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Research Article – Botany

Morphological and biochemical response to salinity stress on *Setaria italica* seedlings

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Abstract

Salinity is considered as the most important abiotic stress limiting the crop production. The present investigation was made to study the impact of different concentrations of sodium chloride on growth, biochemical constituents and antioxidant enzymes of the seedlings of *Setaria italica*. Seeds were grown at different concentrations of NaCl [(0, 25, 50, 75 and 100 mM] for twenty five days. Salt stress influenced a significant modification in the level of osmolyte accumulation**.** The accumulation level of osmolytes such as proline, glycine betaine, phenol and antioxidant enzyme such as catalase (CAT) and hydrogen peroxide increased significantly with increasing salt stress conditionwhen compared to the control. A statistically significant decrease of seed germination percentage, root and shoot length, photosynthetic pigments like chlorophyll *a,* chlorophyll *b* and proteins when higher concentration of NaCl added were recorded. From this experiment it was found that the foxtail millet crops can be sustained in optimum (75 mM) salinity condition. It was concluded that these osmolytes play a key role in generating tolerance against salt stress.

Key words: Salt stress, *Setaria italica,* osomolytes, antioxidant, sodium, abiotic stress

Introduction

Salinity is considered as the most important abiotic stress limiting the crop production. Worldwide, 20% of total cultivated and 33% of irrigated agricultural lands are exacerbated by high salinity. There is a serious competition for fresh water so that high quality water is often used for industrial or domestic purposes and saline and polluted water is allocated for cultivated lands (Bouwer, 2002). Phenomena like low precipitation, high surface evaporation, irrigation with saline water, weathering of native rocks, and poor agricultural practices have increased the rate of soil salinization to 10% per annum (Pooja and Rajesh, 2015).The greatest cause of salinity may be due to the use of poor quality irrigation water (Sifola and Postiglione, 2002). Salts also affect a number of physiological processes such as photosynthesis, stomata conductance, osmotic adjustment, ion absorption, protein and nucleic acid synthesis, enzymatic activity and hormone balance (Hernandez *et al*., 2000). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 2002). If under saline stress plants are not capable of photosynthetic transformation of all the solar energy they receive, the energy excess may produce an increase in singlet and triplet forms of chlorophyll and singlet oxygen. The decrease of $NADP^+$ pool with excess excitation energy causes an increase in the flow of electrons from the donor part of photosystem I (PSI) to oxygen, generating reactive oxygen species (Johnson *et al*., 2003), produced principally in chloroplasts, which provoke metabolic disorders such as oxidation of membrane lipids, proteins and nucleic acids (Imlay, 2003). Reactive oxygen species (ROS) generated under oxidative stress at dangerous levels are detrimental to cellular components, like membrane lipids, proteins, and nucleic acids (Halliwell and Guteridge 1989). Anumber of plant species have evolved antioxidant

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defense mechanisms to combat the devastating effects of oxidative stress. The ability to tolerate salinity by plants is often related to qualitative and quantitative changes in antioxidant systems. Enzymatic ROS - scavenging system includes Hydrogen peroxidases (POX), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR). Elevated levels of H_2O_2 and malondialdehyde (MDA) reflect altered balance in ROS production and detoxification. Salinity induced osmotic stress is also countered by plants through metabolic adjustments, such as synthesis of osmoprotectant like proline (Zhang *et al*., 2014). In response to various environmental stresses, plants have developed different physiological and biochemical mechanisms such as accumulation of compatible solutes like proline, glycine betaine and increase the activities of antioxidant to adapt or to tolerate stress (Rahnama and Ebrahimzadeh, 2005; Faical*et al*., 2009). Limited success in improving crop salt tolerance has been mainly achieved due to the lack of knowledge on the plant-NaCl interaction affecting fundamental physiological, biochemical and cellular processes which inturn affect plant growth and development (Chinnusamy*et al*., 2005; Munns and Tester, 2008).

India is the largest producer of many kinds of millets, which are often referred to as coarse cereals. *Setaria italica* L. is a grass that belongs to the family Poaceae that has been reported to have comparable tolerant level to drought and salinity, thus it became an important food crop in the arid and semi-arid regions. This plant is originated from Northern China and it has been widely cultivated in Asia and Europe. The short life cycle of foxtail millet has made this plant suitable as a second food or fodder crop after wheat or barley. Although foxtail millet is a potential crop to be grown in the saline affected areas where high salinity levels prevent crop production, considerable variation for salt tolerance has been reported within foxtail millet genotypes. Identification of genetic materials contrasting in tolerance level to salinity stress is an important step in generating salt tolerant varieties in an efficient breeding program. However, there is only limited information available for response of foxtail millet to salinity at germination and seedling stages. The present investigation helps to understand the mechanism that regulate form and function and the significance of those processes to plant physiology, ecology and agriculture must include knowledge of plant stress physiology.

Materials and Methods

Foxtail millet was chosen for the investigation and the seeds were obtained from the Agriculture Cooperative Society, Union Office, Somarasam-pettai, Tiruchirappalli. The experiments were carried out in Botanical Garden and Laboratory of Botany Department, Bishop Heber College, Tiruchirappalli, India. The seeds were soaked in distilled water and sown in polythene bags filled with soil as growth medium and were allowed to grow for 20 days by treated with normal water. After 20 days the salt treatment was imposed for further studies. The seeds of *Setaria italica* were irrigated with distilled water as a control and NaCl in different concentration 25 mM, 50 mM, 75 mM and 100 mM. After 5 days root and shoot length was measured and performed various biochemical and antioxidant enzyme activities.

Germination percentage

Germination percentage was calculated depending on the germination ability of the treated seeds and this was done after 24 hours of sowing. Germination percentage for each treatment was calculated.

Growth parameters

Root length was recorded by measuring below the point of root-shoot transition to the fibrous root and the length of lateral roots was taken as total root length. The root lengths are expressed in centimeters per plant. The length between shoot tip and point of the root shoot transition region was taken as shoot length. The shoot lengths are expressed in centimeters per plant.

Photosynthetic pigments

Chlorophyll and carotenoid contents were measured from the *S. italica* leaves according to Arnon (1946). Fresh leaves (100 mg) were extracted with 80% acetone (v/v) and chlorophyll *a*, chlorophyll *b* and carotenoid contents were estimated at 663, 645 and 470 nm using a spectro-photometer and were expressed in terms of mg chlorophyll present g^{-1} fresh mass.

Biochemical constituents

Total protein content

Total protein were extracted and estimated by following the method of Lowery *et al*., (1951). Fresh samples (250 mg) were homogenized in 2.5 ml of phosphate buffer (pH 7.0). The extract was centrifuged at 5000 g for 15 min at 4°C and the supernatant was transferred to a tube containing a mixture of 20 ml acetone and 14 ml β- Mercapto-ethanol for precipitation of protein. The sample tubes were stored at 0°C for 5 h and then centrifuged at 10000 *g* for 20 min. The supernatant was discarded and the pellet was dissolved in 2.5 ml 1 N sodium hydroxide solution. Aliquot of 0.2 ml from this sample was used to prepare the reaction mixture. The intensity of blue color developed was recorded at 660 nm and protein concentration was measured using bovine serum albumin as standard.

Proline estimation

Free proline was assayed spectrophotometrically by the ninhydrin method. The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of acid ninhydrin and glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with toluene, and absorbance was read at 520 nm using L-proline as standard.

Glycinebetane

Glycinebetaine was estimated by the method of Grieve and Grattan. Briefly, finely ground dried plant tissue (0.5 g) was stirred with 20 cm^3 distilled water for 24 h and filtered. The filtrate was diluted with equal volume of 1 M H_2SO_4, made into aliquots of 0.5 cm^3 in micro centrifuge tubes, cooled over ice for 1 h and to each of these were added 0.2 cm^3 cold KI-I_2 reagent. The reactants were gently stirred, stored at 4°C overnight and centrifuged at 12 000 g for 15 min at 4°C to get the precipitated per iodide crystals. The crystals were dissolved in 1,2-dichloroethane, and absorbance was measured at 365 nm after 2 h. Glycinebetaine dissolved in 1 M H_2SO_4 served as standard.

Total phenol

Total phenol contents were estimated by following Malick and Singh (1980). Total phenols were extracted from 500 mg of fresh roots and shoot tissues separately in 80% (v/v) ethanol and estimated by Folin-Ciocalteau reagent. The absorbance of the reaction was measured at 650 nm wavelength on spectrophotometer. Total phenols were calculated by using standard graph of catechol.

Antioxidant enzymes activity

Catalase activity

CAT (EC 1.11.1.6) was measured according to the method of Chandlee and Scandalios (1984) with small modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mMH_2O_2, and 0.04 ml of enzyme extract. The decomposition of H_2O_2, is followed by the decline in absorbance at 240 nm. The enzyme activity is expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Hydrogen peroxide

The content of H_2O_2 was determined according to Velikova *et al*. (2000). Plant tissues were homogenized in 0.1% (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,100×g, 15 min, 4 and 200 ml of the supernatant was added to 200 ml of 100 mM potassium phosphate buffer (pH 7.0) and 800 mlof 1M KI. The absorbance was read at 390 nm. H_2O_2 content for all samples was determined using H_2O_2 as a standard.

Metabolic enzymers

α-amylase

The assay of α-amylase activity was performed from 1 g of tissues (Tarrago and Nicolas, 1976) after inactivating β-amylase by heating at 70°C for 5 min with 9 mM $CaCl_2$ and performing the assay following the standard method (Chrispeels and Varner, 1967). Each unit of activity is defined as the number of μmoles of maltose released per minute.

Results and Discussion

Germination percentage

The foxtail millet seeds were irrigated with distilled water as control and treated at 25 mM, 50 mM, 75 mM and 100 mM of NaCl. Germination percentage of *S. italic* was recorded everyday and

day after sowing. The germination percentage was found to be more in control when compared to NaCl treatments and the lowest seed germination was found in 100 mM of NaCl. The seed germination percentage was found significantly affected by the different concentration of salt treatments (Table 1). Jamil *et al*. (2012) also found that different treatments showed different germination pattern in control and seeds stressed with NaCl. Increasing salt levels had detrimental effects on germination percentage. Seed germination percentage was found to be highest in control (Distilled water). Rate of germination of wheat cultivars were significantly affected due to salt stress from 75mMNaCl salt concentration onwards. According to the previous work it can be concluded that seeds of five different wheat cultivars were susceptible to higher concentrations of salt solutions in germination stage which was supported by the works of Ungar *et al.,*(1996) and Gul *et al.,* (1999).The reduced level of seed germination may be due to loss of viability at higher salinity level, delaying germination of seeds at salinities that cause some stress to but not germination percent as reported by Gulzar *et al*., (2001) and also due to salinity induced high oxidative stress for halophytic seeds as reported by Amor *et al*., (2005). A similar report of reduced level of germination of *Suaeda salsa* seeds under increased salinity level was also reported (Duan, 2007).

Table 1. Effect of salt stress on seed germination percentage of *Setaria italica* of 10 days old seedlings

Treatments	Seed Germination %
Control	88
25mM	81
50mM	76
75mM	72
100mM	53

Root and shoot length

Root and shoot length of *Setaria italica* was measured both in control and NaCl treatment and it was found that the root and shoot length was higher in control when compared to other concentration [Figure 1(a) and 1(b)]. But at higher concentration of NaCl *i.e*., 75 mM and 100 mM, root and shoot length was affected. Salinity caused a significant reduction on root length and shoot length at the higher NaCl concentration. Increase in the salinity from 0 to 25mM of NaCl had no effect on plant root and shoot length, while further increase from 50 mM onwards significantly reduced the root length and shoot length. These results are in agreement with those obtained by Orabi *et al*., (2013) in faba bean, Meloni *et al*., (2001) in cotton, Neves *et al*. (2004) in umbu plants. The reduction in root and shoot development may be due to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings (Datta *et al*., 2009). High level of salinity may have also inhibit the root and shoot elongation due to slowing down of water uptake for overall osmotic adjustments of the plant body under high salt stress condition. Bukhari *et al*., (2012) studied salinity stress in pearl millet *(Pennisetum glaucum*) found the root and shoot length of the pearl millet decreases with the increase in the salinity level. Halima *et al*., (2014) found in Oat (*Avena sativa*) that the root and leaf lengths were adversely affected due to NaCl treatments when compared to control. There was a gradual decrease in the root and shoot lengths with an increase in NaCl level. The present study results were registered as well, including the study done by Mathur *et al*. (2006)on moth bean, Jamil *et al*. (2007) on radish plant, Taffouo *et al*. (2009) on cowpea and Kapoor and Srivastava (2010) on *Vigna mungo* L. They found that increasing the concentrations of NaCl developed a decline in the lengths of the plants. The study noticed decrease in the length of the stem, also due to treatment with sodium chloride solution, could be due to the negative effect of this salt on the rate of photosynthesis, the changes in enzyme activity (that subsequently affects protein synthesis), and also the decrease in the level of carbohydrates and growth hormones, both of which can lead to inhibition of the growth (Mazher *et al*., 2007).

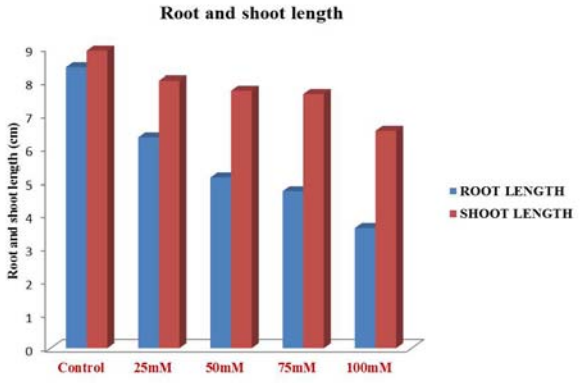

Fig. 1a. Effects of different concentration of NaCl on root and shoot length (cm) in *Setaria italica*

Fig. 1b. Effects of different concentration of NaCl on root and shoot length (cm) in *Setaria italica* on 10th days

Pigment content

Chlorophyll a, b and carotenoids

In this experiment the result showed that the 25 mM of NaCl concentration in chlorophyll *a* was increased when compared to control and other concentration of NaCl in foxtail millet [Figure 2(a)]. This is due to enhance the enzymatic activities to increase the chlorophyll *a*. The seeds of foxtail millet were treated with distilled water as a control and different concentration of NaCl *i.e.*25 mM, 50 mM, 75 mM and 100 mM [Figure 2(b)]. The highest content of chlorophyll *b* was found in control compared to the different concentration of NaCl. This is due to influence of NaCl in enzyme activities. The carotenoid content of foxtail millet was higher in control compared to the different concentration of NaCl *i.e.* 25 mM, 50 mM, 75 mM and 100 mM. The higher concentration of NaCl reduced the carotenoid content in *S. italica* [Figure 2(c)]. The reduction of pigment content such as chlorophyll *a,* chlorophyll *b* and carotenoids are due to enzyme activities suppressed by NaCl. Chlorophyll *a* content enhanced in 25mM concentration of NaCl and chlorophyll *b* suppressed the rate of photosynthetic pigment in foxtail millet. Chlorophyll *a* and *b* molecule supressed the carotenoids pigment which is shown in Figure 2 (a, b & c).Singh *et al*., (2015) studied the maize (*Zea mays*) under the salt stress and found that the content of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), especially chlorophyll *a* decreased sharply with increasing stress levels. High doses of NaCl appeared with deleterious effects on the content of chlorophyll *a.* Chlorophyll *b* also decreased sharply with increasing stress levels. According to Moradi and Ismail (2007), reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic. Ali *et al*., (2013) studied salt stress in Jojoba *(Simmondsia chinensis)* and found the effect of salinity concentrations on the chlorophyll content of jojoba leaves revealed that increasing salinity concentrations significantly decreased chlorophyll *a*, *b* and total chlorophyll ionic stress. These results are in good agreementswith those obtained by Atlassi-Pak *et al.* (2009) in rape, and El-Khallal *et al.* (2009)in maize plant, Taie *et al*. (2013) in faba bean, and Bahari *et al*. (2013) in wheat plant. The inhibitory effect of salinity stress on the photosynthetic pigments may be due to the effect of salinity on the activities of photosynthetic enzymes and this may be a secondary effect mediated by the reduced CO_2 partial pressure in the leaves caused by stomatal closure (DeRidder and Salvucci, 2007).

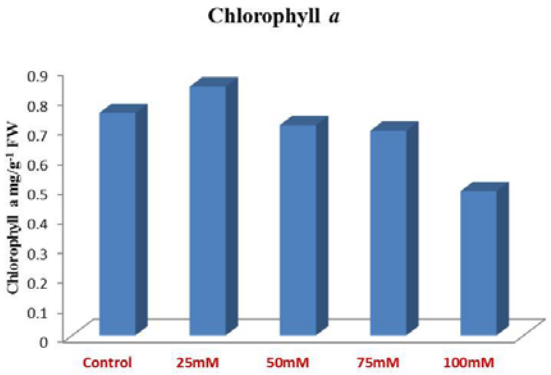

Fig. 2a. Estimation of Chlorophyll a after treatment

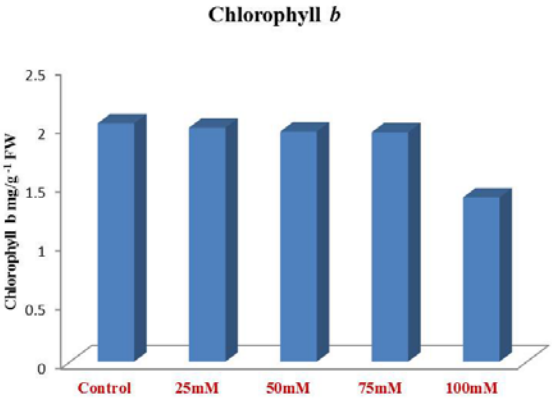

Fig. 2b. Estimation of Chlorophyll b after treatment

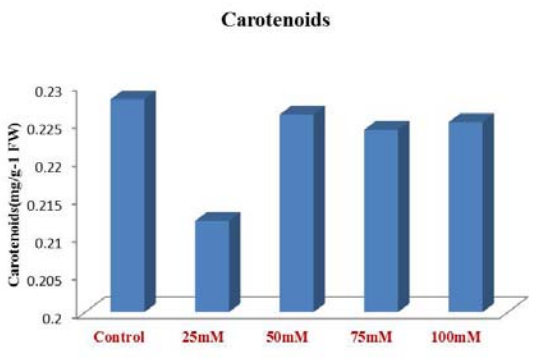

Fig. 2c. Estimation of Carotenoids after treatment

Sairam *et al*., (2002) showed higher decrease in pigment contents of wheat genotypes under salinity at the three stages. Carotenoids are responsible for quenching of singlet oxygen hence their comparative levels in a variety may determine its relative tolerance (Knox and Dodge, 1985). The observed decrease of Chl and Car content in the plants grown under saline conditions may be attributed to both of the increased degradation and the inhibited synthesis of that pigment (Garsia *et al*., 2002).

Protein content

The *Setaria italica* seedlings irrgated with 100mM concentration of NaCl showed the lower protein content compared to control and other concentration (Figure 3). Singh *et al*. (1987) found higher level of salt stress showed that there was proteolytic activity that occurred due to stress condition for the synthesis of osmoprotectant. Other possibility is that if accumulation of proteins appeared in plants under salinity stress then it may provide nitrogen in storage form that is again utilized for biosynthesis of chlorophyll which is directly correlated with photosynthesis and helps in osmotic adjustment. Osman *et al*., (2007) studied salt stress on response of *Catharanthus roseus* shoots to salinity and drought in relation to vincristine alkaloid content and it was observed that proteins content in *Catharanthus roseus* have significantly decreased along with increasing NaCl concentrations. In lentil, Ashraf and Waheed reported that leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance. In *Bruguiera parviflora* a decrease in the intensity of some polypeptides was reported by Parida *et al*. (2011). These results are conjectured by Khosravinejad *et al*. (2009) as they reported that treatment with sodium chloride reduced protein concentration in the plant seedlings. Jamil *et al*., (2012) studied salt stress in *Oryza sativa* and it showed that the protein content was high in control and significantly diminished in plants grown under salt stress. At 100 mM salt stress, protein content was reduced in all varieties, which showed that rice plants were severely affected at high salt concentration. In contrast, Kapoor and Srivastava (2010) as they observed an increase in protein concentration with increasing salt concentration. Ashraf and Harris (2004) observed that the higher content of soluble proteins has been observed in salt tolerant cultivars of barley, sunflower, rice, sugarcane (Pagariya *et al*., 2012) and (Patade *et al*., 2009).

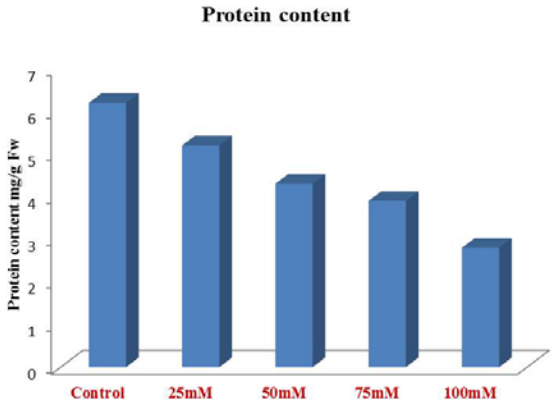

Fig. 3. Estimation of Protein after treatment

Proline content

Proline content was induced by the NaCl treatments over the foxtail millet. A higher proline content was recorded in 100 mM concentration. Proline content of the salt-stressed and control plants are illustrated in Fig. 4. Proline is known to play as an osmoprotectant in plants subjected to osmotic stresses resulted from drought and soil salinity. A positive correlation between proline accumulation and osmoticstress tolerance has been reported by Muthulakshmi *et al*., (2013),Abraham *et al*., (2003), Abdelhamid *et al*. (2013), Khattab (2007), Amirjani (2010), Sadak and Dawood (2014) and Taie *et al*. (2013). Remarkable increase in proline content under stress conditions could be due to changes in proline metabolism profile under salinity stress, with an increased expression of proline synthetic enzymes and breakdown of proline-rich protein (Tewari and Singh, 1991). Jia *et al*., (2011) studied salt stress with when salinity was raised above 0.45 M, the proline contents increased with

rising salinity in *Simarouba glauca.* According to Kaouther *et al*. (2012) investigations with Chili pepper (*Capsicum frutescens*) obtained results showing significant increase in proline in all cultivars with the increase of salt concentration in irrigation water. The accumulation of osmolyte compounds is often proposed as a solution to overcoming the negative consequences of water deficits in crop production which has been proposed as an adaptive mechanism for drought and salt tolerance. Indeed, osmolyte accumulation (OA) in plant cell results in a decrease of the cell osmotic potential and help in the maintenance of water absorption and cell turgor pressure, which might contribute to sustaining physiological processes, such as stomatal opening, photosynthesis and expansion growth (Kaouther *et al*., 2012).

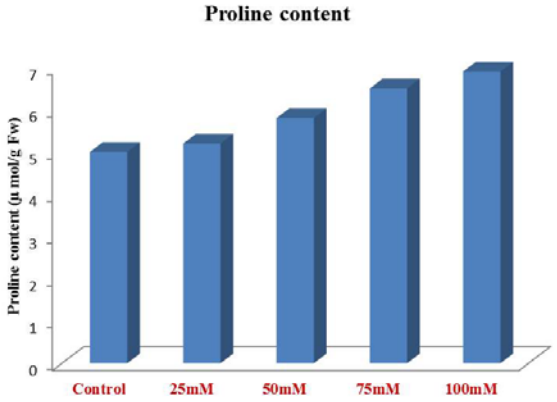

Fig. 4. Estimation of Proline after treatment

Glycine betaine

In the present study revealed that the glycine betaine content also increased with the increasing the concentration of salt stress in *Setaria italica* and 100 mM of salt treatment showed that the higher content of glycine betaine was increased when compared to control and 25 mM, 50 mM and 75 mM of salt concentration (Figure 5). Glycine betaine is regarded as an effective compatible solute that accumulates in the chloroplast of plants, when exposed to environmental stresses (Sawahel, 2004). The accumulation of glycine betaine assumed to have constructive functions in relation to the maintenance of membrane integrity and the constancy of other cellular structures under salt and drought stress has been reported in *Atriplex halimus* (Martínez *et al*., 2005). GB accumulates in response to stress in many crops, including spinach, barley, tomato, potato, rice, carrot and sorghum (Yang *et al*. 2003). Several investigators have noticed that accumulation of glycine betaine under salt stress was found to be high in salt tolerant species (Jagendorf and Takabe, 2001). Besides osmoregulation, glycine betaine stabilizes the oxygen evolving activity of photosystem-II protein complexes at high concentration of NaCl. The major role of glycine betaine might be to protect membranes and macromolecules from damaging effects of stress (Sawahel, 2004). Ranganayakulu (2013) in groundnut (*Arachis hypogaea*) cultivars namely cv. K-134 and cv. JL-24 found that the level of glycine betaine content was significantly increased in both cultivars at all stress regimes. The rate of increase in glycine betaine content was found to be higher at severe stress level (150 mM NaCl). Salt stress episode shows no significant effects on glycine betaine content of tomato. Similarly, according to Elayaraj *et al*.,(2015) with *Ceriops roxburghiana* the glycine betaine content was also increased significantly at all concentrations of NaCl.

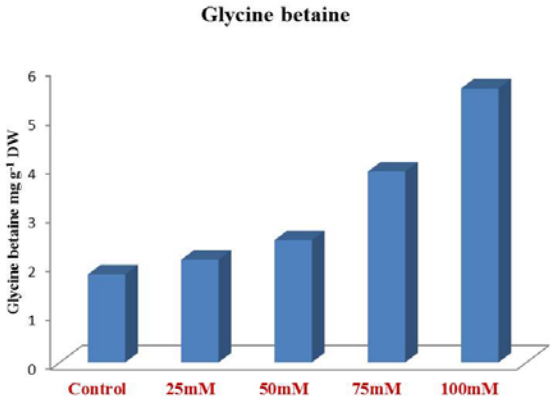

Fig. 5. Estimation of Glycine betaine after treatment

Total phenols

In this investigation the phenol content studied in both control and 25, 50, 75 and 100 mM concentration of NaCl. The result showed that content of phenol was increased and the phenols content was found more in 100 mM of NaCl compared to control and the other concentrations (Figure 6). Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or by

preventing the decomposition of hydroperoxides into free radicals (Pokorny *et al*., 2001). The degree of cellular oxidative damage in plants exposed to abiotic stress is controlled by the capacity of the plants to produce antioxidant agents. Therefore, salt tolerance seems to be favored by the increase in plant antioxidant levels to detoxify the reactive oxygen species produced under these conditions (Noctor and Foyer, 1998). Rajakumar (2013) showed the results that total phenol content was gradually increased with progressing salt stress, however contrasting results were evidenced lower magnitude of increase in phenol contents. According to Muthulakshmi *et al*., (2013) in *Solanum nigrum* the studies found that the phenol value increases as the salt concentration increases. Phenol are synthesized in the leaves and then carried to other tissues and organs. Similarly, Pandey *et al*., (2015) in Cumin plant (*Cuminum cyminum*) found total phenolic and flavonoid contents were decreased initially to some extent at 50 mM and thereafter increased at 100 mM concentration under salt stress condition.

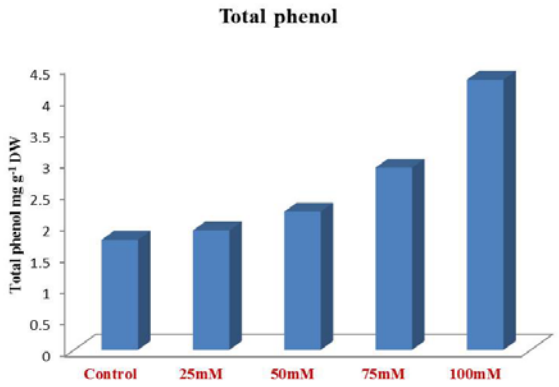

Fig. 6. Estimation of Total phenol after treatment

Catalase activity

The foxtail millet seeds irrigated with control and different concentration of 25, 50, 75 and 100 mM of NaCl was found that as increased the concentration and the amount of catalase also increased (Fig. 7). Higher activity of CAT decrease H_2O_2 level in cell and increase the stability of membranes and CO_2 fixation because several enzymes of the calvin cycle within chloroplasts are extremely sensitive to H_2O_2. CAT activity mostly increased in salt stressed plants. Jin *et al*., (2011) was investigate that the CAT activity of the control in *Kalidium foliatum* was relatively high and the activity of catalase (CAT) increased with increasing sodium. Unal *et al*., (2014) studied in barley (*Hordeum vulgare*) and found CAT activity increased by increasing salt concentration. Chernane *et al*., (2015) in Wheat (*Triticum durum*) and observed CAT activity increased in treated plant compared to control.

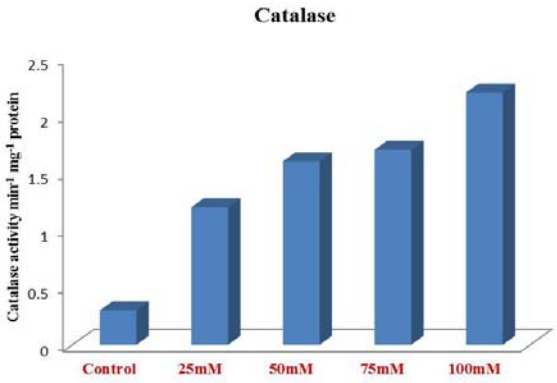

Fig. 7. Estimation of catalase activity after treatment

Hydrogen peroxidase

The activity of hydrogen peroxidase increased significantly in *Setaria italica* with increasing NaCl of different concentration (Figure 8). The increase of H_2O_2 activity in treated plants was accompanied by elevated lipid peroxidation as evidenced by the change in MDA levels. Salt stress caused increases of H_2O_2 content in foxtail leaves, indicating that salt stress could cause damages to the integrity of the cellular membrane and to cellular components that were sensitive to oxidative stress.

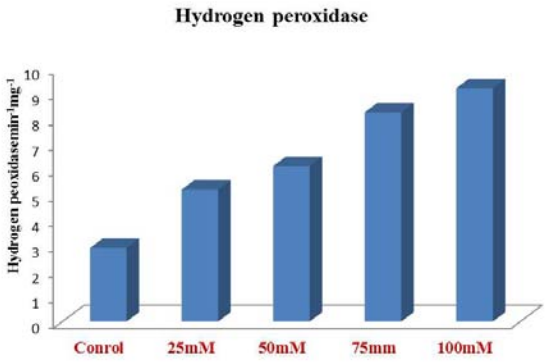

Fig. 8. Estimation of Hydrogen peroxide after treatment

According to Hernandez *et al*., (2010) different concentration of NaCl treatments are significantly increase of H_2O_2 compared to control. Weisany *et al.* (2012) studied salt stress in Soybean (*Glycine max*)

the hydrogen peroxide concentration of leaf tissue was significantly increased with increasing salinity, so that the most hydrogen peroxide concentration was observed under the highest salinity level. Sairam *et al*., (2002) investigatedthe effect of salinity in wheat cvs, the H_2O_2 activity in KRL 19 over Kharchia 65 increased with increasing salinity level. Other authors observed similar effects in rice (Lee *et al*., 2001), cumin (Pandey *et al.,* 2015) and barley (Li *et al*., 2008).

α-Amylase activity

There was significant decrease in alpha amylase activity in the presence of different concentration of NaCl compared to control in *Setaria italica* (Figure 9). Studies conducted by Lúcia *et al*. (2009) in *Plantago ovata* and it showed that the water potentials induced by NaCl led to lower amylase activity. NaCl induced potential and a significant reduction in alpha amylase which was detected under $CaCl_2$, which indicates that only the latter influenced amylase activity due to both its osmotic effect and toxic levels in the cells. Sakil *et al*. (2016) studied that alpha-amylase activity in the germinating seeds of Rice was reduced significantly due to salt stress which is also mentioned by Ben and Denden (2010). Oprica and Marius (2014) in Soybean (*Glycine max*) they found after the NaCl treatment the α-amylase activity in soybean seedlings had the same trend. Thus, at four days after treatment, the decrease in α-amylase activity was observed at 50 mM and 100 mM concentrations but the higher inhibition rate was at 100 mM.

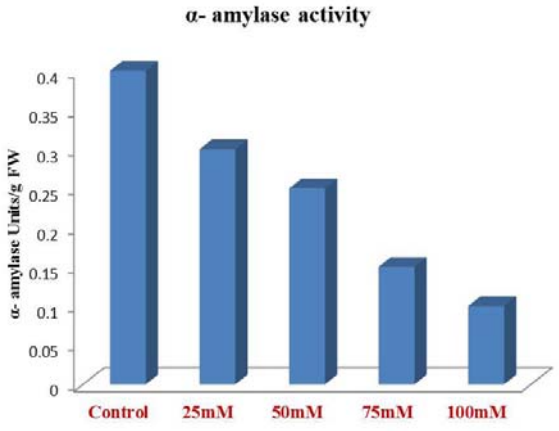

Fig. 9. Effect of salt stress in α-amylase of *S. italica*

Conclusion

In the present investigation, *Setaria italica* was found to survive in NaCl concentration up to 75 mM. It was found that the seeds treated with different concentration of NaCl at 100 mM treatment showed the reduction in some parameters such as germination percentage, root and shoot length, protein content and α–amylase. Salt stress reduces crop growth in different ways. Under normal condition the osmotic pressure in plant cells is higher than that in salinity or soil solution and salt stress condition the osmotic pressure in the soil solution exceeds the osmotic pressure in plant cells due to the presence of high salt, and thus, reduces the ability of plants to take up water and minerals. On the other hand, Na^+ and Cl^- ions can enter into the cells and have their direct toxic effects on cell membranes, as well as on metabolic activities in the cytosol. As a result, in extreme case plants may die under salt stress. It was also observed that with higher concentration of NaCl there was an increase in the proline content, glycine betaine, total phenol, catalase and hydrogen peroxide because the accumulation of proline and the enzyme acts as a strategy against the salt stress. Accumulation of proline, GB and antioxidant enzyme under stress protect the cell by balancing the osmotic strength of cytosol with that of vacuole and external environment. In addition to osmolytes may interact with cellular macromolecules such as enzymes and stabilize the structure and function of such macro-molecules. Some solutes perform an extra function of protection of cellular components from dehydration injury and are called osmoprotectants.

Plants always experience the fluctuation of environment that cause stress and leads to crop loss worldwide. One of the most important problems is to develop stress tolerant plants with maximum yield. Since the development of modern biotechnology, a vast research has been carried out to understand the various approaches that plants have adopted to overcome the environmental stress.

References

Abdelhamid, M.T., Sadak, M.S.H., Schmidhalter, U.R.S., El-Saady, A.K.M., 2013. Interactive Effects of Salinity Stress and Nicotinamide on Physiological and Biochemical Parameters of Faba Bean Plant. Acta biologica colombiana. 18, 3-14.

Abraham, E., Rigo, G., Szekely, G., Nagy, R., Koncz, C.,Szabados, L., 2003. Light dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*. Plant Molecular Biology. 51, 363–372.

Ali, E.F., Bazaid, S., Hassan, F.A.S., 2013. Salt Effects on Growth and Leaf Chemical Constituents of *Simmondsia chinensis* (Link) Schneiderv. Journal of Medicinal Plants Studies.1, 22- 34.

Amirjani, M.R., 2010. Effects of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. American Journal of Physiology. 5, 350-360.

Amor, N.B., Hamed, K.B., Debez, A., Grignon, C., Crabbedly, C., 2005. Physiological and anti-oxidant responses of perennial halophyte *Crithmum maritimum* to salinity. Plant Science. 4, 889-899.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Betavulgaris*. Plant physiology. 24, 1-15.

Ashraf, M., Waheed, A., 1993. Responses of some local/exotic accessions of lentil (*Lensculinaris* Medic.) to salt stress, Archives of Agronomy and Soil Science.170, 103–11.

Ashraf, M., Harris, J.C., 2004. Potential bio-chemical indicators of salinity tolerance in plants. Plant Science, 166, 3–16.

Atlassi-Pak, V., Meskarbashee, M., 2009. Effect of salt stress on chlorophyll content, fluorescence, Na^+ and K^+ ions content in Rape Plants (*Brassicanapus* L.). Asian Journal of Agriculture Research. 3, 28-37.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid Determination of Free Proline for Water-Stress Studies. Plant and Soil. 39, 205-207.

Ben, D. B., Denden,M., 2010. Salt stress induced changes in germination, carbohydrate, starch and enzyme of carbohydrate metabolism in *Abelmoschus esculentus* (L.) Moench seeds. African Journal of Agricultural Research.5, 1412-1418.

Bouwer, H., 2002. Integrated water management for the 21st century: problems and solutions. Journal of Irrigation and Drainage Engineering. 28, 193-202.

Bukhari, I.A., Saba, K., Tayaba, W., Sana, A., Fakhra, J.,Muhammad, U.R., 2012.Effect of NaCl on the morphological attributes of the pearl millet (*Pennisetum glaucum*). International Journal of Water Resources and Environmental Sciences. 1, 98-101.

Chandlee, J.M., Scandalios, J.G., 1984. Analysis of variants affecting the catalase development program in maize scutellum. Theoretical and Applied Genetics. 69, 71-77

Chernane, H., Salma. L., Mounir, M., Mimoun, E.K., 2015. Salt stress tolerance and antioxidative mechanisms in wheat plants (*Triticum durum* L.) by seaweed extracts application. Journal of Agriculture and Veterinary Science.8, 36-44.

Chinnusamy, V., Jagendorf, A., Zhu, J.K.,2005. Understanding and improving salt tolerance in plants. Crop Science. 45, 437–448.

Chrispeels, M.J., Varner, J.E., 1967. Gibberellic acid -enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. Plant Physiology. 42, 398-406.

Chrispeels, M.J., Varner, J.E., 1967. Hormonal control of enzyme synthesis on the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiology, 41, 1008.

Datta, J.K., Nag, S., Banerjee, A., Mondal, N.K., 2009. Impact of salt stress on five varieties of Wheat (*Triticum aestivum* L.) cultivars under laboratory condition. Journal of Applied Sciences and Environmental Management. 13, 93-97.

DeRidder, B.P., Salvucci, M., 2007. Modulation of Rubisco activase gene expression during heat stress in cotton (*Gossypium hirsutum* L.)

involves post-transcriptional mechanisms. Plant Science. 172, 246-252.

Dolatabadian, A., Saleh Jouneghani, R.J., 2009. Impact of exogenous ascorbic acid on antioxidant activity and some physiological traits of common bean subjected to salinity stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 37, 165-172.

Duan, D.Y., Li, W.Q., Liu, X.J., Ouyang, H.P., 2007. Seed germination and seedling growth of *Suaedasalsa* under salt stress. Annales Botanici Fennici44, 161-169.

Elayaraj, B., Selvaraju, M., Dhanam, S., 2015. Physiological and biochemical responses of *Ceriopsroxburghiana* Arn. seedling under salt stress conditions. Journal of Aridland Agriculture, 1: 20-30.

El-Khallal, S.M., Hathout, T.A., Ashour, A.A., Kerrit, A.A., 2009. Brassinolide and salicylic acid induced growth, biochemical activities and productivity of maize plants grown under salt stress. Research journal of agriculture and biological sciences. 5, 380-390.

Faical, B., Imen, A., Kaouther, F., Moez, H., Habib, K., Khaled,M., 2009. Physiological andmolecular analyses of seedlings of two Tunisian durum wheat (*Triticum turgidum* L. subsp. Durum [Desf.]) varieties showing contrasting tolerance to salt stress. Acta Physiologiae Plantarum.31, 14 -22

Garcia-Plazaola, J.I., Hernandez, A., Errasti, A., Becerril, J.M., 2002. Occurrence and operation of the lutein epoxide cycle in Quercus species. Functional Plant Biology. 29, 1075–1080.

Garsia-Sanchez, F., Jufon, J.L., Carvaial, M., Syverstem, J.P., 2002. Gas exchange, chlorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. Plant Science. 162, 705-712.

Grieve, C.M., Grattan, S.R., 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil. 70, 303- 307.

Gul, B., Weber, D.J., 1999. Effect of salinity, light, and temperature on germination in *Allenrolfea occidentalis*.Canadian Journal of Botany.77, 240-246.

Gulzar, S., Khan, M.A., 2001. Seed germination of a halophytic grass *Aeluropus logopoides*. Annals of Botany. 87, 319-324.

Halima, N.B., Saad, R.B., Slima, A.B., Khemakhem, B., Fendri, I., Abdelkafi, S., 2014. Effect of Salt Stress on Stress-associated Genes and Growth of *Avena sativa* L. Journal of Science and Technology, 10, 73-80.

Halliwell, B., Gutteridge, J.M.C., 1989. Freeradicals in biology and medicine. 2nd ed. Oxford : Clarendon Press.

Hernandez, J.A., Jimenez, A., Mullineaux, P., Sevilla, F., 2000. Tolerance of pea (*Pisum sativum* L.) to long term salt stress is associated with induction of antioxidant defenses. Plant Cell Environmental.23, 853-862.

Hernandez, M., Garcia, N.F., Vivancos, P.D., Olmos, E., 2010. A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in *Brassica oleracea* roots. Journal of Experimental Botany. 61, 521–535.

Imlay, J.A. 2003.Pathways of oxidative damage. Annual Review of Microbiology.57, 395-418.

Jagendorf, A.T., Takabe, T., 2001. Inducers of glycinebetaine synthesis in barley. Plant Physiology 127, 1827-1835.

Jamil, M., Bashir, S., Anwar, S., Bibi, S., Bangash, A., Ullah, F., Shikrha, E., 2012. Effect of salinity on physiological and biochemical characteristics of different varieties of rice. Pakistan Journal of Botany.44, 7-13.

Jamil, M., Rehman, S.U., Lee, K.J., Kim, J.M., Rha, H.K., 2007. Salinity reduced growth ps2 photochemistry and chlorophyll content in radish. Scientia Agricola (Piracicaba, Braz.). 64, 111–1.

Jin, J.,Cui, X., Wu, J., Wang, J., Wnag, G., 2011. Physiological and biochemical responses of halophyte *Kalidiumfoliatum* to salt stress. African Journal of Biotechnology. 10, 11468-11476.

Johnson, S.M., Doherty, S.J., Croy, R.R.D., 2003. Biphasic superoxide generation in potato tubers: a self amplifying response to stress. Plant Physiology.13, 1440-1449.

Kaouther, Z., Ben, F. M., Mani, F.,Hannachi, C., 2012. Impact of salt stress (NaCl) ongrowth, chlorophyll content and fluorescence of Tunisian cultivars of chili pepper (*Capsicum frutescens* L.).Journal of Stress Physiology & Biochemistry.8, 236-252.

Kapoor, K., Srivastava, A., 2010.Assessment of salinity tolerance of *Vinga mungo* var. Pu-19 using ex vitro and in vitro methods. Asian Journal of Biotechnology. 2, 73-85.

Khattab, H., 2007. Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline conditions. Australian Journal of Basic and Applied Science.1, 323-334.

Khos ravinejad, F., Heydari, R., Faboodnia, T., 2009. Growth and inorganicsolute accumulation of two barley varieties in salinity. Pakistan Journal of Biological Sciences. 12, 168-172.

Knox,J.P., Dodge, A.O., 1985. Singlet oxygen and plants, Phytochemistry. 24, 889- 896.

Lee, D.H., Kim, Y.S., Lee, C.B., 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa*). Journal of Plant Physiology. 158, 737–745.

Li, Q.Y., Niud, H.B., Yind, J., Wang, M.B., Shao, H.B., Dengd, D.Z., Chend, X. X., Rend, J.P., Li, Y.C., 2008. Protective role of exogenous nitric oxide against oxidative-stress induced by salt stress in barley (*Hordeumvulgare*). Colloids and Surfaces B: Biointerfaces, 65, 220–225.

Lowry, O.M., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. of Biological Chemistry. 193, 265–275.

Lúcia F.B., Marcilio, P.S., Leonardo, C.F., Maria, E.D., Ana, C.C., Joao, F.B., 2009.Proline level and amylase and ascorbate peroxidase activity in the germination of (*Plantago ovate*) Forsk (Plantaginaceae) seeds. Journal of Agricultural and Biological Science.4, 6-14.

Mallick, C.P., Singh, M.B., 1980. Plant enzymology and Histoenzymology. Kalyani publishers, New Delhi. 286.

Martinez, J.P., Kinet, J.M, Bajji, M., Lutts, S., 2005. NaCl alleviates polyethylene glycol-induced water stress in the halophyte species (*Atriplex halimus* L). Journal of Experimental Botany. 56, 21-31.

Mathur, N., Singh, J., Bohra, S., Bohra, A., Vyas, A., 2006. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. American Journal of Plant Physiology. 1,210–221.

Mathur, S. R., Shukla, K. B., Sharma, M.K., 2006. Effect of cadmium on seedling growth, lipid peroxidation and photosynthetic pigments of mothbean cultivar, International Journal of Plant Sciences. 1, 200–201.

Mazher, A.M.A., El-Quesni, E.M.F., Farahat, M.M., 2007. Responses of ornamental and woody trees to salinity. World Journal of Agriculture Science. 3, 386-395

Meloni, D.A., Oliva, M.A., Ruiz, H.A., Martinez, C.A., 2001. Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. Journal of Plant Nutrition. 24, 599-612.

Moradi, M., Ismail, A.M., 2007. Responses of Photosynthesis, chlorophyll fluorescence and ROS-Scavenging systems to salt stress. During seedling and reproductive stages of Rice. Annals of Botany.99, 1161-1173.

Munns, R., Tester M., 2008. Mechanisms of Salinity Tolerance. Annual Review of Plant Biology, 59, 651-681.

Munns, R.C., 2002. Comparative physiology of salt and water stress. Plant Cell Environment. 25, 239-250.

Muthulakshmi, S.M., Gurulakshmi, G.S., Rajathi, S., 2013. Effect of Salt Stress on Physiological and Biochemical Characteristics in *Solanum nigrum* L. International Journal of Science and Research.4, 12-20.

Neves, O.S.C, Sa, J.S, Carvalho, J.G., 2004. Crescimento e sintomas visuais de deficiência de micronutriente em umbuzeiros. Revista

Brasileira de Fruticultura, Jaboticabal. 26, 306-309.

Noctor, G., Foyer, C., 1998. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology. 49, 249-279.

Oprica, L., Marius, S., 2014. Evaluation of Morphological and Biochemical Parameters of Soybean Seedlings Induced by Saline Stress. Romanian Biotechnological Letters. 19, 67-78.

Orabi, S.A., Mekki, B.B., Sharara, F. A., 2013. Alleviation of Adverse Effects of Salt Stress on Faba Bean (*Viciafaba* L.) Plants by Exogenous Application of Salicylic Acid. World Applied Sciences Journal. 27, 418-427

Osman, M.E.H., Elfeky, S.S., Abo El-Soud, K., Hasan, A.M., 2007. Response of *Catharanthus roseus* shoots to salinity and drought in relation to vincristine alkaloid content. Asian Journal of Plant Science. 6, 1223-1228.

Pagariya, M.C., Devarumath, R.M., Kawar, P.G., 2012. Biochemical characterization and identification of differentially expressed candidate genes in salt stressed sugarcane. Plant Science. 184, 1-13.

Pandey, S., Manish, K.P., Avinash, M., Bhavanath, J., 2015. Physio-Biochemical Composition and Untargeted Metabolomics of Cumin (*Cuminumcyminum* L.) Make It Promising Functional Food and Help in Mitigating Salinity Stress. Plus one. 10, 7-18.

Parida, A., Das, A.B, Das, P., 2002. NaCl Stress Causes Changesin Photosynthetic Pigments, Proteins and Other Metabolic Componentsin the Leaves of a True Mangrove, *Bruguiera parviflora*, in Hydroponic Cultures. Journal of Plant Biology.45, 28-36.

Patade, V.Y., Bhargava, S., Suprasanna, P., 2009. Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. Agriculture, Ecosystems and Environment. 13, 424–428.

Patade, V.Y., Suprasanna, P., Bapat, V.A., 2008. Effects of salt stress in relation to osmotic adjustment on sugarcane (*Saccharumo fficinarum* L.) callus cultures. Plant Growth Regulation. 55, 169-173.

Pokorny, J., Korczak, J., 2001. Preparation of natural antioxidant, in Antioxidants in Food: Practical Applications, 1st ed., Pokorny, J., Yanishlieva, N. and Gordon, M., Eds., Woodhead Publishing Limited, Abington, Cambridge, England.7: 311-330.

Pooja, S., Rajesh, K., 2015. Soil Salinity: A Serious Environmental Issue and Plant Growth Promoting Bacteria asvOne of the Tools for Its Alleviation. Saudi Journal of Biological Sciences. 22, 123-131.

Rahnama, H.,Ebrahimzadeh,H., 2005. The effect of NaCl on antioxidant enzyme activities in potato seedling. Biologia Plantarum. 49,93-97.

Rajakumar, K., 2013.A study on effect of salt stress in the seed germination and biochemical parameters of rice (*Oryza sativa*.) under *in vitro* condition. Asian Journal of Plant Science and Research, 3, 20-25.

Ranganayakulu, G.S., Veeranagamallaiah, G., Chinta, S., 2013. Effect of salt stress on osmolyte accumulation in two groundnut cultivars (*Arachis hypogaea* L.) with contrasting salt tolerance. African Journal of Plant Science. 7, 586-592.

Sadak, M.S.H., Abdelhamid, M.T., El-Saady, M., 2010. Physiological responses of faba bean plant to ascorbic acid grown under salinity stress. Egyptian Journal of Agronomy. 32, 89-106.

Sadak, M.S.H., Dawood, M.G., 2014. Role of ascorb ic acid and α tocopherol in alleviating salinity stress on flax plant (*Linumusi tatissimum* L.). Journal of Stress Physiology & Biochemistry. 10, 93-111.

Sairam, M., Shayla, S., Bishan, L.D.C., Mohammad, A.H., Muhammad, J.H.B., 2016.Mitigation of salt stress in rice plant at germination stage by using methyl jasmonate. Asian Journal of Medical and Biological Research.2, 74-81

Sairam, R.K., Rao, K.V., Srivastava, G.C., 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science. 163, 1037- 1046.

Sawahel, W., 2004. Improved performance of transgenic glycine betaine-accumulating rice plants under drought stress. Biologia Plantarum. 47, 39-44.

Sifola, M.I., Postiglione, L., 2002. The effect of increasing NaCl in irrigation water on growth, gas exchange and yield of tobacco Burley type. Field Crops Research. 74, 81-91.

Singh, N.K., Bracker, C.A., Hasegawa, P.M., Handa, A.K., Buckel, S., Hermodson, M.A., Pfankock, E., Regnier, F.E., Bressan, R.A., 1987. Characterization of osmotin. Athumatin-like protein associated with osmotic adaptation in plant cells. Plant Physiology. 85: 126- 37.

Singh, P.K., Shahi, S.K., Singh, A.P., 2015. Effects of salt stress on physic-chemical changes in Maize (*Zea mays* L.) plants in response to salicyclic acid. Indian Journal of Plant Sciences.4, 69-77.

Taffouo, V.D., Kouamou, J.K., Ngalangue, L.M.T., Ndjeudji, B.A.N., Akoa, A., 2009. Effects of salinity stress on growth, ions partitioning and yield of some cowpea (*Vigna ungiuculata* L., walp) cultivars. International Journal of Botany, 5, 135–144.

Taie, H., Abdelhamid, M.T., Dawood, M.G., Nassar, R.M., 2013. Pre-sowing seed treatment with proline improves some physiological, biochemical and anatomical attributes of faba bean plants under sea water stress. Journal of applied sciences research. 9, 2853-2867.

Tarrago, J.F., Nicolas, G., 1976. Starch degradation in the cotyledons of germinating lentils. Plant Physiology. 58, 618–621.

Tewari, T.N., Singh, B.B., 1991. Stress studies in lentil (*Lensesculenta* Moench). II. Sodicity – induced changes in chlorophyll, nitrate, nitrites reductase, nucleic acids, proline, yield and yield components in lentil. *Plant Soil.,* 135, 225-250.

Unal, B.T., Aktas, L.Y., Guven, A., 2014. Effects of salinity on antioxidant enzymes and proline in leaves of barley seedlings in different growth stages. Bulgarian Journal of Agricultural Science. 20, 883-887.

Ungar, I.A., 1996. Effects of salinity on seed germination, growth, and ion accumulation of *Atriplex patula* (Chenopodiaceae). American Journal of Botany. 83, 62-67.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative Stress and Some Antioxidant System in Acid Rain Treated Bean Plants: Protective Role of Exogenous Polyamines. Plant Science. 151, 59-66.

Weisany, W., Yousef, S., Gholamreza, H., Adel, S., Kazem, G.G., 2012. Changes inantioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics J.,* 5,60-67.

Yang, W.J., Rich, P.J., Axtell, J.D., Wood, K.V., Bonham, C.C., Ejeta, G., Mickelbart, M.V., Rhodes, D., 2003. Genotypic variation for glycinebetaine in sorghum. Crop Science. 43, 162–169.

Zhang, L.Q., Niu, Y.D, Huridu, H., Hao, J.F., Qi, Z., Hasi, A., 2014. *Salicorniaeuropaea* L. Na^+/H^+ antiporter gene improves salt tolerance in transgenic alfalfa (*Medicago sativa* L.). Genetics and Molecular Res., 13, 5350–5360.