flowers 1999).

Salt stress triggers an increased formation of reactive oxygen species (ROS), which results in cellular damage (Ramachandra Reddy and others 2004; Molassiotis and others 2006). To minimize oxidative damage, plants have evolved various enzymatic and nonenzymatic defense mechanisms to detoxify free radicals and reduce oxidative stress. The antioxidant defense system includes enzymes such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD), whereas nonenzymatic antioxidants include glutathione, ascorbate, proline, and so on. Moreover, ROS-induced damage of cell membranes is regulated by NO signaling (Delledonne and others 1998). NO as a plant defense signal can react with free radicals such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), and could potentially abolish signaling through these compounds (Delledonne and others 1998).

Microwave irradiation has been shown to have the potential to replace chemical treatment for pest or fungal control in sorghum (More and others 1992), wheat (Shayesteh and Barthakur 1996), and nuts (Wang and others 2002). Low-intensity microwave radiation enhances enzymatic activities (Chen and others 2005) and can protect seedlings of *Isatis indigotica* from enhanced UV-B

damage (Chen 2006). To test whether microwave pretreatment can enhance tolerance of wheat seedling to salt stress, we determined the effect of this treatment on seedling growth and on parameters related to free radical suppression and the alleviation of oxidative stress (for example, the concentration of MDA, NO, GSSG, and GSH and the activities of NOS, CAT, POD, SOD, and GR) in the presence of salt stress. We show that indeed pretreatment with low-energy microwave radiation partially restores growth and enhances protection against ROS of wheat seedlings in 200 mM NaCl.

Materials and Methods

Plant Materials

Equal-sized and plump seeds of wheat (zhengmai No. 9023) were sterilized for 10 min in 0.05% $HgCl_2$, washed for 30 min in running water, and then were air dried.

To determine the microwave doses that did not significantly affect seed germination and were not detrimental to seedling growth, seeds were randomly divided into six batches (500 seeds per batch) and placed in the center of the microwave board. Seed batches were irradiated for 0, (0S), 5 (5S), 10 (10S), 15 (15S0, 20 (20S), or 25 (25S) s. Microwave treatment was carried out with a 700-W (power output) experimental prototype microwave oven with variable power at 2450 MHz (wavelength = 125 mm, Shunde Electron Industries Ltd., Guangzhou, China). The power density at the board's center was 126 mW cm^{-2} with low-power radiation. After treatment, seeds from each batch were sown in six plastic dishes (each containing 60 seeds) with wet filter paper soaked with distilled water and grown in an artificial greenhouse maintained at 25°C, 70% relative humidity, 400 μmol mol^{-1} CO_2, and 1500 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR). When the seedlings were 3 days old, the root length and seedling height were measured and the mean values determined for the 60 seedlings. The experiment was repeated three times.

To determine the effect of microwave irradiation on survival of salt stress, the seeds were randomly divided into four groups: (1) the control group (no treatment, CK), (2) seeds exposed to microwave irradiation only (M), (3) seeds exposed to 200 mM NaCl treatment only (Na), (4) seeds exposed to microwave irradiation followed by 200 mM NaCl treatment (MNa). Each replicate experiment comprised six dishes, each containing 60 seeds. The seeds of groups M and MNa were irradiated for 10 s based on the results of the preliminary experiments to determine the dose rate, and then the four groups were sown separately in plastic dishes (20 cm) with filter paper soaked with distilled water and grown in a greenhouse maintained at 25°C, 70% relative humidity, and 400 μmol mol^{-1} CO_2 in the dark. After 2 days the Na and MNa seedlings were treated with 200 mM NaCl in Petri dishes for 4 days and the groups CK and M were irrigated with 30 ml distilled water per day. Seedlings were grown with a 10-h photoperiod (1500 μmol m^{-2} s^{-1} PAR).

Determination of Biochemical and Physiological Characters

MDA concentration was measured according to Predieri and others (1995). Leaf samples of 0.2 g fresh weight were taken from 7-day-old seedlings and immediately frozen at –70°C. The frozen leaf tissues were then homogenized in 5 ml phosphate buffer (pH 6.7), followed by centrifugation for 15 min at 8000 *g*. A 0.5-ml aliquot of the supernatant was combined with an equal volume of thiobarbituric acid (TBA) reagent [5% TBA (w/v) in 20% trichloroacetic acid (w/v)] and boiled for 20 min. Absorbance was determined at 532 and 600 nm. MDA concentration was expressed in nmol mg^{-1} protein.

Leaf samples (5 g fresh weight) of 7-day-old seedlings were used for enzyme extraction. The samples were homogenized in 10 ml 0.05 M phosphate buffer (pH 6.7) and centrifuged for 10 min at 10,000 *g* at 0°C. Extraction was performed at 4°C. The supernatant was then stored at –20°C until used for assaying. Activities of SOD, POD, CAT, and GR were each determined according to Giannoplitis and Ries (1977), Nakano and Asada (1981), Cakmak and Marschner (1992), and Parida and others (2004), respectively, and expressed as U mg^{-1} protein.

Protein concentration was measured according to Bradford (1976). Wheat leaf samples of 7-day-old seedlings (0.5 g fresh weight) were homogenized in 2.5 ml 0.1 M Tris-HCl (pH 8.0, containing 0.5 M sucrose, 0.06 M L-ascorbic acid, and 0.005 Mβ-mercaptoethanol) at 0°C. After thorough grinding, the samples were removed to 5-ml centrifuge tubes and centrifuged for 15 min at 8000 *g*. For each sample, 0.15 ml supernatant, 0.85 ml distilled water, and 5 ml 0.1 g L^{-1} G-250 Coomassie Brilliant Blue were added. After 15 min the absorbance was determined at 595 nm. Concentration of soluble protein was expressed as mg g^{-1} FW.

The concentrations of oxidized glutathione (GSSG) and reduced glutathione (GSH) were measured using the GSH and GSSG kit as described in the manufacturer's protocol (Nanjing Jiancheng Bioengineering Reagent Co., Ltd., China).

NOS activity was determined according to Murphy and Noack (1994). Leaf samples (5 g) were homogenized in 10 ml buffer (50 mM triethanolamine hydrochloride [pH 7.5] containing 0.5 mM EDTA, 1 μM leupeptin, 1 μM pepstatin, 7 mM glutathione, and 0.2 mM phenylmethylsulfonyl

fluoride). After centrifuging at 10,000 *g* for 20 min (4°C), the supernatant was collected and recentrifuged at 100,000 *g* for 45 min and then used for analysis.

NO content was determined as described by Murphy and Noack (1994). Leaf samples (5 g) were incubated with 100 U of CAT and 100 U of SOD for 5 min to remove endogenous ROS before addition of 10 ml oxyhemoglobin (5 mM). After 2 min of incubation NO was measured spectrophotometrically by measuring the concentration of methemoglobin.

Determination of Root and Shoot Height

When the seeding was 3 days old (for experiments to determine dose) and 7 days old (for experiments to determine the effect of microwave irradiation on survival of salt stress), the root and shoot heights were measured with a ruler.

Statistical Analysis

Samples were arranged in completely randomized designs with three to five replications. The data were presented as the mean ± standard errors (SE). Results from different treatments were compared using one-way ANOVA (analysis of variance). Following ANOVA, *post hoc* comparisons of means were made using Duncan's multiple range tests. Statistical significance was determined at $p < 0.05$.

Results and Discussion

Selection of Appropriate Microwave Irradiation Dosage

Compared with the control (CK), microwave pretreatment for 5, 10, 15, and 20 s caused a significant increase of 6, 25, 24, and 12.5%, respectively, in the root length and 6.5, 25.5, 23, and 10%, respectively, in seedling height measured 3 days after treatment (Fig. 1). However, microwave pretreatment for 25 s resulted in an insignificant decrease of 3% in the root length and seedling height (Fig. 1). The results showed that suitable doses of microwave radiation increase plant growth, with a 10-s treatment giving the largest stimulation. This dose was used for further experiments.

Protective Role of Microwave Radiation

Some studies estimate that 20–50% of all irrigated croplands are affected by high salt concentration, resulting in considerable economic losses (Flowers 1999). Salinity affects several physiological and biochemical processes (for example, membrane functions, photosynthesis, stomatal function, biosynthesis of photosynthetic pigments) and is a major problem for agriculture (Karabal and others 2003). Salt stress triggers an increased formation of ROS, which affect plant metabolism in different ways and bring about cellular damage (Ramachandra Reddy and others 2004). ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids (Imlay 2003). Lipids contain a high percentage of polyunsaturated fatty acid (PUFA) residues and are thus susceptible to peroxidation (Mittler 2002). The content of MDA, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Meloni and others 2003). Increases in various members of the plant defense pathway, such as the enzymes SOD, POD, and CAT, as well as in GSH and AsA will detoxify free radicals, lowering the level of MDA and limiting oxidative stress. Treatment with 200 mM NaCl caused an increase in the concentrations of MDA and GSSG compared with the control (Fig. 2a, c). Microwave pretreatment alone caused a decrease in MDA and GSSG concentrations. When irradiated seeds were treated with NaCl, the concentrations of MDA and GSSG were close to those in the control group. NaCl treatment of nonirradiated seeds caused a significant decrease in the concentration of NO (Fig. 2d), in the activities of NOS, SOD, CAT, POD, and GR (Fig. 3), and in the lengths and fresh weights of shoots and roots (Fig. 4a). There was a small, but not significant, decrease in the concentration of GSH (Fig. 2b). Microwave pretreatment in the absence of NaCl caused a significant

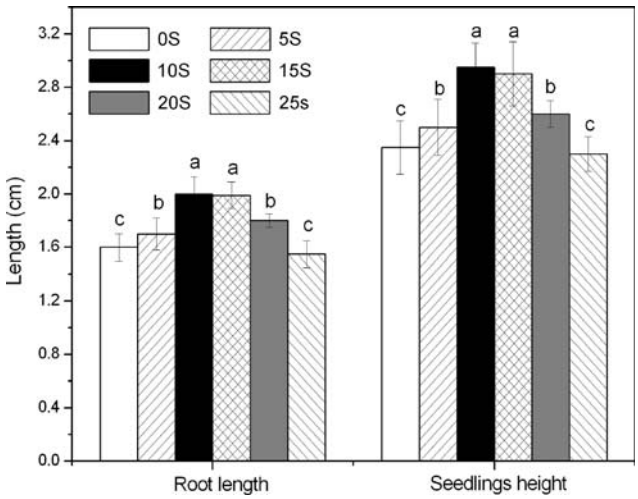

Fig. 1 Effects of microwave pretreatment of seeds on root and shoot growth in 3-day-old wheat seedlings. The experiment consisted of six treatments: 0 s microwave radiation (control), 5 s microwave radiation (5S), 10 s microwave radiation (10S), 15 s microwave radiation (15S), 20 s microwave radiation (20S), 25 s microwave radiation (25S). Data are the means with standard errors from three completely independent experiments. Columns with different letters are significantly different at the 0.5 level ($n = 3$) according to Duncan's multiple range test

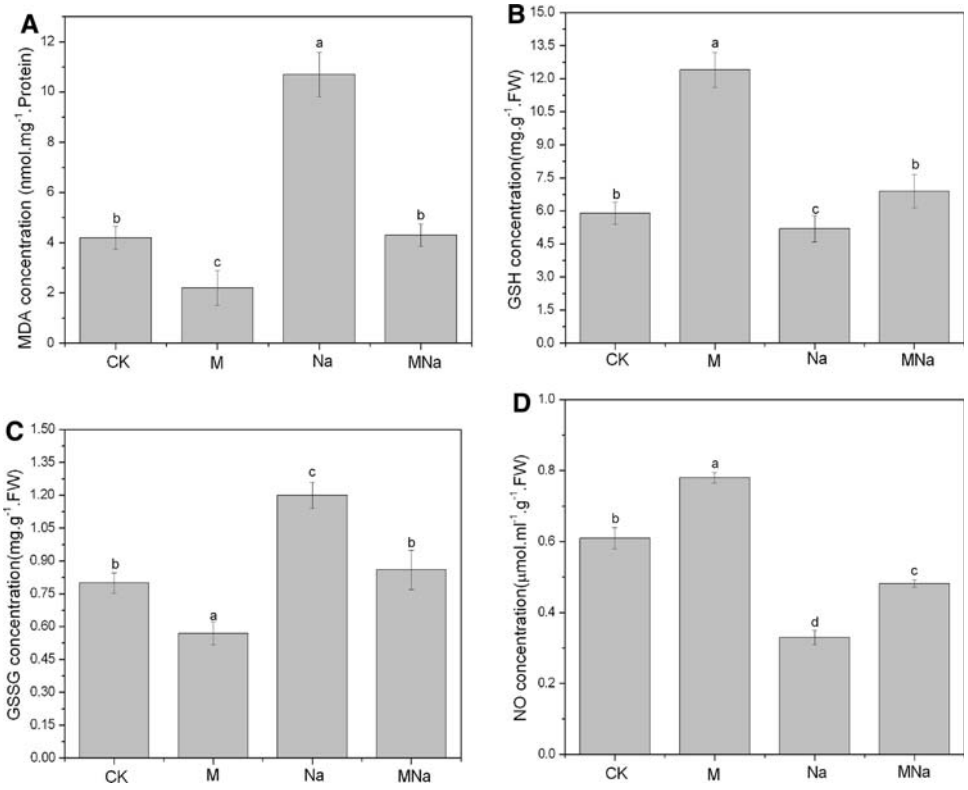

Fig. 2 Effects of microwave pretreatment of seeds on the concentration of MDA (**a**), GSH (**b**), GSSG (**c**), and NO (**d**) in leaves of 7-day-old wheat seedlings treated with 200 mM NaCl. The experiment consisted of four treatments: the CK (no treatment), microwave radiation (M), 200 mM NaCl treatment (Na), and microwave radiation plus 200 mM NaCl treatment (MNa). Data are the means with standard errors from three independent experiments. Columns with different letters are significantly different at the 0.5 level ($n = 3$) according to Duncan's multiple range test

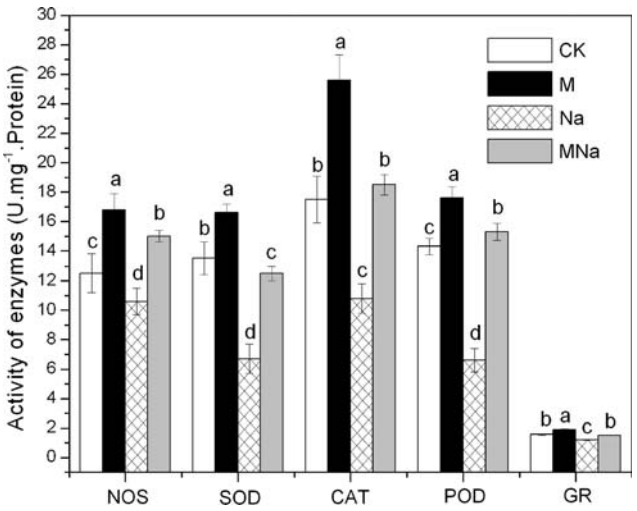

Fig. 3 Effects of microwave pretreatment of seeds on the activities of NOS, SOD, CAT, POD, and GR in 7-day-old wheat seedlings treated with 200 m MNaCl. The experiment consisted of four treatments: the CK (no treatment), microwave radiation (M), 200 mM NaCl treatment (Na), and microwave radiation plus 200 mM NaCl treatment (MNa). Data are the means with standard errors from three independent experiments. Columns with different letters are significantly different at the 0.5 level ($n = 3$) according to Duncan's multiple range test

increase in the concentrations of NO and GSH, in the activities of NOS, SOD, CAT, POD, and GR, and in the lengths and fresh weights of shoots and roots compared with nonirradiated plants (Fig. 4). Microwave pretreatment of seeds prior to NaCl treatment resulted in higher concentrations of NO and GSH, increased activities of NOS, SOD, CAT, POD, and GR, and greater lengths and fresh weights of shoots and roots than for NaCl-stressed plants without microwave treatment. The results indicate that microwave radiation can protect cells of wheat seedlings from salt stress by activating the antioxidant defense system, which involves NO signaling and ROS suppression. Although we have not yet investigated the effects of the microwave seed treatment on the later stages of growth and on grain yields, this method has the potential to increase plant growth and decrease yield losses under saline conditions.

Conclusion

This study demonstrated that the ROS-induced decrease in root and shoot development and growth of wheat seedlings under saline conditions can be ameliorated by microwave treatment of the seeds. The results support the hypothesis that microwave treatment can enhance tolerance of wheat seedlings to salt stress by stimulating the antioxidant defense system through enhancing the production of NO and enzymatic and nonenzymatic antioxidants. This treatment could potentially benefit agriculture by increasing plant growth and decreasing yield losses under saline

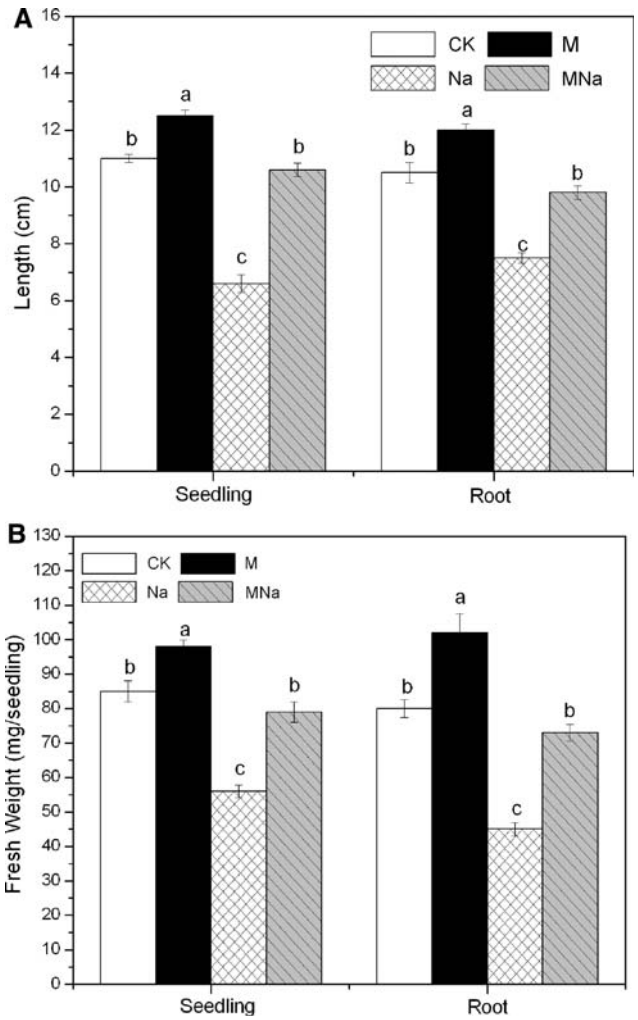

Fig. 4 Effects of microwave pretreatment of seeds on root and shoot growth in 7-day-old wheat seedlings treated with 200 mM NaCl (**a** is root and shoot length, **b** is root and shoot fresh weight). The experiment consisted of four treatments: the CK (no treatment), microwave radiation (M), 200 mM NaCl treatment (Na), and microwave radiation plus 200 mM NaCl treatment (MNa). Data are the means with standard errors from five independent experiments (20 seedlings were measured in each experiment). Columns with different letters are significantly different at the 0.5 level ($n = 5$) according to Duncan's multiple range test

conditions. The mechanism by which microwave treatment may protect against salt stress will be investigated further.

Acknowledgment This work was supported by State Fund of China (40599422) and the West China Star of CAS (2007YB04).

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