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Modulation of NaCl induced DNA damage and oxidative stress in mungbean by pretreatment with sublethal dose

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Abstract

Salinity is one of the most severe problems in worldwide agricultural production. The effect of salt on dry mass, total glutathione content, its regulatory enzymes, and extent of DNA damage in growing mungbean (*Vigna radiata* L. Wilczek) seedlings was investigated. The salt stress decreased a dry mass accumulation in the seedlings. A total glutathione (GSH) content and the activities of the enzymes of GSH metabolism were adversely affected by the salt stress. The enhanced accumulation of reactive oxygen species under the NaCl stress caused an increase in DNA damage, measured using a comet assay, in both roots and leaves of the mungbean seedlings. The pretreatment of mungbean seeds with a sublethal dose of NaCl was able to overcome the adverse effects of the salt stress to variable extents by exhibiting significant alterations of all tested parameters, imparting better growth and metabolism of the mungbean seedlings.

Additional key words: comet assay, glutathione metabolism, hardening, reactive oxygen species.

Introduction

Among abiotic stresses, salinity is one of the most severe environmental factors limiting the productivity of agricultural crops (Pessarakli and Szaboles 1999). Salt stress effectively decreases the availability of water in soil to plants, and hence there is a substantial overlap between plant responses to drought and to salinity. However, in addition to affecting water balance of plant, the excess accumulation of ions in cells is toxic. The increased content of Na^+ and Cl^- impairs enzymes function, inhibits protein synthesis, affects the structure and permeability of cell membranes, inhibits photosynthesis, and leads to the production of reactive oxygen species (ROS). In mungbean, salt stress causes decline in seed germination, shoot and root lengths, fresh mass, and seedling vigor (Misra *et al*. 1996, Promila and Kumar 2000, Misra and Dwivedi 2004). Some of the pronounced salt induced injury symptoms on mungbean are enhanced chlorosis, necrosis, and decreased content of chlorophyll (Chl) *a*, Chl *b*, and carotenoids. The content of proline increases with an increasing NaCl concentration to adjust the osmotic potential of cells (Hanson and Hitz 1982, Arora *et al.* 1995, Nandwal *et al.* 2000). The effect of salinity stress on plants is mediated at least in part by an enhanced generation of ROS like a superoxide radical, hydroxyl radical, and hydrogen peroxide (Alscher *et al.* 1997) particularly in chloroplasts and mitochondria (Mittler 2002), which causes lipid peroxidation, membrane injury, protein degradation, and enzyme inactivation (Imlay and Linn 1988). Plants have developed a complex antioxidant system which mitigates and repairs the damage initiated by ROS.

Glutathione and glutathione metabolism play a key role in protecting the cell against ROS. Glutathione is an abundant low molecular mass thiol (Rennenberg 1982). Under normal conditions, it is predominantly present in its reduced form (GSH), with only a small proportion of oxidized form (GSSG; Noctor *et al.* 2002). GSH within the plant cell functions as storage of reduced sulphur and

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Abbreviations: CDNB - 1-chloro-2,4-dinitrobenzene; Chl - chlorophyll; DTNB - 5,5'-dithiobis-(2-nitrobenzoic acid); EDTA - ethylene diaminetetraacetic acid; GPx - glutathione peroxidase; GR - glutathione reductase; GSH - glutathione (reduced); GSSG - glutathione (oxidized); GST - glutathione-S-transferase; MDA - malondialdehyde; PMSF - phenylmethane-sulphonyl fluoride; ROS - reactive oxygen species; SSA - sulphosalicylic acid.

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substrate for glutathione-S-transferases (GST) which is involved in the removal of toxic compounds and in other key metabolic reactions (Noctor *et al.* 2002). The biosynthesis of GSH in plants takes place in two steps. Firstly, L-cysteine is conjugated to L-glutamate by γ-glutamylcysteine synthetase, forming γ-glutamyl-cysteine. In the second step, glycine is added by glutathione synthetase. Moreover, glutathione reductase (GR) is responsible for maintaining the reduction state of GSH (Murgia *et al.* 2004), as GSSG synthesised upon reduction of dehydroascorbate is reduced to GSH by GR (Yu and Facchini 2000). The main biological role of glutathione-S-transferase (GST) and glutathione peroxidase (GPx) is to protect the organism from oxidative damage. The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GPx and GR utilize GSH to play an important role in a plant defence mechanism (Anderson and Davis 2004).

Excess of ROS can damage cellular components, such as DNA, proteins, and lipids (Lopez *et al.* 2006). All primary components of DNA (sugar residues, phosphodiester linkages, purine and pyrimidine bases) can suffer from damage. Resulting DNA-lesions that vary from innocuous molecular changes to highly mutagenic or genotoxic alterations in the genome lead to genomic instability or genotoxic stress which may result in senescence, aging, change in gene expression, and decrease in biodiversity and crop yield. Although most DNA damages are repaired, error-prone DNA repair or DNA damage beyond repair triggers programmed cell death (Bray and West 2005). However, less evidence has been given about the relationship between oxidative stress and DNA damages induced by salinity in plants.

A number of genotoxic endpoints or markers in plants ranging from chromosome aberration (CA), micronuclei (MN), sister chromatid exchange (SCE) to single cell gel electrophoresis (SCGE), or comet assay are currently in vogue to assess genotoxic stress (Panda and Panda 2002). Of these genotoxicity assays, the comet assay is gaining popularity because the assay is fast and simple to perform. Comet assay mostly carried out employing mammalian cells *in vivo* and *in vitro* has been extensively reviewed (Brendler-Schwaab *et al.* 2005), but information available with respect to comet assay using plant cells is sparse.

Plants can improve their physiological ability to adapt to various environmental stresses. This phenomenon is known as acclimation, and there have been reports on acclimation to cold, drought, salinity, and other environmental stresses (Amzallag *et al.* 1990, Umezawa *et al.* 2000, Djanaguiaraman *et al.* 2006). The analysis of the physiological changes associated with acclimation may help in advancing the study of plant tolerance to environmental stresses. Several workers have been trying to develop more effective methods to create stress tolerant plants. Attempts can be made towards hardening the sensitive cultivars, so that they can tolerate salinity induced stress conditions by a certain physiochemical manipulation. It has been established from our laboratory that a pretreatment with a sublethal dose of NaCl ameliorates the injurious effects of salt stress to some extent through enhancements in growth and photosynthetic pigments, modifications of activities of antioxidant enzymes, a reduction of oxidative stress markers like malondialdehyde and H_2O_2 content, an accumulation of osmolytes for osmotic adjustments, and improvements of ion composition and oxidative metabolism of mungbean seedlings (Saha *et al.* 2010, 2012).

In the present study, we investigated the degree of oxidative damages caused by a salt stress, and its possible amelioration by a pretreatment with a sublethal concentration of NaCl in mungbean seedlings by studying the extent of DNA damage, changes of total glutathione (GSH) content, and activities of GSH-associated enzymes.

Materials and methods

Plants and treatments: Mungbean (*Vigna radiata* L. Wilczek) cv. B 105 seeds obtained from the Pulse and Oilseed Research Station, Behrampore, West Bengal, India, were surface sterilized with sodium hypochlorite solution (5 %, m/v) and washed thoroughly in distilled water. About 25 seeds were placed on each glass plate lined with blotting papers dipped in different concentrations (0, 50, 100 and 150 mM) of sodium chloride purchased from *Merck*, Mumbai, India. In the second phase, mungbean seeds were pretreated with 50 mM NaCl for 2 h prior to the treatment with the different concentrations of NaCl. In each set, a minimum of five glass plates were maintained, with one set as control. The seeds were kept in a well aerated place at a temperature of 28 ± 2 °C, a 16-h photoperiod, an irradiance of 260 μmol m^{-2} s^{-1}, and a relative humidity of 70 %. The seedlings were harvested after seven days and data obtained from the non-pretreated and pretreated samples were compared with the non-pretreated water control. Roots and shoots of each set were separated and allowed to dry in an oven at 100 °C for 1 d and then at 70°C for 4 d to obtain a dry mass.

Estimation of GSH content and exzyme activities: Total glutathione content from root and shoot samples of 7-d-old mungbean seedlings was extracted using a mortar-pestle in 5 % (m/v) sulphosalicylic acid (SSA) containing 1 mM ethylenediamine-tetraacetic acid disodium salt (Na_2EDTA), and centrifuged at 10 000 *g* for 20 min. Total thiols were measured in a 100 mM phosphate buffer (pH 7.5) containing 1 mM Na_2EDTA, 6 U cm^{-3} GR, 10 mM 5,5'-dithiobis-(2-nitrobenzoic acid)

(DTNB), and 0.16 mg cm^{-3} NADPH. Absorbance was recorded at 412 nm (Sedlak and Lindsay 1968) using a spectrophotometer *Hitachi 2 000* (Tokyo, Japan).

Further, root and shoot samples were extracted using a mortar-pestle in a 100 mM phosphate buffer (pH 7.5) containing 0.5 mM Na_2EDTA and centrifuged at 15 000 *g* and 4 °C for 20 min. GR (EC 1.6.4.2) activity was assayed in a 100 mM phosphate buffer (pH 7.5) containing 0.5 mM Na_2 EDTA, 0.75 mM DTNB, 0.1 mM NADPH, and 1 mM oxidized glutathione (Smith *et al.* 1988). The reaction mixture was incubated at 35 °C, meanwhile absorbance at 412 nm was measured for up to 5 min. The activity was calculated using the coefficient of absorbance (ε) of 6.22 mM^{-1} cm^{-1}.

GPx (EC 1.11.1.9) was extracted in a 220 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose, 50 mM potassium chloride, 1 mM magnesium chloride, 160 mM β-mercaptoethanol, and 0.57 mM phenylmethanesulphonyl fluoride. The GPx activity was assayed in a 20 mM sodium acetate buffer (pH 5), 30 mM H_2O_2, and 2 mM guaiacol. Absorbance at 470 nm was recorded and the activity was calculated using ε = 26.6 mM^{-1} cm^{-1} (Ranieri *et al.* 1997).

For GST assay, root and shoot samples were extracted in a 100 mM Tris-HCl buffer (pH 7.5) containing 2 mM Na_2EDTA, 14 mM β-mercaptoethanol, and 7.5 % (m/v) polyvinylpyrrolidone. After centrifugation at 15 000 *g* for 15 min, the activity of GST (EC 2.5.1.18) was measured in a 100 mM phosphate (pH 6.5), 5 mM GSH, and 1 mM 1-chloro-2,4-dinitrobenzene mixture based on absorbance at 340 nm. The activity of GST was calculated using ε = 9.6 mM^{-1} cm^{-1} (Ando *et al.* 1988). Protein content was estimated from assay samples according to Lowry *et al.* 1951.

Analysis of DNA damage using comet assay: It was done according to Singh *et al.* (1988) with slight modifications. After harvest, roots and leaves were washed three times with redistilled water, blotted dry with filter paper, and placed on ice. All operations were conducted under dim or yellow light to avoid DNA damage. For isolation of nuclei, tissues were placed in 60-mm Petri plates containing 0.2 cm^3 of cold phosphate buffered saline (130 mM NaCl, 7 mM disodium hydrogen phosphate, 3 mM sodium dihydrogen phosphate, and 50 mM Na_2EDTA; pH 7.5). Using a razor blade, the roots and leaves were finely and gently sliced to allow the isolation of nuclei. The isolated nuclei were collected in the buffer. Then the suspension of nuclei was mixed with low melting point (42 °C) agarose to obtain a 1 % (m/v) final concentration, and 0.1 cm^3 was immediately spread onto an observation area of a slide. The slides prepared in triplicates per concentration were immersed in a cold lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM *Trizma* base, and 1 %, v/v, *Triton X-100*; pH 10) and kept at 4 °C overnight. After that, the slides were placed in an alkaline electrophoresis buffer (300 mM sodium hydroxide and 1 mM Na_2EDTA; pH > 13) for 10 min to allow unwinding DNA in a horizontal gel tank (*GeNei*, Bangalore, India) followed by electrophoresis at 4 °C, 26 V and 300 mA for 15 min adjusted by the buffer level in the tank. The slides were neutralized in a 0.4 M Tris-HCl buffer, pH 7.5, for 5 min and finally rinsed in distilled water. Each experiment was repeated twice.

The slides were stained with ethidium bromide (20 µg cm^{-3}) and rinsed in water to wash off excess stain. The slides were scored using an image analysis system (*Kinetic imaging*, *Andor Technology*, Nottingham, UK) attached to a fluorescence microscope (*Leica*, Wetzlar, Germany) equipped with appropriate filters (N2.1). The microscope was connected to a computer through a charge-coupled device (CCD) camera to transport images to a software (*Komet 5.5*) for analysis. The final magnification was 100×. Among the comet parameters, we report the percent of DNA in the tail [tail DNA (%)]. This gave us a clear indication of the extent of DNA damage induced by the NaCl treatment. Images of 75 (25 × 3) cells per concentration were analyzed for both roots and leaves of the mungbean seedlings. Median values of each concentration with respect to the comet parameter were calculated.

Statistical analysis: The experiments were carried out in a completely randomized design with five replicates and repeated two times; each replication comprised a single glass plate containing an average of 25 seeds. Each data point was expressed as mean ± SE of five independent replicates. All data were subjected to the analysis of variance using the *PASW Statistics 18* software (*SPSS Inc.*, Chicago, IL, USA) and the least significant difference (LSD) test (α = 0.05) was used to compare the mean values of the different treatments.

Results

The NaCl treatment caused a significant ($P \leq 0.05$) reduction in root and shoot dry masses (26 and 23 %, respectively) compared to the non-pretreated water control (Table 1). However, when the seeds were pretreated with 50 mM NaCl prior to germination and the treatment with the various concentrations of NaCl, decreases in dry masses were 23 and 19 % (Table 1).

Due to the increasing NaCl concentration, the total glutathione content decreased to about 65 % in roots and slightly increased in shoots of the non-pretreated seedlings in comparison with the control (Table 1). The pretreatment of the mungbean seeds with 50 mM NaCl prior to germination in the different concentrations of NaCl altered the total glutathione content in both roots and shoots of the mungbean seedlings. In the pretreated seedlings the total glutathione content decreased by about

Table 1. The effect of NaCl on dry mass and total glutathione content in roots and shoots of 7-d-old non-pretreated and pretreated (50 mM NaCl) mungbean seedlings. Means ± SE, *n* = 5. Different letters indicate statistically significant differences between treatments at $P \leq 0.05$.

Treatment	NaCl [mM]	Dry mass [mg seedling^{-1}] root	Dry mass [mg seedling^{-1}] shoot	GSH [µmol g^{-1}(f.m.)] root	GSH [µmol g^{-1}(f.m.)] shoot
Non-pretreated	0	3.2 ± 0.003 a	12.3 ± 0.010 a	3.83 ± 0.07 a	6.53 ± 0.03 b
	50	2.7 ± 0.004 ac	10.1 ± 0.002 bc	1.79 ± 0.06 a	6.96 ± 0.07 b
	100	2.3 ± 0.003 ac	9.3 ± 0.003 b	1.28 ± 0.04 a	7.00 ± 0.06 b
	150	2.1 ± 0.003 bc	9.1 ± 0.003 b	0.92 ± 0.04 a	7.19 ± 0.09 b
Pretreated	0	3.0 ± 0.004 ac	11.4 ± 0.005 acd	3.74 ± 0.06 a	6.90 ± 0.09 b
	50	2.8 ± 0.003 ac	10.4 ± 0.004 bd	3.36 ± 0.06 a	6.97 ± 0.03 b
	100	2.4 ± 0.003 ac	9.9 ± 0.003 b	6.39 ± 0.07 a	7.15 ± 0.10 b
	150	2.2 ± 0.004 ac	9.6 ± 0.004 b	7.35 ± 0.08 a	7.33 ± 0.08 b

12 % in 50 mM NaCl and increased by about 67 and 92 % in 100 and 150 mM NaCl, respectively, in roots, whereas in shoots, the total glutathione content slightly increased compared to the non-pretreated plants (Table 1).

In the non-pretreated seedlings, the GR activity in roots showed a significant ($P \leq 0.05$) increase of about 85 % in 50 mM and insignificant one of about 10 % in 100 mM NaCl, but decreased by about 11 % in 150 mM NaCl over the water control, whereas in shoots, the GR activity tended to increase linearly with the increasing NaCl concentration (Fig. 1*A*). In the pretreated seedlings, the GR activity in roots increased, on average of about 69 % in 50 mM, but decreased in 100 and 150 mM NaCl, whereas in shoots, the GR activity further increased significantly ($P \leq 0.05$) of about 205 % on average over the non-pretreated water control (Fig. 1*A*).

The activity of GPx in roots and shoots of the non-pretreated mungbean seedlings significantly ($P \leq 0.05$) increased in an average of about 214 and 144 %, respectively, over the water control, whereas in the pretreated seedlings, the rate of increments in an average were 94 and 111 % respectively, (Fig. 1*B*), but the changes were not always significant.

In the non-pretreated seedlings, the GST activity showed a significant ($P \leq 0.05$) increase over the water control in roots of about 49 and 90 % in 100 and 150 mM NaCl, respectively, and in shoots of about 43 % in 100 mM NaCl. In the pretreated seedlings, the enzyme activity further increased in an average of about 72 and 62 % in roots and shoots, respectively (Fig. 1*C*).

The NaCl treatments induced a dose dependent DNA damage in both roots and leaves of the non-pretreated seedlings. The comet parameter (tail DNA %) significantly ($P \leq 0.05$) increased at 100 and 150 mM NaCl for roots and at 150 mM NaCl for shoots. However, the pretreatment of the mungbean seeds with 50 mM NaCl prior to germination in the different concentrations of NaCl ammeliorated the extent of DNA damage in both roots and leaves (Fig. 2).

Discussion

Reduction in growth is generally observed in plants exposed to salinity stress. This may be partly due to a lower water potential in cells which, in turn, causes stomatal closure and limits CO_2 assimilation. The salt treatments caused a reduction in dry mass of the mungbean seedlings (Table 1). Similar reductions in dry mass were also observed earlier in salt stressed maize (Khodary 2004) and sugar beet (Ghoulam *et al.* 2001). The enhanced growth in the pretreated over non-pretreated seedlings at the lethal dose indicates that the low concentration of the NaCl pretreatment had a stimulative effect on acclimation process. The pretreatment with 50 mM NaCl was also found to be beneficial in improving rice seedling growth under cadmium stress (Ma *et al.* 2013).

The present study shows that the content of GSH as well as the activities of enzymes of GSH metabolism, such as GR, GST, and GPx, altered considerably in response to the salinity stress. GSH plays an active role in protecting membranes against free radical damage (Rennenberg 1995) as it is implicated in the ascorbate/GSH cycle and in the protein thiol-disulphide redox state regulation (De Pinto *et al.* 2000). The GSH content considerably decreased in roots, whereas increased a little in shoots of the NaCl non-pretreated seedlings (Table 1). However, the pretreatment with a sublethal dose of NaCl enhanced the content of GSH in both roots and shoots of the mungbean seedlings over the non-pretreated seedlings (Table 1), which might be important for a detoxification process (Aravind and Prasad 2005). GSH was reported to be an essential component of a thiol pool and plays important roles in oxidative stress control and protection against xenobiotics and heavy metals (Mendoza-Cozatl and Moreno-Sánchez 2006). Also the enzymes of GSH metabolism participate in the H_2O_2-scavenging pathway, thereby maintaining a

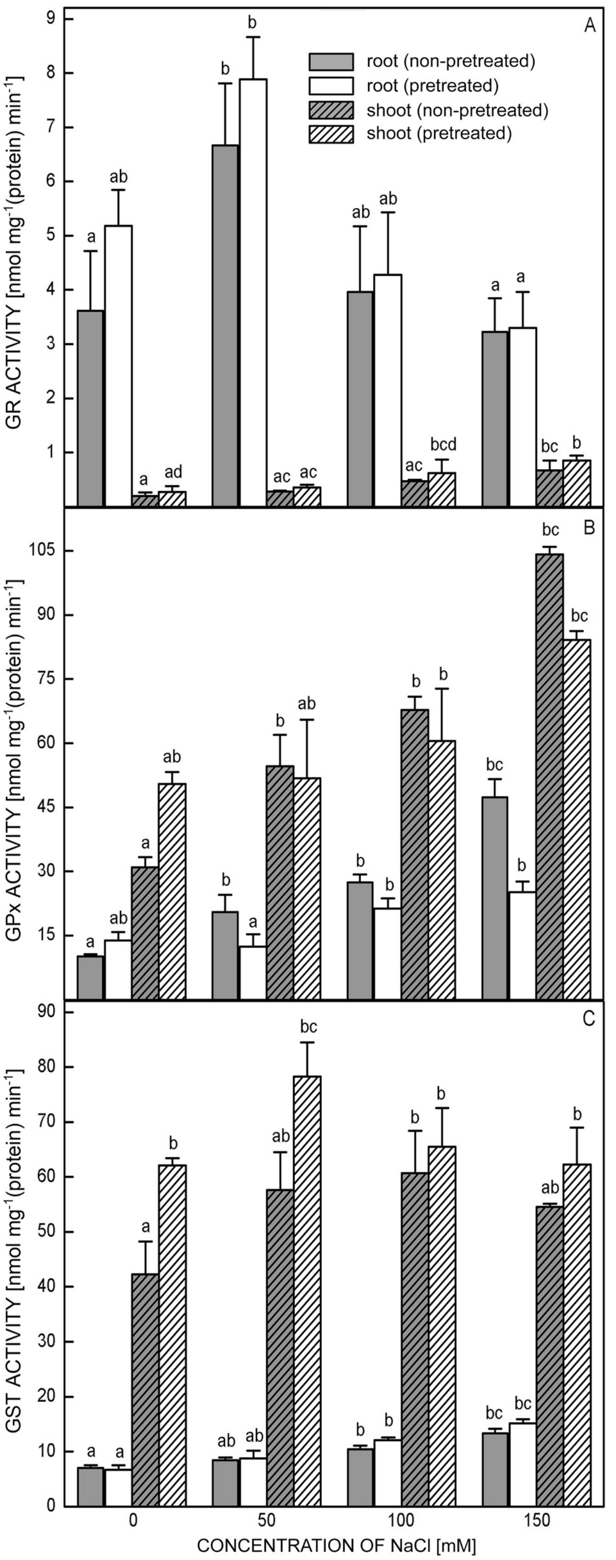

Fig. 1. The effect of NaCl on glutathione reductase (GR; *A*), glutathione peroxidase (GPx; *B*), and glutathione-S-transferase (GST; *C*) activities in roots and shoots of 7-d-old non-pretreated and pretreated mungbean seedlings. Means ± SE, n = 5. Different letters indicate significant differences between the treatments at $P \leq 0.05$.

cellular GSH pool (Rauser 1999).

GR plays an essential role in the defence of cells against ROS and xenobiotics by sustaining the reduced status of GSH (Yoon *et al.* 2005). The activity of GR decreased in roots and increased in shoots under the NaCl treatments in the test seedlings (Fig. 1*A*). GSH is oxidized to GSSG by oxy-radicals generated in the presence of excess NaCl, and GR activity may be a limiting factor for recycling GSH. Both roots and shoots of the pretreated seedlings showed an increased GR activity in comparison with the non-pretreated seedlings (Fig. 1*A*), which helped to maintain a proper ratio of GSSG to GSH. It was reported that a salt tolerant wheat cultivar exhibits a greater GR activity than a sensitive cultivar (Mandhania *et al.* 2006), which may be related to an increased pool of antioxidants acting as scavengers of various ROS (Foyer *et al.* 1997, Panda and Upadhyay 2003).

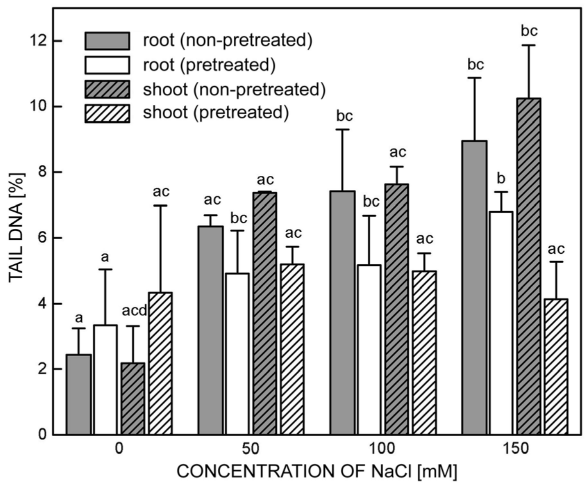

Fig. 2. The effect of NaCl on DNA damage [% tail DNA] in roots and shoots of 7-d-old non-pretreated and pretreated mungbean seedlings. Mean ± SE, n = 3. Different letters indicate significant differences between treatments at $P \leq 0.05$.

H_2O_2 is scavenged by GPx in the cytoplasmic compartment (Drotar *et al.* 1985). It is one of the protective enzymes present in plants when oxidative stress leads to lipid peroxidation. In the mungbean seedlings, the GPx activity increased in the presence of 50, 100, and 150 mM NaCl (Fig. 1*B*). However, the pretreatment of seeds with a sublethal dose of NaCl reduced the activity of GPx in the pretreated seedlings over the non-pretreated ones. H_2O_2 content increases with the increasing concentration of NaCl (Saha *et al.* 2010), which may serve as stimulus for the activation of the enzyme, whereas a decreased content of H_2O_2 in pretreated seedlings (Saha *et al.* 2010) results in a reduced GPx activity. An elevated production of harmful peroxides that catalyze peroxidative chain reactions in the presence of NaCl may be the reason for the activation of the enzyme. Recent studies have shown that in some plants, GPx can reduce peroxides more efficiently when using GSH as reductant (Herbette *et al.* 2002, Tanaka *et al.* 2005). It has been also reported that the activity of GPx in various organisms is affected by different stress conditions (Agrawal *et al.* 2002).

GSTs are ubiquitous enzymes catalyzing the addition of GSH to electrophilic substrates, which tag them for vacuolar sequestration (Edwards *et al.* 2000). GSTs have cytoprotective activities and they are essential for the protection of plants against environmental and biotic stresses (Marrs 1996). GSTs catalyze the conjugation of GSH, which suggests its role in protection against oxidative stress. In the present study, an increase in the GST activity was observed in both roots and shoots of the non-pretreated seedlings, especially in shoots at 100 mM NaCl and in roots at 150 mM NaCl treatments (Fig. 1*C*). From earlier reports, it has been concluded that the GST mediated conjugation of GSH is enhanced under stress conditions to increase plant defence (Jablonkai and Hatzios 1993). However, shoots of the pretreated seedlings tended to have higher GST activities than the non-pretreated seedlings (Fig. 1*C*). It was reported that transgenic tobacco lines which overexpress genes encoding GST and GPx shows a substantial improvement in seed germination and seedling growth under stress (Roxas *et al.* 2000).

The present study shows that DNA damage was induced in response to the salinity stress. The oxidative stress can induce many kinds of negative effects including membrane peroxidation, loss of ions, protein cleavage, and even DNA strand breakages (Collins and Harrington 2002, Mittler 2002). We detected DNA damage using the comet assay which is fast, reliable, and simple (Koppen and Verschaeve 1996). The alkaline version of the comet assay can quantitatively measure DNA damage including single strand breaks, double strand breaks, alkali labile sites (primarily apurinic and apyrimidinic sites), incomplete excision repair sites, and DNA crosslinks (Procházková *et al.* 2013). In the present study, the enhancement of DNA damage was correlated to oxidative stress induced by the NaCl treatments. With the increasing concentration of NaCl, the mungbean seedlings accumulated more ROS (indicated by a higher content of H_2O_2 and MDA; Saha *et al.* 2010), which resulted in a larger DNA damage. Similar results were obtained in *Vicia faba* under cadmium stress (Lin *et al.* 2007). But, the pretreated seedlings showed a decrease in the H_2O_2 and MDA content compared to the non-pretreated seedlings (Saha *et al.* 2010), thus resulting in a lower extent of DNA damage in roots and leaves of the pretreated seedlings compared to the non-pretreated ones (Fig. 2).

Salinity induced oxidative stress accompanied with the accumulation of ROS altering antioxidant scavenging machinery and ascorbate-glutathione cycle. The enhanced accumulation of ROS also resulted in an increased extent of damage to DNA. The pretreatment with a sublethal dose of NaCl ameliorated the injurious effects of the NaCl stress to some extent by improving the GSH content and activities of some enzymes leading to efficient scavenging ROS and also reducing DNA damage. Therefore, plants can acclimate to a lethal salinity by a pretreatment with a sublethal dose of NaCl.

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