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Impact of Salt Stress on Morphological and Biochemical Parameters of *Zea Mays*

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ABSTRACT

This research was conducted in order to evaluate the effect of salinity stress on the growth and biochemical parameters of *Zea mays*. The salt concentrations of 25, 50, 75 and 100mM were compared to the blank which was without any salt stress. There was not a visible decline in growth as is shown in increased fresh and dry weights. The biochemical parameters like chlorophyll and Carotenoid contents as well as soluble protein showed a decrease owing to the salt stress. Soluble sugar showed an increase with stress which might be because of accumulation of proline, which is used in osmotic adjustment of plants.

Keywords : Salinity, *Zea mays*, Salt stress, Proline

I. INTRODUCTION

The crops in arid and semi-arid regions are usually prone to undesirable environmental factors like high soil salinity. Salinity is one of the major environmental factors challenging plant growth and productivity (Bacha et al., 2017).The damaging effect of salt stress on plant growth can be seen at the whole-plant level as the plant death or decrease in its productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells (Gupta and Huang, 2014).Salt stress in plants affects all the major plant processes like photosynthesis, protein synthesis as well as energy and lipid metabolism. The quickest response is the decrease in the leaf surface expansion rate, followed by a termination of this expansion as the stress increases (Ahmadi et al. 2018). Growth resumes when the stress is relieved. Salt stress affects plant growth in three ways: (i) The reduction of water availabity because of salt stress (ii) Toxicity of the Na^+ and Cl^- ions (iii) Disturbance in the uptake of essential nutrients which results in nutritional imbalance (Munns, 2002; Munns and Tester, 2008).

Tolerance to different stresses varies according to the species and varieties, of plants (Jabeen 2018). The various stresses in plants result in activation of various defense systems which include powerful antioxidant systems such as enzymatic (e.g., superoxide dismutase, catalase, glutathione reductase, several peroxidases) and non-enzymatic (Jabeen et al., 2009). Another adaptive response to salt stress in plants is the release of phytohormones like abscisic acid, salicylic acid and jasmonates which might have a role in mitigation of salinity stress (Yoon et al. 2009). Studies have suggested a rising confirmation that plant hormones like methyl jasmonate and jasmonic acid (JA) have an mitigating effect on various plant species subjected to salt stress (Yoon et al., 2009; Manan et al., 2016).

Agriculture is of important role in the economical development of various countries like Saudi Arabia. However, the salinity which has affected majority of the soils in the kingdom is one of the major obstacles

limiting the expansion of the agricultural area or the enhancement in agricultural production of many crops of economic importance. Given the huge consumption of *Zea mays* for food and the salinity of most arable lands in Saudi Arabia, our objective was to study the effect of salinity stress on grwoth and biochemical characteristics of *Zea mays.*

II. METHODS AND MATERIAL

Material:

The experiments were carried out at college of science and arts, Addarb, Jizan University, using (*Zea mays* L.). The seeds were obtained from the local market.

Treatments:

15-day old plants were treated with Sodium chloride treatments of 25, 50, 75 and 100mM while as one without any sodium chloride was used as blank. After 10 days of treatments, the plants were sampled for various growth and biochemical parameters.

Methods:

1. Fresh and Dry Weights

Fresh weight of the plants was taken and recorded as fresh weight (FW) and then the same samples were dried and the samples were weighed on an electronic top pan balance to obtain the dry weight (DW) of plants, which were expressed in grams.

2. Pigment content:

Requirements: Plant leaves, Acetone, Spectrophotometer

Hiscox and Israelstam's (1979) method was used to estimate the pigment concentration in the samples.

Procedure:

The method involves the estimation of plant pigments without maceration. Leaves were washed with DDW and chopped. 100 mg of chopped leaf material was taken in vials in triplicates and 10 ml of DMSO was added to each vial. The vials were then kept in oven at 65˚ C. After 30 min, the vials were taken out and the absorbance of the solution was recorded at 663, 645, 510 and 480 nm on Uv-Vis spectrophotometer.

Values of optical densities (ODs) were used to compute the chlorophyll a, cholorophyll b and total chlorophyll contents by using the following formulae:

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ fw)} = \frac{12.3\,(A_{663}) - 0.86\,(A_{645})}{d \times 1000 \times W} \times V$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{ fw)} = \frac{19.3\,(A_{645}) - 3.60\,(A_{663})}{d \times 1000 \times W} \times V$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ fw)} = \frac{20.02\,(A_{645}) + 8.02\,(A_{663})}{d \times 1000 \times W} \times V$$

$$\text{Carotenoids (mg g}^{-1}\text{ fw)} = \frac{7.6\,(A_{480}) - 1.49\,(A_{510})}{d \times 1000 \times W} \times V$$

Where,

d = distance traveled by the light path

W = weight of the leaf material taken

V = volume of the extract

OD = Optical density

3. Soluble Sugar Content:

Soluble sugar content in the leaf samples were estimated by the method of Dey (1990).

Reagent preparation:

(a) 90 % Ethanol

It was prepared by mixing 90 ml of pure ethanol in 10 ml of DDW.

(b) 5 % phenol

It was prepared by mixing 5 ml of phenol in 95 ml of DDW.

Method:

0.1 g of fresh leaf sample was taken to which 10 ml ethanol was added and the mixture was incubated at 60º C for one hour. Final volume was made up to 25 ml with ethanol. From this 1 ml aliquot was taken and 1 ml of phenol was added to it and mixed thoroughly. 5 ml of sulphuric acid was added to the reaction mixture, which was then cooled in air. Optical density was measured at 485 nm on uv-vis spectrophotometer (Model DU 640 B, Beckman, USA).

The corresponding concentration of sugar was determined against the standard curve of sugar prepared by glucose ($C_6H_{12}O_6$) solution. The amount of sugar was expressed as mg g^{-1} Fw.

4. Soluble Protein Content

The total soluble protein content of the leaves was estimated by following the method of Bradford (1976).

Reagent Preparation

- 10% Trichloroacetic acid (TCA):
  10 g of TCA was dissolved in DDW to a final volume of 100 ml
- 0.1N Sodium hydroxide (NaOH):
  0.4 g of NaOH was dissolved in DDW to a final volume of 100 ml.
- Extraction buffer (0.1M Phosphate buffer, pH 7.5):

0.1M potassium phosphate (pH 7.5) was used as extraction buffer. It was prepared in the following manner:

Solution A: 1.36 g of KH_2PO_4 was dissolved in DDW and the volume was made up to 100mL.

Solution B: 1.74 g of KH_2PO_4 was dissolved in DDW and the volume was made to 100mL.

Solution A and B were mixed in appropriate ratio to adjust pH at 7.5. EDTA (0.5 mM) and ascorbic acid (1 mM) were added to the buffer solution.

Bradford's reagent :

A 50 mL of 90% ethanol was mixed to 100 mL of O-phosphoric acid (85%). Its volume was made up to 1 L by adding 850 mL of DDW. 100 mg of Coomassie Brilliant Blue G-250 (Sigma) was added to it which was stirred well on a magnetic stirrer in dark conditions. This solution was filtered carefully by using the Whattmann filter Paper No. 1 to remove the un-dissolved particles of dye, and stored in dark condition. The resultant solution is called Bradford's reagent.

Procedure

Extraction: A0.5 g of fresh and chopped leaf material was homogenized in 5 mL of 0.1 M- phosphate buffer (extraction buffer) in cooled condition with the help of a pre-cooled mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 min at 4ºC. One millilitre chilled 10% TCA was added to 1.0 mL 0f the supernatant, which was again centrifuged at 3300 rpm for 10 min. The supernatant was discarded and the pellet was washed with acetone. It was then dissolved in 1.0 ml of 0.1 N NaOH.

Estimation: To 1.0 ml aliquot, 5 ml of Bradford's reagent was added and vortexed. The tubes were kept for 10 min for optimum color development. The absorbance was then recorded at 595 nm on Uv-vis spectrophotometer (λ BIO 20, Perkin Elmer, Germany). The soluble protein content was quantified with the help of a standard curve prepared from the standard of BSA and the protein content was expressed in mg g^{-1}.

5. **Proline Content:** The proline content was determined using the method of Bates et al. (1973). Fresh material (300 mg each sample) was homogenized in 10 ml of 30% aqueous sulfosalicylic acid. The homogenate was centrifuged at 9000g for 15min. A 2ml aliquot of the supernatant was mixed

with an equal volume of acetic acid and acid ninhydrin (1.25 g ninhydrin in 30 ml acetic acid and 20 ml of 6N H_3PO_4) and incubated for 1 h at 1008C.

The reaction was terminated in an ice bath and extracted with 4ml of toluene. The extract was vortexed for 20 sec. The chromatophore-containing toluene was then aspirated from the aqueous phase, and its absorbance determined spectrophotometerically at 520nm (using toluene as a blank.

III. RESULTS AND DISCUSSION

Fresh and Dry Weight

There is an increase in the fresh and dry weights of the plants in general as compared to the blank. The maximum increase in fresh and dry weight, after 3 days of the treatment, was achieved at 75mM and 25 mM NaCl concentrations. Same results were observed by Qadoos (2011) in *Vicia faba.* Our results are in line with Ahmadi et al (2018), who found an increase in shoot and root fresh and dry weights with an increase in salt stress up to 200 mM.

Pigment Contents

We found a decreasing trend in all the pigments under all the concentrations of salt stress. Our results were in line with Khavarinejad et al (2009) who found that the contents of total chlorophyll, Chl-*a*, and β carotene decrease by NaCl stress in leaves of barley. The reduction in photosynthesis under salinity can be attributed to a decrease in chlorophyll content (Ahmadi et al, 2008). Loss of chlorophyll content because of salt stress is seen very commonly in salt-sensitive plants owing to the salt toxicity which results in the burning of leaves as well as the loss of pigments. On the other hand, the salt-tolerant species protect themselves from salt toxicity and the resultant pigment loss (Wang and Nil 2000).

Soluble Sugar Content

Soluble sugar content in the leaves of *Zea mays* were found to be increasing with all the concentrations of NaCl. There are many ways in which plants deal with salinity stress which include the buildup of compatible solutes like proline, soluble sugars, sugar alcohols and glycine betaine (Hare et al., 1998; Hasegawa et al., 2000). Ahmad and John (2005) found a considerable increase in sugar content in Salt treated pea plants.

Soluble Protein Content

Zea mays leaves showed a decrease in soluble protein contents under NaCl stress. The maximum decrease was found at 100 mM concentration. Chen et al. (2007) studied the effect of salt stress in *Vigna unguiculata* (L.) plants, and found a decrease in protein content with 75 mM of sodium chloride. Alamgir and Ali (1999) also noticed a decrease in soluble protein contents of leaves when subjected to salt stress.

Proline Content

Proline content showed a gradual increase with increase in NaCl concentrations. The highest proline content was found at 100mM NaCl. An increase in Proline content with increased salt stress was found by Heidari (2012) in *Ocimum basilicum* L. plants. Proline in addition to sucrose and other organic sugars are said to contribute to osmotic adjustment in stress conditions and guard the macromolecular structure and the structure of membranes while the plant is facing extreme dehydration (Prado et al., 2000). Melonid et al (2001) opined that proline is a vital source of nitrogen in plant metabolism, as well as a common source of energy and a reducing agent.

Fig 1. Effect of different NaCl concentrations on Fresh Weight of plants

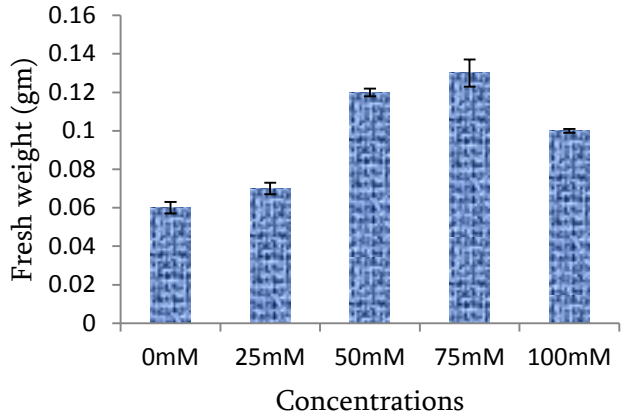


Fig 2. Effect of different NaCl concentrations on dry Weight of plants

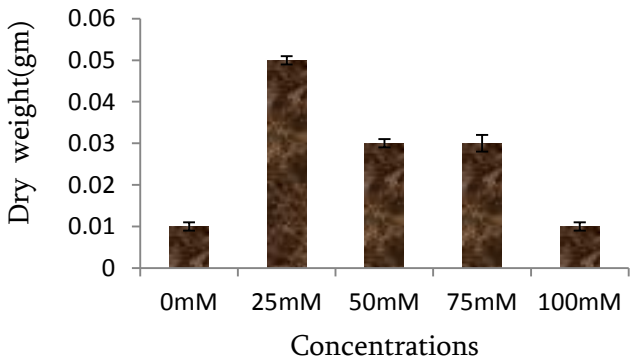


Fig 3. Effect of different NaCl concentrations on Chlorophyll a content of plants

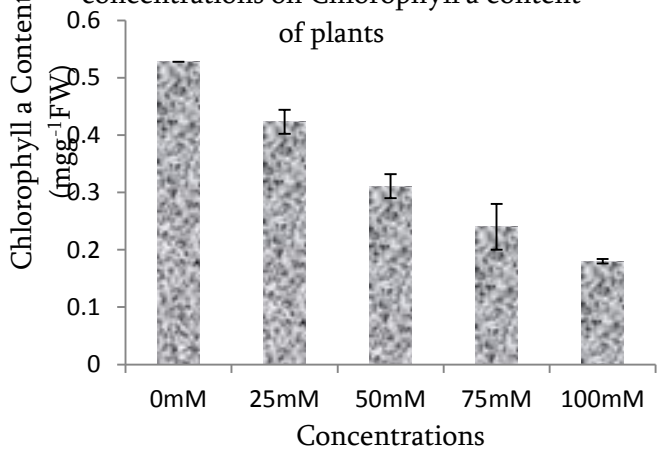


Fig 4. Effect of different NaCl concentrations on Chlorophyll b content of plants

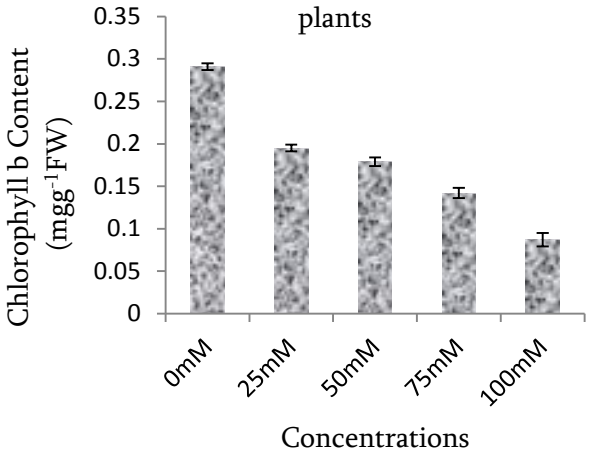


Fig 5. Effect of different NaCl concentrations on Total Chlorophyll Content of plants

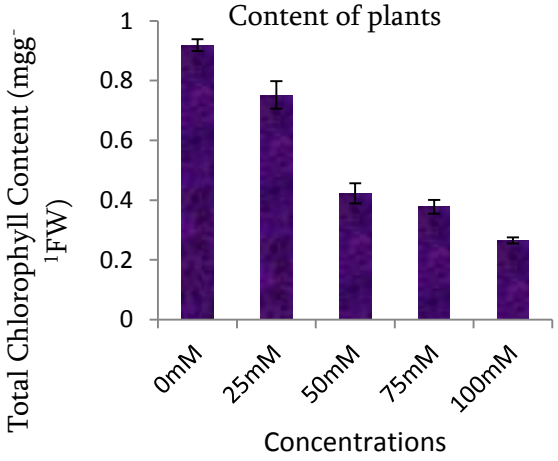
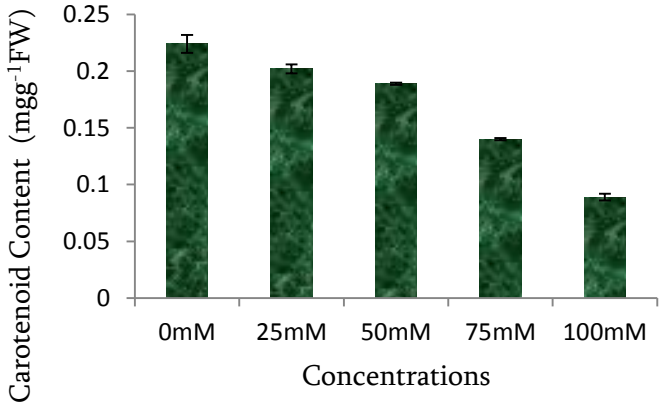
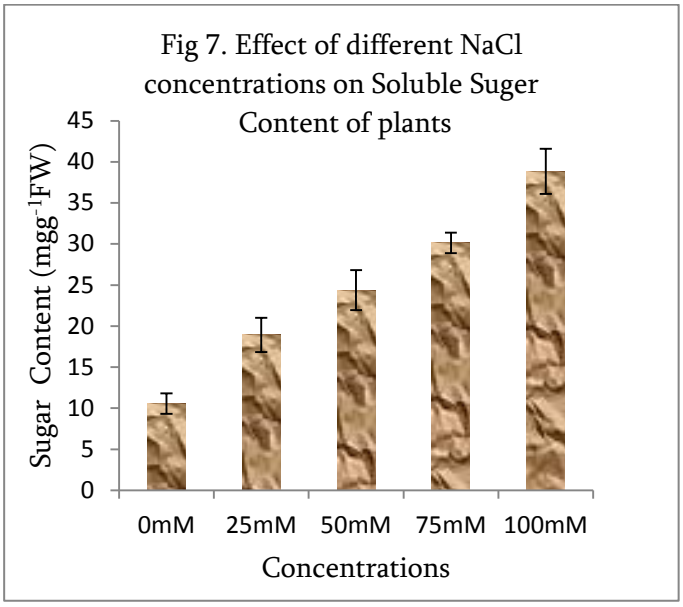
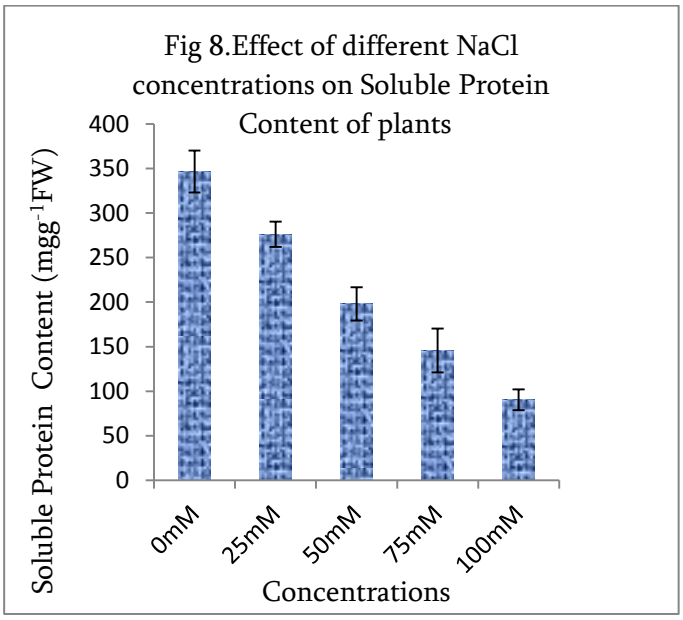
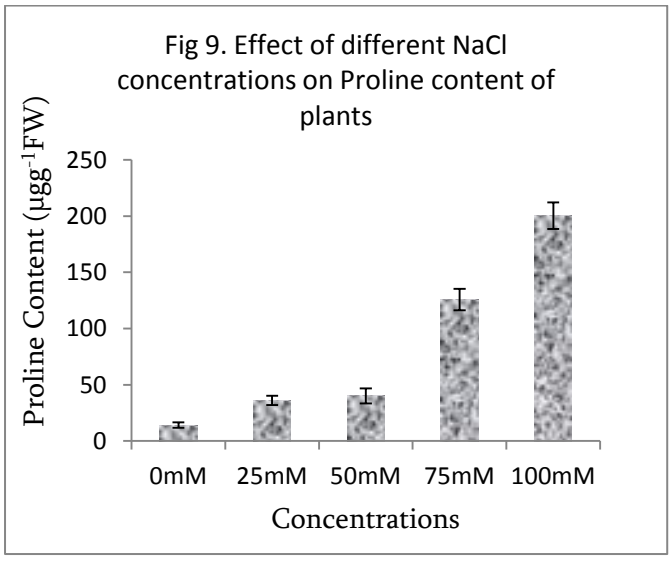


Fig 6. Effect of different NaCl concentrations on Carotenoids of plants









IV. SUMMARY AND CONCLUSIONS

The rapidly growing industries have resulted in environmental changes, increase in sea level as well as soil and water salinity threatening the survival of many important crop species. Considering this urgency, our study was aimed at studying the effect of salt stress on different growth and biochemical parameters on Zea mays. We have found out that even though the plant is not affected visibly manifested by unaffected rather an increasing fresh and dry weights, the biochemical parameters like pigment and proteins are decreased. The soluble sugar content is increased owing to the increase in proline content, which is known to be increased under salinity stress. In conclusion, salinity is the most serious threat to agriculture and to the environment in many parts of the world and key molecular factors that can be used for genetic engineering of salt-tolerant plants.

V. ACKNOWLEDGMENT

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