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EFFECT OF SELENIUM ON THE BIOCHEMISTRY OF *ZEA MAYS* UNDER SALT STRESS

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Abstract

It is well known that salinity badly effect plant growth all over the world and greatly reduces crop production in the affected regions. Selenium functions as antioxidant in plants and in low concentration it also promotes plant growth and produce tolerance against stress. The present study was designed to check the effect of salt and selenium on the biochemistry of *Zea mays* like chlorophyll a, chlorophyll b, carotenoids, carbohydrates, reducing sugars, non-reducing sugars, total antioxidants, flavonoids, total phenols and proteins. *Zea mays* was grown in pots and maize plants were irrigated with different concentration of NaCl (0, 40 mM and 80 mM). Different concentrations of selenium (0, 5 mM, 10 mM and 15 mM) were applied foliarly after one month of germination.

An increase in salt concentration considerably decrease the chlorophyll a, chlorophyll b and carotenoids, reducing sugars, non-reducing sugars, carbohydrates, and total proteins while flavonoids, phenols and total antioxidants showed significant increase with elevated salt concentration. Foliar application of different concentrations of selenium showed an increase in total proteins, reducing sugars, non-reducing sugars, phenols and flavonoids under saline as well as non-saline conditions. It is concluded from this project that salt has an inhibitory effect on the biochemistry of *Zea mays* as photosynthetic pigments, carbohydrates, proteins, reducing sugars, non-reducing sugars showed a decline under salt stress, while total antioxidants phenols and flavonoids showed an increase under salt stress. After the application of selenium spray, the proteins, reducing sugars, non-reducing sugars, phenols and flavonoids showed an increase under both (saline and non-saline) conditions. It is suggested that selenium should applied at 10 mM as it showed better performance on different biochemical attributes in both saline and non-saline conditions.

Key words: Salinity, Selenium, Chlorophyll, Proteins, Carbohydrates, Flavonoids, Phenols.

Introduction

Salinity is one of the most widely known stress which badly effect crop production and it is estimated that about 20% of cultivated land is negatively affected by salinity (Flowers & Yeo, 1995; Shinwari *et al*., 1998; Narusaka *et al*., 1999). Simply salinity can be defined as the presence of salts in soil in a concentration that is detrimental to crops and this problem is increasing day by day due to the use of poor quality water for irrigation and poor drainage. Salt stress inhibits seed germination, plant growth and development, (Dash & Panda, 2001; Nakashima *et al*., 2000), seedling development (Ashraf *et al.,* 2002), inhibit enzyme action (Seckin *et al.,* 2009), disturb DNA, RNA and protein synthesis (Anuradha & Rao, 2001; Narusaka *et al*., 2003). It also affect the processes of cell division specially mitosis (Tabur & Demir, 2010).

Maize plant has separate male and female flowers. It is a common staple food the color of its grain varying from yellow and sometime ranging to black. To some extent due to its significant potassium content, corn silk is a useful diuretic and other problem of the urinary system. Corn silk relaxes the lining of the urinary tubules and bladder also reduces inflammation and help in urine flow and elimination. It is also observed that corn silk have a positive effect on the kidneys, prevent kidney stone formation and reduce some of the symptoms of existing stones. Corn silk is also helpful in treatment of chronic cystitis.

Selenium acts as an essential trace element for animals and humans or act as toxicant to the environment, the boundary between the two is narrow and depends on its chemical form, concentration, and other environmentally regulating variables (Fan *et al*., 2002; Shardendu *et al*., 2003). Selenium exists in very small amounts in humans, plants, animals and microorganisms. Although it has an importance as micro element and exists in small amounts but its toxicity occurs at high concentrations due to replacement of sulphur with selenium in amino acids resulting in incorrect folding of the protein and consequently nonfunctional proteins and enzymes. It has the ability to increase the plant tolerance to UV-induced oxidative stress, delay senescence, and promote the growth of ageing seedlings (Xue *et al*., 2001; Pennanen *et al*., 2002). Recent studies showed that selenium has the ability to regulate the water status of plants under conditions of drought (Kuznetsov *et al*., 2003). Hartikainen *et al*. (2000) reported that selenium has growth promoting effect in ryegrass. Other studies evaluated the effect of pH, temperature, hardness etc. on selenium toxicity. Mostly studies have been carried out on sulphate in relation to selenium uptake and toxicity in aquatic and terrestrial organisms (Sappington, 2002). Sulphur and selenium has very similar chemical properties and their uptake and assimilation proceed through common pathways (Eapen & D'Souza, 2005). The present work was designed to check the effect of different selenium concentration on some biochemical attributes of *Zea mays* grown under NaCl induced salt stress.

Material and Methods

The present project was designed to check the effect of selenium spray on total phenols, total flavonoids, total antioxidants, total carbohydrates, total proteins, total chlorophyll total reducing sugars and non-reducing sugars of *Zea mays* grown under salt stress. Seeds of *Zea mays* were obtained from the local market of Mardan, Khyber Pukhtunkhwa. The experiment was composed of 48 pots

which were divided into 4 sets and these were treated with different concentration of salt selenium. The details of these sets were as follow:

Set 1 (Control): Plants sprayed with distilled water (Without selenium application) and irrigated with different doses of salt [control (non-saline), 40mM and 80mM].

Set 2: Plants sprayed with 0.5mM selenium solution and irrigated with different doses of salt [control (non-saline), 40mM and 80mM].

Set 3: Plants sprayed with 1mM selenium solution and irrigated with different doses of salt [control (non-saline), 40mM and 80mM].

Set 4: Plants sprayed with 2mM selenium solution and irrigated with different doses of salt [control (non-saline), 40mM and 80mM].

These 4 sets have 12 pots in each set with 3 replicates per treatment. In each set the first 3 pots were kept as control and in other different concentration of NaCl solution was applied (40 mM and 80 mM). The plastic pots were 15.7 cm in diameter and were 6.5cm deep. Each pot was filled with equal amount of clay loam. Surface sterilization was done with 1% mercuric chloride for one minute after that rinse 2 to 3 time with distilled water and were kept in distilled water for 30 minutes. Five seeds were placed in each pot and these pots were provided with uniform amount of tap water. The 48 pots were placed orderly in the Botanical Garden, Department of Botany, Abdul Wali Khan University, Mardan. When seeds germinated and on reaching three leaf stage, the plants were irrigated and the soil saturated with hoagland solution. After that pots were treated with different NaCl concentrations i.e. 40 mM and 80 mM. Plants were foliarly applied with different concentrations of selenium (0, 5 mM, 10 mM and 15 mM) after 1 month of germination.

Biochemical analysis: Leaves sample were collected at a grand period of growth for the estimation of phenols, carbohydrates, antioxidants, flavonoids, chlorophyll a, chlorophyll b, total chlorophyll, reducing sugars, proteins, carotenoids.

Extraction and estimation of chlorophyll: Chlorophyll content was estimated by Maclachlam & Zalik (1963) method. Fresh leaves were taken and grind with three mL of 80 percent acetone and centrifuged at 1000 rpm for 5 mints. The plant material was centrifuged 3 times by using one mL of 80 percent acetone each time. The liquid part was poured into test tube and finally making the volume upto seven mL with distilled water. Optical densities were recorded at different wavelength i.e. 663nm, 645nm, 510nm and 480nm.

Equation

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

Determination of reducing and non-reducing sugars: Reducing sugars was determined by the method proposed by Nelson (1944), Somogyi (1952). Take 100 mg of the plant material and the sugar was extracted from it by the addition of five mL of hot 80% ethanol two times. The supernatant was collected and then evaporate it on water bath at 80^{o}C and add 10mL water in order to dissolve the sugars. After that Pipette out 0.1 or 0.2 mL aliquots into separate test tube. Similarly Pipette out 0.2, 0.4, 0.6, 0.8 and one mL from working standard solutions into a separate series of test tubes. Finally raise the volume of both standard and sample test tubes to two mL with distilled water. Take 2 mL of distilled water in a separate test tube to use as a blank. One mL of alkaline copper tartarate reagent is added to each tube. These tubes are then place in boiling water for ten minutes, cool the tubes and added one mL of arsenomolybolic acid reagent.

Extraction and estimation of total carbohydrates: Extraction and estimation of total carbohydrates was performed following Anthrone method (Yemm & Willis, 1956).

Extraction: Fresh leaves (0.1 gm) was crushed in a mortar with 5 mL of distilled water. This material was centrifuged at 1000 rpm for about ten minutes. The liquid part was then collected in another test tubes and residue was discarded.

Estimation: Take 0.5 mL of plant extract in a test tube and add 5 mL of anthrone reagent into it. Test tubes were then placed on water bath for about 15 minutes for heating and directly cooled in ice cold water. Appearance of green color was noted and optical density was recorded at 620 nm. Anthrone reagent was taken as the reagent blank.

Determination of soluble proteins: Extraction and estimation of soluble proteins was performed by Bradford's Assay reagent (Bradford, 1976).

Extraction of soluble proteins: Homogenize 0.1 gm of leaves in 5 mL of Potassium phosphate buffer (0.1M, pH7) with ice chilled pestle and mortar. The homogenate or extract filter with cheese cloth or with glass wool. The extract placed in freezer for chilling. After chilling the extract centrifuged at 4000 rpm for 10 minutes (centrifuge at 12,000 rpm for 20 minutes if high speed refrigerated centrifuge is available). The supernatant transferred in a test tube and rise the volume five mL with buffer. For dilution, take 0.2ml of this extract and add 4.8 ml buffer (this extract will give dilution factor as 20).

Estimation of soluble proteins: Take 0.1 mL of diluted extract in test tube then add five mL Bradford assay reagent and shake. For reagent blank, take 0.1 ml of buffer and then add 5 ml Bradford assay reagent. Measurement of optical density (O.D) was taken at 595 nm in a spectrophotometer. The amount of soluble proteins is determined by standard curve.

Total phenol estimation: Total phenolic contents of leaves were calculated by the method described by Malik and Singh (1980). Take aliquots of the extracts in a ten ml glass tube and make up the volume upto three mL with distilled water. Add 0.5 mL folinciocalteau reagent (1:1 with water) and two mL Na2CO3 (20%) in each tube. A blue color was appear in each tube. The test solutions were then warmed for one minute immediately cooled and OD was calculated

at 650 nm against the catechol reagent used as a blank. A standard calibration curve was generated at 650 nm by using different concentrations of catechol. Phenols contents in the test samples were determine from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Total flavonoids estimation: Total flavonoid content of the sample extracts was measured by aluminum chloride method (Mervat *et al.,* 2009). In this method five gm of sample was dissolved in 50 mL of 80% ethanol. The sample was then placed in shaking incubator for 24 hours. This extract was then centrifuged at 10,000 rpm at 25ºC for 15 mints. After centrifugation the supernatant was stored in 50 mL falcon tube as it containing flavonoid at 4ºC. Estimation was carried out by a spectrophotometric assay as described by Lillian *et al*., (2007). 250 µl supernatant was mixed with 1.25 mL of distilled water and 75 µl of 5% $NaNO_2$ solution. The solution was leave for 5 min and then adds 150 µl of a 10% $AlCl_3$.H2O and placed in incubator for 6 min. finally 500 µl of 1M NaOH and 275 µl of distilled water were added to the mixture. At the end solution was mixed well and optical density was measured at 415 nm against blank that is 80% ethanol. Quercetin different concentrations (15 µg- 500 µg) were prepared to make standard curve.

Total antioxidants: The ferric ion reducing power of samples was determined by using method of Yen and Chen (1995). 750µl extract of each sample was mixed with phosphate buffer and 1% potassium ferrciyanide. The sample was then incubated at 50 ºC for 20 minutes and then added 10% of an equal amount of trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. 1.5 mL of liquid was separated from the surface and mixed with an equal amount of distilled water and 0.1 mL $FeCl_3$ solution. The absorbance was measured at 700 nm. Greater the absorbance greater will be the reducing power capability of sample.

Experimental design and statistical analysis: The experimental design was completely randomized design by using two salt levels and three replicates. The data was analyzed statistically by using SPSS to analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($p<0.05$).

Results and Discussion

Chlorophyll Contents: In this study plants treated with different concentration of NaCl (0, 40 Mm and 80 mM) in all sets showed significant ($p<0.01$) decrease in total chlorophyll contents as compared to their control due to salt stress (Figs. 1-4). The outcome of previously conducted researches supports our current findings. According to the work of Jaleel *et al.* (2007), salt stress affects different morphological parameters of plants. Mainly its affect is concentration dependent as at low concentration it slightly decrease the chlorophyll a and b, and total chlorophyll content but it elevated concentration it greatly reduced the photosynthetic pigment (Masood *et al*., 2005). This affect was observed in *Catharanthus* plants under salt stress. The work of Senay *et al.,* (2011) also supported my result who worked on four pumpkin genotypes. The effect of salt stress on the chlorophyll content in pumpkin genotypes exhibited decreased. Similar studies were also carried out by Akca & Samsunlu (2012) who observed decrease in chlorophyll contents in three walnut cultivars. Reduction in chlorophyll content was the result of increasing salt applications. It is estimated that application of 5 dS/m salt reduced the chlorophyll a and chlorophyll b content 38, 27% and 32% respectively as compare to control. In the present study plants treated with different concentration selenium (5mM ,10mM and 15mM) showed non significant decrease as compare to control plants. The result is in accordance of Pedrero *et al.,* (2007), who noted reduction in chlorophyll content in Broccoli (*Brassica oleracea*) plants subjected to cadmium exposure. The addition of selenium alleviated cadmium-induced stress in the leaves.

Carotenoids: In the present study plants treated with different concentration of sodium chloride showed significant decrease in total carotenoids compare to control plants (Fig. 5). The outcome of previously conducted researches supports our result. Saikachout *et al*. (2009) work on two varieties of *A. hortensis* and investigated that NaCl toxicity reduced carotenoids contents of plant. Similar results were reported in *Zea mays* genotypes (Singh *et al*., 2008) and *Triticum aestivum* genotypes (Sairam *et al*., 2002). Sali *et al.,* (2015) perform research to study the effect of salinity stress on seed germination and chlorophyll content in maize. He investigated considerably lower content of carotenoids at elevated concentrations of treatments. The work of Nirmala *et al.,* 2015 also supported this fact who worked on the effect of salt stress on two mungbean varieties. During this study he noted that salinity decrease chlorophyll and carotenoid contents which resulted chlorosis and necrosis in leaves. Plants treated with different concentrations of selenium (5 mM, 10 mM and 15 mM) showed significant ($p<0.001$) variation.

Reducing and non-reducing sugars: Plants treated with different concentration of sodium chloride showed significant ($p<0.001$) reduction in total reducing sugars and non-reducing sugars as compared to control plants (Figs. 6-7). Hakim *et al.,* (2013) observed that Reducing sugar and total sugar decreased up to $8dSm^{-1}$ and decreased up to $12dSm^{-1}$. Non reducing sugar decreased with increasing the salinity levels in all varieties in *Oryza sativa*. Kaur *et al.,* (2013) also observed total soluble sugars content was decreased with increased concentrations of NaCl as compared to control. Plants treated with different concentrations of selenium (5 mM, 10 mM and 15 mM) showed significant ($p<0.001$) increase at different concentration of salt (80 mM and 40 mM) and control sets except 80mM salt treated plants of 5mM selenium applied set which showed decrease as compare to their control set.

Carbohydrates: In the present study plants treated with sodium chloride different concentration showed significant reduction in total proteins as compare to their control plants (Fig. 8). Findings of Amel *et al.,* (2011) revealed that carbohydrate contents were reduced at salinity extremes in *D. salina* and *D. tertiolecta*. Ben-Amotz *et al*., (1985) reported that *Botryococcus braunii* treated with 0.5 M NaCl show decrease carbohydrates contents in their cells. Similarly Rejeskova *et al.,* (2007) observed that salinity

caused a decrease in total carbohydrates in *Oleaeuropaea* shoots. This decrease is not only due osmotic stress but also due to the presence of toxic Na^+ and Cl^- ions. Mostafa (2004) reported that even at low concentration sugar and total carbohydrates contents of plants are decreased because most of the plants are sensitive to salt stress. Plants treated with different concentrations of selenium showed great increase in this parameter in saline as well as non-saline medium except control plants in the set applied with 15mM selenium as compare to control set.

Total proteins: In the present study different concentration of sodium chloride (40Mm and 80mM) in all sets showed great reduction in total proteins contents of plant as compare to their control (Fig. 9). Datta *et al*. (2009) worked on the impact of salt stress on different varieties of Wheat (*Triticum aestivum* L.) cultivars under laboratory condition and reported that there was a significant decreased in protein contents of leaves with increase in the salt concentrations as compared to the control. Similar result was reported by Parvaneh *et al*., (2011) while working on Purslane (*Portulaca oleracea* L.) leaves and observed decreasing of leaf proteins concentrations with increasing of salinity stress. Mahboobeh & Akbar (2013) also noted during their work on effect of salinity on the protein content of transgenic *Nicotiana plumba ginifolia* over expressing P5CS gene and find out that protein content of the non-transgenic plants reduce when salinity was increased and at 200 and 250mM it is much lower than the other salt concentrations (Merril, 1990). Kumari & Vishnuvardhan (2014) worked on the effect of salinity on protein in three Kodo Millet (*Paspalum scrobiculatum*) Germplasm and find out that as NaCl concentration increases the availability of proteins seems to be decrease in all accessions. Protein content in *Catharanthus roseus* had been significantly decreased along with NaCl concentrations (Osman *et al.,* 2007). Plants treated with different concentrations of selenium showed considerable increase in this parameter in saline as well as non-saline medium except control plants in the set applied with 15mM selenium as compare to control set.

Total phenols: In the present study plants treated with NaCl showed high increase in total phenols as compare to their control plants due to salt stress (Fig. 10). The work of Mohammad Al-Hassan *et al.* (2015) supported my result who observed total phenols increased in cherry tomato under salt stress, particularly those that are treated with elevated salt solutions. Simply salt stress significantly increases phenols contents in leaves. Singh *et al.,* (2015) observed that content of total phenolics was increased by 64%, in *Zea mays* the plants after salt treatment. Abd EL-Azim *et al*., 2009 indicated that in *Achillea fragratissima* the content of phenols increased significantly along with increasing salinity. Plants treated with different concentrations of selenium under salinity (5Mm, 10mm and15mm) showed significant ($p<0.05$) decrease as compare to control plants. The work of Põldmai *et al.,* (2013) showed an increase in total phenolics in onion bulbs with selenium treatment. Sellappan *et al.,* (2002) have reported total polyphenol content in white onions to be 7.3mg of $GAg^{-1}$of DM. Considering that average DM content of our experimental onions was 11%, the content of total phenolics in our onions was higher. Se 50 treatment increased total phenolic content significantly in both years and Se10 treatment in 2009, whereas the greatest increase (26%) was caused by Se 50 treatment in 2008.

Total antioxidants: In the present study different concentration of NaCl showed great increase in total antioxidants as compare to their control plants due to salt stress (Fig. 11). The work of Mohamad Al-Hassan *et al.,* (2015) is in agreement with my study who observed an increase in antioxidant content in cherry tomato plant under salt stress. Ebtihal M. AbdElhamid *et al.* (2014) also observed that the performance of different antioxidant enzymes greatly increased due to salinity stress in wheat cultivars. The activity of antioxidant enzymes i.e., superoxide dismutase, catalase, peroxidase, polyphenol oxidase, ascorbate peroxidase and glutathione reductase increased due to salinity stress. Gao *et al.,* (2008) work on the effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings and observed a significant increase in antioxidant enzymes superoxide dismutase, peroxidase, catalase and phenylalanine ammonia-lyase. Suraj (2015) worked on effect of NaCl on antioxidant enzymes of Isabgol (*Plantago ovata* Forsk.) Genotypes and observed higher activity of superoxide dismutase, catalase and ammonia-lyase leaves of all the isapgol genotypes under stress conditions. In the this study plants treated with different concentrations of selenium (5Mm, 10mm 15mm) showed significant ($p<0.05$) decrease under salinity as compare to control plants. The work of Põldma *et al.* (2013) supported our results and observed decrease in total antioxidant contents in the bulb of the onions while treatment with selenium. He demonstrated that Total antioxidant capacity (TAC) of onion bulbs in the current experiment was affected by Se treatment. It appeared from the current experiment that for onions, the largest decrease in TAC also occurred when lower rates of Se were used.

Flavonoids: In the present study different concentration of NaCl showed an increase in total flavonoids as compare to their control plants (Fig. 12). The work of Rajamane & Gaikwad (2014) also support my result and they observed that in control plants of *S. glauca* flavonoids are relatively low as compared to salt stressed plants. The flavonoids of leaf tissue *S. glauca* are increased with increasing salinity. Ali & Abbas (2003) studied effect of salt stress (50 and 100 mM NaCl) on flavonoid content in shoots and roots of barley. They noticed great increase in flavonoid content in radical and plumule of barley in due to salt stress. Miladinova *et al*., (2013) noticed increased in total flavonoid content in leaves of the Paulownia clones (TF_{01} and EF_{02}) with increasing salt stress (50, 100 and 200 mg/L NaCl treatment). HalaEzzat Mohamed Ali *et al*., (2014) also noted great increase in the total flavonoid contents of tomato fruits under salt stress by about 16.5%, 93.5%, and 97.7%, respectively as compared to the control. Plants treated with different concentrations of selenium (5 mM ,10 mM and 15 mM) showed significant ($p<0.01$) increase as compare to control plants.

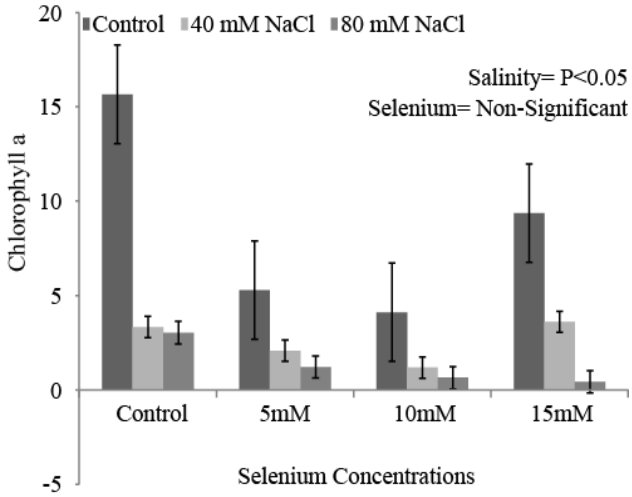

Fig. 1. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on chlorophyll a (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

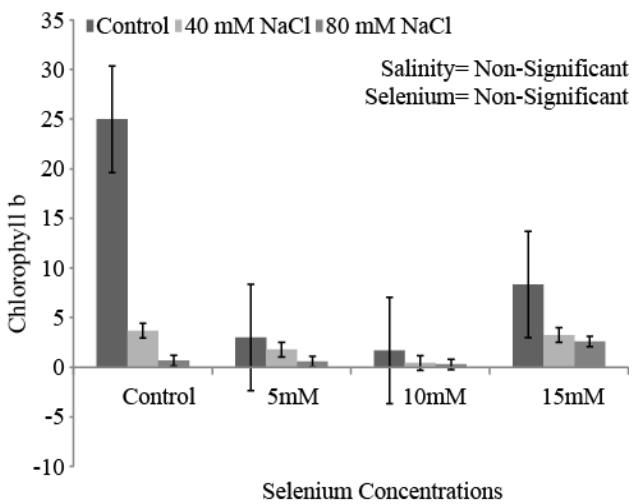

Fig. 2. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on chlorophyll b (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

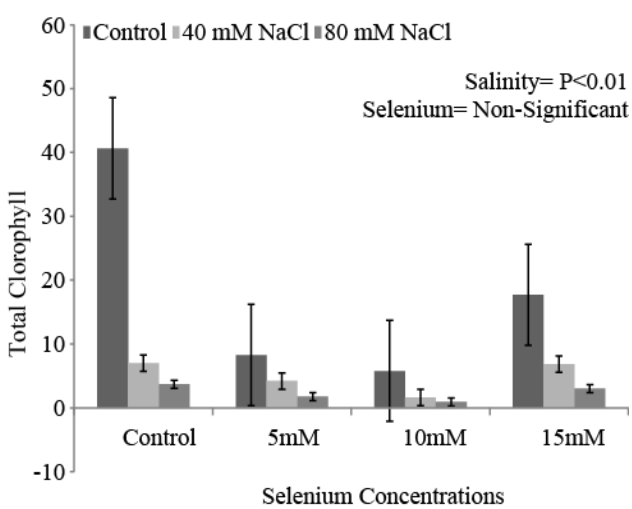

Fig. 3. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total chlorophyll (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

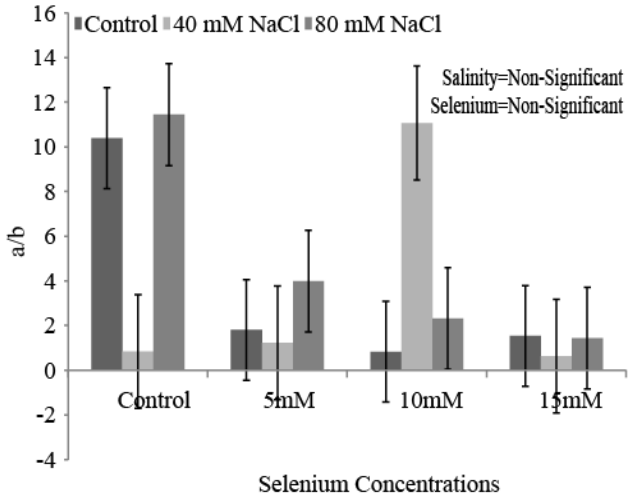

Fig. 4. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on chlorophyll a/b in leaves of *Zea mays* grown under salt stress.

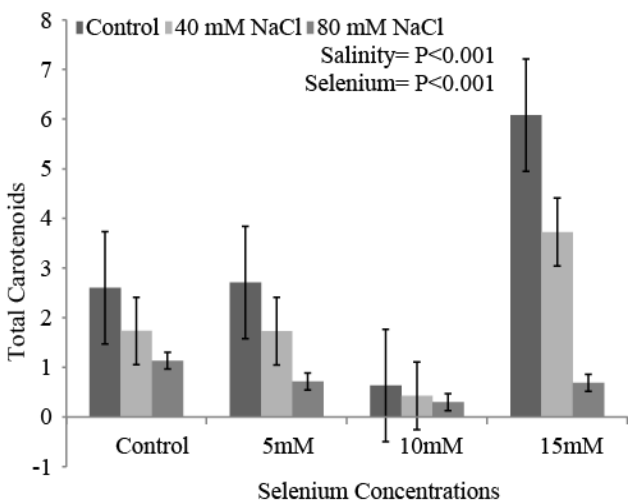

Fig. 5. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total carotenoids (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

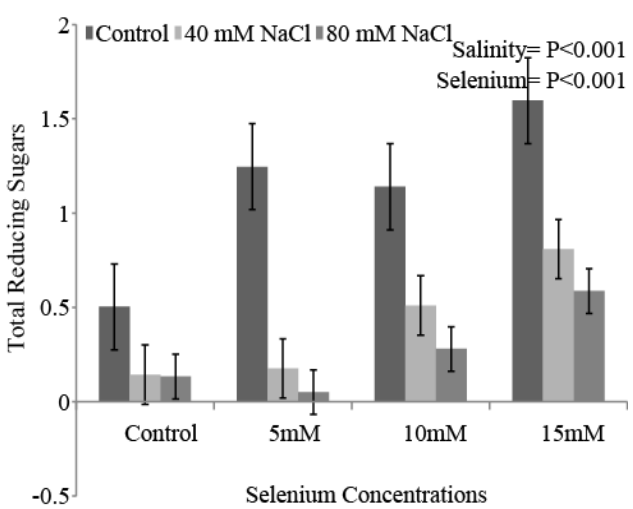

Fig. 6. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total reducing sugar s (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

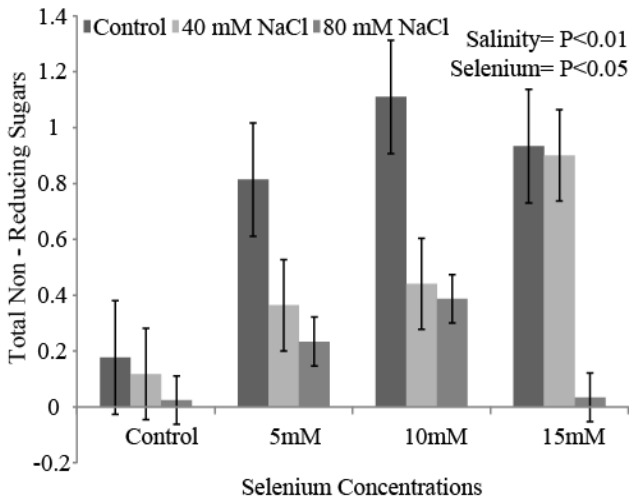

Fig. 7. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total non-reducing sugars (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

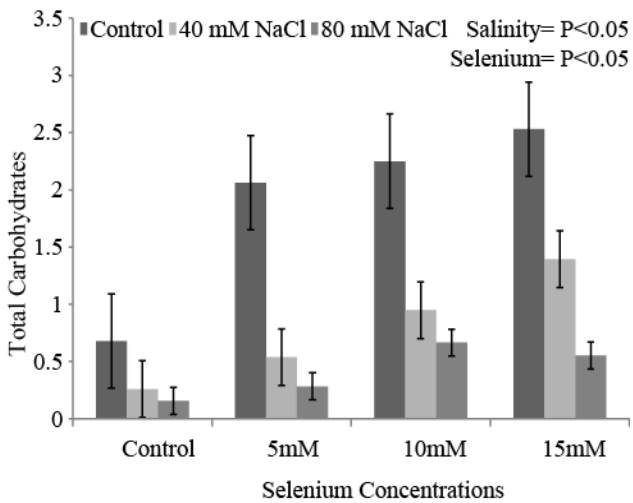

Fig. 8. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total carbohydrates (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

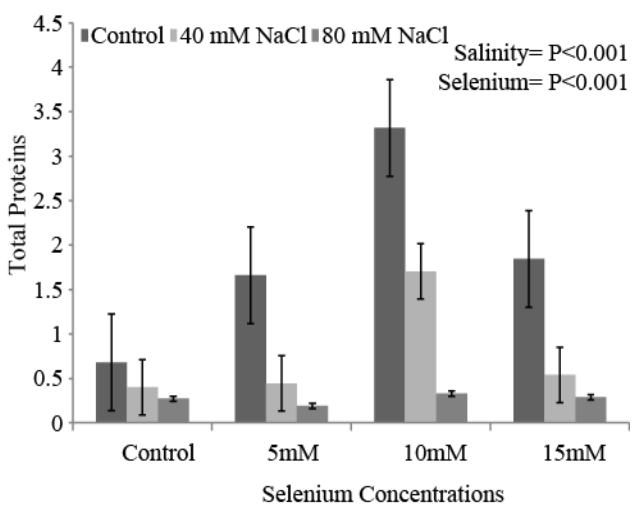

Fig. 9. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total proteins (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

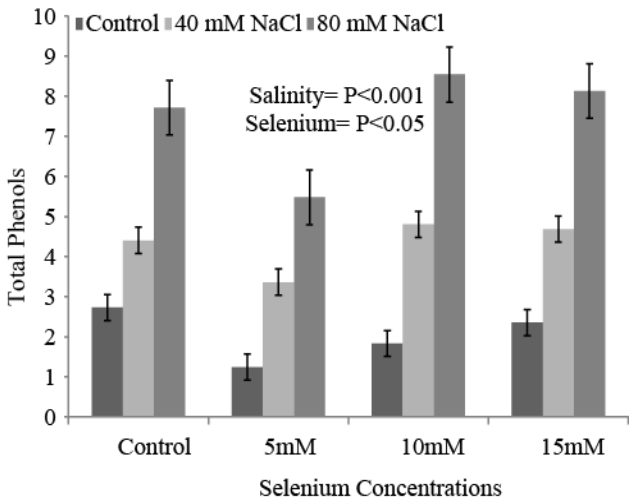

Fig. 10. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total phenols (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

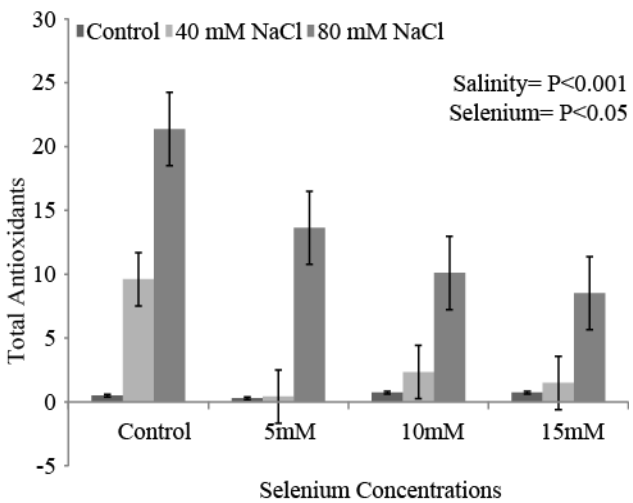

Fig. 11. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total antioxidants (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

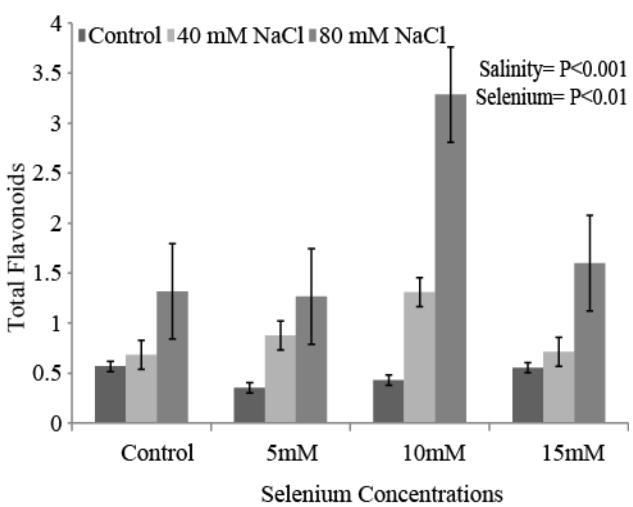

Fig. 12. The impact of different concentrations of selenium (5mM, 10mM and 15mM) on total flavonoids (mg/gm fresh wt.) in leaves of *Zea mays* grown under salt stress.

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