学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名： **Anisur Rahman**
Name

学位論文題目： Title of Dissertation

Calcium and Manganese-Induced Abiotic Stress Tolerance in Rice by Coordinated Action of Antioxidant Defense, Glyoxalase System and Nutrient Homeostasis
（カルシウムおよびマンガンにより誘導されるイネの非生物的ストレス耐性は抗酸化防御、グリオキサラーゼ系および栄養恒常性による協調作用に依存している）

学位論文要約：
Dissertation Summary

Abstract

The present studies were investigated the regulatory role of exogenous calcium (Ca) and manganese (Mn) in developing abiotic stress tolerance in hydroponically grown rice seedlings. Exposure of Cd in rice seedlings caused disruption of ion homeostasis, and the antioxidant defense and glyoxalase systems through over accumulation of Cd, ROS production, and MG formation, respectively. Higher accumulation of Cd also caused water imbalance, chlorosis and growth inhibition. The level of Cd-induced damage increased with increasing the dose of Cd. In contrast, exposure of salt caused ionic and osmotic stress by higher accumulation of Na and water imbalance. Salt-induced ionic and osmotic stress facilitate imbalance between ROS and antioxidants. Salt stress also caused higher formation of MG. Over accumulated Na and overproduced ROS and MG also cause oxidative stress, chlorosis and growth inhibition. The level of salt-induced damage increased with increasing the duration of salt stress. However, exogenous application of Ca and Mn mitigate Cd and salt-induced oxidative stress by increasing detoxification of ROS and MG. Applying Ca and Mn reduce Cd and salt toxicity by decreasing Cd and Na uptake, respectively, that partially improve nutrient balance. Supplementation with Ca and Mn mitigate Cd and Salt-induced damage by the coordinated actions of nutrient homeostasis, antioxidant defense and glyoxalase systems of rice seedlings.

Background of the study

Usually plants experience various kinds of environmental stresses (salinity, drought, heat, cold, flooding, heavy metals, ozone, UV radiation, etc) which affect plant growth, yield and productivity. Abiotic stresses become the major threat to crop production due to gradual change of climate and environment. The severity of environmental stresses has been increasing day by day and it is reported that only 3.5% of the global land area remains unaffected by environmental stress (Cramer et al. 2011; Hasanuzzaman et al. 2012). Abiotic stresses are serious and unavoidable constraints to crop production as crop plants are sessile organism and most of them are grown under field conditions. In addition, it is almost impossible to grow crop in completely abiotic stress free condition. If the severity of stress becomes very high and continues for extended period it may lead to an intolerable metabolic load on cells that consequently causes growth inhibition and even death of plant (Taiz and Zeiger 2006). Though it is difficult to get accurate estimations of the effects of abiotic stress on crop production, it has been estimated that more than 50% yield reduction is the direct result of abiotic stresses (Rodríguez et al. 2005; Acquaah 2007).

Among the abiotic stresses, toxic metals and/or heavy metals become serious environmental issues in worldwide due to fast industrialization including the production and release of considerable amounts of toxic metals into the environment (Sarma 2011; Hasanuzzaman and Fujita 2012). Growing of plants in toxic metal contaminated soil resulted in higher accumulation of toxic metal and disruption of nutrient balance. Higher accumulation of toxic metal alters physiological and biochemical functions of plant that destroys the normal growth of the plant by disturbing absorption, translocation, metabolism and synthesis processes. Gradually, the normal growth of the plant is prohibited and ultimately the plant is died (Wei and Zhou 2008; Hasanuzzaman and Fujita 2012). In addition, heavy metal contamination is also a threat to health of wildlife and humans as toxic metal can easily enter into the food chain and most of the toxic metals are carcinogens (Hasanuzzaman and Fujita 2012).

Of the environmental factors, salinity is one of the most brutal abiotic stress, because most crop plants are sensitive to salt stress (Hasanuzzaman et al. 2012). The salt affected area has been increasing day by day and assumed that 50 % of cultivable land will be salt affected by the middle of the twenty first century (Mahajan and Tuteja 2005; Munns and Tester 2008). High salinity affects plant by both ionic and osmotic stresses that cause membrane damage, nutrient imbalance, enzymatic inhibition, metabolic dysfunction, photosynthesis inhibition and hamper other major physiological and biochemical processes that ultimately leads to growth inhibition or death of the plant (Mahajan and Tuteja 2005; Ahmad and Sharma 2008; Hasanuzzaman et al. 2013; Nahar et al. 2016a).

The response of plant to abiotic stresses is very complex and it is both plastic and elastic (Cramer et al. 2011). The general responses of plant to abiotic stresses are alteration of morphological, physiological, biochemical, and molecular functions that adversely affect plant growth and productivity (Wang et al. 2001). Production of reactive oxygen species (ROS) in plant is unavoidable. Plants have antioxidant defense system to maintain ROS level in cell. However, the most common consequences of abiotic stresses are overproduction of ROS including singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) due to excitation or incomplete reduction of molecular oxygen. Though lower concentration of ROS act as signaling molecules for the activation of defense responses under stresses but higher concentration of ROS are toxic and cause oxidative stress. Reactive oxygen species can pose a threat to cells by oxidative stress through the peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of the programmed cell death pathway and ultimately cell death (Mittler 2002; Sharma and Dubey 2005, 2007). In addition, the cytotoxic compound methylglyoxal (MG) also causes oxidative damage through degradation of protein synthesis under abiotic stress condition (Yadav et al. 2005).

Detoxification of ROS and MG, maintenance of nutrient homeostasis, and reduction of salt and toxic metal uptake comprise some stress tolerance mechanisms within the plant (Hasanuzzaman et al. 2013; Nahar et al. 2015a,b; Ahmad et al. 2015; Rahman et al. 2016). In addition, the plant produces proline (Pro) or other compatible solutes to maintain proper water balance and stabilize the protein complex for ionic and osmotic homeostasis to alleviate abiotic stress (Iqbal et al. 2015; Reddy et al. 2015).

Plants have well equipped antioxidative system composed of non-enzymatic and enzymatic antioxidants for scavenging ROS. Non-enzymatic antioxidants include glutathione (GSH), ascorbate (AsA), carotenoids, tocopherols, and flavonoids which are crucial for detoxification of ROS (Gill and Tuteja 2010). The antioxidant enzyme include 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) located in different sites of cell and work together to detoxify ROS (Gill and Tuteja 2010; You and Chan 2015). The equilibrium between ROS and antioxidative capacity determine the fate of plant under oxidative stress condition (Hasanuzzaman et al. 2012). Simillarly, glyoxalase system composed of glyoxalase I (Gly I) and glyoxalase II (Gly II) enzymes that act coordinately with GSH to detoxify overproduced MG under abiotic stress condition (Yadav et al. 2005).

To cope with adverse environmental condition, regulation of the antioxidant defense and glyoxalase systems, improvement of nutrient homeostasis, and reduction of Na^+, Cl^- and toxic metal uptake by applying exogenous phytoprotectants may be important strategies for abiotic stress tolerance (Hasanuzzaman et al. 2013; Ahmad et al. 2015; Rahman et al. 2016). To regulate antioxidant defense and glyoxalase systems and nutrient homeostasis, the essential plant element calcium (Ca) and manganese (Mn) are important candidates for alleviating abiotic stress (Ahmad et al. 2015; Srivastava et al. 2014; Millaleo et al. 2010; Sebastian and Prasad 2015).

As an essential macronutrient, Ca plays important roles including stabilizing cell walls and membranes, improving the metabolic processes of other nutrients, regulating enzymatic and hormonal processes, and other essential functions. Calcium also acts as a secondary messenger that mediates many aspects of cell and plant development, as well as the stress-resistance response (White and Broadley 2003; Jaleel et al 2007a,b; Jaleel et al. 2009). In addition, several studies have also revealed that exogenous application of Ca in plant growth medium helps to develop abiotic-stress tolerance by maintaining ion homeostasis (Wu and Wang 2012), enhancing the antioxidant defense system and other physiological and biochemical attributes (Manivannan et al 2007; Talukdar 2012; Srivastava et al 2014; Ahmad et al 2015).

The essential trace element Mn plays crucial role in several metabolic process including photosynthesis, respiration, synthesis of ATP, fatty acid, amino acids, lipids, proteins, flavonoids and hormone activation (Lidon et al. 2004; Millaleo et al. 2010). Besides these, Mn also plays a vital role as co-factor including Mn-SOD, Mn-CAT which participates in the plant defense against oxidative stress. In addition, several studies also revealed that, supplemental Mn plays an important role in the adaptive responses of plant under various environmental stresses. Simultaneous application of Mn improves stress tolerance and alleviates oxidative stress by reducing metal accumulation, Na uptake, lipid peroxidation and improving biomass, chlorophyll content, carotenoid content and antioxidant defense system under salt and toxic metal stress (Cramer and Nowak 1992; Pandey et al. 2004; Pal'ove-Balang et al. 2006; Peng et al. 2008; Sebastian and Prasad 2015).

Considering the strategies discussed, the present studies were conducted

i. To investigate the response of rice seedlings in terms of physiological and biochemical changes under abiotic stress (Salt stress and toxic metal) conditions
ii. To investigate the regulatory role of supplemental Ca and Mn-induced abiotic stress tolerance in rice seedlings
iii. To understand the coordinated actions of antioxidant defense, glyoxalase systems and nutrient homeostasis in conferring abiotic stress tolerance in plants

To fulfill these objectives, a series of experiments were conducted and the findings are described in four different titles as follows:

i. Exogenous calcium alleviates cadmium-induced oxidative stress in rice (*Oryza sativa* L.) seedlings by regulating the antioxidant defense and glyoxalase systems
ii. Calcium supplementation improves Na^+/K^+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings
iii. Manganese supplementation reduces cadmium toxicity in rice seedlings by maintaining nutrient homeostasis and antioxidant defense
iv. Manganese-induced salt stress tolerance in rice seedlings: regulation of ion homeostasis, antioxidant defense and glyoxalase system

Experiment-I

Exogenous calcium alleviates cadmium-induced oxidative stress in rice (*Oryza sativa* L.) seedlings by regulating the antioxidant defense and glyoxalase systems

Materials and Methods

Plant materials, growing Condition and treatments

Rice (*Oryza sativa* L. cv. BRRI dhan29) seeds were surface sterilized with 70 % ethanol for 15 min followed by washing several times with sterilized distilled water and soaked in distilled water in a dark place for 48 h. 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 48 h. Uniformly germinated seeds were then transferred to a growth chamber (light, 350 μmol photon−m^{-2}–s^{-1}; temperature, 25 ± 2°C; relative humidity, 65–70 %) with the same pot providing a diluted (7500 times) commercial hydroponics 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 twice a week. Fourteen-day-old rice seedlings were exposed to Ca (2.5 mM $CaCl_2$) and Cd (0.25 mM and 0.5 mM $CdCl_2$) separately and in combination. Control plants were grown in Hyponex solution only. Therefore, our experiments consisted of six treatments as follows: control, 2.5 mM $CaCl_2$ (Ca), 0.25 mM $CdCl_2$ (Cd1), 0.25 mM $CdCl_2$+2.5 mM $CaCl_2$, 0.5 mM $CdCl_2$ (Cd2), and 0.5 mM $CdCl_2$ + 2.5 mM $CaCl_2$. The experiment was repeated three times under the same conditions. Data were taken after 3 days of treatment.

Determination of Physiological and Biological Parameters

Different physiological and biochemical parameters including Fresh weight (FW), Dry weight (DW), relative water content (RWC) of leaf, Ca Content, Cd content, chlorophyll (chl) content, Pro content, MDA content, H_2O_2 content, Histochemical detection of ROS in leaf, lipoxygenase (LOX) activity, non-enzymatic antioxidants (ASA and GSH content), enzymatic antioxidants (activities of APX, MDHAR, DHAR, GR, SOD, CAT, GPX and GST), glyoxalase components (MG content, activities of Gly I and Gly II) were measured following standard methods.

Statistical analysis

The data were subjected to one way analysis of variance (ANOVA) and the mean differences were compared by Fisher's LSD using XLSTAT v. 2013 software (Addinsoft 2013). Differences at $P \leq 0.05$ were considered significant.

Results and summary

Exposure to Cd caused chlorosis, leaf rolling symptoms, and growth inhibition. A higher concentration of Cd in the growth medium resulted in higher Cd accumulation, which induced oxidative stress through overproduction of ROS by disrupting the antioxidant defense system. Cadmium treatment increased the MG level. Calcium supplementation in the Cd-treated growth medium reduced Cd uptake. Application of Ca also significantly increased the AsA content, increased the activities of SOD, CAT, GST, MDHAR and DHAR in the antioxidant system, and increased the Gly I and Gly II activities in the glyoxalase system in rice seedlings exposed to both levels of Cd. Exogenous Ca regulates the antioxidant defense and glyoxalase systems, which reverses overproduced ROS and detoxifies methylglyoxal, which in turn reduces Cd toxicity.

Experiment-II:

Calcium supplementation improves Na^+/K^+ ratio, antioxidant defense and glyoxalase systems in salt-stressed rice seedlings

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 place for 48 h. 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 (like as Experiment I) with the same pot providing a diluted (5000 times) commercial hydroponics nutrient solution (Hyponex, Japan; nutrient composition was like Experiment I). The nutrient solutions were renewed twice a week. Each pot contained approximately 60 seedlings. Thirteen-d-old rice seedlings were exposed to salt stress (200 mM NaCl) in the presence and absence of Ca (2 mM $CaCl_2$) with nutrient solution to verify the role of Ca under salt-stress conditions. We also applied the Ca scavenger ethylene glycol tetraacetic acid ($C_{14}H_{24}N_2O_{10}$) (EGTA) (Ammoaghaie and Moghym, 2011) together with NaCl+$CaCl_2$ and alone to determine the role of Ca under salt stress conditions. Control plants were grown in Hyponex solution only. Therefore, our experiment consisted of six treatments as follows: control, 2 mM $CaCl_2$ (Ca), 2 mM EGTA (EGTA), 200 mM NaCl (Salt), 200 mM NaCl + 2 mM $CaCl_2$ (Salt+Ca), and 200 mM NaCl + 2 mM $CaCl_2$ + 2 mM EGTA (Salt+Ca+EGTA). The experiment was repeated three times under the same conditions. Data were taken after 3 days of treatment.

Determination of Physiological and Biological parameters

Along with the parameters, those were observed in Experiment I, plant height, Na and other mineral (K, Mg, Mn and Zn) contents and histochemical staining of root for revealing loss of plasma membrane integrity and lipid peroxidation were also observed following standard methods.

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 (Addinsoft 2015). Differences at $P \leq 0.05$ were considered significant.

Results and summary

The salt stress caused growth inhibition, chlorosis and water shortage in the rice seedlings. The salt-induced stress disrupted ion homeostasis through Na^+ influx and K^+ efflux, and decreased other mineral nutrient uptake. Salt-induced stress caused oxidative stress in rice seedlings through lipid peroxidation, loss of plasma membrane integrity, higher ROS production and MG formation. The salt-stressed rice seedlings supplemented with exogenous Ca recovered from water loss, chlorosis and growth inhibition. Calcium supplementation in the salt-stressed rice seedlings improved ion homeostasis by inhibition of Na^+ influx and K^+ leakage. Exogenous Ca also improved ROS and MG detoxification by improving the antioxidant defense and glyoxalase systems, respectively. On the other hand, applying EGTA along with salt and Ca again negatively affected the rice seedlings as EGTA negated Ca activity. It confirms that, the positive responses in salt-stressed rice seedlings to exogenous Ca were for Ca mediated improvement of ion homeostasis, antioxidant defense and glyoxalase system.

Experiment III

Manganese supplementation reduces cadmium toxicity in rice seedlings by maintaining nutrient homeostasis and antioxidant defense

Plant materials and treatments

Rice (*Oryza sativa* L. cv. BRRI dhan29) 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 place for 48 h. 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 (like Experiment I) with the same pot providing a diluted (5000 times) commercial hydroponics nutrient solution (Hyponex, Japan). The nutrient solutions were renewed twice a week. Each pot contained approximately 60 seedlings. Fourteen-day-old seedlings were exposed to Cd stress (0.3 mM $CdCl_2$) in presence and absence of supplemental Mn (0.3 mM $MnSO_4$) with nutrient solution to verify the role of exogenous Mn under Cd stress condition. Control plants were grown in Hyponex solution only. Therefore, our experiment is consisted of four treatments as follows: control, 0.3 mM $MnSO_4$ (Mn), 0.3 mM $CdCl_2$ (Cd), 0.3 mM $CdCl_2$ + 0.3 mM $MnSO_4$ (Cd+Mn). The experiment was repeated three times under the same conditions. Data were taken after 3 days of treatment.

Determination of experiment parameters

In addition to the physiological and biochemical parameters that were measured in Experiment I and II, total phenol contents also measured in present experiment following the standard method.

Statistical analysis

Data were analyzed following Experiment II

Results and Summary

Exposure of 14-d-old seedlings to 0.3 mM $CdCl_2$ for three days caused growth inhibition, cholorosis, nutrient imbalance, and higher Cd accumulation. Higher amounts of Cd uptake caused oxidative stress through lipid peroxidation, loss of plasma membrane integrity, and overproduction of reactive oxygen species (ROS) and methylglyoxal (MG). Exogenous application of 0.3 mM $MnSO_4$ to Cd-treated seedlings partly recovered Cd-induced water loss, chlorosis, growth inhibition, and nutrient imbalance by reducing Cd uptake and its further translocation to the upper part of the plant. Supplemental Mn also reduced Cd-induced oxidative damage and lipid peroxidation by improved antioxidant defense and glyoxalase systems through enhancing ROS and MG detoxification, respectively.

Experiment-IV

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

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 ± 2 °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 with the same pot providing a diluted (500-times) commercial hydroponics nutrient solution (Hyponex, Japan). 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 $MnSO_4$ (Mn), 150 mM NaCl (Salt), and 150 mM NaCl + 0.5 mM $MnSO_4$ (Salt + Mn). Data were taken after 3 days and 6 days of treatment. The experiment was repeated three times under the same conditions.

Determination of different physiological and biochemical parameters

Different physiological and biochemical parameters were measured following Experiment I, II and III. In addition, flavonoid contents and osmotic potential were also measured following standard methods.

Statistical analysis

Data were analyzed following Experiment II

Results and Summary

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 antioxidant defense and glyoxalase system through overproduction of ROS and MG, respectively. The salt-induced damage increased with the increasing duration of stress. However, exogenous application of Mn partially recovered growth inhibition, 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 phenolic, flavonoid and AsA contents and increasing MDHAR, DHAR, SOD and CAT activities in salt-treated seedlings. Supplemental Mn also reinforced MG detoxification by increasing the activities of Gly I and Gly II in salt-affected seedlings. Thus, exogenous application of Mn conferred salt stress tolerance by coordinated action of ion homeostasis, antioxidant defense and glyoxalase system in salt-affected seedlings.

Overall summary

Our results suggest that, exposure of Cd in rice seedlings causes oxidative stress, ionic stress and growth inhibition. The level of Cd-induced damage increased with increasing the dose of Cd. In contrast, exposure of salt causes ionic stress, osmotic stress, oxidative stress and growth inhibition. The level of salt-induced damage increased with increasing the duration of salt stress. However, supplementation with Ca and Mn mitigate Cd and Salt-induced damage by the coordinated actions of nutrient homeostasis, antioxidant defense and glyoxalase systems of rice seedlings.

Referrences

Acquaah G (2007) Principles of plant genetics and breeding. Blackwell, Oxford, p 385

Addinsoft (2013) XLSTAT 2013: Data analysis and statistics software for microsoft excel, Addinsoft, Paris, France

Addinsoft (2015) XLSTAT v. 2015.1.01: Data analysis and statistics software for Microsoft Excel. Addinsoft; Paris, France

Ahmad P, Sarwat M, Bhat NA, Wani MR, Kazi AG, Tran LP (2015) Alleviation of cadmium toxicity in *Brassica juncea* L. (Czern. & Coss.) by calcium application involves various physiological and biochemical strategies. PLoS One 10(1):e0114571. doi:10.1371/journal.pone.0114571

Ahmad P, Sharma S (2008) Salt stress and phyto-biochemical responses of plants. Plant Soil Environ 54:89–99

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, Urano K, Delort S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163. doi: 10.1186/1471-2229-11-163

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Hasanuzzaman M, Fujita M (2012) Heavy metals in the environment: current status, toxic effects on plants and possible phytoremediation. In: Anjum NA, Pereira MA, Ahmad I, Duarte AC, Umar S, Khan NA (eds.) Phyto- technologies: Remediation of Environmental Contaminants. CRC Press, Boca Raton, pp 7–73

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 C, Mandapaka M (eds) Crop Stress and its Management: Perspectives and Strategies. Springer, Berlin, pp261–316

Hasanuzzaman M, Nahar K, Fujita M (2013) 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

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

Jaleel CA, Azooz MM (2009) Exogenous calcium alters pigment composition, γ-glutamyl kinase and proline oxidase activities in salt stressed *Withania somnifera*. Plant Omics J 2:85−90.

Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, Panneerselvam R (2007a) Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseu*. Compt Rend Biol330:674–683

Jaleel CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R et al (2007b) Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. Colloids Surf B Biointerfaces60:110–116

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

Manivannan P, Jaleel CA, Sankar B, Somasundaram R, Murali PV, Sridharan R, Panneerselvam R (2007) Salt stress mitigation by calcium chloride in *Vigna radiata* (L.) wilczek. Acta Biol CracoviensiaSer Bot 49:105–109

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 10:476–49

Mittler R (2002) Oxidative stress: antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Munns R, Tester M (2008) Mechanism of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nahar K, Hasanuzzaman M, Alam MA, Fujita M (2015b) Exogenous glutathione confers high temperature stress tolerance in mung bean (*Vigna radiata* L.) by modulating antioxidant defense and methylglyoxal detoxification system. Environ Exp Bot 112:44–54

Nahar K, Hasanuzzaman M, Alam MM, Fujita M (2015a) Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. Biol Plant 59(4):745−756

Nahar K, Hasanuzzaman M, Fujita M (2016) Roles of osmolytes in plant adaptation to drought and salinity. In: Iqbal N, Nazar R, KhanNA (eds) osmolytes and plants acclimation to changing environment: emerging omics technologies. Springer, New Delli, pp37-58

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

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, Nahar K, Hasanuzzaman M, Fujita M (2016) 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

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

Rodríguez M, Canales E, Borrás-Hidalgo O (2005) Molecular aspects of abiotic stress in plants. Biotechnol Appl 22:1–10.

Sarma H (2011) Metal hyperaccumulation in plants: a review focusing on phytoremediation technology. J Environ Sci Technol 4:118–138

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

Sharma P, Dubey RS (2005) Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul 46:209–221

Sharma P, Dubey RS (2007) Involvement of oxidative stress and role of antioxidative defense system in growing rice exposed to toxic levels of aluminium. Plant Cell Rep 26:2027–2038

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

Taiz L, Zeiger E (2006) Plant physiology, 4th edn. Sinaur Associates, Sunderland

Talukdar D (2012) Exogenous calcium alleviates the impact of cadmium induced oxidative stress in *Lens culinaris* Medic. through modulation of antioxidant enzyme activities. J Crop Sci Biotechnol 15:325–334

Wang WX, Vinocur B, Shoseyov O, Altman A (2001) Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. Acta Hort 560:285–292.

Wei S, Zhou Q (2008) Trace elements in agro-ecosystem. In Prasad MNV (Ed.)Trace elements as contaminants and nutrients: Consequences in ecosystems and human health.Wiley, Hoboken p55–79

White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92:487–511

Wu GQ, Wang SM (2012) Calcium regulates K^+/Na^+ homeostasis in rice (*Oryza sativa* L.) under saline conditions. Plant Soil Environ58 (3):121–127

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

You J, Chan Z (2015) ROS regulation during abiotic stress responses in crop plants. Front Plant Sci 6:1092. doi:10.3389/fpls.2015.01092