The Impacts of Salt Stress on Growth Factors and Photosynthesis Pigments in Wheat (*Triticum Aestivum* L.)

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Abstract: Among a biotic stresses, salinity is serious global threat to agriculture and adversely affects on environment. The research intentions identify affection of salt stress of several concentrations (0, 50, 100, 150 mM NaCl) on growing parameters and photosynthesis pigments of wheat under controlled environment. Plants were harvested for measurement of physiology parameters and seedlings were investigated as well. The results showed clear effect of salinity on physiological parameters especially at high salinity concentration (150 mM NaCl) with increasing plant age. By increasing the salinity parameters such as plant fresh weight, plant dry weight, (RGR), (NAR), (RLGR), (SLA) and chlorophylls content decreased. Also end result shows that wheat plant could endure 50 and 100 mM of salt solution. Wheat, showed sensitivity to 150 mM salt solution.

Keywords: Salt Stress, *Triticum Aestivum* L., Chorophyll

1. Introduction

Stress is a force which disrupts the functioning of biotic systems like plant (Jones & Jones, 1989). "The biotic and abiotic stresses threats stability of agriculture industry. The increasing of world population at a high-rate, it is expected to grow up to 6 billion people by 2050" (Mahajan & Tuteja, 2005). Oppositely, the nutrition products are decreasing down because of effects of abiotic tension. Diverse methods are needed to minimize the relevant losses. Salt, frigidity, dryness, and heavy elements impose the highest degree of stress on progress and efficiency of plants. Thus, propagation of resistant products is vitally important (Mahajan & Tuteja, 2005). Salt is one of the main stress factors that are harmful when increases in the farmlands. It is also anticipated that salt will cause the loss of 30% of cultivable lands in the next 25 years, and this figure will be increased to more than 50% by mid 21st century. It affects 7% of fertile areas of the world (Wang et al., 2003). The prohibiting affect of salt on crops stems from the following: 1. osmosis effect 2. ionic toxicity 3. nutrients imbalanced.

"All of the above reduce the efficiency of photosynthesis and physiological parameters" (Almodares et al., 2008). Losing intracellular fluid is a main concern of salt-stress. Plants employ special mechanisms to prevent losing intracellular liquid, and to regulate internal osmosis conditions in salty environment. As a whole, these methods like: the accumulation of metabolites particularly proline, and sugars which are soluble osmolytes, egress of sodium from cell by plasma membrane H^+/Na^+

antiporters, and entrance of extra N^+ into vacuoles by H^+/Na^+ antiporters (Paridaa & Dasa, 2005). The effect of salt is determined by factors such as soil material, weather, plus water management. Salt effects the main organs like leaves, stems, and roots. Roots are located directly with soil, and provide stems with nutrition. This is why measuring physiological parameters of roots and organs in response to salt are so important.

Agriculture plays a vital role in development and economy of most countries, particularly Iraq. Wheat is one of the most significant agricultural products for many people in the world; also a stable source of nutrients in Iraq. Wheat, originated in southwest of Asia, has been a main agricultural product for centuries. About 31.4% of cultivable land in Iraq is devoted to wheat. Dry-land wheat is mostly grown in the northern and central parts of Iraq (Shirazi et al., 2001; Al-Haboby et al., 2014). Unfortunately, salt is one of the barriers restricting growing crops in Iraq, due to density of solvable salt in wash-out water, besides rapid evaporation in hot areas which reduce the water intake and nutrients balance. These factors cause ionic toxicity, and reduce the production and growth of plant life. The purpose of the study is to examine different salt concentrations upon wheat plant in laboratory conditions. Various growth parameters, photosynthesis, and proline were measured, as well.

2. Material and Method

2.1 Preparing and Sowing of Seed

After procuring wheat seeds, 300 uniform and homogenous seeds hand-picked and sterilized by five percent (v/v) sodium hypochlorite solvent for 5 minutes to prevent fungoid contamination. Then they were washed for several times by distilled water. Twenty seeds were sited on a filter paper layer inside petri dishes. Petri dishes were covered by aluminum foil. After three days, the seeds begin to geminate.

Then petri dishes were exposed to light. Finally, seedlings were planted in pots filled with a uniform quantity of a clay/sand mixture which were irrigated by distilled water. There were several holes at the bottom of pots to adjust the amount of water according to field capacity condition. The pots were transferred into light, and they were irrigated by Hoagland solution for a week. Before treatment, 4 plants were taken for preliminary analysis to laboratory.

2.2 Treatments

Treatments started on the 10^{th} day and continued for 10 days. Each treatment combination was applied to a pot containing five plants; there were four replications at 4 different levels (0, 50, 100, and 150 mM). The pots were irrigated with the intended nutrient solution during the treatment. To prevent accumulation of extra ions, half of the nutrient solution left by the bottom of pots in each irrigation. The surfaces of pots were kept humid several times of a day using spray guns. The required light was provided by 100w tungsten, and florescent lights. The temperature was set on 25°c during the days and on 18°c at nights. Day and night periods were fixed respectively on 16 and 8 hours. PH of solution was set on 5.8. To minimize the microclimatic effect of greenhouse environment, the pots were randomly displaced each day. The 20 days-old plants were harvested for biochemical, and physiological tests.

2.3 Determination of Growth Parameters

After harvesting, dried and garden-fresh weight for leaves, buds and roots were weighted. Dry mass (DM) determined after plant parts were dried at 105 °C in oven for 24 hours. The size of special leaf area was determined by a LI-3000 leaf area meter (LI-COR, Inc. Lincoln, NE, USA). "The relative growth rate (RGR (1)), the net assimilation rate (NAR (2)), the relative leaf growth rate (RLGR (3)) and the special leaf area (SLA (4))" were evaluated according to recommended procedures (Watson, 1952; Evans & Hughes, 1962) as follows:

$$\text{RGR} = \frac{LnW_2 - LnW_1}{t_2 - t_1} \ (\text{g kg}^{-1}\ \text{d}^{-1}) \quad (1)$$

$$\text{NAR} = \frac{W_2 - W_1}{L_2 - L_1} \times \frac{LnL_2 - LnL_1}{t_2 - t_1} \ (\text{g m}^{-2}\ \text{d}^{-1}) \quad (2)$$

$$\text{RLGR} = \frac{LnL_2 - LnL_1}{t_2 - t_1} \ (\text{cm}^2\ \text{m}^{-2}\ \text{d}^{-1}) \quad (3)$$

$$\text{SLA} = \frac{L}{LDW} \ (\text{m}^2\ \text{kg}^{-1}) \quad (4)$$

W_1 is the primary complete (shoot + root) arid mass, W_2 the end complete arid mass, L_1 the first leaf zone, L_2 the ending leaf zone, LDW is leaf waterless mass also ($t_2 - t_1$) the differences between the test groups in time interval.

2.4 Determination of Photosynthetic Pigments

Chlorophyll-type a (1) and Chlorophyll-type b (2) has been determined by using Arnon's method (1949). Samples of leaf parts have been weighed and grinded using mortar with 20 ml of 80% acetone solution. The extract was filtered using funnel and placed in a flask. The remains were extracted again by same way till it's transparent. A standard flask was used to place the filtrate then volume finalized by addition 80% acetone. Final volume of diluted extract was noted. The instrument then zeroed by 80% aqueous acetone and afterwards wavelength resetting. Then wave lengths ،8/646 and 2/663 nm used for calculating optical density (O.D.) of the extract to estimate chlorophylls, by Shimadzu spectrophotometer (UV-1700, Tokyo, Japan). Four trials used for every treatment, then calculation of pigment quantity in every one sample done by these following equations:

$$\text{Chl.a } (\text{mgl}^{-1}) = 12.25 \times A663.2 - 2.79 \times A646.8 \quad (1)$$

$$\text{Chl.b } (\text{mgl}^{-1}) = 21.51 \times A646.8 - 5.1 \times A663.2 \quad (2)$$

According to final volume and weight of samples, content of chlorophyll was showed by means of mg g^{-1} F.W.

2.5 Determination of Proline Content

After weighting, homogenizing of samples were made in 3% (w/v) sulphosalycylic acid then homogenate filtered by filter paper. For every 1ml of aliquo, 2 mL acid ninhydrin and 2 mL glacial

acetic acid were added. To end the reaction, samples transferred immediately to ice bath for 20 minutes. Then addition of four ml of toluene to each sample, after taking the absorbance at 520 nm, then determination of proline content was done by calibration curve and showed as µg g^{-1} FW. "Standard curve was obtained by a known concentration of proline" (Bates et al., 1973).

2.6 Statistical Analysis

Each treatment was included four replicates containing six plants in each pot. Data were inspected using two-way ANOVA. Means compared with Least Significant Difference (LSD) ($P< 0.05$). SPSS software (version 19) was used for analysing the data.

3. Result

3.1 Effect of Salt Stress on Growth Factors

Growth factors examined in this study including wet and thirsty weight of shoots and roots were lessened by the high salt concentrations when matched with intact control, but This reduction was significantly detected at 150 mM NaCl for wet and dry mass of shoots also, the means to FW also DW of root were higher at 100, 150 NaCl compared with intact control (Table 1). This reduction was at 150 mM NaCl about 30% and 35% in FW of root, FW of shoot, respectively, as compared to control.

Table 1: Impacts of NaCl concentrations on development criteria in seedlings wheat

Treatment	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)
0	0.99 a ± 0.029	0.094 a ± 0.001	0.17 a ± 0.104	0.018 a ± 0.0004
50 mM NaCl	0.97 a ± 0.017	0.090 a ± 0.001	0.16 a ± 0.009	0.017 a ± 0.0006
100 mM NaCl	0.92 a ± 0.021	0.089 a ± 0.003	0.13 b ± 0.006	0.011 b ± 0.0006
150 mM NaCl	0.69 b ± 0.009	0.061 b ± 0.003	0.11 b ± 0.008	0.008 cb ± 0.0002

FW: fresh weight; DW: dry weight

Standards within each pillar followed by the similar letters are non-significantly different at five %, using least significant difference (LSD) examination. It is trailed by standard errors of means based on four replications.

3.2 Affection of Salt on Physiological Parameters

Variations in NAR, SLA, and RGR are given in Table 2. Salt stress cause decrease in the SLA, RGR, NAR RLGR wheat seedlings related to control, but this lessening in NAR was high at 150 mM salt solution, also SLA was decreased significantly in concentration 100 and 150 mM NaCl as unsalinized control. Increasing of NaCl concentrations (50, 100 and 150 mM) significant slip RGR and RLGR were observed as matched to the control group.

Table 2: Impact of NaCl concentricity on Physiological parameters in seedlings wheat

Treatment	NAR (g m^{-2} d^{-1})	SLA (m^2 kg^{-1})	RGR (g kg^{-1} d^{-1})	RLGR (cm^2 m^{-2} d^{-1})
0	3.3 a ± 0.108	19.25 a ± 0.47	121.1 a ± 4.26	670.2 a ± 23.54
50 mM NaCl	3.1 a ± 0.13	18.75 a ± 0.85	106.87 b ± 2.68	588.7 b ± 23.3
100 mM NaCl	3.05 a ± 0.086	16 b ± 0.40	96 c ± 2.27	461.2 c ± 23.3
150 mM NaCl	1.87 b ± 0.085	11.5 bc ± 0.64	69.5 d ± 2.1	340 d ± 16.8

NAR: net assimilation rate; SLA: special leaf area; RGR: relative growth rate; RLGR: relative leaf growth rate

Standards within each pillar followed by the identical letters are non-significantly different at 5%, by least significant difference (LSD) examination. Means are followed by standard errors of means based on four replications.

3.3 Impact of Salt on Pigments of Photosynthesis

Salinity showed significance ($p< 0.05$) for reduction of Chlorophyll in wheat-leaves in 150 concentration (Fig. 1). A concentration from 0 to 50 mM has non-affection on the Chlorophyll concentrations , while further increase (150 mM) NaCl significantly decreased chla and chlb by 48% and 43%, respectively, as compared to control (fig 1a,b).

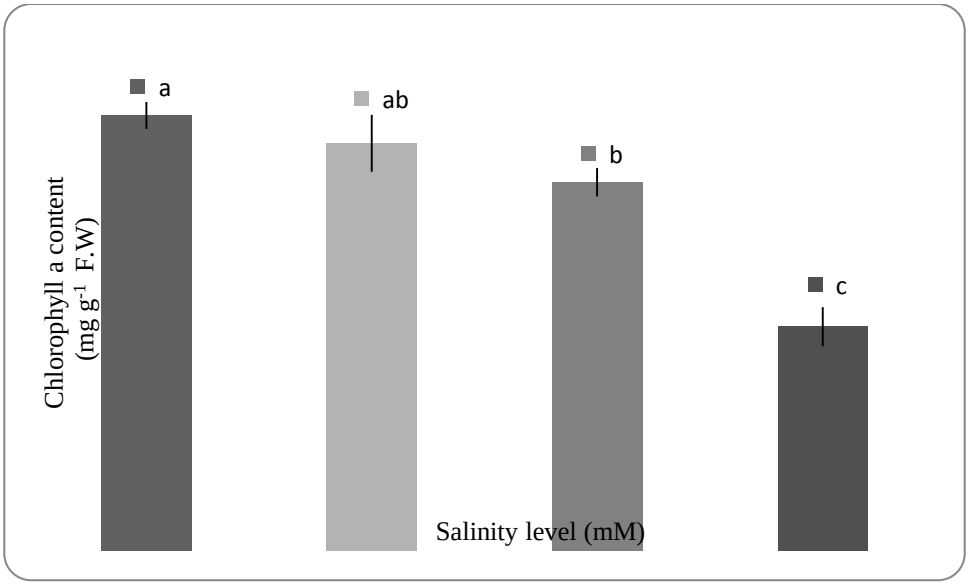

Figure 1a: Impact of concentrations of salt on Chlorophyll amount in seedlings wheat

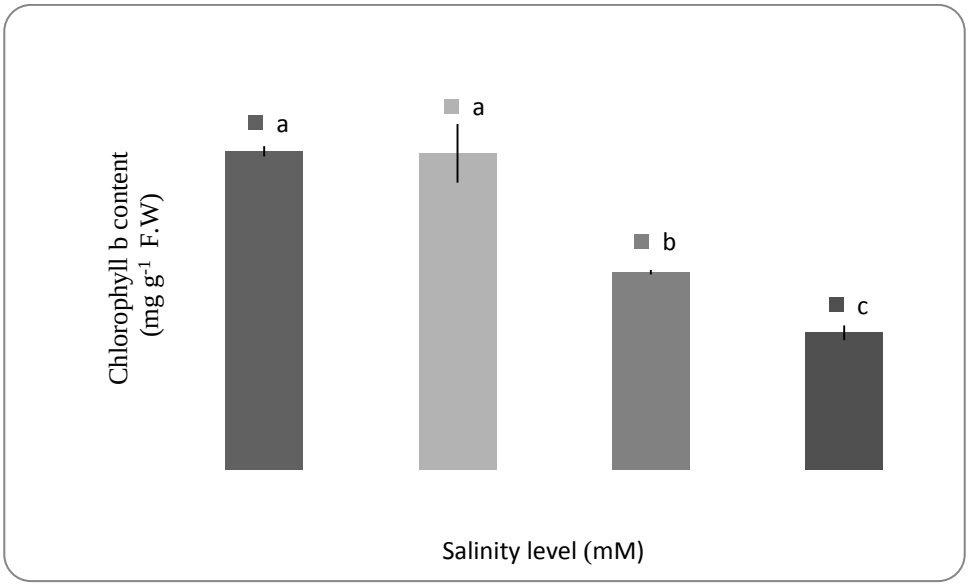

Figure 1b: Influence of concentrations of NaCl to the Chlorophyll b volume in seedlings wheat

The content of proline in the leaf of wheat plants is presented in fig 2. Commensurate With increase in the levels of salinity, the contents of proline had moved higher in the leaf significantly as compared to intact controller. This increase in concentrations100 also 150 mM NaCl was almost twofold and threefold if matched to the control.

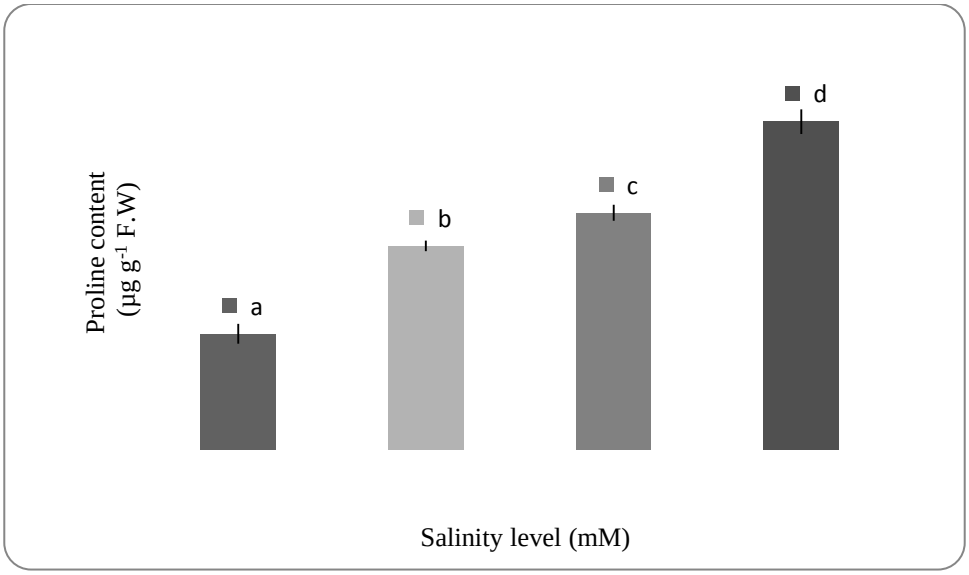

Figure 2: Effect of different concentrations of NaCl on proline content in seedlings wheat

4. Discussion

Plants growth results from regular completion of physiological processes. The physiological functions are influenced by environment that determines the plant reaction to stress. As soon as new cells start lengthening, "High amount of salt alters the cell wall by extraction of different materials which reduce the elasticity of the wall and consequently cell walls become rigid and the turgor

pressure efficiency in cell enlargement will be deceased" (Ali et al., 2004).

"Consistent with our study, growth reduction is reported in many types of plants suffering from salt stress" (Datta et al., 2009; Amira & Qados, 2011). Salt stressed Plants that had less leaf biomass related to control plants that basically caused by Senescence and death of leaves (Jamil et al., 2005). Jamil et al. (2005) states that salt reduces the wet mass of stems and roots, decreases the amount of leaves and leaf biomass. The effect of salt on growth is more evident on aerial organs than roots. "The cause for less developmental shoot and root might be the toxic properties of the NaCl plus dispersed nutrient uptake by the seedlings" (Munns, 2002). A research by Saker and Arafa (2009) indicated that growth reduction in canola, subjected to salt, could be for the following reasons: 1. reduction of meristematic activity or reduction of cellular development 2. harm to growing cells which disrupts cellular functioning 3. restriction of necessary metabolites 4. malfunction of vital photosynthesis parts.

The area of the leaf reflects the growth rate. It can be affected by diverse types of stress such as salinity. Table 2 shows leaves response to salinity. An increase in salt results in reduction in leaf area, and this relation is significant in high concentrations. This is consistent with other studies (Amira & Qados, 2011; Yilmaz & Kina 2008). Leaf surface in treatment plants sooner than other parameters due salinity effects. This action proves first symptom of salt is usually reduction of leaf area. "reduction growth in plant under salt stress as a result decreased production of growth regulators like cytokinin and an elevation in growth prohibitory factors such as abscisic acid (ABA)" (Belaqziz et al., 2009). Drop in leaf zone could be the reduction of stabilized carbon, net amount of photosynthesis, and biomass (Saker & Arafa, 2009). The lessening in leaf surface underneath salt stress might be regarded as "a stress reduction machinery to minimize water loss from closed stomata, this happens in many plant types in osmosis stress" (Munns & Tester, 2008; Miranda et al., 2010). "Under salty environments the reduction in leaf area can be detailed a decreased in turgor Pressure leaves, the alteration of cell wall characteristics or decreased photosynthesis rate" (Ali et al., 2004).

As evident within table 2, salinity reduces NAR, RGR, RLGR physiological parameters. This is more prominent and significant in higher concentrations which consistent with other studies (Neto et al., 2004; Heidari et al., 2011). Reduction in leaf area, decreases the light reception area, photosynthesis, consequences reduce RGR (Lamber et al., 1998). RGR reduction can be due to a straight affection of stress on stomata closing, or photosynthesis. This indicates that "the growth-limiting factor is photosynthesis process" (Rodriguez et al., 2005). RGR reduction under salt stress is clearly accompanied by a drop-in net assimilation rate (NAR)(Suarez & Medina, 2005).

Less chlorophy type ll in salinized groups was observed. Many studies confirmed the salt inhibitory inhibition on chlorophy type-ll a and b with increased concentrations of NaCl have been reported (Taffouo et al., 2010; Amira & Qados, 2011). They indicated that content of photosynthetic pigments in treated plants are decreased by salinity, which are in agreement with our results. This decline may be associated with decreased synthesis or decomposition of chlorophyll (Santos, 2004). Najafi et al. (2006) attested that salt can lead to oxidative stress in chloroplast of plant-leaves. This can result in production of different types of activated oxygen which destroys DNA, proteins, membrane, and chlorophyll. Chlorophyll reduction under salt stress leads to an increase in activity of chlorophyllase

enzyme, and its effect on ferrite enzymes such as cytochrome oxidase (Saker & Arafa, 2009). The stressful condition also increase the concentration of some growth regulators (e.g. Abscisic Acid, and ethylene), this also stimulates the activation of chlorophyllase enzyme (Drazkiewicz, 1994). On the other hand the decrease of chlorophylls content can affect nitrogen metabolism, proline and amino acid synthesis (Rosa & Maiti, 1995).

In our study, NaCl increased the amount of proline like (*Brassica napus* L., Wheat cultivars) by researchers (Rossi et al., 2016; Datta, 2009). Balanced hormone do a significant part in the accumulation of proline concentration under salt or dryness stresses. "Abscisic acid also have important activity in osmotic adjustment" (Marcinska et al., 2013). Rapid concentration of free proline is a kind of response to salt. A powerful relationship is found in the level of proline and the capability to continue in water shortage and high rate of salt, in different organisms from bacteria to higher plants. High concentration of proline due to increased synthesis and reduced decomposition in different stress conditions like salt stress, dryness, etc. are reported (KaviKishor et al., 2005). It regulates osmosis, safety of membrane, stabilizes the enzymes and proteins, maintains the ratio of $NADP^+$ to NADPH, and removes free radicals (Saker & Arafar, 2009; Ali, 2007; KaviKishor, 2005). The concentration of congenial metabolites (e.g. proline) results in cellular osmolarity which facilitates water entrance into the cell and reduces its egress to provide turgor pressure necessary for cellular development.

Biosynthesis of proline in plants takes two routs: glutamate and ornithine. Proline which is accumulated under osmosis stress is usually obtained from glutamic acid (Hare & Cress, 1997; Dubey, 1997). Glutamate is one of the constituents of proline and chlorophyll, and its activity increases under salt stress. Glutamate will be used for proline synthesis resulting in high concentration of proline and a reduction in chlorophyll, consistent with our findings.

5. Conclusion

Our results show that salt has a noticeable effect on growth, physiological parameters, photosynthetic pigments and proline. It hinders the growth and reduces the amount of chlorophylls types A and B, which is more prominent at 150 mM concentration that clearly indicate high sensitivity of plants at this salt level.

6. Recommendation

Salt stress increases the amount of proline, but its function and the function of other growth regulator hormones in salt resistance mechanisms of crops require more investigation.

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