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SALINITY-INDUCED ANTIOXIDANT ENZYME SYSTEM IN MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] CV.) GENOTYPES

HESHAM F. ALHARBY[1*], HASSAN S. AL-ZAHRANI [1], KHALID REHMAN HAKEEM[1*], REIAZ UL REHMAN[2] AND MUHAMMAD IQBAL[3]

[1]Departmentof Biological Sciences, Faculty of Science, King Abdulaziz University, 21589 Jeddah, KSA
[2]Department of Bioresources, University of Kashmir, Hazratbal, Srinagar, India
[2]Botany Department, Jamia Hamdard (Deemed University), Hamdard Nagar, New Delhi, India
[*]Corresponding author's email: khakim@kau.edu.sa ; halharby@kau.edu.sa

Abstract

Salt stress poses a serious challenge to plant growth and development and hence influences the yield and crop productivity. This study investigates the impact of exogenous sodium chloride (NaCl) on the seedlings of six genotypes of mungbean [*Vigna radiata* (L.) Wilczek] with reference to the status of a stress indicator (hydrogen peroxide: H_2O_2) and some antioxidant enzymes, *viz.* superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT)**,** glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase (GST). Ten-day-old seedlings were subjected to salt stress (0-250 mM) given in split application along with the half strength Hoaglands. The salt stress increased the H_2O_2 production in a concentration-dependent manner up to 250mM NaCl in all genotypes. NaCl levels were positively correlated with the production of hydrogen peroxide in leaves as evidenced by the 3, 3-diaminobenzidine (DAB)-mediated tissue fingerprinting. The activity of SOD, CAT, APX, GR, GPX and GST increased significantly upon the NaCl treatments and attained its maximum at 150mM for SOD and CAT, at 200mM for APX, GR and GST and at 250mM for GPX. The pattern of increase in the activity was similar in all the genotypes studied, though the quantitative levels were markedly different. The oxidative stress due to H_2O_2 generationand the antioxidant enzymes activity to combat it may serve as the screening markers for selecting the stress-tolerant genotypes of mungbean.

Key words: Antioxidant enzymes, DAB tissue fingerprinting, Oxidative stress, Sodium Chloride, (NaCl), *Vigna radiate*.

Introduction

Salinity stress in water bodies or soil is a major problem in the arid and semi-arid regions of the world, as it severely limits the plant growth and yield. It affects the physiological and metabolic processes in plants, which ultimately results in the reduced yield. The decline in plant growth and yield is primarily caused by osmotic stress and later by ion toxicity (Rahnama *et al*., 2010, James *et al*., 2011). The responses within the plants varies with severity and duration of salt stress (Rozema & Flowers, 2008, Munns & Tester, 2008). Many studies have revealed the adaptation potential of plants under salt stress but the mechanisms involved are not yet clearly understood (Gupta & Huang, 2014). It is important to understand the plant response towards salt stress at the molecular, biochemical and physiological levels for identifying and developing the tolerant varieties for salt-infested regions (Palao *et al*., 2014). Several mechanisms including the uptake, transport and compartmentalization of ions and the biosynthesis of osmoprotectants and antioxidant enzymes, facilitate plant survival under salinity stress (Roy *et al*., 2014).

Salinity can cause stomatal closure that limit CO_2 availability for carbon fixation, and exposure of chloroplasts to excessive energy excitation leading to ROS generation, which induces oxidative stress (Parida & Das, 2005, Parvaiz & Satyawati, 2008). The ROS include the superoxide radical (O2. A), hydroxyl radical ('OH) and hydrogen peroxide (H_2O_2), which can interact with biomolecules causing damage to membranes within the cell (Perez-Lopez *et al*., 2009, Gill & Tuteja, 2010). For scavenging the abnormal levels of ROS and maintaining a tight control on their extent, the plants possess efficient enzymatic antioxidants (Apel & Hirt, 2004) which include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase (GST). Upregulation of these enzymes during the salt stress has been recorded in several plant species including *Oryza sativa* (Chawla *et al*., 2013), *Zea mays* (AbdELgawad *et al*., 2016), *Triticum aestivum* (Caverzan *et al*., 2016). Mungbean is an important food grain, which is significantly affected by salinity **(**Abd-Alla *et al*., 1998; Ahmad, 2009; Ashraf *et al*., 2015). The present work aims at studying the effect of salinity in different cultivars of this crop plant to check whether they have a differential antioxidant potential.

Material and Methods

Procurement of genotypes: This study is focused on six genotypes of Mungbean [*Vigna radiate* (L.) Wilczek], of which AEM-96 (AzriBhakkar), NCM-1 (NARC-Islamabad) and CM-6 (BARI-Chakwal)] belong to arid regions whereas NFM-12 (NIFA-Peshawar), NM-92 (NIAB-Faisalabad) and NFM-6 (NIFA- Peshawar) are from semi-arid regions. Seeds of all these genotypes were procured from the Pakistan Agriculture Research, Islamabad.

Growth conditions: The seeds were sterilized with 0.2% $HgCl_2$ solution for 5 min and thoroughly washed with tap water followed by another washing with deionized water. The sterilized seeds of each genotype were sown in plastic pots (300mm diameter) filled with moist 3kg of acid-washed autoclaved sand. The pots were moistened (watered) regularly until germination took place. The seedlings were irrigated with half-strength Hogland's nutrient medium at pH 6.5 (Hoagland & Arnon, 1950). All

pots were maintained in a growth chamber at 28 ± 1.5ºC at daytime and at 22 ± 1.5ºC at nighttime. The plants grew at uniform irradiance of photosynthetic photon flux density of 300µmol m^{-2} s^{-1} and relative humidity of 60-70%. The treatments were arranged in randomized block design with three replicates and the random sampling was done 20 days after the start of treatments with sodium chloride (0, 100, 150, 200, 250mM). The sodium chloride treatments prepared in Hoaglands solution were given in the split application of 50 mM from 1-5 days. The randomly collected leaves from the control and treated plants were used for the estimation of antioxidant enzymes.

Estimation of hydrogen peroxide (H_2O_2) content: The accumulation of H_2O_2 in the leaves was determined by the method of Velikova *et al*., (2000) by grinding the leaf tissue (0.5g) in liquid nitrogen. To the fine powder, 5 ml of 0.1% (w/v) TCA was added and the homogenate was centrifuged at 12000rpm for 15 min at room temperature. To the supernatant, 0.5 ml of 1M potassium phosphate buffer (pH 7.0) and 1 ml 1M potassium iodide (KI) were added. The absorbance of the mixture was recorded at 390 nm after vortexing and the amount of H_2O_2 was calculated using the equation H_2O_2 (µmol g^{-1} FW) = 1+227.8×O.D_{390}.

Histochemical detection of H_2O_2: The DAB-mediated tissue fingerprinting for visualizing H_2O_2 in leaves was done according to the method of Orozco-Cardenas & Ryan (1999). Clean plant samples from the control and NaCl-treated lots were immersed in DAB-HCl solution (1mg mL^{-1}, pH 3.8) overnight at 25ºC, and then in the boiling ethanol (70% v/v) for 10 min in order to remove the green background. The deep brown polymerization products formed by the reaction of DAB with H_2O_2 were visualized and the samples were photographed.

Enzyme extraction: The randomly collected fresh leaf tissues (200mg) were extracted with liquid nitrogen at 4°C and homogenized with 3.0ml extraction buffer containing 50mM phosphate buffer (pH 7.8), 1 mM EDTA-Na_2 and 1%PVP. The contents were subjected to centrifugation at 13000 rpm for 30 min at 4°C. The supernatant was used to determine the antioxidant enzyme activities expressed in accordance with the total soluble protein estimated by the Bradford (1976) method.

Determination of superoxide dismutase activity: The superoxide dismutase **(**SOD) activity was estimated by measuring its ability to inhibit the reduction of Nitro Blue Tetrazolium (NBT) with a slightly modified method of Beyer and Fridovich (1987). The assay mixture contained 0.1mM phosphate buffer (pH 7.5), 750µM NBT, 130mM methionine, 0.1mM EDTA, 100µM riboflavin and 300µl enzyme extract. The reaction was initiated by illuminating the tubes for 5 min in closed box containing 25W florescent tubes. In the control tube, the sample was replaced by buffer and the reaction was arrested by switching off the lights and placing the tubes in the dark. The absorbance was measured at 560nm against the blank and the SOD was defined as the amount of enzyme causing the half-maximal inhibition of NBT reduction under the assay conditions.

Determination of catalase activity: Catalase (CAT) activity was determined by the method of Aebi *et al*., (1984). The assay based on the decomposition of H_2O_2 measured the decrease in absorbance at 240nm. The reaction mixture (2.2ml) comprised of 0.5 M sodium phosphate buffer (pH 7.3), 3mM EDTA, 5mM H_2O_2 and an aliquot of enzyme. The unit of CAT activity, defined as the amount of enzyme catalyzing the conversion of 1mM of H_2O_2 min^{-1} was calculated using the extinction coefficient 0.036mM^{-1} cm^{-1}.

Determination of ascorbate peroxidase activity: The ascorbate peroxidase **(**APX) activity was determined according to the method of Nakano & Asada (1981). The reaction mixture (2.22mL) contained 0.1 M potassium phosphate buffer (pH 7.0), 0.5mM ascorbate, 0.1mM EDTA, 1.0mM H_2O_2 and 20µl enzyme extract. The oxidation of ascorbic acid was measured by the decrease in absorbance at 290nm for 3 min. One unit of enzyme is an amount necessary to decompose 1µmol of substrate per minute at 25°C and the enzyme activity was calculated using the enzyme coefficient 2.8mM^{-1} cm^{1}.

Determination of guaiacol peroxidase activity: The guaiacol peroxidase **(**GPX) activity was measured at 25°C by the method of Whitaker & Bernhard (1972). In the presence of H_2O_2, POD catalyzed the transformation of guaiacol to tetraguaiacol (brown product). The reaction mixture contained 10mM sodium phosphate buffer (pH 7.0), 1.0ml of 30% H_2O_2, 1.0ml of 0.05M guaiacol, 2ml double distilled (dd) H_2O and 0.2ml enzyme extract. The oxidation of guaiacol was measured by the increase in absorbance at 470nm for 1mn. One unit of POD is defined as the amount of enzyme required to catalyze the conversion of 1µM of H_2O_2,with guaiacol as hydrogen donor, per minute under specified conditions and was calculated using the enzyme coefficient 26.6mM^{-1} cm^{-1}.

Determination of glutathione reductase activity: The glutathione reductase **(**GR) activity was determined at 25°C according to the method of Cakmak & Marschner (1992) by monitoring the oxidation of NADPH oxidation at 340nm. The reaction mixture (2.31mL) contained 50mM phosphate buffer (pH 7.6), 0.1mM EDTA, 0.12 mM NADPH, 0.5mM oxidized glutathione (GSSG). The reaction was initiated by the addition of soluble aliquot of enzyme extract and the activity was monitored by the decrease in absorbance at 340nm. One unit of GR activity, as defined by its reduction of 1µM of GSSG min^{-1} under standard assay conditions, was calculated using the enzyme coefficient 6.2mM^{-1} cm^{-1}.

Determination of glutathione-s-transferase activity: The glutathione-s-transferase (GST) was determined according to the method of Habig & Jacoby (1981). The reaction mixture (2.9ml) contained 1mM reduced glutathione (GSH), 1mM 1-chloro 2,4-dinitrobenzene (CDNB) and 100mM phosphate buffer (pH 6.5) and the reaction was initiated by the addition of 100µl of the enzyme extract. The enzyme activity was measured at 340nm and calculated using the extinction coefficient of the conjugate 9.6 mM^{-1} cm^{-1}.

Statistical analysis: The data presented as mean ± SE were subjected to one-way ANOVA, using Graph Pad Prism 6.0 software by Tukey's post hoc test ($p<0.05$). All experiments were carried out in triplicates (n=3) and the results of the test are expressed by alphabet letters on the bar graph.

Results

Production of hydrogen peroxide (H_2O_2): The H_2O_2 content increased linearly from 100 to 250mM NaCl treatments in all the genotypes studied (Fig. 1). The increase ranged from 6.76%-49.70% and 9.86%-67.96% at 200mM and 250mM respectively. At 200mM the maximum increase (49.70%) was seen in NM-92, while at 250mM the maximum (67.96%) occurred in NFM-6 as compared to the control. This genotype (NFM-6) showed a slight decrease (-2.48%) with respect to the control at 100mM. Genotype NCM-1 showed the minimum increase (1.79%-9.86%) in the H_2O_2 content at 100mM-250mM NaCl, compared to other genotypes. As visualized with DAB-mediated fingerprinting the H_2O_2 content increased with NaCl treatments (as shown for the representative genotype CM-6). The deep brown polymerization product formed by the reaction of DAB with H_2O_2 can be visualized clearly (Fig. 2).

Superoxide dismutase (SOD) activity: The SOD activity of mungbean genotypes was significantly affected by the salt treatments (Fig. 3A). It increased at all concentrations of NaCl with respect to the control, attaining the peak activity (0.07–1.39 fold) at 150mM. The maximum increase of 1.39 fold was observed in NFM-12. Thereafter the SOD activity started to decline at 200-250mM NaCl in all the genotypes ranging 0.29–0.77 fold at 200mM and (-) 0.248–0.125 fold at 250mM.

Ascorbate peroxidise (APX) activity: The APX activity increased at all NaCl concentrations (100-250mM) in all the genotypes with respect to the control (Fig. 3B). It attained its peak at 200mM and thereafter declined at 250mM. The increase in APX at 200mM ranged from 0.47-3.15 fold in the different genotypes while this range was 0.22-1.18 fold at 250mM. The maximum increase (3.15 fold) at 200mM was observed in AEM-96 the maximum (1.18 fold) at 250mM occurred in NCM-1.

Catalase (CAT) activity: The catalase activity increased at all NaCl concentrations (100-250mM) in all the genotypes with respect to control (Fig. 3C). It attained its peak at 150mM and declined thereafter. The increase among different genotypes was 0.51-2.37 fold at 150mM, with the maximum seen in CM-6. At 200mM the increase was 0.14-1.63 fold, with the maximum in AEM-96 and at 250mM it was 0.09-0.30 fold with maximum in CM-6.

Glutathione reductase (GR) activity: The GR activity increased at all NaCl concentrations (100-250mM) in all the genotypes (Fig. 4A). The increase was linear from up to 200mM salt treatment, followed by a decline at 250mM, the increase was 0.23-1.77 fold, showing the maximum in CM-6. At 200mM, the increase was 0.72-3.51fold with the maximum found in CM-6.

Guaicol peroxidase (GPX) activity: The GPX activity increased linearly at 100-250mM NaCl in all the genotypes except in AEM-96 where the maximum increase was seen at 200mM NaCl, followed by a decline at 250mM (Fig. 4B). The increase in GPX activity among the genotypes ranged 1.06-4.65 fold at 200mM with the maximum seen in AEM-96, and 1.22-4.40 fold at 250mM with the maximum found in NM-92.

Glutathione S-transferase (GST) activity: The GST activity also increased linearly up to 200mM NaCl in all the genotypes and thereafter declined at 250mM (Fig. 4C). The activity ranged from 0.03-1.10 fold among genotypes at 200mM. In NM-92 and NFM-12 the peak activity also occurred at 200mM and thereafter it declined becoming almost at par with the control at 250mM.

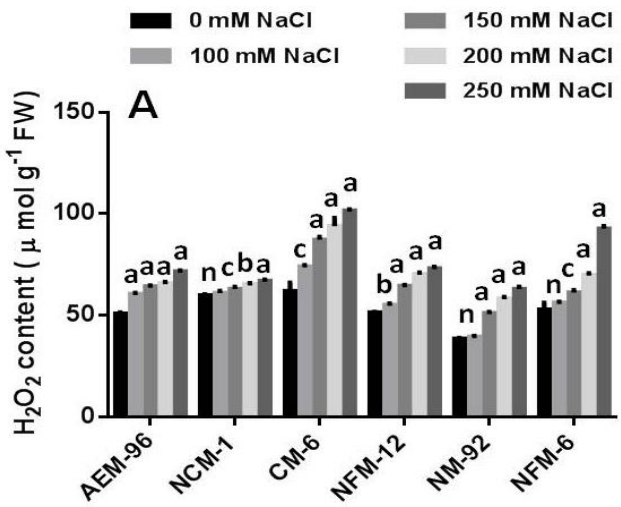

Fig. 1. Effect of NaCl on hydrogen peroxide (H_2O_2) accumulation (A) in mungbean genotypes AEM-96, NCM-1, CM-6, NFM-12, NM-92, NFM-6 respectively. Each of the genotypes were subjected to different NaCl treatments (0, 100, 150,200, 250 mM). The experiment was repeated thrice with mean ± SE (n=3). Different letters within columns represent significant differences ($p<0.05$) between treatments within each genotype respectively. Significance of values at $p<0.05$, a=**** (highly significant), b=*** (moderately significant), c=** (less significant) and ns (not significant) with respect to control of each genotype

Fig. 2. Localization of hydrogen peroxide (H_2O_2) by DAB tissue fingerprinting printing (A). The formation of red-brown pigment is indicative of H_2O_2 accumulation which results from DAB polymerization in the presence of peroxidase enzyme.

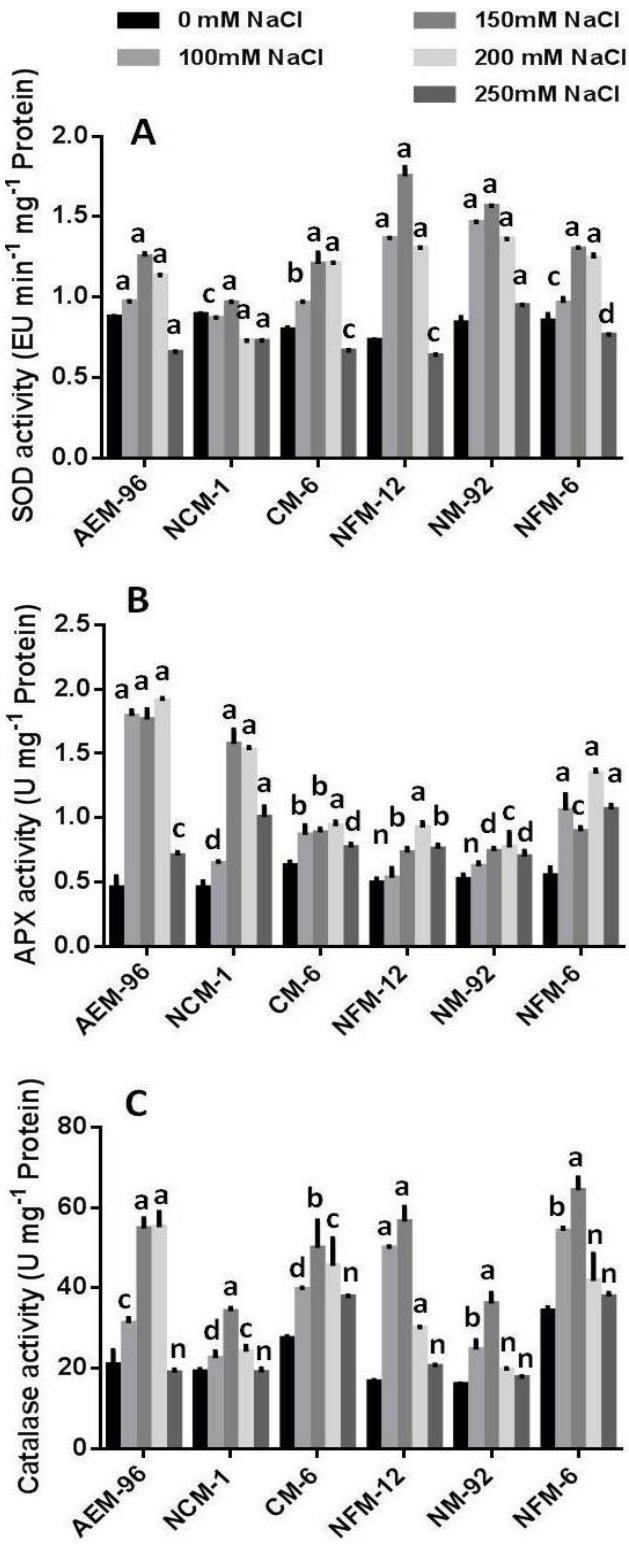

Fig. 3. Effect of NaCl on superoxide dismutase (SOD) (A) ascorbate peroxidase (APX) (B) and Catalase (CAT) (C) in mungbean genotypes AEM-96, NCM-1, CM-6, NFM-12, NM-92, NFM-6 respectively. The enzymes were extracted from leaves from control and salt treated seedlings from each genotype were collected and activity recorded and data is presented as mean ± SE (n=3). Different letters within columns represent significant differences ($p<0.05$) between treatments within each genotype respectively. Significance of values at $p<0.05$, a=**** (highly significant), b=*** (moderately significant), c=** (less significant) and ns (not significant) with respect to control of each genotype

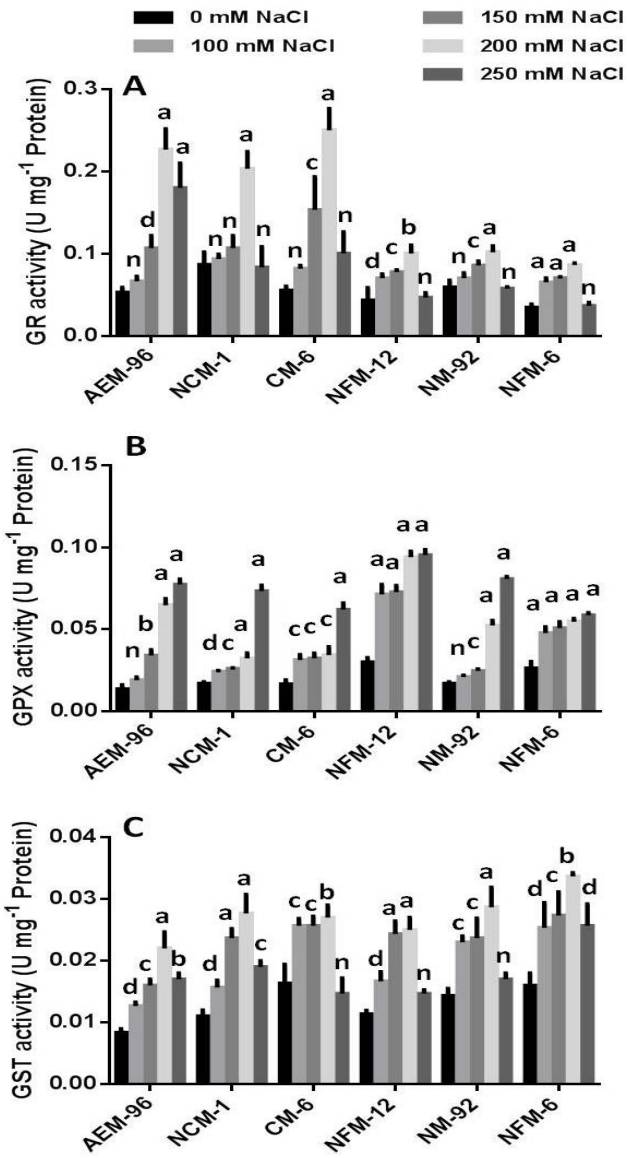

Fig. 4. Effect of NaCl on glutathione reductase (GR) (A) glutathione peroxidase (GPX) (B) and glutathione -s- transferase (GST) (C) in mungbean genotypes AEM-96, NCM-1, CM-6, NFM-12, NM-92, NFM-6 respectively. The enzymes were extracted from leaves from control and salt treated seedlings from each genotype were collected and activity recorded and data is presented as mean ± SE (n=3).Significance of values at $p<0.05$, a=**** (highly significant), b=*** (moderately significant), c=** (less significant) and ns (not significant) with respect to control of each genotype.

Discussion

In general, stresses cause oxidative damage in plants by generating the reactive oxygen species (O_2^-, H_2O_2 and OH) (Van Breusegem *et al*., 2001; Parida & Das, 2005). The ROS damages biomolecules (lipids, proteins, pigments, enzymes, nucleic acids) (Jithesh *et al*., 2006, Anjum *et al*., 2015). For countering the oxidative damages caused by salt stress plants activate antioxidant enzymes (Matamoros *et al*., 2003; Qureshi *et al*., 2011). The antioxidant enzymes form a defensive force, which protect plant cells from cytotoxic effects (Rahnama & Ebrahimzadeh, 2005) including the oxidative damage (Mittler, 2002; Aref *et al*., 2013). In this system

superoxide dismutase, catalase and peroxidases react with the active oxygen forms for keeping them at acceptable levels and glutathione reductase and ascorbate peroxidase regenerate the oxidized antioxidants (Anjum *et al*., 2012; Aref*et al*., 2013). The increased activities of these enzymes have been related to salt tolerance in many plant species (Azevedo Neto *et al*., 2006, Koca *et al*., 2007, Gao *et al*., 2008; Arshi *et al*., 2012).

The role of superoxide dismutase: Superoxide dismutase (SOD) is the key enzyme for coping with the ROS-induced oxidative stress and maintaining the intracellular ROS levels (Mittler, 2002). By way of dismutating the superoxides into H_2O and oxygen it helps to maintain O_2 radical in a steady state (Chen *et al*., 2015; Yousuf *et al*., 2017). A positive correlation between SOD levels and salt stress found in the present study finds support from many earlier studies (Cavalcanti *et al*., 2007; Qureshi *et al*., 2013). The increase in SOD might be due to over production of active oxygen species (AOS) or the overexpression of SOD encoding genes providing stress protection in transgenics (Yiu & Tseng, 2005, Tseng *et al*., 2007; Yousuf *et al*., 2015). The salt stress enhanced the H_2O_2 production in all the mungbean genotypes studied (Fig. 1), suggesting its regulatory role in the cross- talks of redox metabolic signals and stress signaling pathways.

H_2O_2, a by-product of SOD, is toxic at higher concentrations and must be eliminated by conversion to H_2O by subsequent reactions. The SOD activity in mungbean genotypes increased in all genotypes at all treatments attaining the peak at 150mM, but declined at 250mM (Fig. 3A), which could be due to SOD inability to handle AOS species beyond certain level. H_2O_2 is mildly toxic to cellular systems but its toxicity is ascribed to the production of hydroxyl radical (OH) during Fenton reaction (Kumaran & Kurarnakaran, 2007). It is presumed that SOD activity is linked to synthesis of other antioxidant enzymes (APX, CAT, GR, GPX) and their combined effect is required for detoxification of ROS so as to render the plants tolerant to stressed environments. APX, GPX and GR act as the second line of defence for neutralization of H_2O_2 (Aref *et al*., 2013). These antioxidant enzymes act in a chain reaction as the SOD dismutates ROS into H_2O_2, which is subsequently converted to H_2O and O_2 in different proportions by APX, GPX and GR.

Catalase activity: The CAT enzyme decomposes H_2O_2 to H_2O and O_2 (Gill & Tuteja, 2010) and is a most effective enzyme for preventing the oxidative damage (Willekens *et al*., 1995, Mittler, 2002). These proteins are peroxisome-localized and CAT genes respond differently to different stresses (Scandalios, 2002; 2005).The present study revealed CAT activity in mungbean to increase significantly up to 150mM, and decrease slightly at higher concentrations (Fig. 3C). This reduced activity may not imply that the H_2O_2 accumulated at these concentrations would cause a serious damage but the other continued reactions may remove the H_2O_2 for imparting tolerance (Macrae & Ferguson, 1985). This observation suggests that mungbean genotypes have a great ability to cope with oxidative stress caused by NaCl. The increase in CAT activity suggested the increase in substrate for maintaining H_2O_2 levels (Reddy *et al*., 2015). The decrease in CAT activity at higher concentrations (200-250mM) may be due to enzyme inactivation by excessive AOS, or an inhibition of enzyme synthesis (Sahu *et al*., 2012). The present study on *Vigna radiata* and many earlier studies reporting the increased CAT activity under moderate salinity stress in *Oryza sativa* (Chawla *et al*., 2013), *Zea mays* (Azevedo Neto *et al*., 2006), *Hordeum vulgare* (Pérez-López *et al*., 2009), *Cassia angustifolia* (Agarwal & Pandey, 2004), *Sesamum indicum* (Koca *et al*., 2007), and *Jatropha curcas* (Gao *et al*., 2008) have shown a differential salt tolerance, suggesting that CAT activity might depend on species development, metabolic state, duration and intensity of stress (Chaparzadeh *et al*., 2004).

Ascorbate peroxidise (APX) activity: During stressed environment, APX reduces harmful H_2O_2 to H_2O by employing ascorbic acid as a specific electron donor (Majeed *et al*., 2010, Jebara *et al*., 2005). It possesses more affinity towards H_2O_2 than CAT as it could be responsible for modulating H_2O_2 for signalling process (Mittler 2002). The APX genes are modulated differently by different abiotic stresses (Rosa *et al.*, 2010; Caverzan *et al.*, 2012; Caverzan *et al.*, 2014) and the APX enzyme is present in chloroplast, mitochondria, peroxisomes as well as cytosol. In the current study the APX activity in mungbean seedlings increased at all treatments in the genotypes attaining the peak at 150mM and thereafter showing a slight decline at higher concentrations (Fig. 3B). The enhanced APX activity is correlated with adaptations towards elevated ROS levels as reported in different plants viz., rice (Chawla *et al*., 2013), barley (Pérez-López *et al*., 2009). In the mutant lines of *Triticum aestivum* the reduced thylakoid APX activity led to impairment in photosynthesis (Danna *et al.*, 2003). The presence of plethora of antioxidant enzymes can be explained by the fact that in the absence or dysfunctioning of any specific enzyme/s, the others might be taking the function of getting rid of oxidants**.** This has been demonstrated in *Oryza sativa* silenced lines for cytosolic APX, which exhibited high guaiacolperoxidise (GR) activity under abiotic stress conditions (Bonifacio *et al.*, 2011). It is important to have a balance between SODs, CATs and APXs for scavenging of ROSs and any changes in this balance would induce compensatory mechanisms (Apel&Hirt, 2004; Scandalios, 2002; 2005).

Guaicol peroxidase activity: GPX catalyzes the reduction of H_2O_2 to water and the presence of GPX proteins has been confirmed in many organisms including plants (Margis *et al.*, 2008). In the present study the GPX activity in mungbean seedlings increased linearly at all treatments (Fig. 4B), indicating the presence of an efficient detoxification mechanism for resisting the NaCl stress. GPX acts as H2O2 scavanger under salt stress in *Triticum aestivum* plants as the expression of *GPX* genes was altered (Zhai *et al.*, 2013). It was also demonstrated in *Oryza sativa* that expression of *GPX* genes was necessary for redox homeostasis (Passaia *et al.*, 2013, 2014).

Glutathione reductase activity: Glutathione reductase (GR) is one of the major components of ascorbate-glutathione (AsA-GSH) pathway, which plays a significant role in cell protection against ROS (Gill *et al*., 2013). GR is

involved in reduction of oxidised glutathione (GSSG) to reduced glutathione (GSH) and GSH in turn is involved in redox regulation of cell cycle and plays essential role against oxidative stress (Grant 2001, Aref *et al*., 2013). The increase in GR activity in mungbean (Fig. 3A) corroborates the previous reports on many plants including *Triticum aestivum* (Hasanuzzaman *et al*., 2012); *Zea mays* and *Oryza sativa* (Kumar *et al*., 2012); *Cucumis sativus* (Dai *et al*., 2012); *Nicotiana tobaccum* (Tan *et al*., 2011) and *Phaseolus aureus* (Kumar *et al*., 2011).The increase in GR activity might be due to de novo synthesis of the enzyme protein (Baisak *et al*., 1994).

Glutathione-s-transferase activit: The role of GST as an antioxidant agent in plants has not been well characterized (Dixit *et al*., 2011). However, it is said to be induced by stress and its increased levels are used for maintaining the cellular redox homeostasis for protecting plants against oxidative stress (Chen *et al*., 2012). It plays an important role in cellular detoxification of abnoxious toxic chemicals which are the secondary products (such as an alkenal, 4-hydroxynonenal) released during oxidative damage to membranes (Edwards *et al*., 2000). It has been reported that overexpression of GST in *Arabidopsis thaliana* and *Nicotiana tobaccum* improved their tolerance towards oxidative and drought stress (Yu *et al*., 2003; Sharma *et al*., 2014). Several plant GSTs exhibit peroxidase activity and might have a role in according tolerance towards chilling, dehydration and herbicide stress (Bartling *et al*., 1993; Cummins *et al*., 1999; Roxas *et al*., 2000). Overexpression of GST in certain transgenic plants have caused enhanced tolerance towards heavy metals, drought, salt, heat and cold stresses (Kumar *et al*., 2013; Yang *et al*., 2014).

Conclusion

It is important to develop a comprehensive knowledge on responses of plants towards salt (NaCl) stress by combining the physiological, biochemical and molecular approaches, which would help in identifying and raising salt-tolerant varieties for use in the salt-infested areas. Our findings suggest that increased CAT activity coordinated with the changes of SOD and POD activities plays an important protective role in the ROS-scavenging process and that the active involvement of these enzymes are related, at least in part, to tolerance to the salt-induced oxidative stress.

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