Manganese-induced salt stress tolerance in rice seedlings: regulation of ion homeostasis, antioxidant defense and glyoxalase systems

Anisur Rahman^{1,2} · Md. Shahadat Hossain¹ · Jubayer-Al Mahmud^{1,3} · Kamrun Nahar^{1,4} · Mirza Hasanuzzaman² · Masayuki Fujita¹

Received: 23 June 2016 / Accepted: 31 July 2016 / Published online: 17 August 2016
© Prof. H.S. Srivastava Foundation for Science and Society 2016

Abstract Hydroponically grown 12-day-old rice (*Oryza sativa* L. cv. BRR1 dhan47) seedlings were exposed to 150 mM NaCl alone and combined with 0.5 mM MnSO₄. Salt stress resulted in disruption of ion homeostasis by Na⁺ influx and K⁺ efflux. Higher accumulation of Na⁺ and water imbalance under salinity caused osmotic stress, chlorosis, and growth inhibition. Salt-induced ionic toxicity and osmotic stress consequently resulted in oxidative stress by disrupting the antioxidant defense and glyoxalase systems through overproduction of reactive oxygen species (ROS) and methylglyoxal (MG), respectively. The salt-induced damage increased with the increasing duration of stress. However, exogenous application of manganese (Mn) helped the plants to partially recover from the inhibited growth and chlorosis by improving ionic and osmotic homeostasis through decreasing Na⁺ influx and increasing water status, respectively. Exogenous application of Mn increased ROS detoxification by increasing the

content of the phenolic compounds, flavonoids, and ascorbate (AsA), and increasing the activities of monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), superoxide dismutase (SOD), and catalase (CAT) in the salt-treated seedlings. Supplemental Mn also reinforced MG detoxification by increasing the activities of glyoxalase I (Gly I) and glyoxalase II (Gly II) in the salt-affected seedlings. Thus, exogenous application of Mn conferred salt-stress tolerance through the coordinated action of ion homeostasis and the antioxidant defense and glyoxalase systems in the salt-affected seedlings.

Keywords Methylglyoxal · Nutrient homeostasis · Osmotic stress · Oxidative stress · Reactive oxygen species · Trace elements

Introduction

Salinity is one of the most brutal environmental factors that has affected 6 % of the world's total land area. Of the cultivable areas, 20 % of the irrigated land and 2 % of dry land agriculture area are affected by salinity directly or by secondary salinity (Munns and Tester 2008). The salt-affected area is increasing day by day because of climate change, and it is assumed that 50 % of cultivable land will be salt affected by the middle of the twenty-first century (Mahajan and Tuteja 2005). High salinity affects plant growth through the osmotic effect of the salt in the growth medium and the toxic effect of the salt within the plant. The immediate response of plants to high concentrations of salt is osmotic adjustment by reducing cell expansion, cell division, stomatal closure, and gradually reducing leaf area, and thus photosynthesis and growth is reduced. In later stages, plants experience ionic stress after accumulating

✉ Masayuki Fujita
fujita@ag.kagawa-u.ac.jp
Mirza Hasanuzzaman
mhzsauag@yahoo.com

¹ Laboratory of Plant Stress Responses, Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa 761-0795, Japan

² Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh

³ Department of Agroforestry and Environmental Science, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh

⁴ Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh

toxic levels of sodium (Na^+) and chloride (Cl^-). Plants exposed to higher salt result in higher Na^+ accumulation and a higher Na^+ /potassium (K^+) ratio because Na^+ influx causes K^+ efflux and triggers K^+ leakage from plant cells (Cramer et al. 1985; Shabala et al. 2006; Wu and Wang 2012; Rahman et al. 2016a). Higher Na^+ content and a higher Na^+/K^+ ratio in cells cause metabolic toxicity because Na^+ compete with K^+ for binding sites of essential enzymes, and Na^+ cannot be a substitute for K^+ (Bhandal and Malik 1988; Tester and Davenport 2003). In addition, Na^+ influx causes toxicity symptoms (chlorosis and necrosis) in mature leaves and premature senescence of adult leaves by disrupting protein synthesis and interfering with enzyme activity (Carillo et al. 2011; Munns 2002; Munns and Tester 2008). Reduced leaf area caused by osmotic stress and premature senescence of adult leaves caused by ionic stress limit the photosynthetic area available to support continued growth of salt-affected plants (Hasegawa et al. 2000; Munns 2002; Carillo et al. 2011). Salt-induced osmotic stress, ionic toxicity, and a lower rate of photosynthesis increase the formation of reactive oxygen species (ROS), which disrupt the antioxidant defense system and consequently causes oxidative stress (Hasanuzzaman et al. 2013a, b; Mishra et al. 2013). In addition, the cytotoxic compound methylglyoxal (MG) also causes oxidative damage through degradation of protein synthesis under salt stress and other abiotic stress conditions (Yadav et al. 2005; Hasanuzzaman et al. 2014a).

Enhancing ionic and osmotic homeostasis plus detoxifying ROS and MG are some salt-stress tolerance responses in plants (Wu and Wang 2012; Wutipraditkul et al. 2015; Rahman et al. 2016a, b). Usually, plants produce proline (Pro) or other compatible solutes to stabilize osmotic homeostasis by maintaining the water relationship and stabilizing protein and enzyme complexes (Iqbal et al. 2015; Reddy et al. 2015; Nahar et al. 2016a). Besides this, to detoxify overproduced ROS, plants have an antioxidant defense system composed of non-enzymatic antioxidants (ascorbic acid, AsA; glutathione, GSH; phenolic compounds; alkaloids; non-protein amino acids; and α -tocopherols) and enzymatic antioxidants (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; and glutathione *S*-transferase, GST) (Pang and Wang 2008; Gill and Tuteja 2010; Hasanuzzaman et al. 2012; Hasanuzzaman et al. 2013a, b). Similarly, the glyoxalase enzymes (glyoxalase I; Gly I and glyoxalase II; Gly II) act coordinately with GSH to detoxify overproduced MG under salt stress condition (Yadav et al. 2005; Nahar et al. 2015).

Regulating the antioxidant defense and glyoxalase systems to detoxify overproduced ROS and MG, improving

ionic and osmotic homeostasis, and reducing Na^+ influx by applying exogenous phytoprotectants are important strategies to reduce salt-induced damage in plants (Hasanuzzaman et al. 2011a; Wu and Wang 2012; Nahar et al. 2015; Ozfidan-Konakci et al. 2015).

Manganese (Mn) is an essential trace element for plants and plays a crucial role in several metabolic processes including photosynthesis, respiration, synthesis of ATP, fatty acid, amino acids, lipids, proteins, flavonoids, and hormone activation (Lidon et al. 2004; Millaleo et al. 2010). Manganese deficiency is detrimental to plants because it affects the water-splitting step of photosystem II (PS II), which directly provides electrons for photosynthesis. Manganese deficiency also weakens structural resistance against pathogens and reduces tolerance against drought and heat stress (Gherardi and Rengel 2003; Millaleo et al. 2010).

Along with the beneficial roles mentioned above, Mn also plays a vital role as a co-factor including Mn-SOD, and Mn-CAT which participate in plant defense against oxidative stress. Although not clear, it is also assumed that Mn acts as scavenger of O_2^- and H_2O_2 (Ducic and Polle 2005). Several studies also revealed that supplemental Mn plays an important role in the adaptive responses of plants under various environmental stresses. Simultaneous application of Mn improves growth, relative growth rate (RGR), net assimilation rate (NAR), and photosynthetic rate of salt-stressed barley seedlings (Cramer and Nowak 1992; Pandya et al. 2004). Exogenous application of Mn reduces metal toxicity in plants by reducing metal accumulation and lipid peroxidation, and improving biomass, chlorophyll (chl) content, carotenoid content, and the antioxidant defense system (Palove-Balang et al. 2006; Peng et al. 2008; Sebastian and Prasad 2015). However, the role of Mn in reducing salt-induced damage by regulating ion homeostasis, and the antioxidant defense and glyoxalase systems has not been studied.

Considering the strategies discussed, our present study was conducted to investigate the positive role of supplemental Mn in improving ROS and MG detoxification, improving ionic and osmotic homeostasis, and reducing Na^+ uptake capacity in salt-treated rice seedlings.

Materials and methods

Plant materials and treatments

Rice (*Oryza sativa* L. cv. BRRI dhan47) seeds were surface sterilized with 70 % ethanol for 8–10 min followed by washing several times with sterilized distilled water and soaked in distilled water in a dark germinator for 48 h at $28 \pm 2^\circ\text{C}$. The imbibed seeds were then sown on plastic

nets floating on distilled water in 250 mL plastic beakers and kept in the dark at 28 ± 2 °C for 72 h. Uniformly germinated seeds were then transferred to a growth chamber (light, $350 \mu\text{mol photon m}^{-2} \text{s}^{-1}$; temperature, 25 ± 2 °C; relative humidity, 65–70 %) with the same pot providing a diluted (5000-times) commercial hydroponics nutrient solution (Hyponex, Japan). The nutrient solution contained 8 % N, 6.43 % P, 20.94 % K, 11.8 % Ca, 3.08 % Mg, 0.07 % B, 0.24 % Fe, 0.03 % Mn, 0.0014 % Mo, 0.008 % Zn, and 0.003 % Cu. The nutrient solutions were renewed after 3 days. Each pot contained approximately 60 seedlings. Twelve-day-old rice seedlings were exposed to 150 mM NaCl in the presence and absence of 0.5 mM MnSO_4 with nutrient solution to verify the role of exogenous Mn under a salt-stress condition. Control plants were grown in Hyponex solution only. Our experiment consisted of four treatments as follows: control, 0.5 mM MnSO_4 , 150 mM NaCl, and 150 mM NaCl + 0.5 mM MnSO_4 . Data were taken after 3 and 6 days of treatment. The experiment was repeated three times under the same conditions.

Observation of salt toxicity symptoms and seedling growth

Growth and salt toxicity symptoms in the rice seedlings were determined by careful observation and measuring fresh weight (FW) and dry weight (DW). For DW, seedlings were oven dried at 70 °C for 48 h. Fresh weight and DW were expressed as mg seedling^{-1} . Plant height was measured from the base of the shoot to the tip of the longest leaf.

Determination of leaf relative water content

Leaf relative water content (RWC) was measured according to Barrs and Weatherly (1962). Leaf laminae were weighed (fresh weight, FW) then placed immediately between two layers of filter paper and immersed in distilled water in a Petridish for 24 h in a dark place. Turgid weight (TW) was measured after gently removing excess water with a paper towel. Dry weight (DW) was measured after 48 h oven drying at 70 °C. Finally, leaf RWC was determined using the following formula:

$$\text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

Determination of Na^+ , K^+ and Mn contents

The content of Na^+ , K^+ and Mn were determined by using an atomic absorption spectrophotometer (Z-5000; Hitachi, Japan). The plant samples were oven dried at 70 °C for 48 h. The dried samples from the roots and shoots (0.1 g)

were ground and digested separately with acid mixture at 70 °C for 48 h. The acid mixture consisted of HNO_3 :- HClO_4 (5:1 v/v).

Determination of chlorophyll content

Chlorophyll content was measured according to Arnon (1949) using homogenizing leaf samples (0.5 g) with 10 mL of acetone (80 % v/v) followed by centrifuging at $10,000 \times g$ for 10 min. Absorbance was measured with a UV–visible spectrophotometer at 663 and 645 nm for chl *a* and chl *b* contents, respectively. Carotenoid content was also measured spectrophotometrically at wave length 470 nm.

Determination of osmotic potential

Osmotic potential was measured according to Ozfidan-Konakci et al. (2015) with a modification. Leaves were homogenized in an ice cold mortar and pestle and centrifuged two times at $12,000 \times g$ for 10 min. Supernatant was used to measure osmolarity (c) by using a K-7400 semi-micro osmometer. Osmolarity was converted to the osmotic potential according to Van't Hoff equation using the following formula:

$$\Psi_{\Pi}(\text{MPa}) = -c (\text{mOsmol kg}^{-1}) \times 2.58 \times 10^{-3}$$

Determination of proline content

Proline content was determined according to Bates et al. (1973). Leaf samples (0.5 g) were homogenized in 5 mL 3 % sulfo-salicylic acid and the homogenate was centrifuged at $11,500 \times g$ for 12 min. Supernatant (1 mL) was mixed with 1 mL glacial acetic acid and 1 mL acid ninhydrin. After 1 h incubation at 100 °C, the mixture was cooled. The developed color was extracted with 2 mL toluene, and the optical density of the chromophore was observed spectrophotometrically at 520 nm. Proline content was determined by comparing with a standard curve of known concentration of Pro.

Histochemical detection of ROS ($\text{O}_2^{\cdot-}$ and H_2O_2) generation in the leaves; lipid peroxidation and membrane damage in the roots

Localization of $\text{O}_2^{\cdot-}$ in the leaves was detected following Chen et al. (2010) with a slight modification. Leaves were immersed in 0.1 % nitroblue tetrazolium chloride (NBT) solution and incubated at 25 °C. After 12 h, the incubated leaves were immersed in boiling ethanol (90 %) for 15 min to decolorize the leaves and reveal the dark blue spots produced by the reaction of NBT and $\text{O}_2^{\cdot-}$.

Fig. 1 Phenotypic appearance of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and 150 mM NaCl, respectively

Localization of H_2O_2 was detected according to the method of Thordal-Christensen et al. (1997) with a minor modification. Leaves were incubated at 25 °C in a solution containing 1 mg mL^{-1} 3,3-diaminobenzidine (DAB) prepared in HCl-acidified (pH 3.8) water. After 12 h incubation, the leaves were boiled in ethanol (90 %) for 15 min to reveal the reddish brown spots produced by the reaction of H_2O_2 and DAB.

Lipid peroxidation in the roots was visualized by histochemical staining using Schiff's reagent with the modification of Srivastava et al. (2014). Lipid peroxidation-originated aldehydes were detected after 30 min staining when the roots changed color to pink-red. The stained roots were rinsed with sulphite solution (0.5 % [w/v] $\text{K}_2\text{S}_2\text{O}_5$ in 0.05 M HCl) and dipped in the same solution for 10 min to retain the stained color. The loss of plasma membrane integrity in the roots was measured with the slight modification of Schützendübel et al. (2001) by histochemical staining using 0.25 % aqueous Evan's blue solution. After 40 min, the roots were rinsed with distilled water and membrane damage was revealed using glycerine.

Determination of lipid peroxidation and hydrogen peroxide levels

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA) following the method of Heath and Packer (1968). Malondialdehyde content was measured by observing the difference in absorbance at 532 nm using an extinction coefficient of 155 $\text{mM}^{-1} \text{cm}^{-1}$ and expressed as nmol of MDA g^{-1} FW. Hydrogen peroxide content was determined according to Yu et al. (2003) by observing absorbance at 410 nm using an extinction coefficient of 0.28 $\mu\text{M}^{-1} \text{cm}^{-1}$.

Determination of methylglyoxal content

Methylglyoxal was measured following the method of Wild et al. (2012) by extracting plant samples in 5 % perchloric acid. After centrifuging homogenized leaf tissues at 11,000 $\times g$ for 10 min, the supernatant was decolorized by adding charcoal. The decolorized supernatant was neutralized by adding saturated Na_2CO_3 and used to estimate MG by adding sodium dihydrogen phosphate and

Table 1 Effect on plant growth, chl and carotenoids contents of rice seedlings under salt stress with and without exogenous Mn

Duration	Treatments	Plant height (cm)	Fresh weight (mg seedling ⁻¹)	Dry weight (mg seedling ⁻¹)	chl <i>a</i> (mg g ⁻¹ FW)	chl <i>b</i> (mg g ⁻¹ FW)	chl (<i>a</i> + <i>b</i>) (mg g ⁻¹ FW)	Carotenoid (mg g ⁻¹ FW)
Day 3	Control	21.26 ± 0.29 b	138.33 ± 4.93 c	18.26 ± 0.36 c	2.44 ± 0.06 ab	0.77 ± 0.03 a	3.21 ± 0.07 a	0.58 ± 0.02 a
	Mn	21.27 ± 0.36 b	132.00 ± 3.88 cd	17.97 ± 0.34 c	2.50 ± 0.04 a	0.77 ± 0.04 a	3.27 ± 0.08 a	0.56 ± 0.03 a
	Salt	17.54 ± 0.22 d	104.47 ± 4.22 f	15.03 ± 0.23 e	1.86 ± 0.08 d	0.52 ± 0.02 b	2.38 ± 0.07 d	0.38 ± 0.02 d
	Salt + Mn	19.10 ± 0.20 c	125.71 ± 3.03 de	16.68 ± 0.14 d	2.36 ± 0.04 b	0.74 ± 0.12 a	2.96 ± 0.12 b	0.48 ± 0.01 b
Day 6	Control	24.56 ± 0.32 a	167.44 ± 7.28 a	20.78 ± 0.75 a	2.52 ± 0.07 a	0.79 ± 0.02 a	3.32 ± 0.08 a	0.59 ± 0.03 a
	Mn	24.27 ± 0.29 a	151.71 ± 3.41 b	19.43 ± 0.57 b	2.47 ± 0.08 ab	0.77 ± 0.02 a	3.24 ± 0.1 a	0.56 ± 0.01 a
	Salt	19.16 ± 0.31 c	118.10 ± 2.36 e	15.96 ± 0.23 d	1.37 ± 0.05 e	0.41 ± 0.02 c	1.78 ± 0.03 e	0.33 ± 0.02 e
	Salt + Mn	21.09 ± 0.22 b	139.93 ± 3.20 c	18.02 ± 0.38 c	2.20 ± 0.05 c	0.55 ± 0.03 b	2.75 ± 0.03 c	0.43 ± 0.02 c

Here, Mn and Salt indicate 0.5 mM MnSO₄ and 150 mM NaCl, respectively

Means (±SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

N-acetyl-L-cysteine to a final volume of 1 mL. The absorbance was recorded after 10 min at 288 nm, and MG content was calculated using a standard curve of known concentration of MG.

Determination of ascorbate and glutathione

Rice leaves (0.5 g) were homogenized in 3 mL ice-cold 5 % meta-phosphoric acid containing 1 mM EDTA using a mortar and pestle. The homogenates were centrifuged at 11,500×*g* for 15 min at 4 °C, and the collected supernatants were used according to the method of Nahar et al. (2016b) with minor modifications to determine total and reduced AsA. After neutralizing the supernatant with 0.5 M potassium-phosphate (K-P) buffer (pH 7.0), the oxidized fraction was reduced with 0.1 M dithiothreitol. Total and reduced AsA content were assayed spectrophotometrically at 265 nm in 100 mM K-P buffer (pH 7.0) with 1.0 U of ascorbate oxidase (AO). To calculate ascorbate, a specific standard curve of AsA was used. Dehydroascorbate (DHA) was measured using the formula $DHA = \text{total AsA} - \text{reduced AsA}$. Reduced glutathione (GSH), oxidized glutathione or glutathione disulfide (GSSG), and total glutathione (GSH + GSSG) were determined according to Griffiths (1980) based on enzymatic recycling. GSH was measured using the formula $GSH = \text{Total GSH} - GSSG$. Glutathione was removed by 2-vinylpyridine derivatization to determine GSSG.

Determination of phenolic contents

Total phenolic content was measured following Ashraf et al. (2010) with a modification. Leaf samples (0.5 g) were homogenized with 80 % (v/v) acetone followed by centrifuging at 10,000×*g* for 10 min. Supernatant (50 µL) was mixed with Folin phenol reagent, 7 % Na₂CO₃, and distilled water where total volume was 5 mL, and the absorbance was measured at 750 nm. Total phenolic content was measured by comparing with a gallic acid standard.

Determination of flavonoid content

Flavonoid content was measured according to Zhishen et al. (1999) with a modification by homogenizing plant samples (0.5 g) in 80 % aqueous ethanol. The homogenate was centrifuged at 10,000×*g* for 20 min at room temperature. Filtrate (500 µL) was diluted with distilled water (2 mL) and mixed with 150 µL NaNO₂ (5 %). After 5 min, 150 µL AlCl₃ (10 %) was added, and the solution was incubated for 6 min. Finally the mixture was diluted with 1 M NaOH, and volume was made up to 5 mL with distilled water. After vigorous vortexing the color intensity

Table 2 Effect on RWC, Pro content, osmotic potential, MDA content, H₂O₂ content and LOX activity of rice seedlings under salt stress with and without exogenous Mn

Duration	Treatments	RWC (%)	Pro content ($\mu\text{mol g}^{-1}$ FW)	Osmotic potential Ψ_{π} (MPa)	MDA content (nmol g^{-1} FW)	H ₂ O ₂ content (nmol g^{-1} FW)	LOX activity ($\text{nmol min}^{-1}\text{mg}^{-1}\text{protein}$)
Day 3	Control	97.52 $\pm$ 0.81 a	0.18 $\pm$ 0.01 e	−0.23 $\pm$ 0.01 ab	16.11 $\pm$ 1.75 f	19.16 $\pm$ 0.96 f	18.19 $\pm$ 1.55 d
	Mn	96.29 $\pm$ 0.28 a	0.22 $\pm$ 0.01 e	−0.25 $\pm$ 0.01 b	16.80 $\pm$ 2.21 ef	20.16 $\pm$ 0.7 ef	19.11 $\pm$ 1.34 d
	Salt	83.69 $\pm$ 0.89 e	2.29 $\pm$ 0.16 b	−0.57 $\pm$ 0.02 d	29.00 $\pm$ 1.05 c	33.39 $\pm$ 0.71 b	30.70 $\pm$ 2.09 b
	Salt + Mn	91.59 $\pm$ 1.09 c	0.75 $\pm$ 0.12 d	−0.38 $\pm$ 0.01 c	21.01 $\pm$ 1.64 de	25.44 $\pm$ 1.06 d	24.01 $\pm$ 2.28 c
Day 6	Control	93.71 $\pm$ 0.74 b	0.27 $\pm$ 0.02 e	−0.21 $\pm$ 0.01 a	17.95 $\pm$ 0.78 ef	22.14 $\pm$ 1.31 e	21.43 $\pm$ 0.79 cd
	Mn	89.81 $\pm$ 1 c	0.55 $\pm$ 0.05 d	−0.24 $\pm$ 0.01 b	23.16 $\pm$ 2.94 d	27.14 $\pm$ 1.17 cd	24.58 $\pm$ 2.94 c
	Salt	75.03 $\pm$ 1.67 f	4.35 $\pm$ 0.29 a	−0.88 $\pm$ 0.01 e	54.43 $\pm$ 2.29a	42.54 $\pm$ 2.11 a	41.54 $\pm$ 2.42 a
	Salt + Mn	86.01 $\pm$ 0.73 d	1.55 $\pm$ 0.19 c	−0.55 $\pm$ 0.01 d	33.48 $\pm$ 3.63 b	29.23 $\pm$ 0.81 c	32.10 $\pm$ 2.91 b

Here, Mn and Salt indicate 0.5 mM MnSO₄ and 150 mM NaCl, respectively

Means ($\pm$ SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

was measured at 510 nm. Flavonoid content was measured by comparing with a quercetin standard.

Determination of protein

Protein concentration was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard.

Enzyme extraction and assays

Leaves (0.5 g) were homogenized in 50 mM ice cold K–P buffer (pH 7.0) containing 100 mM KCl, 1 mM ascorbate, 5 mM β -mercaptoethanol, and 10 % (w/v) glycerol using a pre-cooled mortar and pestle. The homogenates were centrifuged two times at 11,500 $\times g$ for 15 min, and the supernatants were used to determine protein content and enzyme activity. All procedures were performed at 0–4 °C.

Lipoxygenase (LOX, EC: 1.13.11.12) activity was determined according to the method of Doderer et al. (1992) using linoleic acid as a substrate solution. The increased absorbance was observed at 234 nm and the activity was calculated using an extinction coefficient of 25 mM^{−1} cm^{−1}.

Ascorbate peroxidase (APX, EC: 1.11.1.11) activity was determined according to Nakano and Asada (1981) by observing the decreased absorbance at 290 nm for 1 min and using an extinction coefficient of 2.8 mM^{−1} cm^{−1}.

Monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4) activity was assayed by following the method of Hossain et al. (1984) using an extinction coefficient of 6.2 mM^{−1} cm^{−1}.

Dehydroascorbate reductase (DHAR, EC: 1.8.5.1) activity was determined according to the method of

Nakano and Asada (1981) by observing the change in absorbance at 265 nm for 1 min using an extinction coefficient of 14 mM^{−1} cm^{−1}.

Glutathione reductase (GR, EC: 1.6.4.2) activity was determined according to the method of Foyer and Halliwell (1976) by monitoring the decreased absorbance at 340 nm using an extinction coefficient of 6.2 mM^{−1} cm^{−1}.

Glutathione *S*-transferase (GST, EC: 2.5.1.18) activity was measured as described by Hossain et al. (2006). The activity was calculated by observing the increased absorbance at 340 nm for 1 min using an extinction coefficient of 9.6 mM^{−1} cm^{−1}. The reaction mixture contained 100 mM Tris–HCl buffer (pH 6.5), 1.5 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), and enzyme solution.

Glutathione peroxidase (GPX, EC: 1.11.1.9) activity was determined according to Elia et al. (2003) by monitoring the change in absorbance at 340 nm for 1 min using an extinction coefficient of 6.62 mM^{−1} cm^{−1}.

Superoxide dismutase (SOD, EC: 1.15.1.1) activity was measured based on the xanthine–xanthine oxidase system following the method of El-Shabrawi et al. (2010).

Catalase (CAT, EC: 1.11.1.6) activity was measured as described by Hasanuzzaman and Fujita (2011) using an extinction coefficient of 39.4 mM^{−1} cm^{−1}.

Glyoxalase I (Gly I, EC: 4.4.1.5) and glyoxalase II (Gly II, EC: 3.1.2.6) activities were determined as described by Hasanuzzaman and Fujita (2011) using extinction coefficients of 3.37 and 13.6 mM^{−1}cm^{−1}, respectively.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and the mean differences were compared by Fisher's LSD using XLSTAT v.2015 software (2015). Differences at $P \leq 0.05$ were considered significant.

Fig. 2 Effect on Na⁺ and K⁺ content and their ratio and Mn content in root (a, c, e, g) and leaf (b, d, f, h) of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO₄ and 150 mM NaCl, respectively. Means (±SD) were

calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Results

Phenotypic appearance and plant growth

Salt stress deteriorated phenotypic appearance in the salt-treated rice seedlings (Fig. 1). Treatment of the seedlings with salt caused rolling and burning of leaf tips and yellowing of the whole plant. Treatment of the seedlings with

salt also decreased plant growth in terms of plant height, FW, and DW (Table 1). Growth inhibition and deterioration of phenotypic appearance were more severe after 6 days of treatment, compared with 3 days. However, exogenous application of Mn partially recovered inhibited growth and improved the phenotypic appearance of the salt-treated seedlings.

Effect on leaf RWC, Pro content, and osmotic potential

Treating the seedlings with salt resulted in osmotic stress caused by decreasing leaf RWC and increasing osmotic potential and Pro content (Table 2). Compared with the control seedlings, leaf RWC decreased and Pro content, and osmotic potential increased with the increasing duration of the stress. Supplementation with Mn eliminated salt-induced osmotic stress indicated by decreased osmotic potential and Pro content and increased RWC, compared with the salt alone-treated plants.

Effect on ion homeostasis and Mn uptake

The rice seedlings treated with salt disrupted ion homeostasis by increasing Na^+ uptake and the Na^+/K^+ ratio, and decreasing K^+ accumulation, compared with the control seedlings (Fig. 2). Accumulation of Na^+ was higher in the shoots and increased with the duration of stress. Exogenous application of Mn decreased Na^+ uptake, and the Na^+/K^+ ratio, and increased K^+ uptake in both the roots and shoots, compared with salt treatment alone. Reduction of Na^+ uptake and the Na^+/K^+ ratio was higher in the shoots compared with the roots. Manganese accumulation slightly decreased with the increasing duration of salt stress and markedly recovered with exogenous application of Mn (Fig. 2).

Effect on oxidative stress indicator

Salt stress caused oxidative damage indicated by ROS generation, lipid peroxidation, and LOX activity in the salt-treated seedlings. Salt stress increased lipid peroxidation (indicated by MDA content) by 80 and 203 %, H_2O_2 content by 74 and 92 %, and LOX activity by 69 and 95 % after 3 and 6 days of treatment, respectively, compared with control (Table 2). Histochemical staining also confirmed oxidative stress in the salt-treated seedlings. Salt stress resulted in overproduction of O_2^- and H_2O_2 revealed by dark blue spots and brown spots, respectively, in the leaves of the salt-treated seedlings (Fig. 3a, b). Higher lipid peroxidation and loss of plasma membrane integrity were observed in the roots by the color changing to an intense pink-red and dark blue, respectively, in the salt-affected seedlings (Fig. 4a, b). In contrast, exogenous application of Mn reversed lipid peroxidation, O_2^- and H_2O_2 generation, loss of plasma membrane integrity, and LOX activity in the salt-treated seedlings. However, supplemental Mn slightly increased lipid peroxidation and H_2O_2 content in the non-stressed seedlings after 6 days of treatment.

Fig. 3 Histochemical detection of O_2^- (a) and H_2O_2 (b) in leaf of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and 150 mM NaCl , respectively

Effect on non-enzymatic antioxidants

Application of salt to the rice seedlings decreased AsA content and the AsA/DHA ratio but increased DHA content with the increasing duration of salt stress, compared with the control seedlings (Fig. 5a–c). In contrast, exogenous Mn considerably recovered AsA content and the AsA/DHA ratio in the salt-affected seedlings, compared with salt-alone treatment.

Salt stress resulted in higher GSH and GSSG contents with a lower GSH/GSSG ratio, compared with the control seedlings (Fig. 5d–f). Exogenous Mn reduced GSSG content and increased the GSH/GSSG ratio in the salt-treated seedlings, whereas GSH content remained unchanged

Fig. 4 Histochemical detection of lipid peroxidation (a) and loss of plasma membrane integrity (b) in root of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and 150 mM NaCl, respectively

compared with salt treatment alone. However, application of Mn to the non-stressed seedlings did not change GSH content, GSSG content, or the GSH/GSSG ratio.

Compared with the control seedlings, the phenolic and flavonoid contents decreased in the salt-affected seedlings, which were partially recovered with Mn supplementation (Fig. 6a, b). Applying Mn slightly increased flavonoid content in the non-stressed seedlings, compared with the control seedlings.

Effect on antioxidant enzymes

Salt stress increased the activities of MDHAR, DHAR, and GR after both 3 and 6 days of treatment, whereas APX activity increased only after 6 days of treatment (Fig. 7a–d). However, supplementation with Mn further stimulated the activities of MDHAR and DHAR after both 3 and 6 days of treatment, compared with salt treatment alone. GR activity was stimulated by Mn supplementation only after 3 days of treatment, whereas after 6 days of treatment it remains unchanged, compared with salt-alone treatment.

Salt exposure increased the activities of SOD and GPX with the increasing duration of stress, compared with control (Fig. 8a, c). In contrast, exogenous application of Mn to the salt-treated seedlings further stimulated the activities of SOD and GPX (except GPX activity at 6 days), compared with salt stress alone. On the other hand, treatment of seedlings with salt decreased the activities of CAT and GST with the increasing duration of stress, compared with control (Fig. 8b, d). Exogenous application of Mn to the salt-treated seedlings stimulated CAT activity and did not change GST activity compared with salt stress alone.

Effect on glyoxalase system

Compared with the control seedlings, salt stress increased MG content (Fig. 9c) and Gly II activity (Fig. 9b) with the increasing duration of stress. Gly I activity remained unchanged after 3 days of treatment but increased after 6 days of treatment in the salt-treated seedlings, compared with the control seedlings (Fig. 9a). However, supplementation with Mn decreased MG content and increased

Fig. 5 Effect on AsA content (a), DHA content (b), AsA/DHA ratio (c), GSH content (d), GSSG content (e), GSH/GSSG ratio (f) of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO₄ and 150 mM NaCl, respectively.

Means ($\pm$ SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

the activities of Gly I and Gly II, compared with the salt alone-treated plants.

Discussion

A higher amount of Na⁺ and a higher Na⁺/K⁺ ratio disrupt ion homeostasis and decrease other nutrient uptake (Simaei et al. 2012; Bose et al. 2014; Rahman et al. 2016a). In the present study, salt stress increased Na⁺ content and decreased K⁺ content in both the roots and shoots with the increasing duration of stress because of salt-induced Na⁺ influx and K⁺ efflux. The Na⁺ influx and K⁺ leakage might also result from salt-induced higher ROS production because higher production of ROS can also activate the non-selective cationic channels (NSCC, Demidchick and Maathuis 2007). Accumulation of Na⁺ was higher in the shoots, compared

with the roots. Decreased Mn was also observed under the salt-stress condition due to disrupted ion homeostasis. A similar salt-induced ion imbalance was reported in previous studies (Tuncturk et al. 2008; Wu and Wang 2012). However, in our study, Mn supplementation decreased Na⁺ accumulation and the Na⁺/K⁺ ratio, and increased K⁺ and Mn accumulation as well as improved ion homeostasis in both the roots and shoots under the salt-stress condition. The reduction in Na⁺ accumulation was higher in the shoots, compared with the roots, which might be due to the reduction in Na⁺ uptake, reduction in further translocation of Na⁺ from the roots to the shoots, and lower ROS production by Mn supplementation. This result corroborates previous findings (Demidchick and Maathuis 2007; Rahman et al. 2016a), which reported that a reduction in ROS production under salt stress reduced Na⁺ uptake and inhibited further translocation from the roots to the shoots.

Fig. 6 Effect on total phenol content (a) and flavonoid content (b) of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and 150 mM NaCl, respectively. Means ($\pm$ SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Salinity-induced osmotic stress inhibits growth by reducing water uptake capacity and photosynthesis efficiency (Shabani et al. 2013). In our experiment, salt stress resulted in osmotic stress indicated by osmotic potential, which increased with the increasing duration of stress. This higher osmotic potential was due to higher accumulation of Pro and lower RWC in the salt-affected seedlings. This result corroborates previous studies (Hasanuzzaman et al. 2014a; Nahar et al. 2015; Ozfidan-Konakci et al. 2015), which reported salinity increased osmotic potential and decreased water retention capacity with higher Pro for osmotic adjustment. Salt stress decreased growth in terms of FW, DW, and plant height in the salt-affected seedlings. Similar salt-induced growth inhibition has been reported in previous studies (Tuncturk et al. 2008; Munns 2011). However, supplementation with Mn to the salt-stressed seedlings restored biomass loss, improved water status, and reduced Pro content and osmotic potential. These results corroborate previous findings in which Mn supplementation restored growth loss (Cramer and Nowak 1992; Pandya et al. 2004). Salinity-induced ionic toxicity and osmotic stress decreased chlorophyll content by increasing the activity of chlorophyllase and overproduction of ROS (Saha et al. 2010; Hasanuzzaman et al. 2014a, b) or both. In

Fig. 7 Effect on APX (a) MDHAR (b), DHAR (c) and GR (d) activities of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and

150 mM NaCl, respectively. Means ($\pm$ SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

our study, salt stress decreased chl *a*, chl *b*, chl (*a* + *b*), and carotenoid contents, which were partially recovered by Mn supplementation. This restoration of photosynthetic pigments might be due to lower production of ROS, lower Na⁺ uptake, and upregulation of Mn uptake by Mn supplementation. These results are in agreement with previous studies (Sebastian and Prasad 2015) in which Mn supplementation restored chl and carotenoid contents by lowering ROS production under an abiotic stress condition.

Overproduction of ROS causes oxidative stress through lipid peroxidation, protein oxidation, and enzyme inhibition, resulting in cell death (Gill and Tuteja 2010; Hasanuzzaman et al. 2013a, b; Mishra et al. 2013). Oxidative stress results from higher ROS production and increased LOX activity, which also causes lipid peroxidation (Molassiotis et al. 2006). In our study, the seedlings exposed to salt resulting in higher production of ROS such as O₂^{•−} and H₂O₂ might be due to salt-induced ionic toxicity and osmotic stress. Production of higher ROS resulted in oxidative stress by increasing lipid peroxidation (indicated by MDA content), loss of plasma membrane integrity, and increasing LOX activity. However, exogenous Mn reduced salt-induced oxidative damage by reversing ROS production and lipid peroxidation, improving plasma membrane integrity, and reducing LOX activity. These results are supported by Sebastian and Prasad (2015) who reported that supplemental Mn reduced abiotic stress-induced oxidative stress by reversing ROS production and lipid peroxidation. These results also corroborate previous studies (Hasanuzzaman et al. 2014a, b; Wutipraditkul et al. 2015), which showed that a decrease in ROS production reduced salt-induced oxidative damage.

The non-enzymatic antioxidant components including AsA, GSH, and phenolic compounds play an important role in protecting cell organelles and biomolecules from oxidative damage by scavenging ROS (Gill and Tuteja 2010). The steady ratios of AsA and DHA, and GSH and GSSG are crucial in maintaining a cellular redox state under environmental stress conditions (Hasanuzzaman et al. 2011b; Sharma et al. 2012). In our study, salt stress decreased AsA content and the AsA/DHA ratio, and increased DHA content with the increasing duration of stress. The overproduced ROS and increased oxidation might be aided by the lower AsA content and AsA/DHA ratio. Salt-induced lower AsA content and AsA/DHA ratio were also reported in previous studies (Hasanuzzaman et al. 2011a; Mishra et al. 2013). However, supplementation with Mn increased the AsA content and AsA/DHA ratio through decreased DHA content and caused AsA regeneration by increasing the activities of MDHAR and DHAR. Similar Mn-induced AsA regeneration was observed under a Cd-stress condition (Sebastian and Prasad

2015). Glutathione plays a potential role in salt-stress tolerance by reacting with ROS and helping AsA regeneration (Foyer and Noctor 2003; Huang et al. 2005). In our study, salt stress increased GSH and GSSG contents and decreased the GSH/GSSG ratio with the increasing duration of stress. Increased oxidation of GSH due to overproduced ROS might be responsible for a lower GSH/GSSG ratio and higher GSSG content. Similar findings were also reported in previous studies (Nahar et al. 2015; Rahman et al. 2016a) in which salt stress increased GSSG content and decreased the GSH/GSSG ratio. In contrast, exogenous application of Mn in the salt-treated seedlings decreased GSSG content and increased the GSH/GSSG ratio, which might result from Mn-induced lower ROS production. Phenolic compounds are non-enzymatic antioxidant and secondary metabolites that can increase or decrease under abiotic stress and exhibit antioxidant properties by scavenging ROS (Amarowicz et al. 2004). It is also evident that Mn is involved in synthesizing flavonoids (Millaleo et al. 2010), which are ROS scavengers (Ferreira et al. 2012). In this study, salt stress decreased total phenol and flavonoid content because of overproduced ROS, which was restored by Mn supplementation. This restored phenolic and flavonoid content might be because of Mn supplementation, which probably plays a role in scavenging salt-induced ROS. A similar decreasing flavonoid content under salt stress was reported by Simaei et al. (2012).

In the AsA–GSH cycle, the antioxidant enzymes APX, MDHAR, DHAR, and GR work together with AsA and GSH to detoxify ROS and regenerate AsA and GSH (Asada 1992; Mishra et al. 2013). In our study, salt stress decreased AsA content and increased the activities of APX, MDHAR, DHAR, and GR along with GSH content. Similar results were also found by Mishra et al. (2013) under a salt-stress condition. However, simultaneous application of Mn in the salt-treated seedlings further stimulated the activities of MDHAR and DHAR. The increased activities of MDHAR and DHAR correlate with restored AsA content and decreased DHA content because AsA is regenerated from DHA through the increased activities of MDHAR and DHAR (Noctor et al. 2002; Hasanuzzaman et al. 2012; Mishra et al. 2013).

Superoxide dismutase is considered the first line enzymatic defense against ROS, which converts O₂^{•−} to H₂O₂ (Gill et al. 2015). Sequentially, CAT activity is involved in converting H₂O₂ to H₂O and O₂, which reduces ROS (Sánchez-Casas and Klesseg 1994). In the present study, SOD activity increased and CAT activity decreased in the salt-affected seedlings along with the increase in duration of stress because of overproduced ROS. These findings corroborate previous reports (Hasanuzzaman et al. 2011a;

Fig. 8 Effect on SOD (a), CAT (b), GPX (c) and GST (d) activities of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and 150 mM NaCl ,

respectively. Means ($\pm$ SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Fig. 9 Effect on Gly I (a) and Gly II (b) activity, and MG content (c) of rice seedlings under salt stress with and without exogenous Mn. Here, Mn and Salt indicate 0.5 mM MnSO_4 and 150 mM NaCl ,

respectively. Means ($\pm$ SD) were calculated from three replicates for each treatment. Values with different letters are significantly different at $P \leq 0.05$ applying the Fisher's LSD test

Mishra et al. 2013) in which salt stress increased SOD activity, decreased CAT activity, and overproduced ROS. In contrast, exogenous application of Mn increased the

activities of SOD and CAT under a salt-stress condition because Mn is a co-factor in Mn-SOD and Mn-CAT, which might play a role in decreasing ROS content (Ducic and

Polle 2005). These results are in agreement with Sebastian and Prasad (2015) who reported Mn supplementation increased SOD activity under an environmental stress condition. GPX uses GSH as a substrate to scavenge H_2O_2 (Noctor et al. 2002), and GST together with GSH produces less toxic and water-soluble conjugates by catalyzing the binding of different xenobiotics (Edwards et al. 2000). In our experiment, salt stress increased GPX activity and decreased GST activity, which correlates with increased H_2O_2 content and corroborates previous studies (Tammam et al. 2011; Hasanuzzaman et al. 2014a). However, simultaneous application of Mn increased GPX activity after 3 days of treatment, whereas GST activity remained unchanged in the salt-treated seedlings after Mn supplementation.

Stimulation of the activities of Gly I and Gly II increases tolerance to abiotic stresses by increasing MG detoxification in many plant species (Yadav et al. 2005; Singla-Pareek et al. 2008). In the present study, salt stress increased the production of MG along with increasing the activities of Gly I and Gly II, which are consistent with previous studies (Hasanuzzaman et al. 2014a; Rahman et al. 2016a, b). Simultaneous application of Mn with salt stress improved MG detoxification by increasing the activities of Gly I and Gly II. These results are supported by previous studies (Hasanuzzaman et al. 2014a; Rahman et al. 2015; Nahar et al. 2016b) in which up-regulation of Gly I and Gly II increased MG detoxification and increased oxidative stress tolerance.

Conclusion

Considering the above, our results suggest that salinity caused osmotic stress by over accumulation of Na^+ and water imbalance. Consequently, salt-induced ion toxicity and osmotic stress resulted in oxidative stress by disrupting the antioxidant defense and glyoxalase systems through overproduction of ROS and MG, respectively. Salt-induced damage increased with the increase in the duration of stress. However, exogenous application of Mn reduced salt-induced damage by improving ion homeostasis and the antioxidant defense and glyoxalase systems in the salt-affected seedlings

Acknowledgments This research was funded by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. We are grateful to Prof. Kazuhiro Fukada, Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, for allowing us to use his laboratory to determine some important parameters. We thank Mr. Dennis Murphy, the United Graduate School of Agricultural Sciences, Ehime University, Japan, for English editing of the manuscript. We also thank Mr. Mazhar Ul Alam, Ms. Taufika Islam Anee and Ms. Tasnim Farha Bhuiyan, Laboratory of

Plant Stress Responses, Faculty of Agriculture, Kagawa University, Japan, for critical readings of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Addinsoft (2015) XLSTAT v. 2015.1.01: data analysis and statistics software for Microsoft Excel. Addinsoft, Paris
- Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA (2004) Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem* 84:551–562
- Arnon DT (1949) Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 24:1–15
- Asada K (1992) Ascorbate peroxidase—a hydrogen peroxide-scavenging enzymes in plants. *Physiol Plant* 85:235–241
- Ashraf MA, Ashraf M, Ali Q (2010) Response of two genetically diverse wheat cultivars to salt stress at different growth stage: leaf lipid peroxidation and phenolic contents. *Pak J Bot* 42(1):559–565
- Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust J Biol Sci* 15:413–428
- Bates LS, Waldren RP, Tearei D (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
- Bhandal IS, Malik CP (1988) Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants. *Int Rev Cytol* 110:205–254
- Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. *J Exp Bot* 65:1241–1257
- Bradford MM (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
- Carillo P, Annunziata MG, Pontecorvo G, Fuggi A, Woodrow P (2011) Salinity stress and salt tolerance. In: Shanker A (ed) *Abiotic stress in plants—mechanisms and adaptations*. InTech, Croatia, pp 2–35
- Chen F, Wang F, Wu F, Mao W, Zhang G, Zhou M (2010) Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. *Plant Physiol Biochem* 48:663–672
- Cramer GR, Nowak RS (1992) Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. *Physiol Plant* 84:600–605
- Cramer GR, Läuchli A, Polito VS (1985) Displacement of Ca^{2+} and Na^+ from the plasma lemma of root cells. *Plant Physiol* 79:207–211
- Demidchick V, Maathuis FJM (2007) Physiological role of nonselective cation channels in plants: from salt stress to signaling and development. *New Phytol* 175:387–404
- Doderer A, Kokkelink I, Van der Veen S, Valk B, Schram A, Douma A (1992) Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim Biophys Acta* 112:97–104
- Ducic T, Polle A (2005) Transport and detoxification of manganese and copper in plants. *Braz J Plant Physiol* 17:103–112
- Edwards R, Dixon DP, Walbot V (2000) Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. *Trends Plant Sci* 5:193–198

- Elia AC, Galarini R, Taticchi MI, Dorr AJM, Mantilacci L (2003) Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. *Ecotoxicol Environ Saf* 55:162–167
- El-Shabrawi H, Kumar B, Kaul T, Reddy MK, Singla-Pareek SL, Sopory SK (2010) Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. *Protoplasma* 245:85–96
- Ferreira MLF, Rius SP, Casati P (2012) Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Front Plant Sci* 3:222. doi:10.3389/fpls.2012.00222
- Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta* 133:21–25
- Foyer CH, Noctor G (2003) Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol Plant* 119:355–364
- Gherardi M, Rengel Z (2003) Genotypes of lucerne (*Medicago sativa* L.) show differential tolerance to manganese deficiency and toxicity when grown in bauxite residue sand. *Plant Soil* 249:287–296
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Gill SS, Anjum NA, Gill R, Yadav S, Hasanuzzaman M, Fujita M et al (2015) Superoxide dismutase-mentor of abiotic stress tolerance in crop plants. *Environ Sci Pollut Res* 22:10375–10394
- Griffiths OW (1980) Determination of glutathione and glutathione disulphide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* 106:207–212
- Hasanuzzaman M, Fujita M (2011) Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed. *Biol Trace Elem Res* 143:1758–1776
- Hasanuzzaman M, Hossain MA, Fujita M (2011a) Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnol Rep* 5:353–365
- Hasanuzzaman M, Hossain MA, Fujita M (2011b) Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in rapeseed seedlings. *Biol Trace Elem Res* 143:1704–1721
- Hasanuzzaman M, Hossain MA, Teixeira da Silva JA, Fujita M (2012) Plant responses and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Bandi V, Shanker AK, Shanker K, Mandapaka M (eds) *Crop stress and its management: perspectives and strategies*. Springer, Berlin, pp 261–316
- Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013a) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int J Mol Sci* 14:9643–9684
- Hasanuzzaman M, Nahar K, Fujita M (2013b) Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmed P, Azooz MM, Prasad MNV (eds) *Ecophysiology and responses of plants under salt stress*. Springer, New York, pp 25–87
- Hasanuzzaman M, Alam MM, Nahar K, Jubayer-Al-Mahmud Ahamed KU, Fujita M (2014a) Exogenous salicylic acid alleviates salt stress-induced oxidative damage in *Brassica napus* by enhancing the antioxidant defense and glyoxalase systems. *Aust J Crop Sci* 8(4):631–639
- Hasanuzzaman M, Alam MM, Rahman A, Hasanuzzaman M, Nahar K, Fujita M (2014b) Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *Biomed Res Int* 2014:757219. doi:10.1155/2014/757219
- Hasegawa P, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annu Rev Plant Physiol Plant Mol Biol* 51:463–499
- Heath RL, Packer L (1968) Photo peroxidation in isolated chloroplast: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125:189–198
- Hossain MA, Nakano Y, Asada K (1984) Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol* 25:385–395
- Hossain MZ, Hossain MD, Fujita M (2006) Induction of pumpkin glutathione *S*-transferase by different stresses and its possible mechanisms. *Biol Plant* 50:210–218
- Huang C, He W, Guo J, Chang X, Su P, Zhang L (2005) Increased sensitivity to salt stress in ascorbate deficient *Arabidopsis* mutant. *J Exp Bot* 56:3041–3049
- Iqbal N, Shahid U, Khan NA (2015) Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). *J Plant Physiol* 178:84–91
- Lidon FC, Barreiro M, Ramalho J (2004) Manganese accumulation in rice: implications for photosynthetic functioning. *J Plant Physiol* 161:1235–1244
- Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. *Arch Biochem Biophys* 444:139–158
- Millaleo R, Reyes-Diaz M, Ivanov AG, Mora ML, Alberdi M (2010) Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *J Soil Sci Plant Nutr* 10:476–494
- Mishra P, Bhoomika K, Dubey RS (2013) Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive indica rice (*Oryza sativa* L.) seedlings. *Protoplasma* 250:3–19
- Molassiotis A, Sotiropoulos T, Tanou G, Diamantidis G, Therios I (2006) Boron induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the apple rootstock EM9 (*Malus domestica* Borkh). *Environ Exp Bot* 56:54–62
- Munns R (2002) Comparative physiology of salt and water stress. *Plant, Cell Environ* 25:239–250
- Munns R (2011) Plant adaptations to salt and water stress: differences and commonalities. *Adv Bot Res* 57:1–32
- Munns R, Tester M (2008) Mechanism of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015) Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. *Biol Plant* 59:745–756
- Nahar K, Hasanuzzaman M, Alam MA, Rahman A, Fujita M (2016a) Polyamine and nitric oxide cross talk: antagonistic effects on cadmium toxicity in mungbean plants through up regulating the metal detoxification, antioxidant defense and methyl glyoxal detoxification systems. *Ecotoxicol Environ Saf* 126:245–255
- Nahar K, Hasanuzzaman M, Fujita M (2016b) Roles of osmolytes in plant adaptation to drought and salinity. In: Iqbal N, Nazar R, Khan NA (eds) *Osmolytes and plants acclimation to changing environment: emerging omics technologies*. Springer, New Delhi, pp 37–58
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880
- Noctor G, Gomez L, Vanacker H, Foyer CH (2002) Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. *J Exp Bot* 53:283–304
- Ozfidan-Konakci C, Yildiztugay E, Kucukoduk M (2015) Protective roles of exogenously applied gallic acid in *Oryza sativa* subjected to salt and osmotic stresses: effects on the total antioxidant capacity. *Plant Growth Regul* 75:219–234

- Palove-Balang P, Kisova A, Pavlovkin J, Mistrik I (2006) Effect of manganese on cadmium toxicity in maize seedlings. *Plant Soil Environ* 52:143–149
- Pandya DH, Mer RK, Prajith PK, Pandey AN (2004) Effect of salt stress and manganese supply on growth of barley seedlings. *J Plant Nutr* 27:1361–1379
- Pang CH, Wang BS (2008) Oxidative stress and salt tolerance in plants. In: Lüttge U, Beyschlag W, Murata J (eds) *Progress in botany*. Springer, Heidelberg, pp 231–245
- Peng K, Chunling L, Wuxin Y, Chunlan L, Xiangdong L, Shen Z (2008) Manganese uptake and interactions with cadmium in the hyperaccumulator-*Phytolacca americana* L. *J Hazard Mater* 154:674–681
- Rahman A, Mostofa MG, Nahar K, Alam MM, Hasanuzzaman M, Fujita M (2015) Calcium mitigates arsenic toxicity in rice seedlings by reducing arsenic uptake and modulating the antioxidant defense and glyoxalase systems and stress markers. *Biomed Res Int* 2015:340812. doi:10.1155/2015/340812
- Rahman A, Nahar K, Hasanuzzaman M, Fujita M (2016a) Calcium supplementation improves Na^+/K^+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings. *Front Plant Sci* 7:609. doi:10.3389/fpls.2016.00609
- Rahman A, Mostofa MG, Nahar K, Hasanuzzaman M, Fujita M (2016b) Exogenous calcium alleviates cadmium-induced oxidative stress in rice (*Oryza sativa* L.) seedlings by regulating the antioxidant defense and glyoxalase systems. *Braz J Bot* 39:393–407
- Reddy PS, Jogeswar G, Rasineni GK, Maheswari M, Reddy AR, Varshney RK, Kishor PVK (2015) Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. *Plant Physiol Biochem* 94:104–113
- Saha P, Chatterjee P, Biswas AK (2010) NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). *Ind J Exp Biol* 48:593–600
- Sánchez-Casas P, Klesseg DF (1994) A salicylic acid-binding activity and a salicylic acid-inhibitable catalase activity are present in a variety of plant species. *Plant Physiol* 106:1675–1679
- Schützendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Hyser R, Godbold DL et al (2001) Cadmium induced changes in antioxidative systems, hydrogen peroxide content and differentiation in scot pine (*Pinus sylvestris*) roots. *Plant Physiol* 127:887–892
- Sebastian A, Prasad MNV (2015) Iron-and manganese-assisted cadmium tolerance in *Oryza sativa* L.: lowering of rhizotoxicity next to functional photosynthesis. *Planta* 241:1519–1528
- Shabala S, Demidchick V, Shabala L, Cuin TA, Smith SJ, Miller AJ, Davies JM, Newman IA (2006) Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from Arabidopsis root and leaf cells by controlling plasma membrane K^+ -permeable channels. *Plant Physiol* 141:1653–1665
- Shabani A, Sepaskhah AR, Kamgar-Haghighi AA (2013) Growth and physiologic response of rapeseed (*Brassica napus* L.) to deficit irrigation, water salinity and planting method. *Int J Plant Prod* 7:569–596
- Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanisms in plants under stressful conditions. *J Bot* 2012:217037. doi:10.1155/2012/217037
- Simaei M, Khavari-nezad RA, Bernard F (2012) Exogenous application of salicylic acid and nitric oxide on the ionic contents and enzymatic activities in NaCl-stressed soybean plants. *Am J Plant Sci* 3:1495–1503
- Singla-Pareek SL, Yadav SK, Pareek A, Reddy MK, Sopory SK (2008) Enhancing salt tolerance in a crop plant by overexpression of glyoxalase II. *Transgenic Res* 17:171–180
- Srivastava RK, Pandey P, Rajpoot R, Rani A, Gautam A, Dubey RS (2014) Exogenous application of calcium and silica alleviates cadmium toxicity by suppressing oxidative damage in rice. *Protoplasma* 252:959–975
- Tammam AA, Fakhry EM, El-sheekh M (2011) Effect of salt stress on antioxidant system and the metabolism of the reactive oxygen species in *Dunaliella salina* and *Dunaliella tertiolecta*. *Afr J Biotechnol* 10:3795–3808
- Tester M, Davenport R (2003) Na^+ tolerance in higher plants. *Ann Bot* 91:503–507
- Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB (1997) Subcellular localization of H_2O_2 in plants, H_2O_2 accumulation in papillae and hypersensitive response during barley powdery mildew interaction. *Plant J* 11:1187–1194
- Tuncturk M, Tuncturk R, Yasar F (2008) Changes in micronutrients, dry weight and plant growth of soybean (*Glycine max* L. Merrill) cultivars under salt stress. *Afr J Biotechnol* 7:1650–1654
- Wild R, Ooi L, Srikanth V, Münch G (2012) A quick: convenient and economical method for the reliable determination of methylglyoxal in millimolar concentrations: the *N*-acetyl-L-cysteine assay. *Anal Bioanal Chem* 403:2577–2581
- Wu GQ, Wang SM (2012) Calcium regulates K^+/Na^+ homeostasis in rice (*Oryza sativa* L.) under saline conditions. *Plant Soil Environ* 58:121–127
- Wutipraditkul N, Wongwan P, Buaboocha T (2015) Alleviation of salt-induced oxidative stress in rice seedlings by proline and/or glycinebetaine. *Biol Plant* 59:547–553
- Yadav SK, Singla-Pareek SL, Reddy MK, Sopory SK (2005) Methylglyoxal detoxification by glyoxalase system: a survival strategy during environmental stresses. *Physiol Mol Biol Plants* 11:1–11
- Yu CW, Murphy TM, Lin CH (2003) Hydrogen peroxide-induces chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Funct Plant Biol* 30:955–963
- Zhisen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64:555–559