



Effect of Sodium Chloride on Response of Two Wheat Cultivars (*Triticum aestivum* L.) at Germination and Early Seedling Stages

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Abstract: An experiment was conducted in pots to study the effect of sodium chloride levels (0,50,100,and 150)mg.L⁻¹ on two wheat cultivars AL-Hussein and AL-Rasheed in terms of germination percentages, seedling vigour index, lengths and dry weights of shoot and root, phytotoxicity of shoot and root, stress intensity, stress tolerance index, electrolyte leakage percent and chlorophyll stability index. Salinity treatments were applied from sowing till the end of the experiment. Factorial experiment with CRD design with three replicates was used. Results revealed that, AL-Hussein cultivar gave the highest response to salinity compared with AL-Rasheed in terms of germination percentage., seedling growth, shoot and root dry weights, seedling vigour index, stress tolerance index and chlorophyll stability index. Meanwhile, AL-Rasheed cultivar gave the highest values of shoot and root phytotoxicity stress intensity and electrolyte leakage percent. Sodium chloride significantly affected all studied characteristics, but the highest (level 150 mg.L⁻¹) gave higher increase or decrease in studied characteristics compared with the control. These results indicated that, tolerance of AL-Hussein cultivar to salinity was correlated with stress tolerance index and chlorophyll stability index under salinity stress.

Keywords: Salinity, Nacl, Wheat, Growth, Salinity Tolerance

1. Introduction

The salinity is considered the main limiting problem of the plant production in irrigated area especially in arid and semi-arid zones. It causes negative effects on germination percent, growth and productivity of field crops [1]. Salinization effect in many irrigated areas is caused by using saline irrigation where more than 45 million of irrigated land are influenced by salinity. Yearly, one million and a half hectare are discarded form the production as a result of increasing salinity levels in the soil [2]. High salinity influences plant growth and production in many ways such as water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, plasma membrane disturbance and cell division and expansion [3]. Plants tolerate salinity via discard of salt from cells or tolerate its presence inside the cell [4].

Finding salt - tolerant genotypes through mechanism enable plant to tolerate salinity is considered one of the

main treatment for this problem from one side and to alleviate the negative effects on the growth and production of different crops from another side. Wheat (the first crop in the world in cultivation area and production) is suffering from salinity problem although it is considered semi - tolerant plant [5]. The tolerance for salinity is complex phenomenon controlled by many genes [4].

Water quality, climate conditions and crop variety may affect the tolerance of crops to salinity. The main features of salinity effect are the reduced of seed germination, seedling and root and shoot growth. Testing plants possess such a mechanism for Na discarding, ionic balance and high K absorption would be of importance. Therefore, this experiment was carried out to test two varieties of wheat namely: AL - Hussein which is recently selected (Jaddoa, 2015, Pers. comm.) and AL- Rasheed (which is widely cultivated in Iraq).

2. Materials and Methods

A pot experiment was conducted on Nov. 15th, 2015 in the Botanical Garden of College of Science, University of Kerbala. A completely Randomized Design (CRD) in a factorial manner with three replicates was adapted. The experiment included two factors i.e. two varieties of wheat namely AL -Hussein and AL -Rasheed, and four levels of Na cl (0,50,100 and 150) meq.L⁻¹. Ten seeds from each variety were sown in 20 kgs. pot capacity, irrigated with the salinity treatments at full field capacity for 30 days early seedling stage, plants were carefully lifted from the pots, thoroughly washed to remove soil particles from the roots then divided into roots and shoots. The following characteristics were determined:

1. The germination percent: germination percent was calculated for emerged seeds 10 days after sowing [6].
2. Seedlings were thinned to five seedlings after calculation of seed germination. After 15 days from sowing half dose of recommended nitrogen fertilizer was added. After 30 days from sowing, shoot height and root height were measured using a ruler. Roots and shoots were dried at 70°C for one night and their weights were recorded.
3. The stress intensity (SI) according to the method of [7] on dry matter basis was calculated using the following equation :

$$\text{Stress intensity} = 1 - (y_{si} / y_{pi})$$

Where:

Y_{si}: seedling dry weight of saline treatment.

Y_{pi}: seedling dry weight of control treatment.

4. Measurement of stress tolerance index (STI) : stress tolerance index was calculated according to the equation of [8].

$$\text{STI} = (Y_{pi} \times Y_{si}) / y_{pi}^2$$

Where STI: stress tolerance index.

Y_{pi}: seedling dry weight of control treatment.

Y_{si}: seedling dry weight of saline treatment.

Y_{pi}²: square of seedling weight of control treatment.

5. Measurement of Nacl toxicity percent in root and shoot was calculated according to the following equation described by [9].

(%) Phytotoxicity of shoot = shoot length in treatment / shoot length in control
(%) Phytotoxicity of root = root length in treatment / root length in control.

6. Seed vigor index calculated according to the following equation mentioned by [10]:

Seedling vigor index = % Germination × Seedling dry weight (g).

7. Electrolyte leakage percent.

Electrolyte leakage percent was measured according to [11] as follow:

Five hundred mg of seedling leaves tissue were taken after washing by deionized water, cut into slices 2mm in diameter placed in test tubes containing 10 deionized H₂O then put on the shaker incubator at 5°C for 24 hrs. The electrical conductivity (EC) was measured by using EC - meter, samples were then placed inside the autoclave at 120°C for 20 minutes, the EC was measured (EC₂) after cooling to room temperature. The electrolyte leakage percentage was finally calculated as follow :

$$\% \text{ Electrolyte leakage} = (\text{EC}_1 / \text{EC}_2) \times 100.$$

8. Chlorophyll stability index was calculated according to the equation described by [12].

Chlorophyll stability index = (chlorophyll content in stress treatment) / (chlorophyll content in control treatment) × 100.

9. Statistical analysis: Data were statistically analyzed, treatment were compared by using least significant difference at 0.05 probability level [13].

3. Results

3.1. The Effect of Salinity Stress on the Germination Percent of Two Wheat Cultivars

Data in table (1) revealed that, increasing salt concentration significantly decreased the percentage of germination. Seeds treated with 150 meq. L⁻¹ Nacl gave 53.75% of seed germination. Whereas, the control treatment gave the highest value (95.00%) after 10 days from sowing.

Wheat cultivar varied significantly. AL- Hussein cultivar gave the highest percent of seed germination (84.13%) compared with AL- Rasheed cultivar which gave 78.13%. AL- Hussein cultivar was higher than AL- Rasheed in germination percentage by 7.6%. The interaction between these two factors had a marked effect on the germination percent where the highest value was obtained from AL- Hussein cultivar at 50 meq.L⁻¹ Nacl giving 98%) while at the highest salinity level the lowest value was associated with the AL- Rasheed cultivar grown at 150 meq. L⁻¹ Nacl giving 51.70% of germination.

Table (1). Effect of Nacl level on the germination percent of two wheat cultivars.

Nacl meq.L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	97.20	98.00	85.50	55.80	84.13
AL-Rasheed	92.80	94.30	73.90	51.70	78.18
Mean	95.00	96.15	79.70	53.75	
L.S.D(0.05)	Na cl = 1.37 cultivar = 0.97 Interaction = 1.94				

3.2. The Effect of Salinity Stress on the Plant Height (cm) of Two Wheat Cultivars

The plant height was significantly influenced by Na cl levels treatments (Table 2). The plant height decreased significantly with increasing the Na cl level. The highest

reduction was at 150 meg. L⁻¹. Also the results showed that AL- Hussein cultivar was taller than AL- Rasheed by 28.9% when grown under salinity condition. On the other hand, while the interaction between these two factors, showed there is not significant effect on plant height.

Table (2). Effect of Na cl levels on the plant height of two wheat cultivars.

Na cl meg. L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	30.87	32.10	24.60	14.40	25.49
AL-Rasheed	21.50	30.90	15.30	11.80	19.78
Mean	25.68	31.50	22.45	15.60	
L.S.D(0.05)	N a Cl =2.78 cultivar =1.54 Interaction =N.S				

3.3. Effect of Salinity Stress on the Roots Length (cm) of Two Wheat Cultivars

Table (3) showed that, neither the cultivar nor the interaction had a significant effect on roots length. On the other hand, increasing Na cl levels significantly affected roots length where the roots length decreased by 40.2% as Na cl increased from 0 to 150 meg. L⁻¹.

Table (3). Effect of NaCl levels on the roots length (cm) of two wheat cultivars.

Na cl meg. L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	8.47	9.80	6.10	6.50	7.72
AL-Rasheed	13.10	8.80	7.73	6.40	9.01
Mean	10.78	9.30	6.91	6.45	
L.S.D(0.05)	NaCl =3.38 cultivar =N.S Interaction =N.S				

3.4. The Effect of Salinity Stress on the Shoot Dry Weight (g) of Two Wheat Cultivars.

Table (4) showed that, the highest shoot dry weight was achieved by AL- Hussein cultivar giving 0.129 g whereas, AL- Rasheed cultivar gave 0.054, shoot dry weight of AL- Hussein was 135.2% higher than AL- Rasheed cultivar The salinity also showed a significant effect on the shoot dry weight where it decreased from 0.073 to 0.036 g giving 50.7% reduction due to increasing Na cl from 0 to 150 meg. L⁻¹.

Table (4). Effect of Na cl levels on shoot dry weight (g) of two wheat cultivars.

Na cl meg.L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	0.085	0.261	0.093	0.039	0.127
AL-Rasheed	0.061	0.080	0.043	0.033	0.054
Mean	0.073	0.170	0.068	0.036	
L.S.D (0.05)	Na cl =0.091 cultivar =0.062 Interaction =N.S				

3.5. The Effect of Salinity Stress on Roots Dry Weight (g/plant) of Two Wheat Cultivar

Increasing Na cl level up to 150 meg. L⁻¹ decreased the

root dry weight by 96.8%. No significant differences between cultivars in their roots dry weight. (Table 5).The interaction between the cultivars and Na cl also affected this trait significantly. The highest root dry weight was obtained by at AL- Hussein cultivar the control treatment giving 0.075 g while the lowest value resulted from both cultivars at 150 meg. L⁻¹ Nacl giving 0.002 g. Also the results showed that there is not significant differences between cultivars in their roots dry weight.

Table (5). The effect of NaCl Levels on the roots dry weight (g) of two wheat cultivars.

Na cl meg.L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	0.075	0.032	0.021	0.002	0.032
AL-Rasheed	0.050	0.062	0.007	0.002	0.030
Mean	0.063	0.047	0.014	0.002	
L.S.D(0.05)	Na Cl =0.005 cultivar =N.S Interaction =0.006				

3.6. The Effect of Viability Coefficient of Two Wheat Cultivars Seedlings

Data in (table 6) revealed that, a marked reduction in viability coefficient was obtained due to increasing Na Cl levels from 0 to 150 megL⁻¹ reaching 88%. AL- Hussein cultivar gave a profound increase than AL- Rasheed cultivar giving 144.6% increase. AL- Hussein cultivar at 0 meg. L⁻¹ Na cl gave the highest value (26.215) whereas AL- Rasheed cultivar at 150 meg. L⁻¹ Na cl gave the lowest (1.826).The viability coefficient of both cultivars decreased with increasing salinity level (table 6), but the lowest reduction were at 150 meg L⁻¹.

Table (6). The effect of Na Cl levels on the viability coefficient of two wheat cultivars.

Na cl meg.L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	26.215	19.141	6.726	2.345	13.607
AL-Rasheed	8.508	8.765	3.152	1.826	5.563
Mean	17.361	13.953	4.939	2.086	
L.S.D(0.05)	Na cl =0.653 cultivar =0.462 Interaction =0.924				

3.7. The Effect of Salinity Stress on, the Shoot Phytotoxicity Percent of Two Cultivars

Table (7). The effect of salinity stress on the roots phytotoxicity percent of two wheat cultivars.

Na cl meg.L ⁻¹ cultivars	0	50	100	150	Mean
AL-Hussein	0.136	0.138	0.227	0.489	0.248
AL-Rasheed	0.151	0.166	0.233	0.366	0.229
Mean	0.144	0.152	0.230	0.428	
L.S.D(0.05)	Na cl =0.002 cultivar =0.001 Interaction =0.002				

Table (7) showed that phytotoxicity significantly increased from 0.144 to 0.152, 0.230 and 0.428% due to increasing salinity from 0 to 50, 100 and 150 meg. L⁻¹ giving an increment of 197.2%. AL- Hussein cultivar gave higher value of phytotoxicity coefficient than AL- Rasheed cultivar. There was a significant effect of the interaction on this trait. AL-

Hussein cultivar grown with 150 meq. L⁻¹ gave the highest value 0.489% whereas the lowest value was gained from the same cultivar grown without salinity (0.136%).

3.8. The Effect of Salinity Stress on the Roots Phytotoxicity Percent of Two Wheat Cultivars

Data in Table (8) revealed that Na cl level significantly affected the toxicity percentage of root where the highest value was associated with 150 meq. L⁻¹ treatment (0.945). Cultivars differed significantly in this trait. AL- Hussein cultivar was less affected than AL- Rasheed where the difference was 12.2%. There was a significant effect due to the interaction between Na cl and cultivar, where the highest value accompanied AL- Rasheed cultivar grown with 150 meq. L⁻¹ whereas the lowest value was obtained from AL- Hussein cultivar grown either without Na cl or with 50 meq. L⁻¹ Na cl giving 0.196%.

Table (8). The effect of Na cl levels on the roots phytotoxicity percent of two wheat cultivars.

Na cl meq.L-1 cultivars	0	50	100	150	Mean
AL-Hussein	0.196	0.196	0.427	0.479	0.324
AL-Rasheed	0.201	0.328	0.405	0.511	0.361
Mean	0.198	0.262	0.416	0.495	
L.S.D(0.05)	Na cl =0.050 cultivar =0.036 Interaction =0.071				

3.9. The Effect of Salinity Stress on the Stress Tolerance Index (STI) of Two Wheat Cultivars

Table (9) showed that cultivars were differed significantly in the (STI), the (STI) was higher in AL- Hussein cultivar giving an increment of percent reached 50.6%. Stress tolerance index decreased with increasing Na cl levels where it decreased from 1.05 at the control to 0.267 at 150 meq.L⁻¹ Na cl. The interaction effect on (STI) was significant. AL- Hussein cultivar grown with 50 meq. L⁻¹ gave the highest value (1.437) whereas AL- Rasheed cultivar gave the lowest value (0.256), when grown in 150 meq.L⁻¹.

Table (9). The effect of Na cl levels on the stress tolerance index (STI) of two wheat cultivars.

Na cl meq.L-1 cultivars	0	50	100	150	Mean
AL-Hussein	1.323	1.437	0.512	0.278	0.887
AL-Rasheed	0.778	0.779	0.542	0.256	0.589
Mean	1.050	1.108	0.527	0.267	
L.S.D(0.05)	Na cl=0.100 Cultivar =0.071 Interaction=0.142				

3.10. The Effect of Salinity Stress on the Stress Intensity (IS) of Two Wheat Cultivars

Higher stress intensity was recorded with AL-Rasheed cultivar (0.430) (Table 10). Increasing salinity caused a marked increase in This trait, where control treatment gave 0.196 whereas, plant grown with 150 meq. L⁻¹ gave 0.731. The interaction between the studied factors also affected the stress intensity significantly where AL- Hussein cultivar grown with 50 meq. L⁻¹ gave the lowest value (0.189) while

the same cultivar grown with 150 meq. L⁻¹ gave the highest value (0.740).

Table (10). The effect of Na cl levels on the stress intensity (IS) of two wheat cultivars.

Na cl meq.L-1 cultivars	0	50	100	150	Mean
AL-Hussein	0.191	0.189	0.579	0.740	0.425
AL-Rasheed	0.200	0.197	0.603	0.721	0.430
Mean	0.196	0.193	0.591	0.731	
L.S.D(0.05)	Na cl=0.043 cultivar = 0.030 Interaction = 0.060				

3.11. The Effect of Salinity Stress on the Chlorophyll Stability Index of Two Wheat Cultivars

Table (11) revealed that, Na cl treatment and the interaction significantly affected the chlorophyll stability index. Higher value was obtained from control treatment (1.095) and lower value was obtained from 150 meq. L-1 Na cl treatment, recording 63.3% reduction. AL- Hussein cultivar was superior where increased by 15.3% over AL- Rasheed cultivar. However. AL- Hussein cultivar grown with 150 meq. L-1 Na cl gave lower value 0.357 whereas the same cultivar grown with 50 meq. L-1 Na cl gave the highest value (1.220).

Table (11). The effect of Na cl levels on the chlorophyll stability index of two wheat cultivars.

Na cl meq.L-1 cultivars	0	50	100	150	Mean
AL-Hussein	1.201	1.220	0.572	0.357	0.838
AL-Rasheed	0.990	0.899	0.572	0.447	0.727
Mean	1.095	1.060	0.572	0.402	
L.S.D(0.05)	Na cl=0.46 1 cultivar =0.326 Interaction =0.651				

3.12. The Effect of Salinity Stress on Plasma Membrane Injury of Two Wheat Cultivars

The effect of Na cl on the permeability of plasma membrane is shown in (Table 12) AL- Hussein cultivar was less affected than AL- Rasheed cultivar where the reduction was 14.8%. Increasing Na cl from 0 to 150 meq. L⁻¹, the plasma membrane injury increased from 25.559 to 42.187 % i.e. increased by 65.1%. The interaction between their two factors significantly affected the membrane injury value where AL- Hussein cultivar grown with 50 meq. L⁻¹ gave the lowest value (20.110) whereas AL- Rasheed cultivar, grown with 150 meq. L⁻¹ NaCl gave the highest value.

Table (12). The effect of Na cl levels on the membrane injury of two wheat cultivars.

Na cl meq. L-1 cultivars	0	50	100	150	Mean
AL-Hussein	21.821	20.110	36.764	40.840	29.884
AL-Rasheed	29.297	31.447	36.017	43.535	35.074
Mean	25.559	25.779	36.391	42.187	
L.S.D(0.05)	Na cl =2.870 cultivar =2.092 Interaction =4.059				

4. Discussion

The difference between studied wheat cultivars in the germination percent could be attributed to their genetic differences [14]. The reduction in germination percentage due to the salinity treatment may be attributed to the direct effect of osmosis and to the salts toxicity where salts accumulate inside the seed by the imbibition of water leading to negative effect on GA₃ and the activity of α -amylase enzyme [15]. In addition, salinity could inhibit the consequence metabolism of seed germination [16].

The dry weights of vegetative and root parts were also reduced by NaCl treatment (Tables 7 and 8) in both cultivars of wheat. The cultivars differed in their response to salinity and their difference was attributed to the genetic variation [17]. The superiority of AL-Hussein cultivar in terms of salinity tolerance could be due to its mechanism in discarding and / or avoiding Na from active sites and keeping high concentration of K in different plant parts especially the upper leaves to maintenance of metabolic processes and synthesis of dry matter during different stages of growth. Increasing NaCl, however reduced the dry matter and this could be due to the reduction in the stability of chlorophyll (Table 11) and to the reduction in the dry matter accumulation [18]. The direct and indirect effects of salinity caused reduction in all metabolic processes which reduce the plant growth in all plant parts.

The reduction in plants height with increasing salinity in irrigation water is a result of direct negative effect on the enzymes activity in plant cells leading to the precipitation of proteins or to the inhibition of their active sites, this result was agreed with that found by [19] who stated that using saline water 4 to 10.5 ds. cm reduced leaves protein and consequently reduced the plant growth as a result of reducing the division, expansion and differentiation of leaves cells [20]. Moreover, increasing stress intensity and increasing stress tolerance in AL-Rasheed cultivar were important factors for plant to tolerate the salt effect and grow well under salinity conditions. This finding confirmed that, AL-Hussein cultivar possess tolerance mechanisms better than AL-Rasheed cultivar where the tolerance is highly correlated with discarding Na⁺ and Cl⁻ ions from the vegetative part. These results are in accordance with those found by [21] with *Leptochloa fusca* plant. The stability of chlorophyll is considered as an indicator or a parameter for plant tolerance. Therefore, the abundance of chlorophyll in the plants leaves is leading to increase the photosynthesis rate and consequently a high dry matter is produced. All these give evidence for the salt tolerance by the plant [22].

The toxic effect of NaCl on the permeability of the plasma membrane is shown in Table (12). There was an adverse effect of Na on the membrane permeability compared with the control. These results are in accordance with that found by [23]. Under stress conditions, cell membrane will expose to some changes mostly leading to increase the permeability of membrane [24]. The activity of cell membrane in controlling the ions movement into and out of the cell is used

as a test of injury in the cell membrane. Nevertheless, the plasma membrane permeability in term of EC confirmed that, AL-Rasheed cultivar was sensitive to NaCl. According to [25] and [26], plant species respond differently to the oxidative injury and these could be due to the difference in anti-oxidative systems under salinity stress. The tolerance to NaCl toxicity depends on the differences in the uptake of Na⁺ and Cl⁻ and their translocation and discarding.

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