DOI : 10.35124/bca.2019.19.2.4197

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 4197-4202, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

LIPID PEROXIDATION, ANTIOXIDANT DEFENSE SYSTEM AND PROLINE ACCUMULATION IN SEEDLINGS OF *LASIURUS SINDICUS* HENERD IN RESPONSE TO SALT STRESS

Bhuwnesh Goswami, Rekha Rankawat and B. R. Gadi*

Stress Physiology Laboratory, Department of Botany, Centre for Advanced Study (CAS), Jai Narain Vyas University, New Campus, Jodhpur - 342 005, India.

*e-mail: brgadi@rediffmail.com

(Received 7 April 2019, Revised 23 July 2019, Accepted 17 August 2019)

ABSTRACT : ***Lasiurus scindicus*** **Henrard is an endemic and naturally grown in arid region of north-western part of Rajasthan state because of its drought tolerance, however, limited information is available for its responses to salinity . To investigate these mechanisms, seeds of *L.sindicus* were germinated in different level of NaCl (25, 50 and 100mM). Seven days old seedlings were used for estimation of membrane stability, antioxidant system and proline accumulation. Increasing level of NaCl caused reduction in membrane stability index (MSI) and protein content while increased the accumulation of malondialdehyde (MDA) content. All levels of NaCl increased the activities of antioxidant enzymes of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and non enzymatic antioxidant Ascorbate(AsA) and phemnols content, however the changes depending on applied concentration of NaCl. Apart from the activation of antioxidant sytem, accumulation of proline with increasing concentration of NaCl, might play important role in salt stress tolerance of the *L. sindicus* at seedling stage.**

Key words : Antioxidants, *Lasiurus scindicus*, malondialdehyde, membrane stability, proline.

INTRODUCTION

Salinity is the most worldwide abiotic stress and severely affects the distribution, growth and development of plants, especially in arid and semi arid areas. It causes various negative effects on seed germination, mineral uptake and distribution metabolic activities by changing enzyme activities, photosynthesis and yield of plants (Anjum *et al*, 2014; Lu *et al*, 2017). Salinity induced the negative effects on plants through osmotic and ionic effects. High Na^+ level promotes the osmotic stress due to through dehydration and therefore many responses are similar to drought stress except some direct effect of ios (Na+) as ionic stress (Munns and Tester, 2008). Under salinity, plants synthesize and accumulate osmolytes (such as proline, betaine, polyols and sugars) in cytosol for maintaing osmotic potential to water uptake, and thereby protect the cells from dehydration. Proline is an active osmolytes regulates osmotic adjustment and and ROS level in stressed cells (Versules and Sharma, 2010).

Environmental stresses include salinity induced the production of reactive **o**xygen species (ROS) through transfer of electron to oxygen. These ROS such as hydrogen peroxide hydrogen peroxide (H_2O_2), singlet oxygen (O_2^-), superoxide radicals (O_2^-) and hydroxyl radicals (OH^-) are highly active and cause lipid peroxidation, and damage to lipid, proteins and DNA, and ultimately leads to cell death under stress. Plants possess enzymatic and non –enzymatic antioxidant defense system for elimination of ROS to maintain ion homeiosis for growth and survival of plants under stress. The antioxidative system comprises non-enzymatic antioxidants carotenoids, ascorbate, glutathione, a-tocopherols, phenolic compounds and enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) in plants (Gills and Tuteja, 2010). An enzymatic antioxidans system include SOD, POD and CAT play important role in laboratory and field grown plants under salinity (Khan *et al*, 2007; Caverzen *et al*, 2016). AsA and phenols are most important non enzymatic antioxidants can interacts with ROS, reduce the ROS level and thereby enhance oxidative stress tolerance (Gills and Tuteja, 2010). In general, alleviation of oxidative damage and salt tolerance capacity is determined by the ROS removal efficiency of the antioxidant system.

Lasiurus scindicus Henrard (Sewan grass) belongs to family Poaceae is an endemic perennial fodder grass

of harsh Thar ecosystem of India. It is distributed in north –western part of the Rajasthan state where low rainfall (below 200-250mm) and recurring of drought are common problems (Bhandari, 1990). This species has many importance, since it acts as a soil binders species, improve the rangeland production, natural habitat for endangered and threatened fauna of Thar desert. Water shortage, high temperature, high evaporation rate, salinity, poor fertility of soils in this region are some major limiting factors for survival of plants. Due to C4 photosynthetic pathway and morpho-physiological characteristics the grass is well adapted to dry habitat of Thar ecosystem. Our previous study suggest that salinity adversely affect growth, pigments, chlorophyll stability index at early seedling stage (Gadi and Goswami, 2016).

However, little information is available about the antioxidant defense system and osmolyte accumulation under salt stress in this species at early seedling stage. The present study was aimed to changes in its cellular antioxidant system, membrane integrity and osmolyte (proline) in seedlings of the *L. sindicus* grass , response to different level of NaCl.

METERIALS AND METHODS

Viable seeds of *Lasiurus sindicus* were surface sterilized with 0.1% of sodium hypochlorite solution for 5 minutes followed by thoroughly rinsing with distilled water. Then the seeds were then germinated in deionised water (control) and different level of salinity (25, 50 and 100mM NaCl) in petri plates containing double layer of Whatman No. 1 filter paper. Treatment of 10 ml were added to each petri plate on 1st day and after that according to requirement. These petri plates were kept in BOD incubator at 28°C and after 7 days of salt treatment, seedlings were harvested from control and NaCl-treated plants for estimation of biochemical parameters.

Heath and Packer (1968) method was used for estimation of malondialdehyde (MDA) content in terms lipid peroxidation . Membrane stability index (MSI) were determined by measuring the electrolytes leakage in external solution following Sairam et al (1997). Soluble proteins content was estimated using Coomassie Brilliant Blue G-250 (CBBG-250) dye method given by Bradford (1976). Proline was estimated using nin hydrin reaction based on the method of Bates *et al* (1973).

Enzyme extracts were prepared by freezing the weighed amount of plant material (0.1g) in liquid nitrogen followed by crushing with 5 ml of cold extraction phosphate buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA).The homogenate was centrifuged at 15,000 rpm at 4°C for 20 minutes and the clear aliquot was used for the following enzyme assay. SOD activity was determined by measuring the inhibition of formazon formation rate by the enzyme (Dhindsa *et al,* 1981). CAT activity was assayed by monitoring the decomposition of H_2O_2 (Abei, 1984) and POD activity was determined in increase in absorbance based on formation of to tetraguaiacol following Castillo *et al* (1984). Ascorbate was estimated accordingly Ranganna (1977) by titration method. The total phenolic content compounds was estimated using the photometric method with Folin's reagent according to Sigleton *et al* (1999).

All experiments were performed in five replicates. Values indicate mean+SE. Univariate general liner model followed by Tukey'sb test performed to compare the means. IBM SPSS v. 20.0 software at 0.05 significance level were used for all statistical analysis.

RESULTS AND DISCUSSION

Different adverse environmental conditions increase the levels of 'reactive oxygen species' (ROS), highly active and toxic nature of these ROS cause oxidative stress in plants. These ROS can damage to membrane by lipid peroxidation and its last product MDA is thus considered as a reliable oxidative stress markers (Kumr *et al*, 2017). In the present study, MDA content in the seedlings were assayed for evaluation of lipid peroxidation wich was increased by 12.88, 32.86 and 68.21% with 25, 50 and 100 NaCl, respectively. High level of MDA content is related to oxidative damage by elevated ROS in stressed seedlings of *L sindicus* as reported earlier by researchers in many plants under salinity (Zheng *et al*, 2016; Ahmad *et al*, 2017; Kumr *et al*, 2017).

Electrolyte leakage in response to lipid peroxidation is a common phenomenon which reduce the membrane stability index of stressed seedlings (Ram *et al*, 2014). The data showed a reduce MSI (2.88 to 18.60%) with increasing level of NaCl (25-100mM) with compared to the control. Maximum reduction in MSI with 100mM NaCl is reflected the higher electrolytes leakage due to disruption of membrane integrity through oxidative damage. Furtheremore, such reduction in MSI is positively related to high MDA level suggesting that oxidative stress induced lipid peroxidation could be a main reason for reduced MSI through membrane deterioration by salinity (Fig. 1). Recently, Hnilickova *et al* (2019) reported increasing concentration of NaCl reduced membrane stability through high electrolyte leakage in *Lactuca sativa*, *Tetragonia tetragonoides* and *Portulaca oleracea.*

ROS attacks on macromolecules among lipids and proteins are major targets of oxidative modifications in

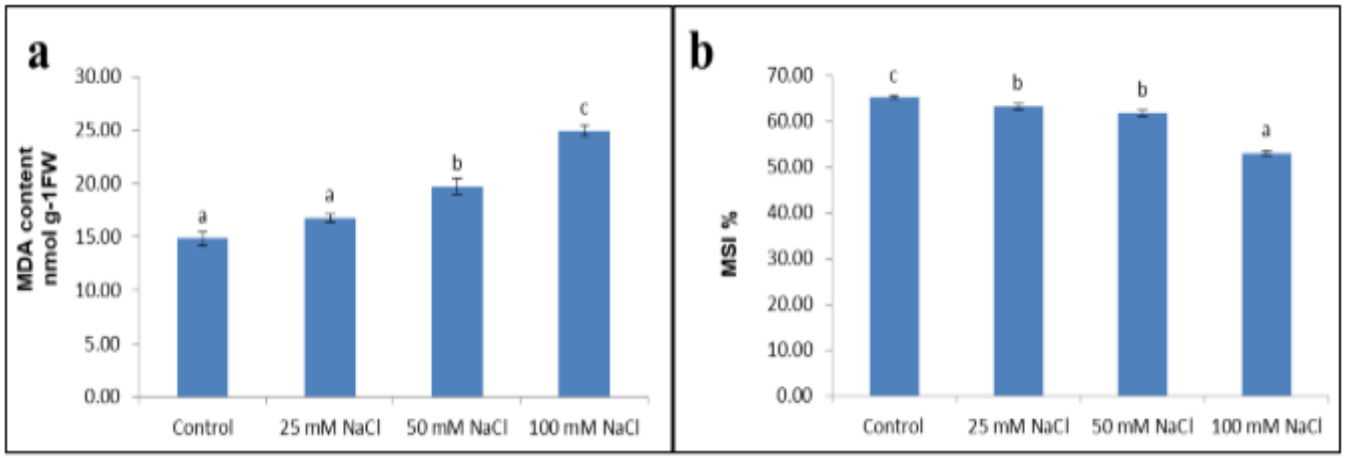

Fig. 1 : Effect of NaCl on MDA content (a) and MSI % (b) in seedlings of *L. sindicus*. Values represent the means of five replications per treatment ± SE. Different letters indicate signiûcant differences according to Tuckey's b test ($p \leq 0.05$).

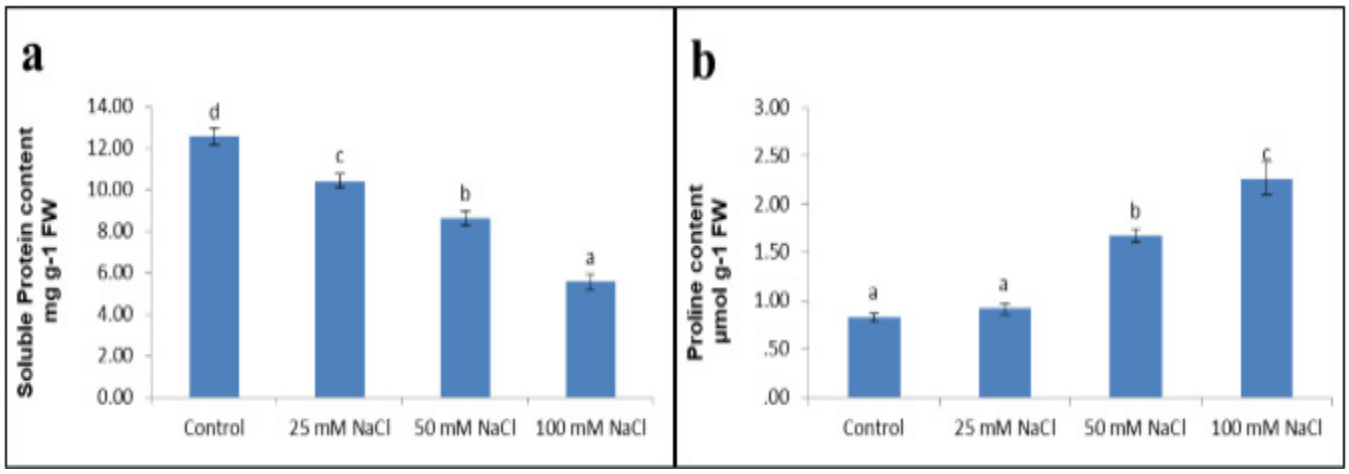

Fig. 2 Effect of NaCl on metabolites contents; soluble protein (a) and proline (b) in seedlings of *L. sindicus*. Values represent the means of five replications per treatment ± SE. Different letters indicate signiûcant differences according to Tuckey's b test ($p \leq 0.05$).

stressed plants, therefore membrane damage and degradation of proteins occurs in stressed plants (Anjum *et al*, 2014). Current study showed that increasing concentration of NaCl (25-100 mM) reduced (16.95-55.69%) and minimum protein content (5.57mg g^{-1} FW) was observed in 100mM NaCl treated *L. sindicus* seedlings (Fig. 2a). It s considered that disruption in protein synthesis as well as to enhanced proteolysis possibly reduced protein content under salinity jn plants (Munns and Temester, 2008; Karimi *et al*, 2009; Godara and Gadi, 2016). High salinity cause osmotic stress in plants, in such dehydrated condition plants responds through the synthesis and accumulation of proline like osmolytes for osmotic adjustment. Fig. 2b shows that all concentrations of NaCl treatment caused a increase in proline content in seedlings relative to control. Proline is one of the major compatible solutes that accumulate following salt induced dehydration to protect the cell and maintain osmotic adjustment for continuous water influx. Accumulation of proline also significant for ROS scavenging and protection of membrane and protein from oxidative damage (Versules and Sharma, 2010; Ahire *et al*, 2013).

In this study, lower level of NaCl (25 mM) was not effective in increasing the proline content as it increased proline about 10.84% relative to control. On the other hand, proline content was remarkably elevated about 2.01 and 2.73 fold with 50 mM and 100 mM NaCl, respectively. The increase in proline content might play important role in osmotic adjustment and regulation of ROS under salt stress. This is consistent with previous studies on desert plants *Ziziphus mauritiana* (Gadi and Gehlot, 2011) and *Calligonum caput-medusae* (Lu *et al*, 2017). The higher level of proline in salt stressed seedlings of *L. sindicus* could be important for osmotic adjustment and oxidative stress regulation.

We have assayed the activities of three antioxidant enzymes generally used by plants for ROS detoxification :SOD,POD and CAT in the *L. sindicus* seedlings under salinity (Fig. 3a-c). SOD is considered the first line of defense against ROS toxicity through dismutation of the superoxide anion to H_2O_2. POD and CAT are

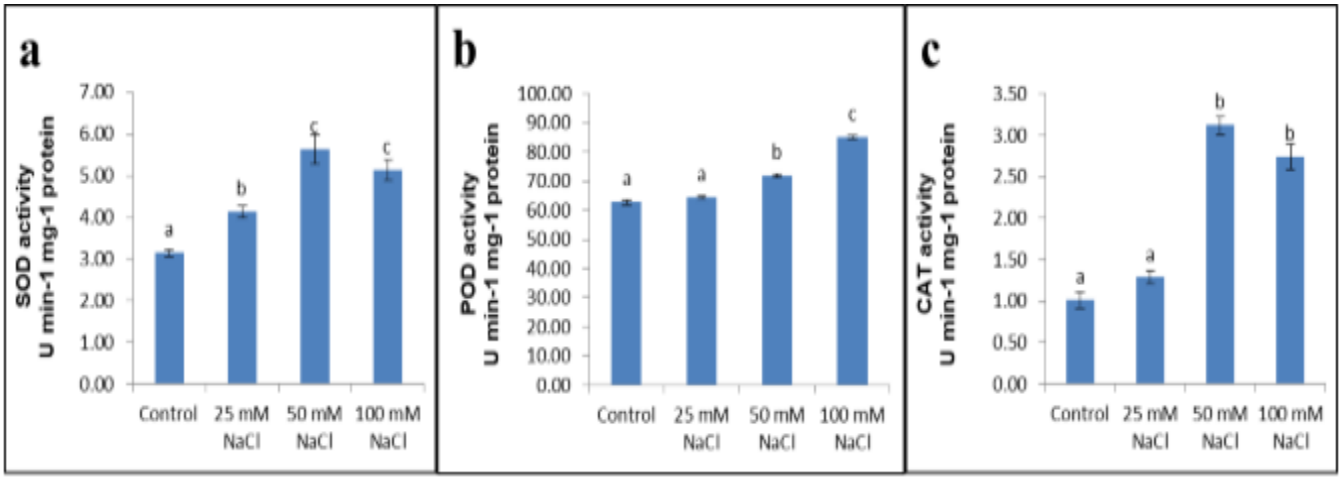

Fig. 3 : Effect of NaCl on antioxidant enzyme activity; SOD (a) POD (b) and CAT (c) in seedlings of *L. sindicus*. Values represent the means of five replications per treatment ± SE. Different letters indicate signiûcant differences according to Tuckey's b test ($p \leq 0.05$).

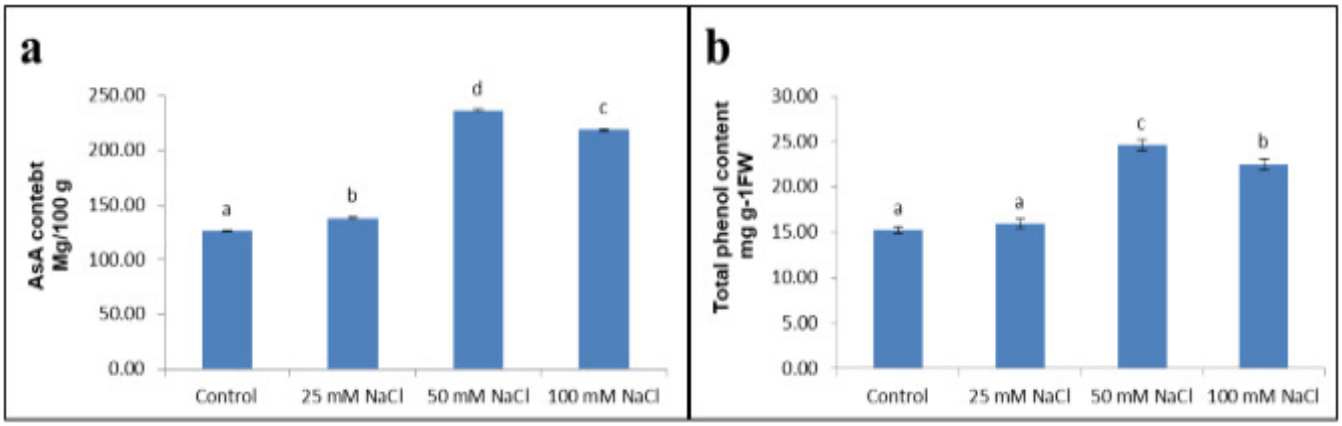

Fig. 4 : Effect of NaCl on non enzymatic antioxidant content; AsA (a) and Phenols (b) in seedlings of *L. sindicus*. Values represent the means of five replications per treatment ± SE. Different letters indicate signiûcant differences according to Tuckey's b test ($p \leq 0.05$).

subsequently detoxify the H_2O_2 into water and oxygen thus removing toxic. Thus, they coordinately regulate the generation of toxic hydroxyl radical by reducing the O^- and H_2O_2 level in plants. Several recent studies have showed that salinity induced SOD, POD and CAT enzymes protect plants from stress injury ; however their activities depends on the plant species, duration and intensity of stress (Khan and Panda, 2008; Caverzan *et al*, 2016; Ahmad *et al*, 2017).

In this study, *L sindicus* seedlings showed drastic changes in activities of SOD, POD and CAT under different NaCl concentrations . Salinity increased the SOD activity up to 50 mM NaCl where maximum (78.16%) activity was reported as compared to control. High level of NaCl (100mM) slightly reduced the SOD activity as compared to 50 mM NaCl while the activity was higher than control**.** Increase of the SOD activity play leading role in ROS detoxification in salinity, water, heat and cold stresses (Caverzan *et al*, 2016; Ahmad *et al*, 2017).

A previous study indicated that antioxidant enzymes show differential responses to salinity as low level of NaCl reduced the activity of SOD, and CAT while POD activity was not affected in *Eurya emarginata* (Zheng *et al*, 2016*).* In contrast to this, Lu *et al* (2016) detected increase in activities of SOD, POD and CAT with 50 mM NaCl and than declined gradually with high level of NaCl. POD activity increased with increasing level of NaCl and at 100 mM NaCl treatment, reached their peak about 35.74% increase as compared to control (Fig. 3b). This is consistent with previous studies where the POD activity increased in salinity stressed plants under salinity (Khan and Panda, 2007; Gadi and Gehlot, 2013). CAT activity remarkably increased by 26.47%, 205.88% and 168.62%, respectively with the 25 mM, 50 mM and 100 mM salt concentrations. Similar to SOD, CAT activity also increased by NaCl up to 50mM NaCl and declined slightly as compared to 50 mM NaCl but the activity was higher than control. Thus, this study indicates that POD can scavenge H_2O_2 at high level of NaCl while CAT was less effective when compared to 50 mM NaCl.

Non enzymatic antioxidant like ascorbate and phenols also play crucial role in oxidative stress by reducing the level of ROS and these molecules major contribute to maintain redox homeiosis (Taibi *et al*, 2016). In our study, seedlings showed NaCl dose dependent changes in the level of both AsA and phenols content; increasing level of NaCl had promontory effect up to 50mm NaCl and therefore Maximum AsA and Phenol content increased as 87.34% and 61.65% in 50 mM NaCl concentration (Fig. 4 a-b). At 100 mM NaCl declined in AsA and phenols content was observed as compared to 50 mM NaCl, while the level was higher than control grown seedlings. Maximum accumulation of these antioxidants at 50 mm NaCl suggest their crucial role at lower level of salinity. Accumulation of AsA in response to extremely high degree of salinity (150mM NaCl) and drought stress (10% PEG -6000) plays important role in oxidative stress tolerance of *Parthenium hysterophorus* (Khan *et al*, 2017). Taibi *et al* (2016) also found that increase in ascorbate and phenols were associated with oxidative stress tolerance in *Phaseolus vulgaris* under salt stress.

CONCLUSION

In summary, NaCl induced oxidative damage is reflected by high level of lipid peroxidation (MDA content) with lower MSI and protein content in *L. sindicus* seedlings. Furthermore, high MDA content is associated with reduction of membrane integrity (MSI) and soluble protein content indicating salinity induced toxicity through peroxidation. Increased activities of antioxidative enzymes (SOD, POD and CAT) and non enzymatic antioxidant (AsA and phenols) suggest protective role depending on different levels of salinity. Moreover, in addition to the antioxidant system, accumulation of proline even at high level of NaCl could play crucial role in salt tolerance during early seedling stage of *L. sindicus.*

ACKNOWLEDGEMENT

Authors are thankful to Science and Engineering Research Board (SERB), Government of India, New Delhi for financial assistance and one of the authors (BG) acknowledge to Council of Scientific and Industrial research(CSIR), New Delhi, India for the granting senior research fellowship (SRF).University Grant Commission, New Delhi for providing facilities under Centre of Advanced Study (CAS) programme.

REFERENCES

Aebi H E (1984) Catalase *in-vitro*. *Methods Enzymol*. **105**, 121-126.

Ahire M L, Walunj P R, Kishor P B K and Nikam T D (2013) Effect of sodium chloride-induced stress on growth, proline, glycine betaine accumulation, antioxidative defence and bacoside A content in *in vitro* regenerated shoots of *Bacopa monnieri* (L.) Pennell. *Acta. Physiol. Plant.* **35**, 1943–1953.

Ahmad J, Bashir H, Bagheri R, Baig A, Al-Huqail A, Ibrahim M M and Quereshi M I (2017) Drought and salinity induced changes in ecophysiology and proteomic profile of *Parthenium hysterophorus*. *PLoS ONE* **12(9)**, e0185118. https://doi.org/10.1371/journal.pone.0185118

Anjum N A, Sofo A, Scopa A, Roychoudhury A, Gill S S, Iqbal M, Lukatkin A S, Pereira E, Duarte A C and Ahmad I (2014) Lipids and proteins-major targets of oxidative modifications in abiotic stressed plants. *Environ. Sci. Pollut. Res.* **2**, 4099–4121.

Bates L S, Waldron R P and Teare I D (1973) Rapid determination of free proline in water stress studies. *Plant Soil* **29**, 205.

Bhandari M M (1990) Flora of the Indian Desert, Jodhpur, India. Desert. Scientific Publishers, Jodhpur.

Bradford M M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-Dye binding. *Anal. Biochem.* **72**, 248-254.

Castillo F I, Penel I and Greppin H (1984) Peroxidase release induced by ozone in *Sedum album* leaves. *Plant Physiol*. **74**, 846-851.

Caverzan A, Casassola A and Brammer S P (2016) Antioxidant responses of wheat plants under stress. *Genet. Mol. Bol*. **39**, 1-6.

Dhindsa R A, Plumb-Dhindsa P and Thorpe T A (1981) Leaf senescence: Correlated with increased permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J .Exp .Bot*. **126**, 93-101.

Gadi B R and Gehlot R K (2011) Effect of bayleton on proline content and peroxidase activity in *psoralia odorata* seedlings under salt stress. *Biochem. Cell. Arch.* **11**(1), 275-278.

Gadi B R and Goswami B (2016) Effect of salt stress on early seedling growth and chlorophyll stability index of *Lasiurus sindicus* Henrard: Endemic to Indian Thar desert. *Biochem. Cell. Arch* **16**, 103-106.

Gill S S and Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem*. **48**(12), 909-930.

Godara R N and Gadi B R (2015) Influence of Uniconazole on biochecimal parameters and nitrate reductase activity in *Ziziphus* seedlings under salt stress. *Biochem.Cell. Arch.* **15(2)**, 461-464.

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

Hnilickova H, Hnilicka F, Orsak M and Hejnak (2019) Effect of salt stress on growth, electrolyte leakage, Na^+ and K^+ content in selected plant species. *Plant Soil Environ.* **65**(2), 90–96.

Karimi S, Rahemi M, Maftoun M, Eshghi L and Tavallali V (2009) Effect of long-term salinity on growth and performance of two pistachio (*Pistacia vera* L.) rootstocks. *Aust. J. Sci.* **3**(3), 1630-1639.

Khan M H and Panda S K (2007) Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta. Physiol. Plant.* **30**, 81–89.

Kumar D, Al Hassan M, Naranjo M A, Agrawal V, Boscaiu M and Vicente O (2017) Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander* L.). *PLoS ONE* **12**(9), e0185017. https://doi.org/10.1371/ journal.pone.0185017.

Lu Y, Lei J Q, Zeng F J, Zhang B, Liu G J, Liu B and Li X Y (2017)

Effect of NaCl-induced changes in growth, photosynthetic characteristics, water status and enzymatic antioxidant system of *Calligonum caput-medusae* seedlings. *Photosynth.* **55**, 96–106.

Munns R and Tester M (2008) Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.* **59**, 651–681.

Ranganna S (1977) Manual of analysis of fruits and vegetable products. Tata Macgrow-Hill Publishing Company Ltd., New Delhi

Ram A, Verma P and Gadi B R (2014) Effect of fluoride and salicylic acid on seedling growth and biochemical parameters of watermelon (*Citrullus lanatus*). *Fluoride* **47**(1), 49–55.

Sairam R K, Shukla D S and Saxena D C (1997) Stress induced injury and antioxidants enzymes in relation to drought tolerance in wheat genotype. *Biol. Planta.* **40**, 357-364.

Singleton V L, Orthofer R and Lamuela-Raventos R M (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*. **299**, 152-178.

Taibi K, Taïbi F, Abderrahim L A, Ennajah A, Belkhodja M and Mulet J M (2016) Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *South Afri. J. Bot*. **105**, 306–312.

Verslues P E and Sharma S (2010) Proline metabolism and its implication for plant-environment interaction. *The Arabidopsis Book* **3**, 1-23.

Zheng J L, Zhao L Y, Shen B, Jiang L H and Zhu A Y (2016) Effects ofn salinity on activity and expression of enzymes involved in ionic, osmotic and antioxidant responses in *Eurya emarginata. Acta. Physiol. Plant*. **38**, 1–9.