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INFLUENCE OF INULIN ON SOME BIOCHEMICAL ASPECTS OF MAIZE UNDER SALT STRESS CONDITION

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ABSTRACT

Salt stress is a major constraint for modern agriculture and crop productivity around the globe. It severely affects seed germination, plant growth, vitality and crop yield. Inulin is one of the fructans and naturally occurring polysaccharides, manufactured by several plant groups instead of starch for storage. Present investigation was based on the ameliorative effect of inulin on maize plant under salt stress conditions. Seeds of *Zea mays* L. were grown in pots and plants were irrigated with various concentrations of NaCl (40 and 80mM). Different concentrations of inulin (0.5, 01 and 1.5mM) were applied on foliar parts after five weeks of germination. Salt stressed plants showed decrease in total chlorophyll, carotenoids, reducing and non-reducing sugars, total carbohydrates, total soluble proteins, and increase in total flavonoids, total phenolic contents and total antioxidants. Foliar application of various concentrations of inulin showed better performance in above mentioned parameters in saline as well as non-saline conditions. Therefore, it is recommended that Inulin should be applied as 1.5mM for the better performance (growth and biochemistry) of *Zea mays* L. in controlled and saline environment.

Keywords: Exogenous application, growth regulator, abiotic stress, Antioxidants.

INTRODUCTION

Soil salinity is a most widespread abiotic stress, limiting plant growth and productivity around the world. Salinity defined as the existence of different inorganic solute i.e. HCO_3^-, SO_4^-, NO_3^-, CO_3^-, Na^+, Ca^{++}, Mg^{++}, K^+, and Cl^- in solution. As applied to soils, it is mentioned as the soluble plus readily dissolvable salts in the soil or, operationally, in an aqueous extract of a soil sample. Soil salinity is increasing gradually due to agricultural malpractices and unavailability of good quality water which drastically affects germination, plant vitality, growth and crop yield productivity (Flowers (2004; Munns and Tester, 2008). Increasing concentrations of salt is soil and water imposed hyper-osmotic stress resulting physiological dryness and nutritional disorders. Overloaded salt accumulation in cytosol ignites the oxidative burst resulting impairment of metabolic mechanisms, loss of membrane integrity, reduced cellular expansion and division and specific ion toxicity are the major challenges for plant survival (Zhu, 2007).

Inulins are a class of naturally occurring polysaccharides synthesized by different plants (Roberfroid, 2005). Most of them are extracted industrially from chicory (Roberfroid, 2007). They are commonly known as fructans which belong to a dietary fibers class and normally found in roots / rhizomes and consumed by some plant for energy storing purpose. This natural polysaccharide has so many pharmaceutical and food applications. The quantity of fructans and inulin is reported to be higher in largest cellular compartment (vacuole) (Livingston and Henson, 1998), It also found in apoplast (Ernst and Pfenning, 2000) and even within xylem and phloem (Van den Ende *et al.*, 2000). Due to its chemical nature, its presence in vacuole and the soluble form in which it is stored enables fructans as a vacuolar sucrose reserve in plants (Sprenger *et al.*, 1995). Fructans storage can be initiated in chicory by incubation in sucrose solutions or by growth inhibition in the absence of nitrogen or moisture (De Roover *et al.*, 2000).

Maize (*Zea mays* L.) is enormously grown worldwide and often referred as "king of grain crops" (GOP, 2005). It belongs to the tribe Maydeae of the grass family Poaceae. It has the ability to reduce pain that's way it having analgesic properties (Owoyele *et al.*, 2010). It is used in treatment of rheumatism. As potassium is one on the main nutrient of corn silk which is consider as a major diuretic. In different countries like Europe (France, Spain and Greece) and in Indo-Pak corn silk is used for urinary tract diseases and for treatment of kidney stones (Lans, 2006). It is also used for blood pressure and for improving liver functioning as well. The decoction of maize cob as tea is used for stomach disorder. Being a good emollient is used for ulcer, injury and swelling. In many regions

decoction of corn silk is used for nausea and vomiting. The present work was designed to study the effect of salt and Inulin on some biochemical parameters i.e. total chlorophyll, carotenoids, reducing and non-reducing sugars, total soluble carbohydrates, total soluble proteins, total phenolic contents, total antioxidants, total flavonoids and Relative water content in leaves of *Zea mays.*

MATERIAL AND METHODS

Seeds of Maize (*Zea mays* L.) were obtained from the local market of Mardan, Khyber Pukhtunkhuwa, Pakistan. This experiment consisted of total 36 pots which were divided into four sets and treated with different doses of salts and Inulin. Details of 4 sets were given below:

Set 1: Without inulin comprised of control and two salinity regimes (40 and 80 mM).
Set 2: Inulin foliar treatment (0.5 mM), the set comprised of control and two salinity regimes (40 and 80 mM).
Set 3: Inulin foliar treatment (1 mM), the set comprised of control and two salinity treatments (40 mM and 80 mM).
Set 4: Inulin foliar treatment (1.5 mM), the set comprised of control and two salinity regimes (40 and 80 mM).

The size of plastic pots was 10 cm in diameter and 15 cm in depth and having basal outlet for drainage. Each set consisted of 9 out of 36 pots and 3 replicates were maintained for each treatment while three treatments were control (non-saline), ii) 40 mM, and iii) 80 mM. Each pot filled with equal amount of thoroughly washed sandy loam soil. Approximately uniform size seeds were selected, surface sterilized with 0.1% mercuric chloride for 1 minute and then rinse with distilled water two to three times. Five seeds were placed in each pot and Hoagland's solution was applied and later tap water was given daily in equal quantity. After the three leaves stage, thinning was carried out and one healthy and uniform seedling / pot was left. Experiment was performed in completely randomized design (CRD) in the Botanical Garden of Department of Botany, Abdul Wali Khan University, Mardan. Various salinity treatments were applied after 15 days of seedling emergence and tap water in case of control was applied twice a week. Different concentrations of Inulin were applied at foliar parts after four weeks of seedling emergence in different sets.

BIOCHEMICAL ANALYSIS

Fresh young leaves were collected from third node of plant and immediately stored in liquid nitrogen for biochemical analysis.

Estimation of Photosynthetic Pigments

Photosynthetic Pigments (chlorophylls and carotenoids) were estimated by a method proposed by Maclachlan and Zalik (1963). Fresh leaves were collected and grind with three ml of 80% acetone and centrifuged three times at 2000 rpm for 5 minutes each time in order to wash all chlorophyll from sample. This supernatant was than collected and volume was maintained up to 6 ml. Absorbance was then measured at 663nm, 645nm, 510nm and 480nm and chlorophyll was calculated by using following equations

Chlorophyll a. (mg/g) = $12.3\ D_{663} - 0.86\ D_{645}$ /d *1000*w*V
Chlorophyll b. (mg/g) = $12.3\ D_{645} - 0.86\ D_{663}$ / d *1000*w*V
Total Chlorophyll = Chl.A + Chl.B
Carotenoids (mg/g) = ($7.6\ D_{480} - 1.49\ D_{510}$ / D*1000*W) * V

Estimation of Reducing and non-reducing sugars

Estimation of reducing sugars was carried out by Nelson-Somogy method (Somogyi, 1952). Take 100 mg of leaf sample and sugar content was extracted in five ml of hot 80% ethanol twice. The resulting supernatant was evaporated on a water bath at 80 ^{o}C and then 10ml water was added to it in order to dissolve the sugar contents. Extracted sample (0.2 ml) was taken in a test tube, and then makes the volume of all test tubes to 2 ml with distilled water. Finally added 1 ml of alkaline copper tartarate reagent to each tube and put them in hot water bath for 10 minutes. After cooling the tubes 1 ml of arsenomolybdic acid reagent was added to all the test tubes and absorbance was measured to calculate reducing and non reducing sugars.

Estimation of Total soluble Carbohydrates

Total soluble carbohydrate was estimated by Anthrone method (Yemm and Willis, 1956). Fresh leaf sample (0.1g) was crushed in a pestle mortar with 5 ml of distilled water. The plant material was centrifuged at 2000 rpm for 10 minutes. The Supernatant was poured into test tubes and residue was discarded. Sample extract (0.5 mL) was pipette out in a test tube and mixed with 5 ml of anthrone reagent. The samples were placed in boiling water bath for 15 minutes and then cooled in ice bath. Absorbance was measured at 620 nm against the standard curve of glucose.

Estimation of soluble Proteins

Estimation of soluble proteins was performed by Bradford's Assay reagent (Bradford, 1976).

Fresh leaf material (0.1 g) was crushed with liquid nitrogen and homogenized in 5 ml of ice chilled Potassium phosphate buffer (0.1 M, pH = 7). The homogenate was filtered with glass wool and then centrifuged at 12,000 rpm for 20 minutes at 4°C. Extract (0.1 mL) was taken in test tube and Added five ml Bradford assay reagent. For reagent blank, take 0.1 ml of buffer and then mix with 5 ml Bradford assay reagent. Absorbance was recorded at 595 nm. The amount of soluble proteins was calculated against the standard curve of Bovine serum albumen.

Estimation of total Flavonoids

Aluminum chloride method was applied to determine the total flavonoid content in fresh leaf samples. Leaf sample (5 g) was dissolved in 50 ml of 80% ethanol and placed at shaking incubator for one day. After incubation sample were centrifuged at 10,000 rpm for 15 minutes at 25 ºC. Supernatant containing flavonoid was collected and store in refrigerator at 4 ºC and the estimation was done by spectrophotometric method as proposed by Barros *et al*, (2007). The flavonoid extract (250 µL) was combining with 1.25 mL of distilled water and 75 µL of 5% $NaNO_2$ solution. Later on after 5 min 150 µL of 10% $AlCl_3.H_2O$ was added and incubated for six minutes. Finally add 500 µL of 1M NaOH and 275 µL of distilled water to samples and mix vigorously. Absorbance was recorded at 415 nm. Quercetin different concentrations (15 µg- 500 µg) were prepared to calculate the standard curve. 80% aqueous ethanol was used as blank.

Estimation of Total Phenolic contents

The total phenolic content of leaves was estimated by the method of Malik and Singh (1980). Aliquots of the extracts were separated in a glass tube and volume was maintained to 3 ml with distilled water then added 0.5 mL folin ciocalteau reagent (1:1 with water) and 2 mL Na_2CO_3 (20%) which developed a blue color. Samples were heated for 1 minute and then cooled and absorbance was calculated at 650 nm against the catechol reagent used as a blank. A standard curve was formulated by using standard curve of catechol.

Total Antioxidants

For ferric ion reducing power capacity of samples was measured by modified method of Yen and Chen, (1995). The extract (750 µL) was blended with equivalent amount of phosphate buffer and 1% potassium ferrciyanide and incubated at 50 ºC for 20 minutes. After that add equal amount of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. 1.5 mL of supernatant was taken from the sample and mixed with an equal amount of distilled water and 0.1% of 0.1 mL $FeCl_3$ solution and optical density was recorded at 700 nm. The same procedure was used for preparation of blank. Higher the absorbance higher will be the reducing power capability of photochemical.

Experimental design and statistical analysis

This experiment was randomly designed, with two salt doses each having three replicas. The data were analyzed statistically by using SPSS for analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Photosynthetic Pigments

Photosynthetic pigments (Chlorophyll and carotenoids) are mainly responsible for photosynthetic activity in plants (XinWen *et al.*, 2008). In our work plants treated with different concentration of NaCl (40 and 80mM) in all sets showed reduction in chlorophyll and carotenoid contents as compared to control plants. Sets treated with 0.5mM, 1mM, 1.5mM Inulin treatment showed non-significant increase in all saline as well as non-saline plants. Gomes *et al.* (2011) stated that the chlorophyll and carotenoid contents decreased significantly in *S. auriculata* when treated with NaCl and Na_2SO_4 (100 and 200 mM) in comparison to control. Similar results were also reported by Meloni *et al*., (2003) who thought that salt stress inhibits the photosynthetic efficiency of leaves and this is due to reduced chlorophyll in leaves. Vajpayee *et al*., (2000) found that one of the reasons for the less chlorophyll synthesis may be due to a decrease of δ-aminolevulinic acid dehydratase enzyme activity under stress conditions. This was proved by the work of Hassanein *et al.* (2009) who observed decrease in Chlorophyll a, b, carotenoids and total pigments in wheat under stress conditions. Sairam *et al*. (2005) found higher chlorophyll deprivation in salt sensitive wheat varieties then those varieties which show tolerance to salt stress. Santos (2004) discovered that decrease in chlorophyll content may be either through chlorophyll deprivation or it may be due to decrease biosynthesis of

chlorophyll. Stepien and Klobus (2006) said that there is strong confirmation that salinity affects different enzyme involved in photosynthesis, chlorophyll and carotenoids biosynthesis. Work done by Siler *et al*., (2007) showed that higher concentration of salt decrease the total chlorophyll contents of different plants like *Teucrium polium, thymus vulgaris, Zataria multiflora*, *Ziziphora clinopodioides* (Koocheki *et al.,* 2008) and *sature jahortensis* (Najafi *et al.*, 2010). Observation of Abd El-Wahab (2006) suggested that the reduction in total chlorophyll and carotenoids may be due to specific ion toxicity that is involved in chloroplast formation and protein synthesis and/or in the breakdown of plastid in fennel. Eryilmaz (2006) also reported that as the time of exposure to NaCl was increased the rate of degradation of chlorophyll pigments also increased.

Reducing and Non Reducing Sugars

Plants treated with different concentration of NaCl showed decline in total Reducing and non-reducing Sugars in comparison to their control due to salt stress. Sets treated with 0.5mM, 1mM , 1.5mM Inulin treatment showed significant (P<0.001) increase in control (non-saline) as well as in salt stressed plants. Patricia *et al*. (1992) observed that the significant decrease in reducing sugar in response to salinity also reduced the photosynthetic efficiency that led to hinder the biosynthesis of carbohydrates (Singh and Dubey, 1995). Irrespective of the salts type Photosynthetic activity is greatly reduced due to salt stress (Misra *et al*., 1997). Kumar (2005) said that beside photosynthesis all other main processes such as protein synthesis and energy and lipid metabolism are also affected by salt stress. Some researchers, such as Antoline and Sauchez-Dais (1992) showed that in alfalfa soluble sugars, proteins and total free amino acids including proline were gradually accumulated along with NaCl level

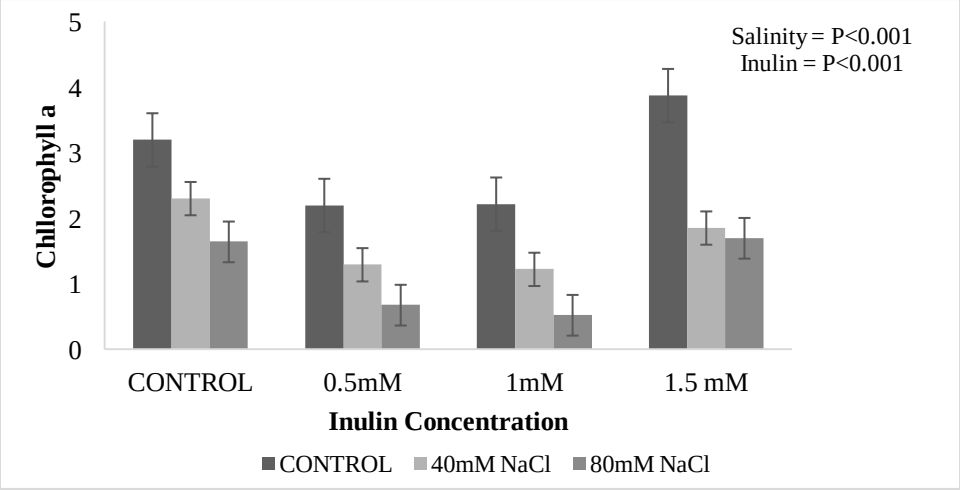

Fig. 1. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on chlorophyll a (mg/g fr.wt) of *Zea mays*.

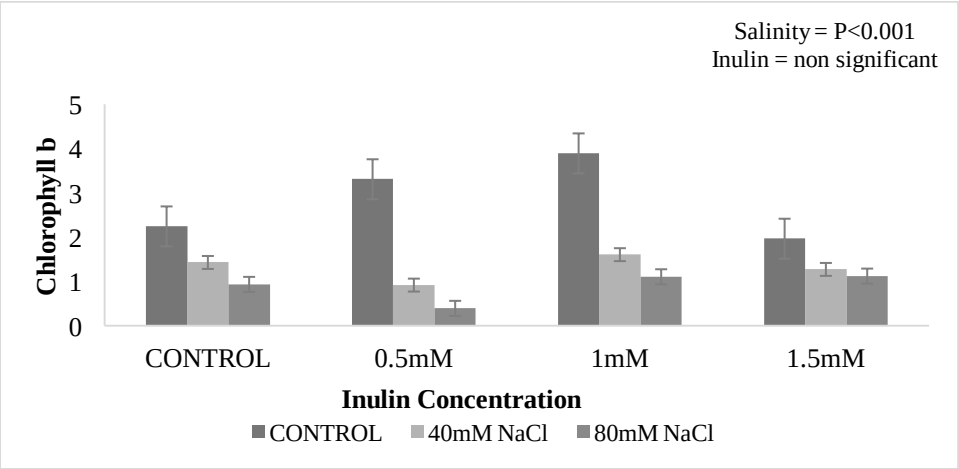

Fig. 2. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on chlorophyll b (mg/g fr.wt) of *Zea mays*.

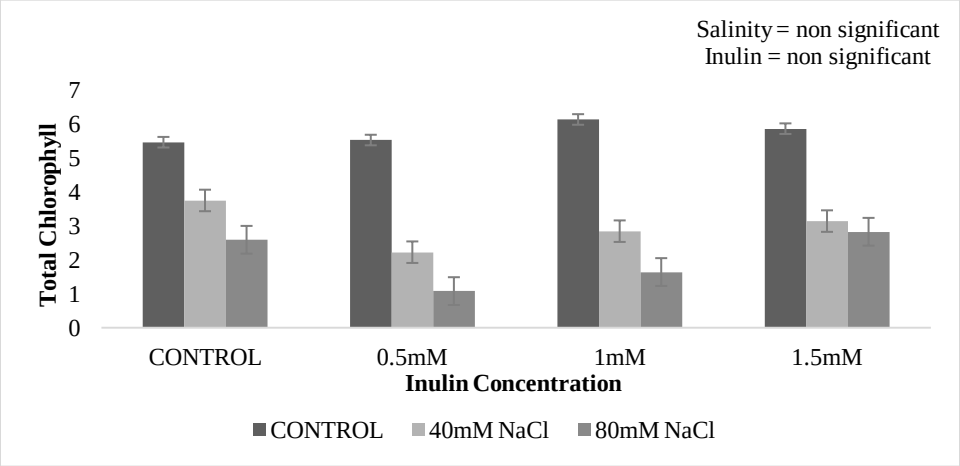

Fig. 3. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total chlorophyll (mg/g fr.wt)of *Zea mays.*

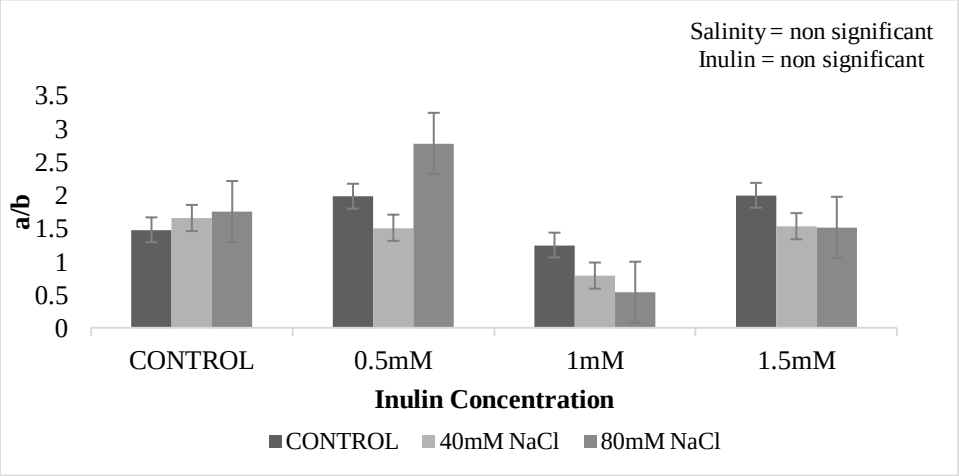

Fig. 4. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on a/b ratio of *Zea mays.*

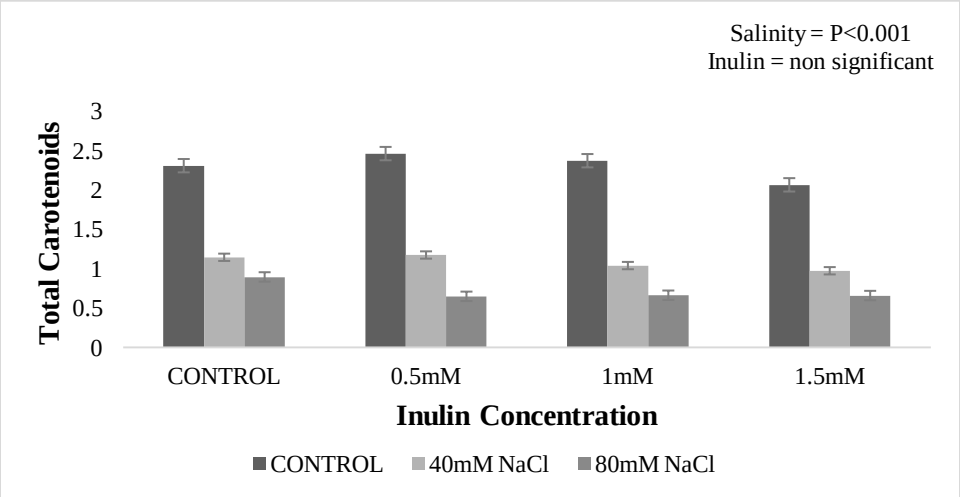

Fig. 5. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total carotenoids (mg/g fr.wt) of *Zea mays.*

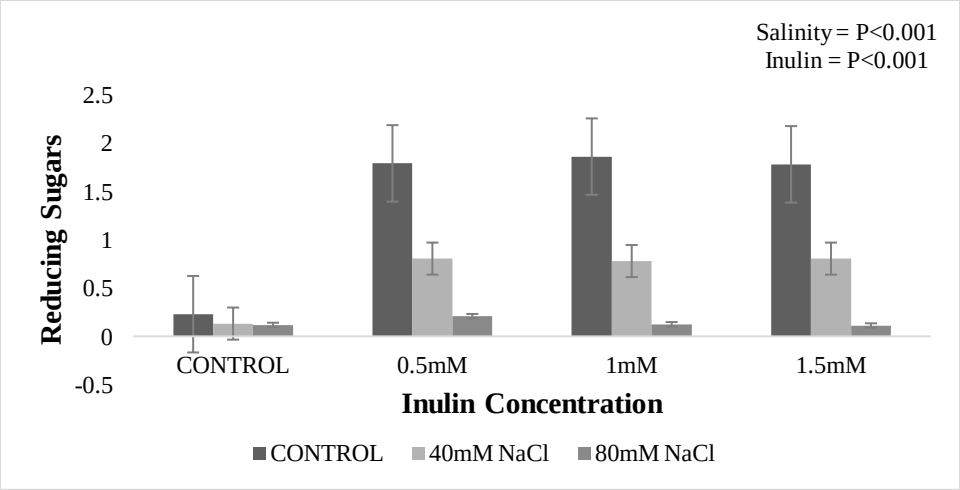

Fig. 6. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on reducing sugars (mg/g fr. wt.) of *Zea mays*.

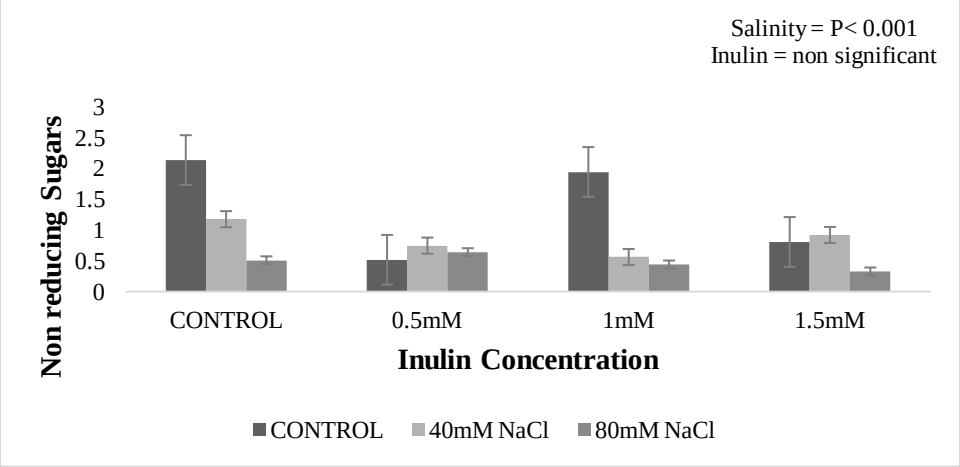

Fig. 7. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on non-reducing sugars (mg/g fr.wt.) of *Zea mays.*

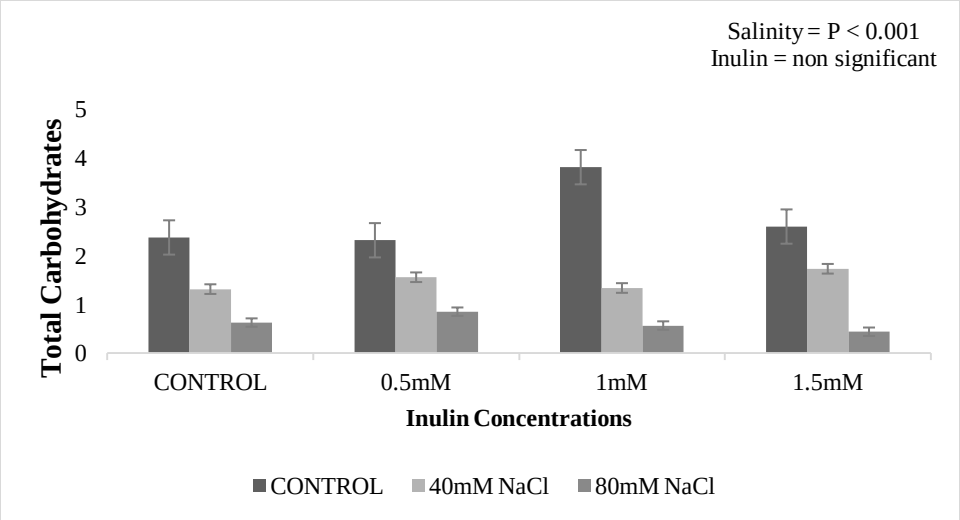

Fig. 8. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total carbohydrates (mg/g fr. wt.) of *Zea mays*.

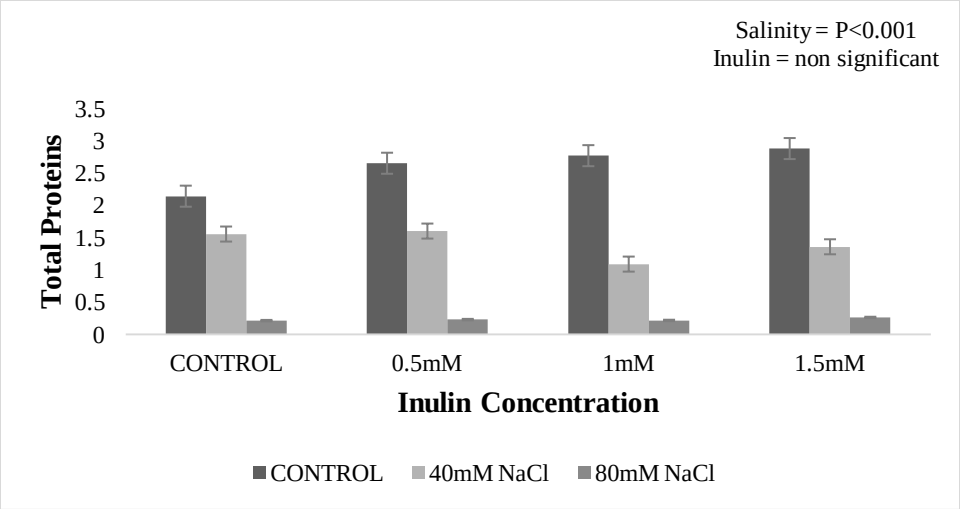

Fig. 9. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total proteins (mg/g fr. wt.) of *Zea mays.*

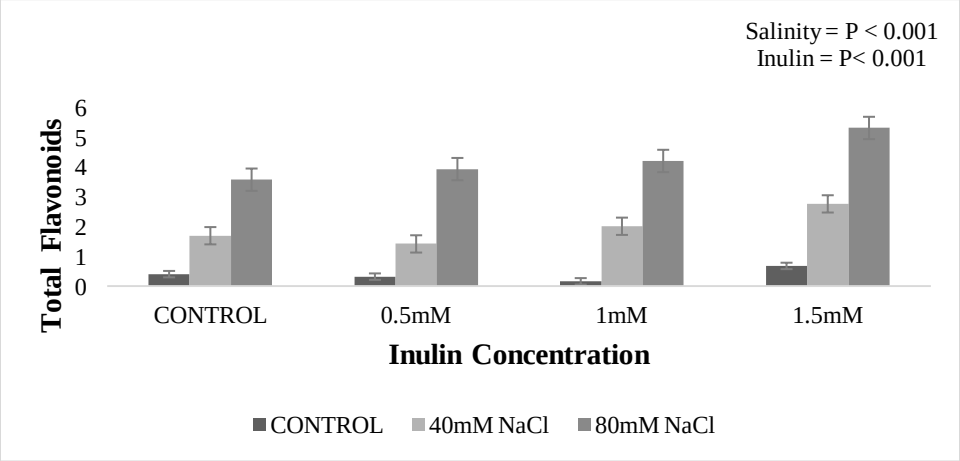

Fig. 10. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total flavonoids (mg/g fr. wt.) of *Zea mays.*

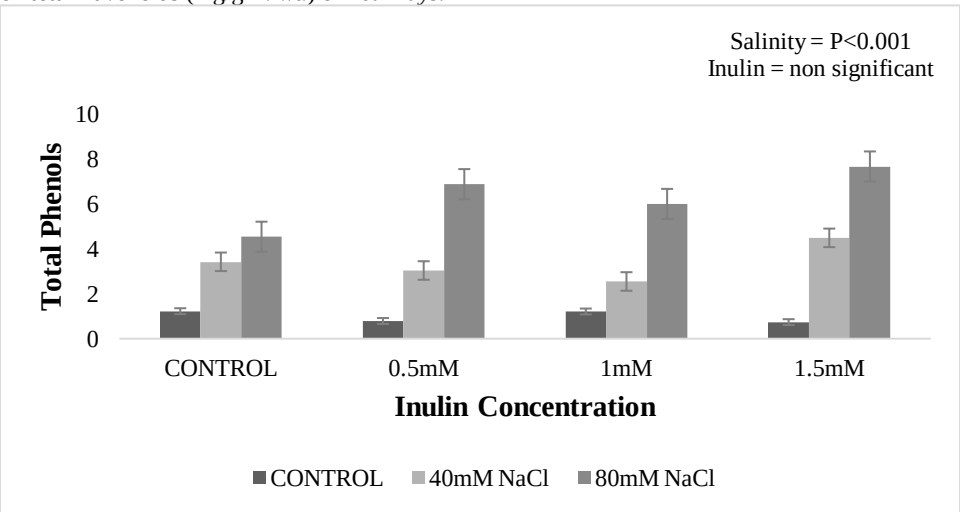

Fig. 11. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total phenols (mg/g fr. wt.) of *Zea mays.*

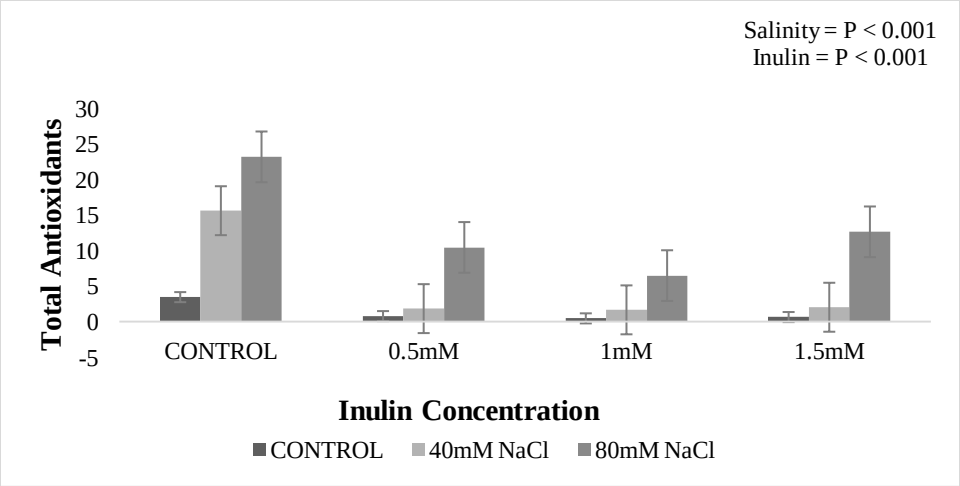

Fig. 12. To investigate the effect of different doses of salt (50mM, 100mM and 150mM) and inulin (0.5mM, 1mM and 1.5mM) on total antioxidants mg/g fr. wt.) of *Zea mays.*

Total Carbohydrates

In the present study plants treated with different concentration of NaCl showed significant (P<0.001) decrease in total carbohydrates as compared to control plants due to salt stress. Set treated with 0.5mM Inulin treatment showed non-significant decrease in control (non-saline) as well as salt stressed plants. Overall Inulin 0.5mM showed better performance as compare to other doses (1.5mM and 1mM) of Inulin. The outcome of some previously conducted researches supports our result as Mostafa (2004) reported that due to salt concentration total carbohydrates are diminish. Parida and Das (2005) noted that plants are sensitive to salt stress so with increasing concentration of salt causes decrease in carbohydrates that are required for cell growth. The main source of carbohydrates is photosynthesis and it was cleared from previous researches that the rates of photosynthesis are low in plants under salt stress.

Total Proteins

In the present study different concentration of NaCl showed significant (P<0.001) decrease in total proteins as compared to control plants. Set treated with different inulin concentrations (0.5mM, 1mM and 1.5mM) showed non-significant increase in control (non-saline) as well as salt stressed plants. Overall Inulin 0.5mM showed better performance as compared to other doses (1.5mM, 1mM) of Inulin. Parida and Das, (2002) and Abed-Latef (2005) highlighted that soluble protein is usually decreased under salt stress. Similar results were obtained by the work performed by several researchers Alaghabary *et al.* (2004); Alamgir *et al*. (1999); Gadallah (1999); Parida and Das (2005); Parvaiz and Satyavati (2008); Wang and Nil (2000) who noted reduce total soluble protein content in *Lycopersicon esculentum*, *Oryza sativa*, *Viciafaba*, *Amaranthus tricolor* and *Brugiera parviflora* plants. Osman *et al.,* (2007) reported significant reduction in protein content in *Catharanthus roseus* treated with NaCl concentrations. Ali *et al.,* (2007) reported decrease level of soluble proteins in salt stressed chamomile and sweet marjoram. Similar result was also found by Abd EL-Azim *et al.* (2009) in *Achillea fragratissima.* Yurekli *et al*., in 2004 noticed that even short-term exposure to salinity stress severely decrease protein content in leaf of *Phaseolus vulgaris*. Similarly, Porgali and Yurekli (2005) reported reduce protein content in salt sensitive tomato (*Lycopersicon esculentum*) plant. Sibole *et al*. (2003) investigated decrease or increase soluble protein content in legumes under salt stress this may be due to some species show tolerance to salt stress.

Total Flavonoids

In the present study plants treated with different concentration of NaCl showed significant (P<0.001) increase in total flavonoids as compare to their control plants. Set treated with different inulin concentrations (0.5mM, 1mM and 1.5mM) showed significant (P<0.001) decrease in control (non-saline) plants and those plants irrigated with 40mM NaCl solution while 80mM salt solutions irrigated plants showed significant (P<0.001) increase as compare to control set. Overall Inulin 1.5mM showed better performance as compare to other doses (0.5mM, 1mM) of Inulin.

The flavonoids of leaf tissue *S. glauca* are increased with increasing salinity in *S.glauca*. Ali and Abbas (2003) studied effect of salt stress (50mM and 100mM NaCl) on flavonoid content in shoots and roots of barley. They noticed significant increase in flavonoid content in root and shoot of barley in response to salt stress. Miladinova *et al*. (2013) noticed increase in total flavonoid content in leaves of the Paulownia clones (TF 01 and EF 02) with

increasing salt stress. This may be due to protective role of flavonoids under stress condition like that of proline compound. Grace and Logan (2000) reported that flavonoids have protective roles in plants and was induced by environmental stress. Moreover the work of Caturla *et al*. (2003) suggested that the modifications of flavonoid structure i.e., glycosylation, prenylation and methylation could affect their antioxidant properties, thus they may help to inhibit lipid peroxidation in stressed-plants.

Total Phenolic Compounds

In the present study plants treated with different concentration of inulin showed significant (P<0.001) increase in total phenols as compared to their control plants due to salt stress. Set treated with different dilutions of inulin (0.5mM, 1mM and 1.5mM) showed non-significant decrease in control (non-saline) plants and those plants irrigated with 40 and 80mM NaCl solution as compare to control set. Overall Inulin 1.5mM showed better performance as compared to other doses (1.5 and 1mM) of Inulin. In the lines of our findings several workers have been observed increased levels of polyphenols under salinity stress in various plants like Parida *et al*., (2004) found the similar results in *Aegiceros corniculatum*. Polyphenol content in leaflets of *S. glauca* seedlings was increased with increasing the salinity treatments. In *Bruguier aparviflora* Parida and Das (2002) noticed an increase in polyphenols with the increasing levels of salinity. Salt stress found to cause increase in total phenolic content in the halophytic species such as *B. parviflora, Agiceras corniculatum, Cakile maritima, Salvadora persica* as conclude by the work of Ksouri *et al*., 2007, Sharma and Ramawat, (2012). Supporting the similar outcome Nouman *et al.,* in (2012) expressed that total polyphenol content increased with increasing salinity in *Moringaoleifera* from 2 dS/m to 12 dS/m. Nikolova*et al*. (2005) studied that secondary plant products are considerably high in plants grown under salt stress as compare to those grown in optimal conditions. For *Nigella sativa* Bourgou *et al.,* (2010) suggested that, salinity improved the biosynthesis of quercetin, apigenin and trans-cinnamic acid. On the other hand in *Achillea fragratissima* tannin content increased significantly under salt stress Abd EL-Azimand Ahmed (2009).

Recent researches elucidate the role of different antioxidant metabolites in plant stress tolerance. Tsai *et al.,* 2002; Posmyk *et al.,* (2009) studied the biological and antioxidant properties of phenolic compounds. Rice-Evans *et al.,* (1996) recommended that the higher activity of phenolics may be due to its high H-donating capability and radical stabilization than other antioxidant metabolites. Polyphenol content in leaflets of S. *glauca* seedlings is increased with increasing the salinity treatments. Several workers have been observed increased levels of polyphenols under salinity stress in plants like *Aegiceros corniculatum* (Parida *et al*., 2004). In *Bruguier aparviflora* Parida and Das (2002) noticed an increase in polyphenols with the increasing levels of salinity. Salt stress found to cause increase in total phenolic content in the halophytic species such as *B. parviflora, Agiceras corniculatum, Cakile maritima, Salvadora persica* studied by (Ksouri *et al*., 2007, Sharma and Ramawat, 2012).

Total Antioxidants

In the present study different concentration of NaCl in all sets showed significant (P<0.001) increase in total antioxidants as compare to their control plants. Sets treated with 0.5mM , 1mM , 1.5mM Inulin solutions showed significant (P<0.001) decrease in comparison to their control plants along with those plants treated with NaCl (40 and 80mM) solutions. Overall Inulin 1.5mM showed better performance as compare to other doses (0.5mM, 1mM) of Inulin. Bartoli *et al.,* (2004) reported that the production of reactive oxygen species increases under salt stress that led to oxidative damage and disturb normal metabolism. The work of Koca *et al*., (2007) showed that plant under salt stress overproduce the reactive oxygen species and the failure of quenching activity of antioxidants resulting in oxidative damage. Demiral and Türkan (2005) also studied the increased activity of catalase in salt tolerant Pokkali under different salt concentrations. Noctor and Foyer, 1998 stated that plant responds to ROS by increasing the synthesis of antioxidant enzymes like SOD, APX and CAT. It was studied in wild beet by Bor *et al.,* (2003) and in tomato by Mittova *et al.,* (2002) and was found induced APX activity in salt tolerant plants. Parida and Das (2005) concluded that salt stress promotes oxidative stress and the activity of antioxidant enzymes and therefore have better tolerance to damage. Foyer and Noctor (2003) reported that production of reactive oxygen species is inhibited by different antioxidant enzymes like superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase and catalase. Neto *et al.* (2006); Athar *et al.* (2008) describe the responses of different species and cultivars that show degree of difference of sensitivity to salt stress illustrate a relationship between salt tolerance and increased activity of the antioxidant system.

REFERENCES

Abdel-Wahab M. A. (2006). The efficiency of using saline and fresh water irrigation as alternating methods of irrigation on the productivity of *Foeniculum vulgare* Mill subsp. *vulgare var*. *vulgare* under North Sinai conditions. *Res. J. Agr. Biol. Sci.,* 2: 571-577.

Abdel Azim W. M. and S. T. Ahmed (2009). Effect of salinity and cutting data on growth and chemical constituents of *Achillea fragratissima forss*, under Ros adr conditions. Research *Journal of Agriculture and Biological Sciences*, 5(6): 1121-1129.

Abdel-Latef A. A. (2005). *Salt tolerance of some wheat cultivars*. Ph.D.Thesis, South valley Univ. Qena, Egypt. 1-159.

Al-Aghabary K., Z. Zhu and Q. Shi (2004). Influence of Silicon supply on chlorophyll content, chlorophyll fluorescence and antioxidative enzyme activities in tomato plants under salt stress. *J. Plant Nut.*, 27: 2101-2115.

Alamgir A. N. M. and M. Y. Ali (1999). Effect of salinity on leaf pigments, sugar and protein concentrations and chloroplast ATPase activity of rice *(Oryza sativa* L.*). Photosynthetica*. 28: 145-149.

Ali R.M., H. M. Abbas and R. K. Kamal (2007). The effects of treatment with polyamines on dry matter, oil and flavonoid contents in salinity stressed chamomile and sweet marjoram. *Plant, Soil and Environment*, 53: 529–543.

Ali, R.M. and H.M. Abbas (2003). Response of salt stresses barley seedlings to phenylurea. *Plant Soil Environ.*, (4): 158–162.

Antoline M. C. and M. Sauchez–Dais (1992). Photosynthetic nutrient use deficiency, nodule activity and solute accumulation in drought stressed alfalfa plants. *Photosynthetica*, 27: 595–604.

Barros, L., P. Baptista and I. C. Ferreira (2007). Effect of Lactarius piperatus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food and chemical Toxicology, 45(9), 1731-1737.

Bartoli C. G., F. Gomez, D. E. Martinez and J. J. Guiamet (2004). Mitochondria are the main target for oxidative damage in leaves of wheat (*Triticum aestivum* L.). *J. Experi Bot.* 55: 1663- 1669.

Bor M., F. O. Zdemir 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(1): 77–84.

Bourgou S., M. E. Kchouk A. Bellila and B. Marzouk (2010). Effect of salinity on phenolic composition and biological activity of Nigella sativa. *Acta Hort*, 853: 57-60.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254.

Caturla N., E. Vera-Samper J. Villalain C. Reyes Mateo and V. Micol (2003). The relationship between the antioxidant and antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. *Free Rad. Biol. Med.*, 34: 648-662.

Darwen C. W. E. and P. John (1989). Localization of the enzymes of fructan metabolism in vacuoles isolated by a mechanical method from tubers of Jerusalem artichoke (*Helianthus tuberosus* L.). *Plant Physiology*, 89: 658–663.

De Roover J., K, Van Den Branden A. Van Laere and W. Van Den Ende (2000). Drought induces fructan synthesis and 1-SST (sucrose: sucrose 1-fructosyltransferase) in roots and leaves of chicory seedlings (*Cichorium intybus* L.). *Planta*, 210: 808–814.

Demiral T. and I. Turkan (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany*, 53(3): 247-257.

Ernst M. and J. Pfenning (2000). Fructan in stem exudates of *Helianthus tuberosus* L. In: Proceedings of the Eighth Seminar on Inulin (ed. A. Fuchs), pp. 56–58. EFA, Stuttgart, Germany.

Eryilmaz F. (2006). The relationships between salt stress and anthocyanin content in higher plants. *Biotechnol Equipment*, 20:47-52.

Flowers T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany*, 55(396): 307-319.

Gadallah M.A.A. (1999). Effects of proline and glycinebetaine on Vicia faba response to salt stress. *Bio. Plant*, 42: 249-257.

GOP. (2005). Economic Survey Govt. of Pakistan 2004-2005, Finance Division, Economic Advisory Wing, Islamabad, Pakistan.

Grace S.C. and B. A. Logan (2000). Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Phil. Trans. Royal Soc. B*, 355: 1499-1510.

Hassanein R.A., F. M. Bassuony, D. M. Baraka and R. R. Khalil (2009). Physiological Effects of Nicotinamide and Ascrobic Acid on *Zea mays* L. Plant Grown Under Salinity Stress. I-Changes in Growth, Some Relevant Metabolic Activities and Oxidative Defense Systems. *Res. J. Agric. Biol. Sci.*, 5: 72–81.

Koca H., M. Bor F. Ozdemir and I. Turkan (2007). Effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot.*, 60: 344–351.

Koocheki A., M. Nassiri-Mahallati and G. Azizi (2008). Effect of drought, salinity, and defoliation on growth characteristics of some medicinal plants of Iran. *J. Herbs Spices Med. Plants*, 14(1-2): 37-53.

Ksouri, R., W. Megdiche A. Debez H. Felleh C. Gringon and C. Abdelly (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritime. *Plant Physiol. Biochem.,* 45: 244-249.

Kumar, P. and A. Das (2005). Salt tolerance and salinity effects on plants. *Ecotoxicology and Environmental Safety*, 60: 324–349.

Lans C. A. (2006). Ethnomedicines used in Trinidad and Tobogo for urinary problems and diabetes mellitus. *J. Ethnobiol. Ethnomed.,* 2(45).

Livingston, D. P. and C. A. Henson (1998). Apoplastic sugars, fructans, fructan exohydrolase, and invertase in winter oat: responses to second-phase cold hardening. *Plant Physiology*, 116: 403–408.

Gomes, M. A. D. C., S. S. Marina D. C. Maura and F. T. Cristiane (2011). Effect of salt stress on nutrient concentration, photosynthetic pigments, proline and foliar morphology of *Salvinia auriculata* Aubl. *Acta Limnologica Brasiliensia*, 23(2): 164-176.

Maclachlan, C. and S. Zalik (1963). Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. *Canadian Journal of Botany*; 41: 1053-1062.

Malik E. P. and M. B. Singh (1980). *Plant Enzymology and Hittoenzymology* (1st Edn). Kalyani Publishers: New Delhi; 286.

Meloni D.A., M. A. Oliva C. A. Martinez and J. Cambraia (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*, 49: 69-76.

Miladinova, K., K. Ivanova T. Georgieva M. Geneva and Y. Markovska (2013). Influence of salt stress on ex vitro growth and antioxidative response of two Paulownia clones. Istitute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. Georgi Bonchev str., bl. 21, Sofia 1113, Bulgaria.

Misra A. N., S. M. Sahu M. Misra P. Singh I. Meera N. Das M. Kar and P. Sahu (1997). Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. *Biologia Plantarum*, 39: 257–262.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.,* 7: 405–410.

Mittova, V., M. Tal M. Volokita and M. Guy (2002). Salt stress induces upregulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. *Plant Physiology*, 115: 393–400.

Mostafa D. M. (2004). Metabolic imbalance and salinity tolerance of two maize cultivars. M.Sc. Thesis. El-Minia Univ. Elminia, Egypt 1-195.

Munns R. and M. Tester (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59: 651-681.

Najafi F., R. A. Khavari- Nejad and M. S. Ali (2010). The effects of salt stress on certain physiological parameters in summer savory (*Satureja hortensis* L.) plants. *J. Stress Physiol. Biochem.* 6: 13-21.

Nikolova M. T. and S. V. Ivancheva (2005). Quantitative flavonoid variations of *Artemisia vulgaris* L. and *Veronica chamaedrys* L. in relation to altitude and polluted environment. *Acta Biol Szegediensis.* 49: 29-32.

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

Nouman W., M. T. Siddiqui S. M. A. Basra T. R. A. Khan Gull M. E. Olson and H. Munir (2012). Response of *Moringa oleifera* to saline conditions. *Int. J. Agric. Biol.,* 14: 757–762.

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

Owoyele B. V., M. N. Negedu S. O. Olaniran S. A. Onasanwo S. O. Oguntoye J. O. Sanya S. A. Oyeleke A. J. Ibidapo and A. O. Soladoye (2010). Analgesic and anti-inflammatory effect of aqueous extract of *Zea mays* husk in male Wistar rats. *J. Med. Food.,* 13(2): 343-47.

Parida A.K., A. B. Das Y. Sanada and P. Mohanty (2004). Effects of salinity on biochemical components of the mangrove. *Aegiceras corniculatum, Aquatic Bot.*, 80 : 77–87.

Parida A. and A. B. Das (2002). NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J. Plant Biol.,* 45: 28– 36.

Parida A.K. and A. B. Das (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety*, 60(3): 324-349.

Parvaiz A. and S. Satyavati (2008). Salt stress and phyto- biochemical responses of plants- a review. *Plant, Soil and Enviro.,* 54: 89-99.

Patricia A., J. C. Thomas D. M. Vernon H. J. Bohnert and R. G. Jensen (1992). Distinct cellular and organismic responses to salt stress. *Plant Cell Physiol.,* 33: 1215–1223.

Porgali Z.B. and F. Yurekli (2005). Salt stress induced alterations in proline accumulation, relative water content and superoxide dismutase (SOD) activity in salt sensitive *Lycopersicon esculentum* and salt tolerant L. *pennellii. Acta Botanica Hungarica,* 47: 173- 182.

Posmyk M. M., R. Kontek and K. M. Janas (2009). Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol. Env. Safety,* 72: 596-602.

Rice-Evans C. A., N. J. Miller and G. Paganga (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids, Free Radical. *Biol. Medi.,* 20: 933-956.

Roberfroid M. B. (2005). Introducing inulin-type fructans. *Br J Nutr.*, 93 Suppl 1: S13–25.

Roberfroid M.B. (2007). Inulin-type fructans: functional food ingredients. Journal of Nutrition, 137 (11 suppl): 2493S–2502S. PMID 17951492.

Sairam R. K., G. C. Srivastava S. Agarwal and R. C. Meena (2005). Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biol. Plant.,* 49: 85-91.

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

Sharma V. and K. G. Ramawat (2012). Salinity-induced modulation of growth and antioxidant activity in the callus cultures of Miswak (*Salvadora persica*), 3 *Biotech,* 13205-012-0064-6.

Sibole J. V., C. Cabot C. Poschenrieder and U. J. Barcel (2003). Efficient leaf ion partitioning; an overriding condition for abscisic acid - controlled stomatal and leaf growth responses to NaCl salinization in two legumes. *J. Experi Bot.,* 390: 2111-2119.

Siler B., D. Misic B. Filipovic Z. Popovic T. Cvetic and A. Mijovic (2007). Effects of salinity on in vitro growth and photosynthesis of common centaury (*Centaurium erythraea* Rafn.). *Arch. Biol. Sci. Belgrade,* 59:129-34.

Singh A. K. and S. S. Dubey (1995). Changes in chlorophyll a and b contents and activities of photosystems 1 and 2 in rice seedling induced by NaCl. Photosynthetica, 31: 489–499.

Somogyi M (1952). Notes on sugar determination. *J. Biol. Chem.,* 195:19–23

Stepien P. and G. Klobus (2006). Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biol. Plant,* 50: 610-616.

Sprenger N., K. H. Bortlik A. Brandt T. Boller and A. Wiemken (1995). Purification, cloning, and functional expression of sucrose: fructan 6-fructosyl transferase, a key enzyme of fructan synthesis in barley. *Proceedings of the National Academy of Sciences*, USA. 92: 11652–11656.

Taiz L. and E. Zeiger (1991). *Plant Physiology*. California: The Benjamin/Cummings Publishing Company, Inc.p346-368.

Tsai P. J., J. Intosh P. Pearce B. Camden and B. R. Jordan (2002). Anthocyanin and antioxidant capacity in Roselle (*Hibiscus sabdariffa* L.) extract. *Food Res. Int.,* 35: 351-356.

Vajpayee P., R. D. Tripathi U. N. Rai, I. M. B. Ali and S. N. Singh (2000). Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. *Chemosphere,* 41: 1075-1082.

Van Den Ende W., A. Michiels D. Van Wonterghem *R.* Vergauwen and A. Van Laere (2000a). Cloning, developmental and tissue-specific expression of 1-SST (sucrose: sucrose 1-fructosyltransferase) from *Taraxacum officinale.* Fructan localization in roots. *Plant Physiology*, 123: 71–80.

Wang Y. and N. Nil (2000). Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. *J. Horti. Sci. Biotech.,* 75: 623-627.

Xinwen X., X. Hailiang W. Yangling W. Xiaojing Q. Yongzhi and X. Bo (2008). The effect of salt stress on the chlorophyll level of the main sand – binding plants in the shelterbelt along the Tarim Desert Highway. *Chinese Science Bulletin,* 53: 109-111.

Yemm, E. W and Willis, A. J. (1956). The respiration of barley plants: IX. The metabolism of roots during the assimilation of nitrogen. *New Phytologist*, 55(2): 229-252.

Yen, G. C., and Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their anti mutagenicity. *J. Agri. Food Chem.* 43(1): 27-32.

Yurekli F., Z. B. Porgali and I. Turkan (2004). Variations in abscisic acid, indole-3-acetic acid, gibberellic acid and zeatin concentrations in two bean species subjected to salt stress. *Acta Bio Cracoviensia Series Botanica*. 46: 201-212.

Zhu J.K. (2007). Regulation of ion homeostasis under salt stress. *Curr. Opin.Plant Biol.*, 6: 441-445.

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