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POTASSIUM AND CALCIUM APPLICATION AMELIORATES GROWTH AND OXIDATIVE HOMEOSTASIS IN SALT-STRESSED INDIAN MUSTARD (*BRASSICA JUNCEA*) PLANTS

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Abstract

The effect of potassium (K) and calcium (Ca) on growth and antioxidant defence system of salt-stressed Indian mustard plants was studied. Twenty-day-old Indian mustard plants grown hydroponically in Hoagland's growth medium were randomly divided into five groups. T0 served as control and did not receive any additional K or Ca (except that present in Hoagland solution), T1 received 150 mM NaCl, T2 was given an additional doze of 6 mM K, T3 was given 5.6 mM Ca as additional doze, while as T4 received a combination of 150 mM NaCl + 6 mM K + 5.6 mM Ca. The response of the plants was studied ten days after treatment. Salt stress inhibited growth parameters including biomass, chlorophyll content, protein content and NR activity. Membrane damage was induced by the salt treatment with a concurrent increase in antioxidant defence system and proline content. Individual application of K and Ca mitigated the negative influence of the stress with the maximum alleviating potential exhibited by the combined application of these nutrients. Results obtained on real time expression of genes encoding enzymatic antioxidants (SOD, APX, CAT and GR), NR and proline supported our findings with biochemical assays. We conclude from the study that maintaining high K and Ca levels may serve as an effective means for regulating the growth and productivity of Indian mustard plants under saline conditions.

Key words: Salinity; Antioxidants; *Brassica juncea*; Reactive oxygen species; Gene expression.

Introduction

The burgeoning human population is expected to cross 9.4 billion by the middle of this century (Anon., 2012). Meeting food demands of the expanding human populations that rely mainly on agricultural productivity is a pressing issue of the hour (Ashraf *et al*., 2012; Hakeem *et al*., 2013). Environmental stresses are the main constraints to crop productivity and salt stress causes specific adverse effects on soil and agriculture (Ozturk *et al.,* 2006, 2012; Ashraf *et al.,* 2009; Rengasamy, 2010) particularly in areas with a high evapo-transpiration rate (Tuteja, 2007; Zhang *et al*., 2011). Accumulation of salts in the soil is on the rise due to anthropogenic activities including deforestation, industrialization, overgrazing and improper irrigation practices. Soil salinization is estimated to cause a loss of at least three hectares of productive land per minute at the global level (Abrol *et al*., 1988). Salinity hampers many cellular and physiological processes in plants including nutrient uptake, transport and metabolism, water absorption and photosynthesis, leading to a decrease in growth and productivity (Pardo, 2010; Aref *et al.,* 2013a, b).

One of the primary effects of salt stress is alteration of redox homeostasis. During salt stress, the electrons flowing through transport chains in chloroplasts and mitochondria reduce oxygen, leading to overproduction of reactive oxygen species (ROS) including superoxide ions, hydrogen peroxide and hydroxyl radicals. These ROS cause oxidation of life-supporting biomolecules including lipids, proteins, nucleic acids and carbohydrates by disrupting their structure and associated metabolic pathways (Arshi *et al*., 2012; Anjum *et al.,* 2014).

Besides oxidative damage, salt stress causes deficiency of mineral nutrients in plants by interfering with their availability (in soil), uptake and transport within the plant body (Maathuis, 2006). Potassium is an essential nutrient, which plays indispensable role in growth and development of plants. It is the most abundant cation in plants that is found mainly in chloroplast and cytosol, and regulates the function of more than 50 enzymes through stabilization of pH, binding to enzymes and functional conformation of enzymes. Besides, it actively participates in different cellular and physiological processes including osmotic adjustment, energy transfer, detoxification of ROS, protein synthesis, stomatal regulation, phloem transport, enzyme functioning and cation-anion balance (Marschner, 2012). Regulation of K^+/Na^+ homoeostasis within cells is an important indicator of salt tolerance in plants (Zhu 2003; Siddiqui *et al*., 2008, 2009). Calcium is another essential element that helps in maintaining structural and functional integrity of membranes, stabilization of cell wall and regulation of ion homeostasis (Arshi *et al.,* 2010; Morgan *et al*., 2014). These two elements seem to be readily displaced from binding sites by sodium and chloride ions, and the functional aspects associated with essential nutrients may become critically impaired. Maintaining sufficient concentrations of K and Ca in saline soil helps plants in overcoming specific ion toxicities, particularly in glycophytes, which are more prone to salt damage (Grattan & Grieve, 1999). In order to overcome the negative impact of high salinity, addition of supplemental K and Ca to the growth medium as an ameliorative agent could be necessary. However, studies on regulation of antioxidant activity by supplying K and Ca to the plants are meagre.

Indian mustard, an important oilseed crop, is grown over a large area in India, which ranks second in its production but is still unable to meet the demand (Shah, 2007). The crop is cultivated in the north-west climatic zone, where existent soil salinity heavily decreases its production. Salinity effect on this crop has attracted the attention of many investigators (Nazir *et al.*, 2001; Jamil *et al.,* 2005a, b; Aziz *et al.,* 2011). The current experiment was conducted to investigate and assess the potential of increased levels of P and Ca and their combination in extenuating salt-induced damage in this important oilseed crop.

Materials and Methods

Plant growth and treatment conditions: Authenticated seeds of *Brassica juncea* (L.) Czern and Coss (genotype CS-54) procured from Genetics Division, Indian Agricultural Research Institute, New Delhi, were washed thoroughly with water, surface-sterilized with 0.1% mercuric chloride for 60-90 seconds and washed again with distilled water prior to sowing in pots containing a mixture of sand and vermiculite (1:1). After germination, ten plants were maintained in each pot and the experiment was set in a randomised design. The plants were grown in Hoagland's growth solution of one-fourth strength for the first ten days, in half-strength for next ten days and in full strength for the last ten days, in a growth chamber under controlled conditions of light (16 h photoperiods), temperature (27˚C) and humidity (60%). Twenty-day-old plants were randomly divided into five groups; T0 served as control and did not receive any additional K or Ca, T1 received 150mM NaCl, T2 was given an additional doze of 6mM K, T3 was given 5.6mM Ca as additional doze while as T4 received a combination of 150mM NaCl + 6mM K + 5.6mM Ca. The leaves of 30-day-old plants were excised and used for experimental analysis in three biological replicates.

Biomass accumulation: At the time of harvest, plant weight was recorded before and after the samples were oven-dried at 65˚C ± 2˚C for 72 hours, when they attained constant weight, for the estimation of biomass accumulation.

Estimation of soluble protein content: Bradford's method (1976) was used for the quantification of soluble protein content. Briefly, fresh leaf material (0.5g) was homogenized with the help of pre-cooled mortar and pestle in 0.1 M phosphate buffer with pH 6.8 at 4˚C. The homogenate was transferred to 2 ml tubes and centrifuged at 5000 × g for 10 min at 4˚C. The supernatant was added with an equal amount of chilled 10% TCA for protein precipitation and then centrifuged at 3300 × g for 10 min. The supernatant was discarded, and the resultant pellet washed with acetone and then dissolved in 1 ml of 0.1 N NaOH. To 1.0 ml aliquot, 5.0 ml of Bradford's reagent (50 mL of 90% alcohol, 100 mL of o-phosphoric acid, 850 mL of double-distilled water, 0.1 g of Coomassie Brilliant Blue G-250) was added and vortexed. Tubes were kept in the dark for 10 min for optimal colour development, and the absorbance noted at 595 nm. Soluble protein content was estimated with the help of standard curve, using Bovine serum albumin (Sigma) as standard. The protein content was expressed in mg g^{-1} FW.

Estimation of chlorophyll content: Chlorophyll content in fresh leaf samples was estimated employing the method of Hiscox & Israelstam (1979). Briefly, 0.1g leaves taken in moist filter paper in an icebox, were washed with cold DDW and chopped. The chopped leaf material was transferred to vials (in triplicates) containing 5 ml of dimethyl sulfoxide (DMSO). The vials were kept in oven at 65ºC for 1h for complete leaching of the pigments. DMSO was further added to make a final volume of 10ml and optical density measured immediately. Absorbance of DMSO containing the pigments was noted at 663 and 645 nm using UV-Vis spectrophotometer. Chlorophyll 'a', and chlorophyll 'b' contents were estimated employing the formulae given by Arnon (1949). Total chlorophyll content was calculated by adding chlorophyll 'a' and chlorophyll "b".

Estimation of proline content: Proline content of leaves was estimated employing the method of Bates *et al*. (1973). A 0.5 g of leaf sample was homogenized in 3% sulphosalicylic acid (10ml) followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant (2ml) was taken in test tube and 2 ml of acid ninhydrin along with 2 ml of glacial acetic acid were added. The mixture was incubated at 100˚C in a water bath for one hour and the reaction terminated by placing the tubes in an ice bath. Toluene (4ml) was added to each and mixed vigorously on a vortex for 10-15 sec. The toluene layer was taken from the mixture and absorbance measured at 520 nm using toluene as blank. The concentration of proline in samples was calculated against the standard curve of proline, expressed in µg g^{-1} fresh wt.

NR activity: Nitrate reductase activity was determined using the method of Robin (1979). Fresh leaf samples were homogenized in 100mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA, 7.5 mM cysteine and 1.5% (w/v) casein. The mixture was centrifuged at 10,000 rpm for fifteen minutes at 4˚C. The supernatant was incubated in a reaction buffer containing 100mM potassium phosphate buffer (pH 7.4) 0.15mM NADH, 10mM EDTA and 0.1 M KNO3 for half an hour at 30˚C. The reaction was stopped by adding 1M zinc acetate (100µl). Absorbance of the supernatant was recorded at 540 nm after adding 5.8mM sulfanilamide and 0.8mM NEDD.

Measurement of lipid peroxidation: Lipid peroxidation was estimated by the method of Heath & Packer (1968), using thiobarbituric acid (TBA) as the primary reagent, and measured from the levels of malondialdehyde (MDA), which is a principal constituent of thiobarbituric acid reacting substance (TBARS). The absorbance was recorded at 532nm and 600nm, and the MDA content was calculated by subtracting the absorbance at 600 nm from that at 532 nm, using an extinction coefficient of 155mM^{-1} cm^{-1}. The MDA content was expressed as nmol g^{-1} fresh weight of the sample.

Analysis of enzymatic antioxidants

a. SOD activity was estimated by its ability to inhibit the formation of formazan from NBT according to the method of Beyer & Fridovich (1987). The reduced NBT was measured at 560nm using the absorbance coefficient of $100mM^{-1}$ cm^{-1}. The SOD activity was expressed in enzyme units per mg of protein.

b. APX activity was determined according to the method of Nakano & Asada (1981) in terms of its ability to catalyze the reduction of hydrogen peroxide to water in the presence of 0.1M phosphate buffer. The decrease in the absorbance was taken at 240 nm, and the APX activity was calculated by using an extinction coefficient of $2.8mM^{-1}$ cm^{-1}.

c. Catalase activity was assayed using the method of Aebi (1984). Absorbance was taken at 240nm of the mixture of 0.1ml enzyme extract and 0.1M phosphate buffer both before and after adding 0.1ml of hydrogen peroxide. Catalase activity was calculated using an extinction coefficient of 0.036 mM^{-1} cm^{-1}.

d. GR activity was determined by the method of Foyer & Halliwell (1976) and modified by Rao (1992), and estimated by monitoring the glutathione-dependent oxidation of NADPH at its absorption maxima of wavelength 340nm. The GR activity was calculated using an extinction coefficient of $6.2mM^{-1}$ cm^{-1}.

Whole tissue RNA isolation and gel electrophoresis: Total RNA was isolated from control and stress-treated plant samples, using the modified method of Chomczynski & Sacchi (1987) and quantified with Nanodrop spectrophotometer (Thermo scientific, USA). Total RNA was diluted with DEPC water and aliquots were taken with concentration of 1µg ml^{-1}. To determine the integrity of the RNA and ensure that equal amounts of RNA were added to each reaction, 1µg of RNA from each sample was separated via gel electrophoresis in formaldehyde gels.

Generation of cDNA pool and standardization of reaction: Total RNA (1 µg) from control and treated samples was used for the first-strand cDNA synthesis, using 20 U/µl molony murine leukaemia virus reverse transcriptase (MuMLV) enzyme (Fermentas,USA) at 42°C for 50 min and at 70°C for 10 min. The reverse transcription reaction primed with 2.5 µM Oligo $(dT)_{18}$ (Fermentas,USA) primer in the presence of 10mM dNTPs was mixed in a total volume of 20µl. Before running the real time PCR, conventional PCR was carried out for standardization of reaction. Locus-specific amplification was tested for both the genes-specific and actin primers. PCR amplification was carried out in 20 µl reaction mixture, containing 10 × reaction buffer, 2 mM dNTPs, 1mM $MgCl_2$, 0.35 µM each of the forward and reverse primers, 10µg cDNA template and 5U *Taq* polymerase (Fermentas, USA) using PCR machine (Master Cycler, Eppendorf, USA). The conditions for PCR amplification were as follows: 94°C for 3 min for initial denaturation, followed by 94°C for 20s, 66°C for 1 min and 72°C for 1 min (40 cycles) with 5 min of final extension at 72°C. Amplified PCR products were analysed on 1% Agarose gel. PCR amplified product of genes and actin gene primer pairs were carefully gel eluted and purified, using the QIAGEN gel extraction kit (Qiagen Inc., USA) and sequenced to confirm the specificity of primers. Purified PCR products were commercially sequenced at Integrative DNA Technology (IDT), India. Primer sequences for target genes are given in Table 1.

Real-time PCR (RT-PCR): Real time PCR (Light cycler 480 II, Roche, Germany) was performed in 96 well reaction plate (Roche, Germany) containing 20ul reaction mixture of 10× reaction buffer, 2mM dNTPs, 1mM $MgCl_2$, 0.35µM each of the forward and reverse primers, 1ul syber green (10×), 10 ug cDNA template and 5 unit Taq polymerase. All quantifications were normalized to Actin DNA fragment amplified by β-Actin forward (5□TCGTACCACAGGTGTTG TGTTGGA-3□) and β-Actin reverse (5□TGTATGTGTAGCACGTTCGGTCA-3□) primers. The actin gene was used as an internal control for evaluating the efficiency of real-time PCR for the particular genes. The reaction conditions for real time-PCR were as follows: 95°C for 3 min for initial denaturation, followed by 95°C for 20s, 66°C for 1 min and 72°C for 1 min (40 cycles) with 5 min of final extension at 72°C. The RT- PCR product was resolved on 1.2% agarose gel. The specificity of amplicons was verified by melting curve analysis (60°C to 95°C) after 40 cycles and agarose gel electrophoresis. All reactions were performed in three biological replicates (with three technical replicates of each), using gene-specific primers and actin primer as an internal control.

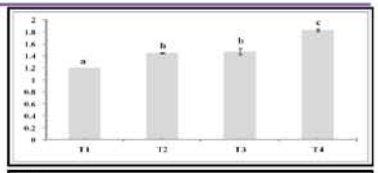
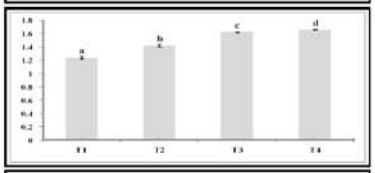
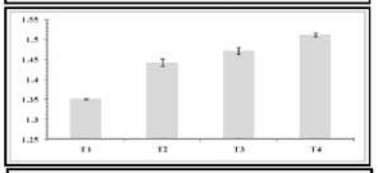
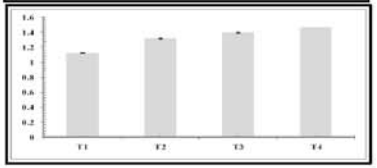
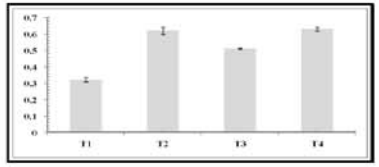
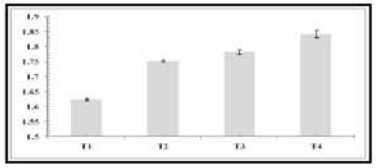
Statistical analysis: Three biological replicates were taken during the study to ensure the reproducibility. The data obtained was statistically analyzed to check the authenticity of results. Means were subjected to Student's *T*-test. ANOVA was performed using Microsoft excel 2007. Post-hoc comparison between the treatment means was done through Tukey-Krammer multiple-comparison test, using Graph Pad Prism software (version 5).

For real time data analysis, ΔΔCt method was used. The data generated from real-time PCR were plotted as the ΔR n fluorescence signal versus the cycle number. The ΔR n was calculated using the equation, $\Delta R\ n = (Rn^+ - Rn^-)$, where Rn^+ is the fluorescence signal of the product at any given time and Rn^- is the fluorescence signal of the baseline emission during cycles 6 to 15. The Ct value is defined as the cycle number at which ΔRn crosses this threshold. The fold change in cDNA (Target gene) relative to the β-actin, an internal control, was determined by ΔΔCt method.

$$\text{Fold change} = 2 - \Delta\Delta Ct$$

where ΔΔCt = (Ct target - Ct actin) at time X - (Ct target - Ct actin) at time 0. Time X is any time point and time 0 represents the 1× expression of each gene.

Table 1. Variation in expression of genes encoding enzymatic antioxidants, proline-synthesizing protein and nitrate reductase in salt-stressed Indian mustard plants due to application of potassium and calcium. Actin gene was used as a reference gene and expression fold was calculated using ΔΔCT method. Values in the graphs are the means of three replicates ± SD. Values with different letters are significantly different from one another at p<0.05.

Gene	NCBI accession number	Forward Primer	Reverse Primer	Expression fold
Cu/Zn -SOD	GI: 1204051	5'-ATGCTGGCGATCTAGGAAAC-3'	5'-AACAGCCCTTCCCACAATAG-3'	
APX	GI: 24421230	5'-GGGAAGATGCCACAAGGATAG-3'	5'-TCAGAGACGAGCTGAAGAAGA-3'	
Catalase	GI: 4336751	5'-GAAGAAGATGCAGTCCGTGTAG-3'	5'-GCTGGATCAATGACCTGGATAA-3'	
GR	GI: 4704610	5'-ACTGGTCGCAAGCCTAATAC-3'	5'-GCCCAGATAGAAGGAACAGATG-3'	
NR	GI: 685274438	5'-TCACGAGGAGATACTGCCTATTA-3'	5'-CTCCACTCTCGTTACCTTCTTC-3'	
Pyrroline-5-carboxylate synthetase	GI: 685364100	5'-CTCACGTTCTCCTCTTCTTCTTC-3'	5'-CATCCTCCATAGCTAGCTTCTTATC-3'	

Results

Biomass accumulation: The biomass accumulation decreased by 1.69 fold during the salt treatment with respect to the control. Application of potassium significantly increased biomass by 1.2 fold compared with salt-stressed plants. A significant upsurge was observed in T3 (1.3 fold) and T4 (1.43 fold) plants, compared to T1 plants (Fig. 1).

Total chlorophyll content: Total chlorophyll content differed substantially between the treatments except between T2 and T3 (Fig. 2); It decreased from 1.1 ± 0.002 mg g^{-1} FW in control to 0.7 ± 0.007 mg g^{-1} FW in T1. Treatments T2 (1.1 fold), T3 (1.1 fold) and T4 (1.29 fold) induced a significant increase in chlorophyll content in contrast to T2.

Protein content of leaves: A pronounced decrease in protein content was noted under salt treatment. Protein content showed a decrease of 1.52 fold in contrast with the control. Application of potassium and calcium, however, resulted in increase of 1.24 and 1.08 fold respectively, with respect to salt-stressed condition. The maximum increase (1.29 fold) was observed during the combined application of both potassium and calcium (Fig. 3).

NR activity of leaves: A considerable decrease in NR activity was recorded in treated samples with respect to control. Among treatments, utmost decrease (1.63 fold) was observed with NaCl treatment. Application of nutrients to NaCl-treated plants increased their NR activity and the maximum increase was observed in T4 (1.53 fold), followed by T2 (1.45 fold) and T3 (1.2 fold), respectively (Fig. 4).

Proline content: Proline, an important amino acid involved in osmotic adjustment, protein stability and other stress adaptations, increased abundantly due to treatments in contrast with the control. Highest levels were recorded in T4 (15.71 ± 2.55) followed by T2 (13.14 ± 2.13), T3 (12.19 ± 1.98) and T1 (11.96 ± 1.94) respectively (Fig. 5). However, variation was not significant between T1 and T2.

MDA content of leaves: MDA content of leaves increased significantly by the treatments; the maximum increase (3.16 fold) was observed in T1 and the minimum (0.872 fold) in T4, compared to control (Fig. 6).

SOD activity: Significant variation in SOD activity was observed under treatment conditions (Fig. 7); There was a considerable enhancement in SOD activities in the leaf tissue by various treatments, with the maximum (1.88 fold) being in T4 and minimum (1.4fold) in T1.

APX activity: Striking differences in APX activities were observed due to treatments (Fig. 8); The APX activity increased by 1.4 fold in T1. It increased further upon treatment of plants with nutrients. The highest activity (1.78 fold) was detected at T4 treatment followed by T2 (1.75 fold) and T3 (1.54 fold).

CAT activity: Salt treatment alone increased the catalase activity by 1.34 fold with respect to the control. Application of K and Ca in the salt-stressed plants ameliorated the catalase activity by 1.5 and 1.7 fold respectively and the maximum activity (1.84 fold) was recorded in salt-stressed plants treated with both K and Ca concurrently (Fig. 9).

GR activity: GR activity showed a linear increase during the treatments (Fig. 10); being the minimum in control plants (2.412 ± 0.005), it increased consistently with NaCl treatment (3.124 ± 0.0412), NaCl + K treatment (3.512 ± 0.014), NaCl + Ca treatment (3.854 ± 0.002), and NaCl + K + Ca treatment (4.111 ± 0.004).

Expression profiling of the enzymes studied: Expression of antioxidants, proline synthesizing protein (pyrroline-5-carboxylate synthetase) and NR at the mRNA level was assayed to check the validity of our results. Although different isoforms of antioxidants are present in the plant cells, the most abundant isoforms related to salt stress was chosen for analysis. Results obtained by the real-time expression studies were parallel to the biochemical assays (Table 1).

Discussion

Salt stress brings about a considerable change in physiological and biochemical processes that play imperative roles in growth, development and productivity of plants. Addition of potassium and calcium in growth medium of salt-stressed Indian mustard plants has helped in restoring the normal growth. Combined effect of K and Ca has proved more effective in combating the adverse effects of salinity than the individual application of these elements.

Biomass accumulation; a significant parameter in agricultural productivity; is one of the primary indicators of plant salt tolerance (Munns & James, 2003; Busemeyer *et al.*, 2013). In Indian mustard, biomass accumulation has decreased substantially under the salt stress. This could be attributed to nutrient deficiency and water stress induced by the salt. An increase in biomass upon fertilization by K and Ca nutrients indicates the differential potential of these nutrients in combating the negative influence of salt. Our results are similar to earlier findings regarding increase in biomass due to K application to salt-stressed plants of sunflower (Akram *et al*., 2009), wheat (Zheng *et al*., 2008), melon (Kaya *et al*., 2007), *Lagenaria siceraria* (Ahmad & Jabeen, 2005) and rice (Cha-um *et al*., 2010). The highest increase in biomass of salt-stressed plants due to combined effect of K and Ca indicates a synergistic effect that minimized the negative effect of NaCl on Indian mustard. Salt stress affected the chlorophyll content.

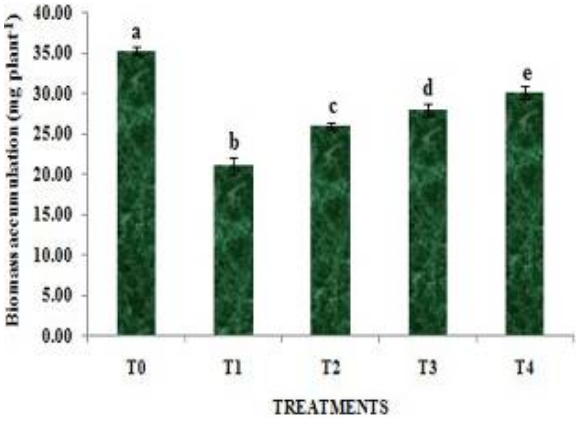


Fig. 1. Variation in biomass accumulation due to salt stress and application of potassium and calcium on salt-stressed Indian mustard plants. Values represent mean ± SD of three replicates. Values marked with different superscript letters are significantly different from one another ($p<0.05$, Tukey).

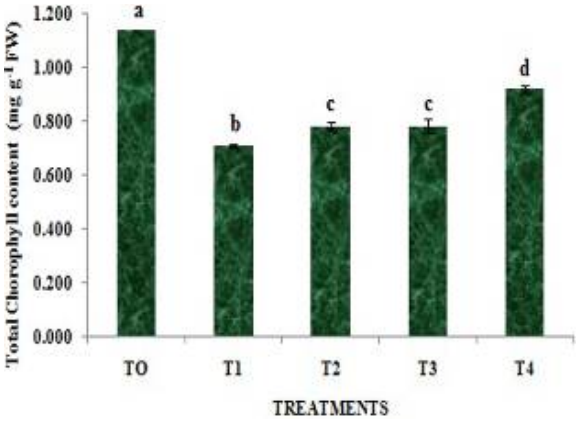


Fig. 2. Changes in total chlorophyll content due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Values represent mean ± SD of three replicates. Values marked by different letters are significantly different ($p<0.05$, Tukey).

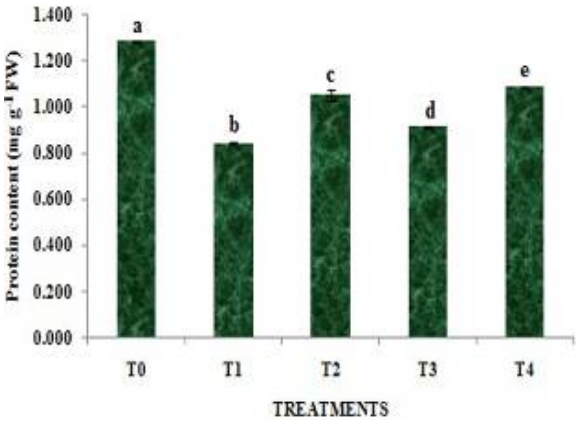


Fig. 3. Variation in the protein content due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Data represent mean ± SD of three replicates. Values with different letters are significantly different ($p<0.05$, Tukey).

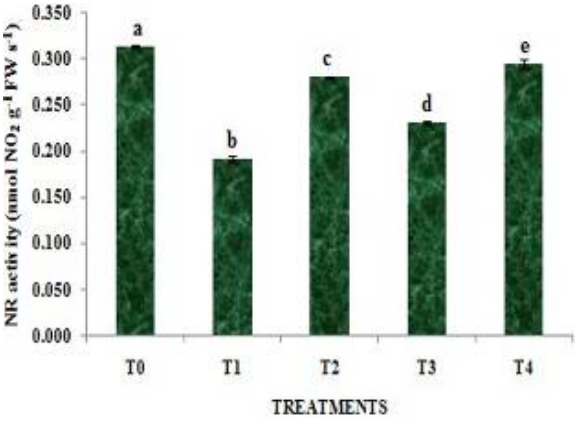


Fig. 4. Variation in the NR activity due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Values represent mean ± SD of three replicates. Values marked with different superscript letters are significantly different ($p<0.05$, Tukey).

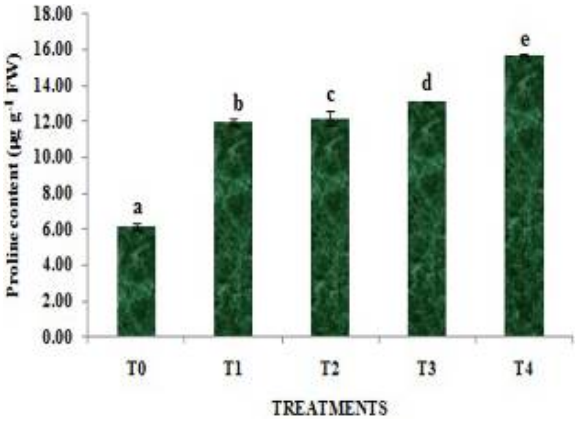


Fig. 5. Changes in proline content due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Values represent mean ± SD of three replicates. Values marked with different letters significantly differ from one another ($p<0.05$, Tukey).

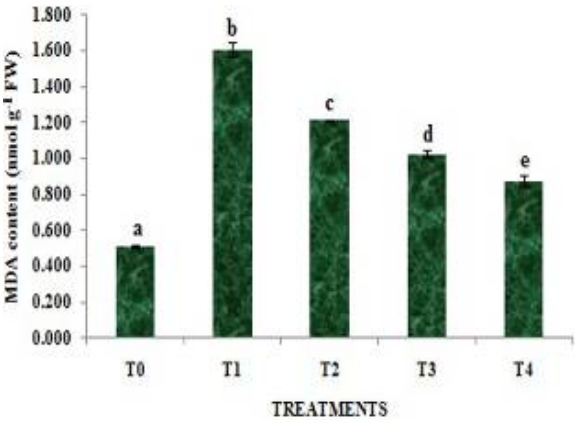


Fig. 6. Variation in MDA content due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Data represent mean ± SD of three replicates. Values with different letters are significantly different ($p<0.05$, Tukey).

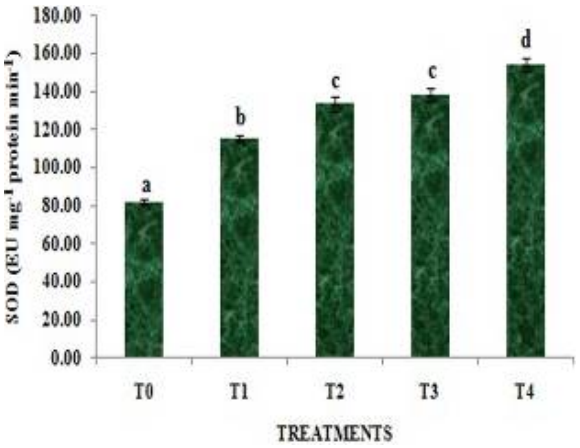


Fig. 7. Variation in the SOD activity due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Values represent mean ± SD of three replicates. Values marked with different superscript letters are significantly different ($p<0.05$, Tukey).

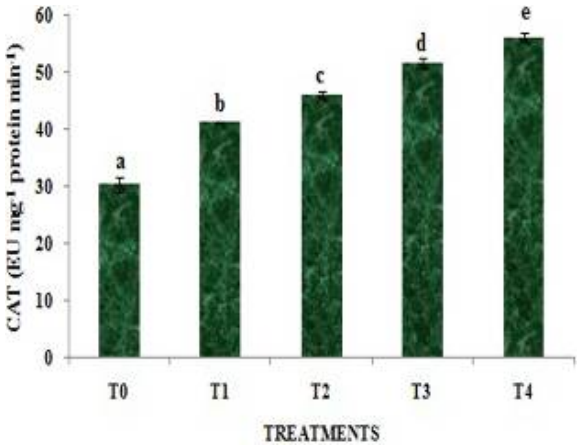


Fig. 9. Variation in the CAT activity due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Values represent mean ± SD of three replicates. Values marked with different superscript letters are significantly different ($p<0.05$, Tukey).

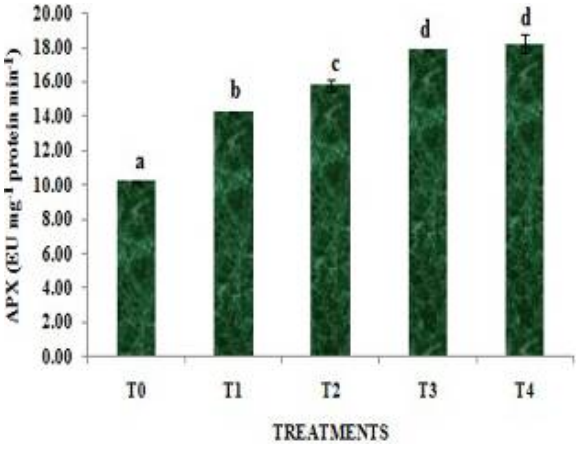


Fig. 8. Variation in APX activity due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Data represent mean ± SD of three replicates. Values marked with different superscript letters are significantly different ($p<0.05$, Tukey).

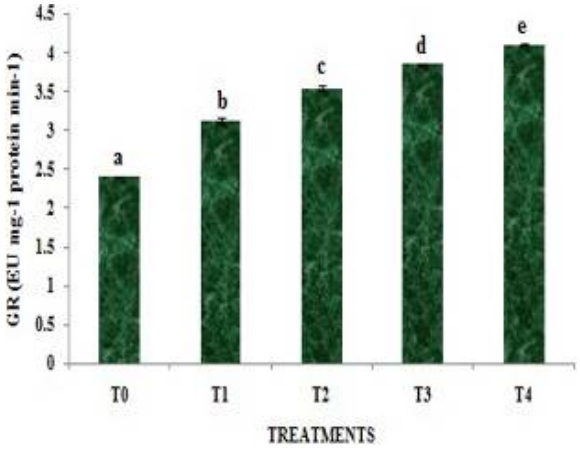


Fig. 10. Changes in GR activity due to salt stress and application of potassium and calcium in salt-stressed Indian mustard plants. Values correspond to the mean ± SD of three replicates. Values marked with different letters are significantly different ($p<0.05$, Tukey).

Decrease in photosynthetic pigments can be associated to (i) the salt-induced deficiency of elements, including magnesium and iron, which play a pivotal role in the synthesis of these pigments and (ii) a probable degradation of chlorophyll (Santos, 2004; Jaleel *et al*., 2008). Our results are supported by earlier findings on pumpkin (Sevengor *et al*., 2011), mustard (Jamil *et al*., 2013), pearl millet (Sneha *et al.,* 2014) and fenugreek (Babar *et al*., 2014). The K and Ca treatments elevated the levels of chlorophyll, suggesting their ability to overcome salt-induced damage of pigments. Potassium helps in regulating the amounts of chlorophyll levels by preventing its decomposition (Umar *et al*., 2011), whereas calcium acts as a secondary messenger in cytokinin-mediated chlorophyll-biosynthetic pathway, besides directly interacting with light during the same pathway (Lechowski & Bialczyk, 1993).

Proteins, the indispensable molecules regarded as determinants of physiological health, decreased in salt-stressed plants, possibly due to inhibition of protein synthesis, as proposed by Kong-Ngern *et al*. (2005). Application of K and Ca fertilizers, however, regained the protein content. K exhibited the highest potential possibly due to its role in triggering the protein synthesis. Similar effect of K treatment (Kaya *et al*., 2007; Zheng *et al*., 2008) and Ca treatment (Manivannan *et al*., 2007; Jaleel *et al.*, 2008; Qados, 2011; Amuthavalli *et al*., 2012) have been observed in some other species.

Salt stress is likely to interfere with nitrate uptake in plants, altering the activity of nitrogen-assimilating enzymes (Grattan & Grieve 1999; Wang *et al.*, 2012). In our study, K and Ca treatments exhibited the potential of reversing the NR activity suppressed by salinity. The maximum NR activity was recorded under the joint effect of these nutrients, signifying an enhanced metabolic

activity related to N assimilation. Potassium was more effective than Ca possibly due to its role in regulating nitrogen-use efficiency of plants.

One of the primary stresses induced by salinity is osmotically-induced water stress (Ozturk *et al.,* 1995; Ashraf & McNeilly, 2004). Plants respond to this condition via synthesis of osmoprotectants that regulate osmotic balance within plants. Proline is one of the main osmoprotectants, which is involved not only in osmotic adjustment but also in antioxidant defense and stabilization of proteins and organelles (Kishore *et al*., 2005; Sumithra *et al*., 2006; Arshi *et al.,* 2010; Anjum *et al*., 2015). In our study, proline levels increased during salinity stress and application of fertilizers further augmented it, possibly by accelerating proline biosynthesis and breaking down proline-rich proteins (Jun *et al.*, 2000). Increased proline levels have been found in salt-stressed plants of *Spinacia oleracea* (Ozturk & Demir, 2003), *Cassia angustifolia* (Arshi *et al*., 2005), *Catharanthus roseus* (Jaleel *et al*., 2007a, b), *Cichorium intybus* (Arshi *et al.*, 2010) and *Artemisia annua* (Qureshi *et al*., 2013), among others.

Lipid peroxidation and antioxidant response: Accumulation of ROS in plants under various environmental stresses including heavy metals, salinity, high temperature and drought stress is well-known (Jabeen *et al*., 2009; Miller *et al*., 2010; Gill & Tuteja, 2010; Anjum *et al.,* 2011; Ansari *et al.,* 2013a,b; Iqbal *et al*., 2015). Salt stress induces divergence of electrons in transport chains from normal pathways to oxygen-reducing ones, leading to overproduction of ROS which oxidize biomolecules including lipids, proteins, nucleic acids and carbohydrates (Anjum *et al*., 2014). Membranes, composed of mainly lipids and proteins, are thus damaged. Lipid peroxidation of membranes by ROS produces aldehydes, of which malondialdehyde is the main type. MDA thus serves as a marker of membrane damage induced by ROS (Hernández & Almansa, 2002; Anjum *et al*., 2014). During our study, salt treatment increased MDA level but application of K and Ca lowered it, signifying their involvement in reducing the production of ROS. This is possibly due to accumulation of ROS-scavenging molecules, including proline and antioxidants that limit lipid-peroxidation-associated membrane damage under salinity stress (Khan *et al*., 2007; Qureshi *et al*., 2013). Our results agree with earlier findings of Siddiqui *et al*. (2012), who observed reduced levels of MDA in broad bean under the effect of K and Ca. At individual level, Ca was more effective than K, possibly because of its specific role in maintaining structural integrity of cellular membranes by linking to lipid-bilayer-stabilizing phospholipids (Hirschi, 2004).

Plants possess a distinct antioxidant defense system that scavenges toxic ROS and protects plants from oxidative damage. The antioxidants, including SOD, APX, GR and CAT act through a well-defined glutathione-ascorbate pathway (Anjum *et al*., 2012, 2015). SOD forms the first line of defence in catalyzing the dismutation of superoxide ion to hydrogen peroxide. In our study salt treatment increased SOD activity, but the increase was more pronounced in salt-stressed plants treated with P and Ca, indicating their potential in conferring superoxide ion-scavenging ability. SOD activity produces hydrogen peroxide, which can cause membrane damage. Plants possess two enzymes; catalase and APX for the regulation of H_2O_2 level in cells. Catalase is mainly located in peroxisomes where it catalyzes breakdown of H_2O_2 to H_2O and O_2 (Arshi *et al*., 2012). Salinity induces upregulation of CAT activities in Indian mustard, as observed earlier in barley (Khosravinejad *et al*., 2008; Seckin *et al*., 2010), tomato (Rodriguez-Rosales *et al*., 1999), mulberry (Sudhakar *et al*., 2001), ryegrass (Hu *et al*., 2012), soybean (Hakeem *et al*., 2012) and sweet annie (Qureshi *et al*., 2013). The CAT activity was ameliorated differentially with the addition of K and Ca, indicating the dependence of CAT activity on the nutrient state of the plant. The analogous function of CAT is carried out by APX in the cellular sites including chloroplasts, mitochondria and cytosol. Increased activity of APX in salt-stressed plants indicated the presence of high levels of H_2O_2; the enhanced activity observed under K and Ca treatments suggest their roles in maintaining ROS level. GR is another antioxidant, which catalyzes the reduction of glutathione disulphide to sulphydryl form GSH, providing reducing potential to APX for scavenging H_2O_2 (Anjum *et al*., 2012; Yousuf *et al*., 2012). The changes in GR activities observed in our study paralleled those in APX activity. Similar observations with reference to potassium fertilization in plants under abiotic stress, including water stress (Jaleel *et al*., 2007a; Hong-Bo *et al*., 2008), metal stress (Siddiqui *et al.,* 2012) and salt stress (Umar *et al*., 2011), have been reviewed by Wang *et al*. (2013).

Conclusion

Our data establish that salt stress reduced the growth of Indian mustard, whereas application of K and Ca to growth environment differentially mitigated the adverse effect of salinity. The combined application of K and Ca showed the maximal mitigating potential to regulate the growth and productivity of the crop by reducing the membrane damage via induction of antioxidants, increase in proline content and regulation of NR activity and the level of chlorophyll and protein contents.

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References

Abrol, I.P., J.S.P. Yadav and F.I. Massoud. 1988. Salt affected soils and their management. *FAO Soils Bulletin*, 39.

Aebi, H. 1984. Catalase in vitro. *Methods Enzymol.,* 105: 121-126.

Ahmad, R. and R. Jabeen. 2005. Foliar spray of mineral elements antagonistic to sodium-a technique to induce salt tolerance in plants growing under saline conditions. *Pak. J. Bot*., 37: 913-920.

Akram, M.S., M. Ashraf and N.A. Akram. 2009. Effectiveness of potassium sulfate in mitigating salt-induced adverse effects on different physio-biochemical attributes in sunflower (*Helianthus annuus* L.). *Flora*, 204: 471-483.

Amuthavalli, P., D. Anbu and S. Sivasankaramoorthy. 2012. Effect of calcium chloride on growth and biochemical constituents of cotton (*Gossypium hirsutum* L.) under salt stress. *Int. J. Res. Bot.,* 2(3): 9-12.

Anjum, N.A., A. Sofo, A. Scopa, A. Roychoudhury, S.S. Gill, M. Iqbal, A.S. Lukatkin, E. Pereira, A.C. Duarte and I. Ahmad. 2015. Lipids and proteins-major targets of oxidative modifications in abiotic stressed plants. *Environ. Sci. Pollut. Res*., 22(6): 4099-4121. doi: 10.1007/s11356-014-3917-1.

Anjum, N.A., I. Ahmad, I. Mohmood, M. Pacheco, A.C. Duarte, E. Pereira, S. Umar, A. Ahmad, N.A. Khan, M. Iqbal and M.N.V. Prasad. 2012. Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids—A review. *Environ. Exp. Bot*., 75: 307-324.

Anjum, N.A., I.M. Aref, A.C. Duarte, E. Pereira, I. Ahmad and M. Iqbal. 2014. Glutathione and proline can coordinately make plants withstand the joint attack of metal (loid) and salinity stresses. *Front. Plant Sci*., 5: 662. doi: 10.3389/fpls.2014.00662.

Anjum, N.A., S. Umar, M. Iqbal and N.A. Khan. 2011. Cadmium causes oxidative stress in mungbean by affecting antioxidant enzyme system and ascorbate-glutathione cycle metabolism. *Russ. J. Plant Physiol*., 58(1): 92-99.

Anonymous. 2012. International Data Base: Total Mid-Year Population for the World (1950-2050). United States Census Bureau (www.census.gov).

Ansari, M.K.A., B.-H. Shao, S. Umar, A. Ahmad, S.H. Ansari, M. Iqbal and G. Owens. 2013a. Screening Indian mustard genotypes for phytoremediating arsenic-contaminated soils. *Clean Soil Air Water* 41(2): 195-201.

Ansari, M.K.A., E. Oztetik, A. Ahmad, S. Umar, M. Iqbal and G. Owens. 2013b. Identification of the phytoremediation potential of Indian mustard genotypes for copper, evaluated from a hydroponic experiment. *Clean Soil Air Water*, 41(8): 789-796

Aref, M.I., A.I. Ahmed, P.R. Khan, H. El-Atta and M. Iqbal. 2013a. Drought-induced adaptive changes in the seedling anatomy of *Acacia ehrenbergiana* and *Acacia tortilis* subsp *raddiana*. *Trees Struct. Func*., 27: 959-971.

Aref, M.I., H. El-Atta, M. El-Obeid, A.I. Ahmed, P.R. Khan and M. Iqbal. 2013b. Effect of water stress on relative water and chlorophyll contents of *Juniperus procera* Hochst. ex Endlicher in Saudi Arabia. *Life Sci. J*., 10(4): 681-685.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. *Plant Physiol.,* 24: 1-15.

Arshi, A., A. Ahmad, I.M. Aref and M. Iqbal. 2010. Effect of calcium against salinity-induced inhibition in growth, ion accumulation and proline contents in *Cichorium intybus* L. *J. Environ. Biol.,* 31(6): 939-944.

Arshi, A., A. Ahmad, I.M. Aref and M. Iqbal. 2012. Comparative studies on antioxidant enzyme action and ion accumulation in soybean cultivars under salinity stress. *J. Environ. Biol.* 33: 9-20.

Arshi, A., M.Z. Abdin and M. Iqbal. 2005. Ameliorative effect of $CaCl_2$ on growth, ionic relations and proline content of senna under salinity stress. *J. Plant Nutr*., 28: 101-125 .

Ashraf, M. and T. McNeilly. 2004. Salinity tolerance in *Brassica* oilseeds. *Critical Reviews in Plant Sciences*, 23(2): 1-18.

Ashraf, M., M. Ozturk and H.R. Athar. (Eds.) 2009. *Salinity and Water Stress: Improving Crop Efficiency*. (Tasks for Vegetation Science 44). Springer Science+Business Media, NY, 244 pp.

Ashraf, M., M. Ozturk, M.S.A. Ahmad and A. Aksoy (Eds.). 2012. *Crop Production for Agricultural Improvement*. Springer Science + Business Media, NY, 796 pp.

Available online: http://www.census.gov/population/ international/ data/idb/worldpoptotal.

Aziz,T., Rahmatullah, M.A. Maqsood, M. Sabir and S. Kanwal. 2011. Categorization of *Brassica* cultivars for phosphorus acquisition from phosphate rock on the basis of growth and ionic parameters. *J. Plant Nutr.*, 34: 522-533.

Babar, S., E.H. Siddiqi, I. Hussain, K.H. Bhatti and R. Rasheed. 2014. Mitigating the effects of salinity by foliar application of salicylic acid in fenugreek. *Physiol. J*., 2014, 869058. doi:10.1155/2014/869058.

Bates, L.S., R.P. Waldren and J.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.

Beyer, W.F. and I. Fridovich. 1987. Assaying for superoxide disniutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.*, 161: 559-566.

Bradford, M.M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72: 248-254.

Busemeyer, L., A. Ruckelshausen, K. Moller, A.E. Melchinger, K.V. Alheit, H.P. Maurer, V. Hahn, E.A. Weissmann, J.C. Reif and T. Wurschum. 2013. Precision phenotyping of biomass accumulation in triticale reveals temporal genetic patterns of regulation. *Sci. Reports*, 3: 2442 doi: 10.1038/srep02442.

Cha-um, S., K. Siringam, N. Juntawong and C. Kirdmanee. 2010. Water relations, pigment stabilization, photosynthetic abilities and growth improvement in salt stressed rice plants treated with exogenous potassium nitrate application. *Int. J. Plant Prod.*, 4(3): 1735-8043.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem*., 162: 156-159.

Foyer, C.H. and B. Halliwell. 1976. The presence of glutathione and glutathione reductase in chloroplasts. A proposed role in ascorbic acid metabolism. *Planta*, 133: 21-25.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem*., 48: 909-930.

Grattan, S.R. and C.M. Grieve. 1999. Salinity-mineral nutrient relations in horticultural crops. *Sci. Hort*., 78: 127-157.

Hakeem, K.R., A. Parvaiz and M. Ozturk (Eds.). 2013. *Crop Improvement-New Approaches and Modern Techniques*. XXVII, Springer Science+Business Media, NY, 493 pp.

Hakeem, K.R., F. Khan, R. Chandna, T.O. Siddiqui and M. Iqbal. 2012. Genotypic variability among soybean genotypes under NaCl stress and proteome analysis of salt-tolerant genotype. *Appl. Biochem. Biotechnol*., 168(8): 2309-2329.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*., 125: 189-198.

Hernández, J.A. and M.S. Almansa MS. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of leaves. *Physiol. Plant*., 115: 251-257.

Hirschi, K.D. 2004. The calcium conundrum. Both versatile nutrient and specific signal. *Plant Physiol*., 136: 2438-2442.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot*., 57: 1332-1334.

Hong-Bo, S., L.Y. Chu and M.A. Shao. 2008 Calcium as a versatile plant signal transducer under soil water stress. *BioEssays*, 30: 634-641.

Hu, L., T. Hu, Z. Xunzhong, P. Huancheng and F. Jinmin. 2012. Exogenous glycine betaine ameliorates the adverse effect of salt stress on perennial ryegrass. *J. Amer. Soc. Hort. Sci.,* 137(1): 38-46.

Iqbal, M., A. Ahmad, M.K.A. Ansari, M.I. Qureshi, M.I. Aref, P.R. Khan, S.S. Hegazy, H. El-Atta, A. Husen and K.R. Hakeem 2015. Improving the phytoextraction capacity of plants to scavenge metal(loid)-contaminated sites. *Environ. Rev*., 23(1): 44-65.

Jabeen, R., A. Ahmad and M. Iqbal 2009. Phytoremediation of heavy metals: Physiological and molecular aspects. *Bot. Rev*., 75: 339-364.

Jaleel, C.A., P. Manivannan, A. Kishorekumar, B. Sankar and R. Panneerselvam. 2007b. Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. *C. R. Biol*., 330(9): 674-683.

Jaleel, C.A., P. Manivannan, B. Sankar, A. Kishorekumar, R. Gopi, R. Somasundaram and R. Panneerselvam. 2007a. Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf. B.*, 60: 110-116.

Jaleel, C.A., R. Gopi, M. Gomathinayagam and R. Panneerselvam. 2008. Effects of calcium chloride on metabolism of salt-stressed *Dioscorea rotundata*. *Acta Biol. Cracov. Ser. Bot*., 50(1): 63-67.

Jamil, M. and E.S. Rha. 2013. NaCl stress-induced reduction in growth, photosynthesis and protein in mustard. *J. Agri. Sci*., 5(9): 114-127.

Jamil, M., D.B. Lee, J.D. Kim, K.Y. Jung, M. Ashraf, S. Rehman, S.C. Lee and E.S. Rha. 2005a.The response of *Brassica* species against NaCl stress during germination and seedling growth. *Korean J. Intl. Agri*., 17(1): 4 3-47.

Jamil, M., S.C. Lee, S.R. Rehman, D.B. Lee, M. Ashraf and E.S. Rha. 2005b. Salinity (NaCl) tolerance of *Brassica* species at germination and early seedling growth. *EJEAFChe*., 4(4): 970-976.

Jun, H.R., L.H. Adam, K.L. Rozwadowski, J.L. Hammerlineli, W.A. Keller and G. Selvaraj. 2000. Genetic engineering of glycine betaine production towards enhancing stress tolerance in plants. *Plant Physiol*., 12: 47-756.

Kaya, C., A.L. Tuna, M. Ashraf and H. Altunlu. 2007. Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. *Environ. Exp. Bot*., 60: 397-403.

Khan, M.N., M.H. Siddiqui, F. Mohammad, M.M.A. Khan and M. Naeem. 2007. Salinity induced changes in growth, enzyme activities, photosynthesis, proline accumulation and yield in linseed genotypes. *World J. Agri. Sci*., 3: 685-695.

Khosravinejad, F., R. Heydari and T. Farboodnia. 2008. Antioxidant responses of two barley varieties to saline stress. *Res. J. Biol. Sci*., 3: 486-90.

Kishore, K.P.B., S. Sangam, R.N. Amrutha, K.R. Srilaxmi, K.R.S.S. Naidu, S. Rao, K.J. Reddy, P. Theriappan and N. Sreenivasulu. 2005. Regulation of proline biosynthesis, degradation, uptake and transport of higher plants: Its implication in plant growth and abiotic stress tolerance. *Curr. Sci*., 88: 424-438.

Kong-Ngern, K., S. Daduang, C.H. Wongkham, S. Bunnag, M. Kosittrakuna and P. Theerakulpisuta. 2005. Protein profiles in response to salt stress in leaf sheaths of rice seedlings. *Sci. Asia*, 31: 403-408.

Lechowski, Z. and J. Bialczyk. 1993. Calcium mediated cytokinin action on chlorophyll synthesis in isolated embryo of Scots pine. *Biol. Plant*., 35: 53-62.

Maathuis, F.J.M. 2006. The role of monovalent cation transporters in plant responses to salinity. *J. Exp. Bot*., 57: 1137-1147.

Manivannan, P., C.A. Jaleel, B. Sankar, R. Somasundaram, P.V. Murali, R. Sridharan and R. Panneerselvam. 2007. Salt stress mitigation by calcium chloride in *Vigna radiata* (L.) Wilczek. *Acta Biol. Cracov. Ser. Bot*., 49(2): 105-109.

Marschner, P. 2012. *Marschner's Mineral Nutrition of Higher Plants*. (3rd ed) Academic Press, London, UK, pp. 178-189.

Mestre, T.C., F. Garcia-Sanchez, F. Rubio, V. Martinez and R.M. Rivero. 2012. Glutathione homeostasis as an important and novel factor controlling blossom-end rot development in calcium-deficient tomato fruits. *J. Plant Physiol*., 169: 1719-1727.

Miller, G., N. Suzuki, S.C. Yilmaz and R. Mittler. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ*., 33: 453-467.

Morgan, S.H., P.J. Maity, C.-M. Geilfus, S. Lindberg and K.H. Muhling. 2014. Leaf ion homeostasis and plasma membrane H^+- ATPase activity in *Vicia faba* change after extra calcium and potassium supply under salinity. *Plant Physiol. Biochem*., 82: 244-253.

Munns and James. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil,* 253: 201-218.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*., 22: 867-880.

Nazir, N., M. Ashraf and E. Rasul. 2001. Genomic relationships in oilseed *Brassicas* with respect to salt tolerance-photosynthetic capacity and ion relations. *Pak. J. Bot. (Special Issue)*, 33: 483-501.

Ozturk, L. and Y. Demir. 2003. Effects of putrescine and ethephon on some oxidative stress enzyme activities and proline content in salt stressed Spinach leaves. *Plant Growth Regul*., 40: 89-95.

Ozturk, M., A.R. Memon, S. Gucel and M.S. Sakcali. 2012. Brassicas in Turkey and their possible role in the phytoremediation of degraded habitats. In: *The Plant Family Brassicaceae: Contribution Towards Phytoremediation*. (Eds.): N.A. Anjum *et al*., Springer Verlag, Environmental Pollution Book Series 21, NY, USA, 265-288 pp.

Ozturk, M., F. Ozdemir, F., B. Eser, O.I. Adiyahsi and H. Ilbi. 1995. Studies on the salt-hormone interactions in the germination and seedling growth of some vegetable species. In: *Biology of Salt Tolerant Plants*, (Eds.): M.A. Khan. & I.A. Ungar. Michigan: Book Crafters, 59-64 pp.

Ozturk, M., Y. Waisel, M.A. Khan and G. Gork (Eds.). 2006. *Biosaline agriculture and salinity tolerance in plants*. Birkhauser Verlag (Springer Science), Basel, 205 pp.

Pardo, J.M. 2010. Biotechnology of water and salinity stress tolerance. *Cur. Op. Biotech*., 21: 185-196.

Qados, A.M.S.A. 2011. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* L. *J. Saudi Soc. Agric. Sci*., 10: 7-15.

Qureshi, M.I., M.Z. Abdin, J. Ahmad and M. Iqbal. 2013. Effect of long-term salinity on cellular antioxidants, compatible solute and fatty acid profile of Sweet annie (*Artemisia annua* L.). *Phytochemistry*, 95: 215-223.

Rao. 1992. Cellular detoxifying mechanisms determine age dependent injury in tropical plants exposed to SO. *J. Plant Physiol*., 140: 733-740.

Rengasamy, P. 2010. Soil processes affecting crop production in salt-affected soils. *Funct. Plant Biol*., 37l: 613-620.

Robin, P. 1979. Etude de quelques conditions d'extraction de la nitrate réductase des racines et des feuilles de maïs. *Physiol. Veg.*, 77: 56-66.

Rodriguez-Rosales, M.P., L. Kerkeb, P. Bueno and J.P. Donaire. 1999. Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane H^+-ATPase and antioxidant enzyme activities of tomato (*Lycopersicon esculentum* Mill.) calli. *Plant Sci*., 143: 143-50.

Santos, C.V. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci. Hort*., 103: 93-99.

Seckin, B., I. Turkan, A.H. Sekmen and C. Ozfidan. 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barley grass) and *Hordeum vulgare* L. (cultivated barley). *Environ. Exp. Bot.*, 69: 76-85.

Sevengor, S., F. Yasar, S. Kusvuran and S. Ellialtioglu. 2011. The effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling. *African J. Agr. Res.*, 6(21): 4920-4924.

Shah, S.H. 2007. Effects of salt stress on mustard as affected by gibberellic acid application. Gen. *App. Plant Physiol.*, 33: 97-106.

Siddiqui, M.H., F. Mohammad and M.N. Khan. 2009. Morphological and physio-biochemical characterization of *Brassica juncea* L. Czern. & Coss. genotypes under salt stress. *J. Plant Interact.*, 4: 67-80.

Siddiqui, M.H., M.H. Al-Whaibi, A.M. Sakran, M.O. Basalah and H.M. Ali. 2012. Effect of calcium and potassium on antioxidant system of *Vicia faba* L., under cadmium stress. *Int. J. Mol. Sci.*, 13: 6604-6619.

Siddiqui, M.H., M.N. Khan, F. Mohammad and M.M.A. Khan. 2008. Role of nitrogen and gibberellin (GA3) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. *J. Agron. Crop Sci.*, 194: 214-224.

Sneha, S., R. Anirudha and C. Subhash 2014. Effect of short term salt stress on chlorophyll content, protein and activities of catalase and ascorbate peroxidase enzymes in pearl millet. *Am. J. Plant Physiol.*, 9(1): 32-37.

Sudhakar, C, A. Lakshmi and S. Giridarakumar. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.*, 161: 613-9.

Sumithra, K., P.P. Jutur, B.D. Carmel and A.R. Reddy. 2006. Salinity-induced changes in two cultivars of *Vigna radiata*: Responses of antioxidative and proline metabolism. *Plant Growth Regul.*, 50: 11-22 .

Tuteja, N. 2007. Mechanisms of high salinity tolerance in plants. *Methods Enzymol.*, 428: 419-438.

Umar, S., I. Diva, N.A. Anjum, M. Iqbal, I. Ahmad and E. Pereira. 2011. Potassium-induced alleviation of salinity stress in *Brassica campestris* L. *Cent. Eur. J. Biol.*, 6(6): 1054-1063.

Wang, H., M. Zhang, R. Guo, D. Shi, B. Liu, X. Lin and C. Yang. 2012. Effects of slat stress on ion balance and nitrogen metabolism of old and young leaves in rice (*Oryza sativa* L.). *BMC Plant Biol.*, 12: 194.

Wang, M., Q. Zheng, Q. Shen and S. Guo. 2013. The critical role of potassium in plant stress response. *Int. J. Mol. Sci.*, 14: 7370-7390.

Yousuf, P.Y., K.U.R. Hakeem, R. Chandna and P. Ahmad. 2012. Role of glutathione reductase in plant abiotic stress. In: *Abiotic Stress Responses in Plants*. (Eds.): Ahmad, P. and M.N.V. Prasad, Springer, New York, pp. 149-158.

Zhang, K., N. Guo, L. Lian, J. Wang, S. Lv and J. Zhang. 2011. Improved salt tolerance and seed cotton yield in cotton (*Gossypium hirsutum* L.) by transformation with betA gene for glycinebetaine synthesis. *Euphytica*, 181: 1-16.

Zheng, Y., A. Jia, T. Ning, J. Xu, Z. Li and G. Jiang. 2008. Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance. *J. Plant Physiol.*, 165: 1455-1465.

Zhu, J.K. 2003. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.*, 6: 441-445.

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