Mitigation effect of exogenous nitric oxide (NO) on some metabolic compounds of maize seedling grown under salt stress

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Abstract. Salt stress is considered as a major limiting factor for plant growth and crop productivity. The present study was conducted to investigate whether using nitric oxide (NO) molecule could alleviate the adverse effects of salt stress in maize (Zea mays L.) seedling. Sodium nitroprusside (SNP) was used at 60 µM concentration, as NO donor in the nutrient solution of maize seedlings grown with three concentrations of NaCl (0.0, 150 and 200 mM). Leaf samples were collected on the 7th and 15th day after NaCl treatment. Chlorophyll contents and lipid peroxidation gave different values under salt stress. The NO treated seedling showed high content of proline, phenolic and flavonoid. Nitric oxide induced an increase in antioxidant enzymes including peroxidase (POD) and catalase (CAT) activities. These data indicated that the exogenous NO application is useful way to mitigate the salinity-induced oxidative stress in maize seedling.

Keywords: Antioxidant enzymes, proline, malondialdehyde, salt stress, signaling messenger

Introduction:

Salt stress as a major adverse factor can lower leaf water potential, leading to reduced turgor and some other responses, and ultimately decrease crop productivity in arid regions. When a plant is exposed to high salinity stress, its major processes such as photosynthesis, protein synthesis, and lipid metabolism are adversely affected. High concentrations of salts lead to damage at the molecular level, arrested growth, and even death (Implay, 2003). Under salinity conditions, tolerant plants typically maintain low sodium (Na) in the cytosol of cells (Jeschke, 1984). The control of Na+ movement across the plasma-membrane and tonoplast to maintain a low Na+ concentration in the cytosol is a key factor to the cell adaptation to salt stress (James et al., 2011). Another common biochemical change occurring when plants are exposed to salt stress is the accumulation of reactive oxygen species (ROS), which unbalances the cellular redox in favor of oxidized forms, thereby creating oxidative stress that can damage DNA, inactivate enzymes and cause lipid peroxidation (Manaa et al., 2013). The
antioxidative enzymatic system includes superoxide dismutases (SOD), catalase (CAT) and peroxidases (PODs) which are involved in the detoxification of superoxide radicals such as \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), \( \text{OH}^- \) as mentioned by (Anjum et al., 2016). Additionally, ascorbate peroxidase and glutathione reductase (GR), as well as reduced glutathione (GSH) are main components responsible for the removal of \( \text{H}_2\text{O}_2 \) in different cellular compartments (Foyer and Noctor, 2011). All these antioxidant components must work coordinately to provide a successful response against oxidative stress. Accordingly, the regulation of these antioxidant constituents by an exogenous substance might mediate the plant tolerance to salt stress. A close correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in cucumber plant (El-Baz et al., 2003). One mechanism utilized by the plants to overcome the salt stress effects might be via accumulation of compatible osmolytes, such as proline (Serraj and Sinclair, 2002). Exogenous application of some antioxidants such as alpha tocopherol, enhanced onion tolerance ability to salt stress (Mohamed and Aly, 2008). Reports have demonstrated that the application of exogenous nitric oxide (NO) provides tolerance to salt stress conditions (Misra et al., 2014). Nitric oxide (NO) is one of the most studied bioactive molecules, due to its involvement in a wide spectrum of physiological processes from bacteria to human. Nitric oxide has been reported to alleviate the oxidative stress in plants generated by abiotic stresses (Crawford and Guo, 2005). In higher plants, NO has an important function in plant growth and development, in processes including seed germination, primary and lateral root growth, flowering, pollen tube growth regulation, senescence, defense response and abiotic stresses, and NO is also a key signaling molecule in different intracellular processes (Corpas et al., 2011). However, the effects of NO application during salt stress on morphological, physiological and biochemical performance of maize are poorly understood. Thus, the present research was aimed to evaluate the role of exogenous NO application in different salt stress regimes of maize seedling.

Material and methods

**Growth condition and NaCl treatments**

Seeds of maize (Zea mays L.) were purchased from JK SURBHI GOLD, (JKMH-1701), Jharkhand state, India. The seeds were surface sterilized for 5 min in 0.5% sodium hypochlorite (NaOCl) solution to prevent fungal infections and then washed three times with distilled water. The experiment was carried out in Central University of Jharkhand, Brambe, Ranchi, India, in a growth chamber (Qiushi, China). Plants were grown under continuous light intensity of photosynthetic photon flux density of 350 µmol photons m\(^{-2}\)s\(^{-1}\), supplied by fluorescent lamps, day/night temperatures of 25/20 \(^\circ\)C, and relative humidity of 70%. Seeds were germinated on filter paper moistened with deionized water for 4 days. Following germination, seedlings were transferred to plastic pots filled with 4 L of nutrient solution, containing 5 mM KNO\(_3\), 3 mM Ca(NO\(_3\))\(_2\), 2 mM NH\(_4\)H\(_2\)PO\(_4\), 2 mM MgSO\(_4\), 25 µM H\(_3\)BO\(_3\), 20 µM FeSO\(_4\), 2 µM MnCl\(_2\), 2 µM ZnSO\(_4\), 0.5 µM [(NH\(_4\))\(_6\)Mo\(_7\)O\(_24\)\(4\)H\(_2\)O], and 0.3 µM CuSO\(_4\) (Hoagland and Arnon, 1950). The solution pH was adjusted to 6.2 with 0.1 M KOH. Growth solutions were continuously aerated and renewed every three days. Sodium nitroprusside (SNP) was used as NO donors. Different concentrations of NaCl (0 mM, 150 mM, 200 mM) without or with SNP (60 µM) were added to the nutrient solution.

The treatments referred as follow (T0 Control, T1 Salinity (150 mM NaCl), T2 Salinity (200 mM NaCl), T3 Salinity + SNP (150 mM NaCl + 60 µm SNP), T4 Salinity+ SNP (200 mM NaCl + 60 µm SNP). At harvest time (After 7 and 14 days of NaCl treatments), plants were firstly photographed, then plants were divided into shoots and roots, extensively washed in distilled water, dried with filter paper and immediately used for analyses or stored at −20°C. For biomass determination, after measurement of fresh weight, dry weight (g/plant), for both shoots and roots were oven-dried at 65°C until reaching a constant weight.
Chlorophyll estimation

The fresh leaves (0.5g) were cut into small pieces; and grinded with 10 ml of 80% chilled aqueous acetone. Leaf homogenate was filtered through Whatman filter paper. The absorbance of the pigment solution was measured at 664, and 647 nm Total Chl (Chl a + Chl b) content were calculated and expressed in mg. g-1 fresh weight (FW) using the method described by Lichtenthaler (1987).

Lipid peroxidation assay

Lipid peroxidation level was measured as the content of malondialdehyde (MDA) according to the methods of Buege and Aust (1978). Content of MDA, which is an end product of lipid peroxidation, was determined using thiobarbituric acid (TBA) reagent. Shoot and root tissue was homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 x g for 10 min. The assay mixture centrifuged at 8,000xg for 10 min, the MDA concentration was determined by (Abs532 – Abs600) and (ε, 155 mM⁻¹ cm⁻¹) and the result expressed as µmol MDA g⁻¹ (d.w.) .

Determination of total phenolic content

The total soluble phenolic was determined by an assay modified from (McDonald et al., (2001). The quantity of 50 mg of dry leaf tissue was immersed in 2.5 ml of 95% ethanol and was kept in the centrifuged at 1000 rpm for 10 min. then, 0.5 ml of sample supernatant was diluted with 0.5 ml of distilled water and was transferred into a test tube with 1 ml of 95% ethanol and 5 ml of distilled water. In each sample, 0.5 ml of 50% (v/v) Folin-ciocalten reagent was added and mixed. After 5 min, 1 ml of 5% Na₂CO₃ was added to the reaction mixture. Absorbance was noted at 725 nm after 1 hrs. A total phenolic content in samples was expressed as µg Gallic acid equivalents (GAE)/g f.w.

Determination of total flavonoid content

A modified method of Chang et al., (2002) was used for determination of total flavonoid contents. 300 µl of each extract dry extract was completed to 2ml with methanol and then mixed thoroughly with 0.1 ml of AlCl₃ and 0.1 ml of 1M sodium acetate. The absorbance of the solution was measured at 415 nm. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 415 nm by using spectrophotometer Total flavonoids in sample were expressed as µg quercetin equivalents (QE)/ f.w.

Determination of proline

The determination was carried out according to the method reported by Bates et al., (1973). Briefly, 0.5gm from leaves tissue with 10 ml of 3% aqueous sulfosalicyclic acid (extraction buffer) was homogenised, then filtered using whatman No.2 filter paper. 2 ml of filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hrs at 100°C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 seconds. The absorbance was read at 520 nm using toluene as a blank. Proline concentration was determined using L-proline as a standard calibration curve and the results expressed as µg proline g⁻¹ FW.

Enzymes extraction and assay (Catalase and peroxidase)

The extraction was carried out according to the method reported by Vitoria et al. (2001). The shoots and root tissue (3 : 1 buffer volume: fresh weight) was homogenized with 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (W/V) insoluble poly-vynilpyrolidone. The homogenate was centrifuged at 10,000 g for 30 min and supernatant were used to determine the protein contents and enzyme activities.
Catalase (CAT) enzyme specific activity

Catalase activity was assayed following the method of Luck (1974), in a reaction medium containing 65 mM K-phosphate buffer (pH 7.2), 12.5 mM H₂O₂ and a sufficient volume of sample to allow a decrease in absorbance, due to H₂O₂ decomposition, from 0.450 to 0.400 at 240 nm in less than 60 sec. The decrease in the absorption was followed for 3 min at 240 nm, and 1 mmol H₂O₂ mL⁻¹ min⁻¹ was defined as 1 U of CAT activity.

Peroxidase (POD) specific activity

The method published previously by El-Beltagi et al. (2011) was adopted for assaying the activity of peroxidase. In the presence of the hydrogen donor pyrogallol, peroxidase converts H₂O₂ to H₂O and O₂. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

Total protein content of (shoots and roots) samples were determined using the Bradford assay (Bradford, 1976). The colour change determined spectrophotometrically at 595 nm for comparison with the colour change in standardized BSA samples.

Statistical analysis: Values presented were means ± one standard deviation (SD) of three replicates.

Results and discussion

Maize plants were treated with 150 mM and 200 mM NaCl for 15 days to assess the effect of increasing salt stress on the indices of stress and antioxidant metabolism. The effect of exogenously supplied of SNP (0.06 mM) was evaluated to salt treated plants to determine the effective of SNP concentration for amelioration of NaCl induced oxidative stress. The addition of NaCl to the nutrient solution resulted in a visually reduction in the growth of maize seedling, this being particularly evident at the highest NaCl concentration (figure 1 C). Treatments of 200 mM of NaCl enhance change in morphological characteristics, such as, change in leaf colour (yellowing), change in root length.

Fig (1): Salinity stress gradually affects the plant growth, control (A) and 150 mM NaCl (B) and 200 mM (C) maize plants grown under different NaCl for 15 days.
Root and shoot dry weight

Root and Shoot dry weight (mg/plant) was decreased upon adding 150 and 200 mM of NaCl. More specifically, following the lowest (150 mM) and the highest (200 mM) level of salt stress treatment roots underwent 1.4 gm/plant and 1.1 gm/plant, whilst shoots were 1.5 gm/plant and 0.4 gm/plant, in comparison with their respective controls, respectively (figure 2). The dry weights of both roots and shoots were increased when SNP adding to the growth media.

![Fig. 2. Effect of salinity on dry weight (g/plant) in Zea mays. Where: T0 Control, T1 Salinity (150 mM NaCl), T2 Salinity (200 mM NaCl), T3 Salinity + SNP (150 mM NaCl + 60 µm SNP), T4 Salinity + SNP (200 mM NaCl + 60 µm SNP).](image)

Total chlorophyll content

Total chlorophyll content was detected at days 7 and 15 at the growth period. NaCl addition to the growth medium resulted in a loss of total chlorophyll which underwent a similar enhancement following the addition of SNP to the nutrient solution. After 7 days, the values were 55.2 (mg/gm fw) for total chlorophyll and 43.5 (mg/gm fw) for total chlorophyll, at 150 and 200 mM NaCl respectively), in accordance with the visual symptoms of yellowing detected at the end of the experiment. However, addition of SNP to NaCl solution enhanced leaf chlorophyll content. Thus; we may postulate that SNP could be responsible in mediating the positive influence on plants that resulted in mitigation of the negative effects of salt stress.

![Fig. 3. Effect of salinity on total chlorophyll content (mg/gm fw) in Zea mays. Where: T0 Control, T1 Salinity (150 mM NaCl), T2 Salinity (200 mM NaCl), T3 Salinity + SNP (150 mM NaCl + 60 µm SNP), T4 Salinity + SNP (200 mM NaCl + 60 µm SNP). Left fig represent chlorophyll content after 7 days where the fig on right represent the chlorophyll after 15 days.](image)
Lipid peroxidation

The content of MDA is an indicator of lipid peroxidation and oxidative damage to membrane. Changes in the MDA content induced by NaCl application and SNP in the nutrient solution in the roots and leaves in maize seedling are presented in Figure (4). Results showed that salt treatment caused an increase in MDA content in comparison with the control whereas SNP treatment slightly inhibited the increase in MDA contents in shoots and roots of maize seedlings induced by salt stress. Increased lipid peroxidation was indeed observed in shoots of plants grown at the highest NaCl concentration (200 mM), the value was (33.3 µmol MDA g⁻¹ DM) in comparison with controls (20.2 µmol MDA g⁻¹ DM). Similar trend was observes in the roots.

![Figure 4. Effect of salinity on lipid peroxidation (MDA content) in Zea mays. Where: T0 Control, T1 Salinity (150 mM NaCl), T2 Salinity (200 mM NaCl), T3 Salinity + SNP (150 mM NaCl + 60 µm SNP), T4 Salinity+ SNP (200 mM NaCl + 60 µm SNP).](image)

Phenolic and flavonoid content

The results of assessment of polyphenolic compounds contents in leaves of maize seedling grown under salt stress indicated an increase in the level of both secondary metabolites after 15 days, in comparison with the control one. When maize seedling exposed to 150 and 200 mM NaCl, an increase in phenolic was recorded (16.2 and 19.7 (µg/g f.w) (Fig. 5). However, in the present work 0.06 mM SNP virtually not enhance phenolic content. It is clear that SNP showed protective effect against NaCl-induced ROS-promoted oxidation of phenolic...
Content of proline

Proline accumulation is a well-known measure adopted for alleviation of salinity stress. Intracellular proline which is accumulated during salinity stress not only provides tolerance towards stress but also serves as an organic nitrogen reserve during stress recovery. After 15 days, sodium chloride addition led to increase in proline content (2.4 µg /g f.w and 2.8 µg /g f.w) at 150 and 200 mM NaCl, respectively, as compared with the control in leaves of Z.mays seedlings. Moreover, application of SNP exogenously led to little enhance in proline content compared with control one (Fig. 6).

Fig. 5. Effect of salinity on shoots phenolic and flavonoids content (µg /g f.w) of Zea mays. Where: T0 Control,T1 Salinity (150mM NaCl),T2 Salinity (200mM NaCl),T3 Salinity + SNP (150mM NaCl + 60 µm SNP), T4 Salinity+ SNP (200mM NaCl + 60 µm SNP).

Fig. 6. Effect of salinity on proline content in fresh leaves of Zea mays. Where: T0 Control,T1 Salinity (150mM NaCl),T2 Salinity (200mM NaCl),T3 Salinity + SNP (150mM NaCl + 60 µm SNP), T4 Salinity+ SNP (200mM NaCl + 60 µm SNP)
Antioxidant enzymes activity

The activities of two antioxidant enzymes (CAT, and POD) in *Zea mays* under different levels of salt treatment without or with SNP were assayed. When *Z. mays* plants were treated with 150 and 200 mM NaCl without SNP, CAT activity in leaves showed high decrease at 200 mM NaCl in comparison with control (Fig. 7). In contrast, roots of plants grown at 200 mM NaCl showed increases in POD activities to that grown at 0 mM NaCl. SNP effects on plant growth and antioxidant activity, the results indicated the activities of antioxidant enzymes in roots of *Z. mays* plants increased when treated with SNP and salt stress in comparison with salt stress treated only (Fig. 8). Treatment with SNP (60 μM) resulted in remarkable increase in the activities of POD in roots and leaves and slight increase in the roots and leaves of the activity of CAT.

![Fig. 7. Effect of salinity on roots and shoots catalase activity of Zea mays. Where: T0 Control, T1 Salinity (150mM NaCl), T2 Salinity (200mM NaCl), T3 Salinity + SNP (150mM NaCl + 60 μm SNP), T4 Salinity + SNP (200mM NaCl + 60 μm SNP)](image-url)
Control 0.06mM SNP 150mM NaCl 150mM NaCl+SNP 200mM NaCl 200mM NaCl+SNP
Control 0.06mM SNP 150mM NaCl 150mM NaCl+SNP 200mM NaCl 200mM NaCl+SNP

Fig. 8. Effect of salinity on roots and shoots peroxidase activity of Zea mays. Where: T0 Control, T1 Salinity (150mM NaCl), T2 Salinity (200mM NaCl), T3 Salinity + SNP (150mM NaCl + 60 µm SNP), T4 Salinity+ SNP (200mM NaCl + 60 µm SNP)

Discussion

Nitric oxide (NO), an endogenous signaling molecule in plants, mediates responses to abiotic and biotic stresses. It was reported to be involved in the responses to different abiotic stresses (Neill, et al., 2003). In our study, the dry weights of both roots and shoots of Zea mays seedlings were increased by exogenous NO treatment. Reductions in growth with increased salinity might be due to the adjustment of osmotic potential (Kerepesi and Galiba, 2002). Plants grown under elevated salt concentration are not able to take up water and minerals like K⁺ and Ca²⁺ that ultimately leads to a reduction in growth. It was found that total chlorophyll level of both salt treatments (T3 Salinity + SNP (150 mM NaCl + 60 µm SNP), T4 Salinity+ SNP (150 mM NaCl + 60 µm SNP) were quite similar. Salt accumulation in leaves might first inhibit photosynthesis by increasing stomatal and mesophyll conductance to carbon dioxide (CO₂) diffusion and is known to impair RuBp caboxylase (Parida and Das, 2005). The degradation of chlorophyll pigments under salt-stress could be linked with increased activity of chlorophyllase (an enhanced enzymatic degradation). In this concern, Turhan and Eris (2004) observed that the variation in total chlorophyll contents caused by salt applications in strawberry is not important statistically. Regardless of the mechanism responsible for chlorophyll loss, such a decrease may lead to severe plant growth inhibition by disturbing photosynthesis. Previous studies have indicated that salt stress induced oxidative stress, which resulted in cellular membrane injuries. Plant tolerance to salt stress should partly depend on the enhancement of antioxidant defense systems including several metabolic mechanisms.
The content of MDA is an indicator of lipid peroxidation and oxidative damage to membrane. Figure 4 showed that salt treatment caused an increase in comparison with the control in MDA content whereas SNP treatment slightly inhibited the increase in MDA contents in shoots and roots of maize seedlings. When plants were subjected to salt stress, AOS attacked membranes resulting in the peroxidation reaction which followed the generation of MDA (Bor et al., 2003). The lower level of MDA concentration in the plant demonstrates that it may have been better defended against oxidative damage under salinity stress. It has been reported that salt-tolerant cultivars exhibited better growth due to their ability to accumulate lower MDA under salt stress (Mohamed and aly, 2008). Phenolics are one of the main groups of plant secondary metabolites, including over 9,000 various compounds. They possess a wide range of biological functions in plants, such as, defense against pathogens, pigmentation to attract pollinators and protection from reactive oxygen species generated under environmental stress. Among them, phenolic compounds such as phenolic acids and flavonoids play an important role in scavenging free radicals generated under salt stress. Higher buildup of phenolics and flavonoids in the plant under salt stress may assist the plant to lighten the salinity-induced oxidative stress (Wahid and Ghazanfar, 2006). Phenolic compounds might play a certain role in salinity tolerance mechanism when they increased in tolerant variety and absent in susceptible variety. The presence of flavonoids under osmotic stress may be related to the strengthening of the plant cell wall and the overall cell elongation. NO also enhanced accumulation of proline concentration as adaptive mechanisms. Whether or not these gene expressions of Zea mays seedlings subjected to salt and SNP treatment were induced remains unclear. Here, our findings provide a perspective on protective roles of NO against salt stress, of Zea mays seedlings subjected to salt and SNP treatment await further elucidation. Antioxidant enzymes can protect the cell structure against the ROS generated by stress condition. In this work, an increase in the activities of POD in roots and leaves of Zea mays seedlings in response to salt stress was clear when compared with control. Increased activities of POD play a crucial role in scavenging ROS during salinity. Interestingly, SNP can improve this role. Here we demonstrate that SNP, when applied exogenously, eliminated the oxidative stress in Zea mays imposed by salt stress. Moreover, the activities of CAT and POD in the presence of SNP under salt stress were much higher than those under salt stress alone. This finding was in agreement with the previous study carried out by Uchida et al., (2002) who found NO induced AOS (active oxygen scavenging) activities in rice under salt stress.

Conclusion

Generally, increased salinity lead to reduced plant growth and affects several metabolic compounds. NO may play a critical role in the salinity tolerant mechanism of maize. However, it should elaborate how much NO can promote salinity tolerance of maize plant grown under salt stress. The positive effects of NO on stress mitigation have been attributed to antioxidant activities and modulation of ROS detoxification system. Improved plant growth under salinity stress by exogenous application of NO was associated with increases in antioxidant enzymes such as CAT, and POD.

References

[1] Anjum NA, Sharma P, Gill SS, Hasanuzzaman M, Khan EA, Kachhap K, Mohamed AA, Thangavel P, Devi GD, Vasudhevan P, Sofo A, Khan NA, Misra AN, Lukatkin AS, Singh HP, Pereira E, Tuteja N (2016). Catalase and ascorbate peroxidase-representative H2O2-detoxifying heme enzymes in plants. Environ Sci Pollut Res. 23, 19002-19029.

[2] Bates, L.S., Waldren, R.P. and Teare, I. D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.
[3] Bor, M., F. Ozdemir and I. Turkan (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and wild beet Beta maritima L. Plant Science 164: 77-84

[4] Bradford MM. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254.

[5] Buege JA, Aust SD. (1978). Microsomal lipid peroxidation. Methods in Enzymolog. 52, 302 – 312.

[6] Chang C, Yang M, Wen H and Chem J (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.

[7] Corpas FJ, Carreras A, Esteban FJ, Chaki M, Valderrama R, del Rio LA, (2008) Localization of S-nitrosothiols and assay of nitric oxide synthase and S-nitrosoglutathione reductase activity in plants. Methods Enzymol; 437: 561-574.

[8] Crawford NM, Guo FQ (2005). New insights into nitric oxide metabolism and regulatory functions. Trends Plant Sci. 10:195-200.

[9] El-Baz, F. K.; Amal A. Mohamed and Amina, A.Aly (2003). Development of biochemical markers for salt stress tolerance in cucumber plants. Pakistan. J. of Biological Sciences, (1): 16 – 22

[10] El-Beltagi , Hossam Saad , Amal Amin Mohamed, Bahaa El-din Bastawy Mmekki (2011). Differences in some constituents, enzymes activity and electrophoretic characterization of different rapeseed (brassica napus l.) cultivars. Analele Universităţii din Oradea - Fascicula Biologie . Tom. XVIII, Issue: 1, pp. 45-52.

[11] Foyer CH, Noctor G (2011). Ascorbate and glutathione: the heart of the redox hub. Plant Physiology 155, 2–18.

[12] Hoagland D.R., D.I. Arnon, (1950). The water-culture method for growing plants without soil. Calif. Agric. Exp. Stat. Circular 347

[13] Implay, J.A. (2003), Pathways of oxidative damage. Annu. Rev. Microbiol., 57: 395-418.

[14] James, R. A., Blake, C., Byrt, C. S., & Munns, R. (2011). Major genes for Na+ exclusion, Na, and decrease Na+ accumulation in bread wheat leaves under saline and waterlogged conditions. Journal of Experimental Botany.

[15] Jeschke WD (1984) K+–Na+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. In Tolerance in Plants: Strategies for Crop Improvement. Wiley-Interscience, New York, pp 37–66.

[16] Kerpehesi, H., and G. Galiba. (2002) Osmotic and salt stress induced alteration in soluble carbohydrate content in wheat seedling. Crop Science 40: 482–487

[17] Lichtenthaler HK. (1987) .Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymolog.; 148,350 – 382.

[18] Luck H. (1974). Methods in Enzymatic Analysis II (ed.) Bergmeyer. (Publ.) Academic Press, New York. 885

[19] Manaa A, Ben Ahmed H, Valot B, Bouchet JP, Aschi-Smiti S, Causse M, Faurobert M (2011). Salt and genotype impact on plant physiology and root proteome variations in tomato. J Exp Bot. 62: 2797–2813.

[20] McDonald S, Prenzler PD, Autolovich M and Robards K (2000) . Phenolic content and antioxidant activity of olive extracts. Food Chem., 73: 73-84.
[21] Misra N, Gupta AK, Dwivedi UN (2006). Changes in free amino acids and stress protein synthesis in two genotypes of green gram under salt stress. J Plant Sci 1: 56-66
[22] Misra AN, Vladkova R, Singh R, Misra M, Dobrikova AG and Apostolova E L (2014). Action and target sites of nitric oxide in chloroplasts. Nitric Oxide 39: 35–45.
[23] Mohamed Amal A. and Aly Amina A. (2008). Alterations of some secondary metabolites and enzymes activity by using exogenous antioxidant compound in onion plants grown under Sea water stress. American-Eurasian Journal of Scientific Research 3 (2): 139-146.
[24] Neill, S.J., Desikan, R., Hancock, J.T., (2003). Nitric oxide signalling in plants. New Phytol. 159, 11–35.
[25] Parida, A. K., and A. B. Das. (2005). Salt tolerance and salinity effects on plant: A review. Ecotoxicology and Environmental Safety 60: 324–349.
[26] Serraj, D. and Sinclair, T. R. (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ., 25: 333-341.
[27] Turhan, E. and A. Eris. (2004). Effects of sodium chloride applications and different growth media on ionic composition in strawberry plant. J. Plant Nutr. 27: 1653-1665.
[28] Uchida A, Jagendorf AT, Hibino T, Takabe T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Science 163(3), 515-523.
[29] Vitoria A.P., Lea P.J., Azevedo R.A. (2001): Antioxidant enzymes responses to cadmium in radish tissues. Phytochemistry, 57: 701–710.
[30] Wahid A., Ghazanfar A., (2006). Possible involvement of some secondary metabolites in salt tolerance of sugarcane, J. Plant Physiol. 163. 723–730.