Effect of NaCl on Some Morphological Characters of Oat (Avena sativa L.) Plants

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Abstract—The experiment was conducted in a field of Biology Department/Faculty of Science and Health/Koya University/ Kurdistan region, located at 44°E, 33°4 N, and 570 m of altitude above sea level. A factorial experiment with randomized complete block design with three replications was conducted to study the combination effect of two oat varieties (POSSUM and ICARDA TALL) and salinity (NaCl) with four concentrations 0 (tap water), 0.5, 1, and 2% on *Avena sativa* L. plants. NaCl stress inhibited plant growth and had a significant effect on the plant height, number of leaves, diameter of plant, leaf area, root length, flag leaf area, length of spike, and shoot dry matter. Furthermore, irrigation with saline water was decreased on some vegetative growth such as leaves chemical content and some stomata characteristics as compared with control. Overall, these results showed that plant growth of ICARDA TALL varieties is a potentially useful candidate gene for salinity tolerance.

Index Terms—Avena sativa L., Morphological response, Salinity, Species.

I. INTRODUCTION

Oat (Avena sativa L.) is an annual grass belongs to the natural order Poaceae family [1]. It is an important and traditional agricultural cereal crop produced in various regions of Europe and North America. It is the third leading crops produced the United States and the fourth most important crop worldwide [2-5]. Oat is one of the most economically and ecologically families in the world. They are the most widely grown plant's considered healthy food being commercially nutritious as well. Furthermore, it is used in external preparations to treat eczema and dry skin. Today, it was used for hay, pasture, green manure, or as a cover crop, which enhances soil life, suppresses weeds, provides erosion control and increases organic component [1].

Salinity is one of the most serious, limiting factors for crop growth and production in the arid regions. About 23% of the world's cultivated lands are saline and 37% is sodic [6]. Soils



can be saline, due to geohistorical processes. Soil salinity in agriculture refers to the presence of high concentrations of soluble salts in the soil moisture in the root zone. These concentrations of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water by the roots [7]. Salinity stress by excess salts in the soil or irrigation water is known to affect various growth processes including photosynthesis, ion regulation, and water relations. Thus, a wide range of physiological and biochemical processes is necessary to re-establish cellular homeostasis [5,8,9].

Nutrient disturbances under salinity reduce plant growth by affecting the availability, transport, and partitioning of nutrients. However, salinity can differently affect the mineral nutrition of plants. Salinity may cause nutrient deficiencies or imbalances, due to the competition of Na+ and Cl– with nutrients such as K+, Ca2+, and NO3–. Under saline conditions, plant growth reduced due to specific ion toxicities (for example, Na+ and Cl–) and ionic imbalances acting on biophysical and metabolic components of plant growth occurs [10]. Increased NaCl concentration has been reported to induce increases in Na and Cl as well as decreases in N, P, Ca, K, and Mg level in fennel [11-14].

The purpose of this experiment was to evaluate the effects of different varieties of oat and irrigation with saline water on the vegetative growth on chemical characteristics and some stomata characteristics of *A. sativa* plant.

II. MATERIALS AND METHODS

A. Plant Materials and Treatment

The experiment was conducted in 2015–2016 in the open field of Biology Department in Koya district. Oat grains (*A. sativa* L.) were obtained from ICARDA organization, two varieties of the oat grains used POSSUM (T_1) and ICARDA TALL (T_2), in each type of oat 180 seeds were taken, these seeds were divided into the 12 plastic pots each pot contained 15 seeds and filled with brought from agriculture research center of Koya city. Oat grains (POSSUM and ICARDA TALL) were sown on January 10, 2016.

B. Evaluation of A. sativa Plants Grown with Salt Stress

A factorial experiment with randomized complete block design with three replications was applied to study the combination of two varieties of oat $(T_1 \text{ and } T_2)$ and irrigation with four saline water by NaCl $(C_0, C_1, C_2, \text{ and } C_3)$. The

irrigation done by water solution containing 0, 5, 10, and 20 g of NaCl dissolved in 1000 ml tap water to obtain concentrations 0 (tap water), 0.5, 1, and 2%, tap water was used as a control. Groups of 360 seeds were germinated after 30 days in 24 plastic pots; seedlings were irrigated with sodium chloride (NaCl) solution every 3 days (250 ml per irrigation). Oat irrigation program was carried out in March–May 2016 under the effect of salt stress. 60 days later, the characteristics were studied on vegetative growth, the chemical content of leaves and some stomata characteristics of *A. sativa* plant.

C. Meteorological Data

Maximum and minimum temperature, the relative humidity and the amount of rainfall in the open field during the planting season are shown in Table I, as recorded by Agrometeorological Station in Koya city.

On (May 1, 2016) the following characteristics were studied:

- A. Vegetative growth included:
 - Plant height (cm): It was measured from the point of stem attachment with soil to the apical point of the main shoot using metric tapeline.
 - Number of leaves/plant: Total numbers of leaves were counted, including those leaves that can be seen by naked eyes.
 - A number of tillers/plant: Total numbers of tillers were counted including branches that can be seen by naked eyes.
 - Root length (cm): It was measured from the point of stem attachment with soil to the apical point of the main root using metric tapeline after washing and rinsing in tap water.
 - Stem diameter (mm): Diameter of main branches was measured by Vernier micrometer.
 - Plant leaf area (cm2/plant): 10 leaves were selected randomly from 15 plants, leaf length (L) measured from the lamina tip to the connected place petiole to a lamina and width (W) from tip to tip at the widest of the lamina, using ruler, leaf area calculated by the formula described by Thomas and Winner [15];

$$La (cm^2) = Length * Width * 0.95$$

• Flag leaf area (cm2/plant): 10 leaves were selected randomly from plants, leaf length (L) measured from a lamina tip to the connected place petiole to the lamina and width (W) from tip to tip at the widest of a lamina, using ruler, leaf area calculated by the formula described by Thomas and Winner [15];

La (cm^2) = Length * Width * 0.95

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- The length of a spike (cm): It was measured from the tip of the stem attached with a spike to the apical point of main a spike using the ruler.
- Number of seed spike/plant: Total numbers of seeds in spike were counted, including that a spike that can be seen by naked eyes.
- Shoots and roots dry matter (%): The dry weight of root and shoot was measured after keeping the fresh plant samples in an oven at 70°C for 48 h until the weight fixed, shoot and root dry matter percentage were calculated using the following formula described by Al-Sahaf [16];

B. Chlorophyll content (mg/100 g fresh weight):

The amount of chlorophyll a, b, and total chlorophyll and total carotenoids were estimated according to Tang *et al.* [17] method. Leaf material was collected, and 0.25 g of fresh leaves of each experimental unit was taken, and then mixed with 10 ml 80% acetone, the extract was placed in a 25 ml glass vial (dark bottle). The glass vials were sealed with par film to prevent evaporation, to avoid photo-oxidation of pigments, and then 1 ml of extract was added to 9 ml of acetone. Chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid were estimated spectrophotometrically at 663 nm, 645 nm, and 440 nm wavelengths, as follows:

Chlorophyll a (mg/L) = $(9.784 \times \text{OD at } 663 \text{ nm}) - (0.99 \times \text{OD at } 645 \text{ nm})$

Chlorophyll b (mg/L) = $(21.426 \times \text{OD at } 645 \text{ nm}) - (4.65 \times \text{OD at } 663 \text{ nm})$

Total chlorophyll (mg/L) = $(5.134 \times \text{OD at } 663 \text{ nm}) + (20.436 \times \text{OD at } 645 \text{ nm})$

Carotenoid (mg/L) = $(4.695 \times 440 \text{ nm}) + 0.268$ (Chlorophyll a + Chlorophyll b)

The following formula used for transferring chlorophyll content from mg/L to mg/100 g fresh weight:

mg chlorophyll /100 g fresh weight =
$$\frac{\text{mg/l}}{1000 \text{ ml}} \times \frac{100 \text{ g}}{\text{g sample}}$$

C. Number, length, and width of stomata on adaxial and abaxial leaf surface:

Three leaves were selected from different plants carefully from each experimental unit and kept in polythene bags during May 10, 2016, and brought to the laboratory. The leaf epidermal peel slides were made by the methods of lasting impressions. In this method, at least one square centimeter on leaf surface was painted by a thick patch of clear nail polish. The nail polish to be allowed to dry completely, then a piece of clear cellophane tape to the dried nail polish

TABLE I

ximum and Minimum Temperature, Relative Humidity and the Amount of RainFall During the Growing S	Season (2015–2016)	

Month (2016)	Air Te	mp. C°	Relative l	Rainfall (mm)	
	Maximum	Minimum	Maximum	Minimum	
January	9.8	5.03	77.8	69.9	100
February	15.8	9.4	68.1	59.3	49
March	18.1	10.7	68.8	60.1	170
April	20.1	15.5	75	71.2	79

patch was placed through carton sealing tape. Gently, the nail polish patch peeled out by pulling a corner of the tape and fingernail polish along with the leaf peel, which was taped on slides and labeled as an adaxial and an abaxial surface. Leaf was examined under least ×100 magnifications by light microscope. Numbers of stomata were counted per square millimeter area. Length and width of stomata guard cells of a leaf were measured in μ m (micrometer) with an ocular micrometer under high power magnifications with the help of Stage-Ocular micrometer [18].

D. Statistical Analysis

Comparisons between means were made using Duncan's multiple range test at 5% level [19].

III. RESULTS AND DISCUSSIONS

Table II shows that oat varieties to affects significantly on vegetative growth, including plant height, number of tillers, leaf area, and root length which recorded the highest value in ICARDA TALL varieties (80.96 cm, 5.78, 43. 02 cm², and 15.78 cm) for these parameters, respectively, whereas the lowest values were recorded in POSSUM varieties (51.55 cm, 4.87, 37.64 cm², and 12.67 cm), respectively, but decreasing in number of leaves and diameter of plants. There were no significant differences between the different varieties of oat and salinity concentration on these parameters. Increasing salinity concentration in irrigation water caused decreases in plant vegetative growth compared to control treatment. Irrigation with saline water and their interactions with different varieties of oat had a significant effect on the plant height, number of leaves, stem diameter, leaf area, and root length.

ICARDA TALL varieties were superior to POSSUM one in stimulating the morphological growth. Table II showed

that in ICARDA TALL varieties all growth parameters significantly increased compared to the POSSUM varieties, this effect may be attributed to the genetic properties of the plants. The growth of plant decreased with increasing the salinity concentration because salinity can also affect plant growth (plant physiological, morphological, biochemical processes growth, and water) because the high concentration of salts in the soil solution interferes with balanced absorption of essential nutritional ions by plants [5,7,9,20]. The results agreed partially with [21] confirmed that irrigation with saline water decreased vegetative growth.

Results in Table III showed that there was a significant difference between two oat varieties and salt concentrations in their effect on the flag leaf area and length of the spike, this effect may be attributed to the genetic properties of the plants. The results agreed partially with the study of Dhanapackiam and Ilyas [22] and Iqbal [23] who confirmed that irrigation with saline water decreased vegetative growth.

The results in Table IV showed significant differences between two oat varieties in the shoot dry matter, the highest value was recorded in ICARDA TALL varieties, whereas the lowest value was recorded in POSSUM varieties. Irrigation with saline water had no significant effect on this parameter. The interaction between different varieties and irrigation with saline water had a significant effect on the shoot dry matter, the highest value was obtained from ICARDA TALL varieties with concentration 2%, whereas the lowest value was obtained from POSSUM varieties.

The saline growth medium caused many adverse effects on plant growth, which was due to the low external water potential (osmotic stress). Under salt stress, plants have to cope with stress imposed by low osmotic potential and with ion toxicity due to the accumulation of ion inside the plants. Fresh and dry weights were also decreased with increasing salinities in shoot and root dry matter. The results agreed

EFFECT OF IRRIGATION WITH SALINE WATER AND TWO OAT VARIETIES AND THEIR INTERACTIONS ON VEGETATIVE GROWTH Treatments Plant height Number of Number of Stem diameter Plant leaf area Flag leaf area Root length leaves/plant tillers/plant (mm) (cm)² $(cm)^{2}$ (cm)(cm) Varieties of oat 4.52ª POSSUM (T₁) 51.55^b 37.64^b 34.59ª 4.87^a 33.48^a 12.67^b ICARDA TALL (T,) 80.96ª 28.98^a 5.78ª 4.09^b 43.02ª 24.77^b 15.78^a Salinity concentration (%) Tap water (C0) 68.00^a 37.10^a 4.77ª 4.42ª 42.77^a 30.88ª 12.50^a 0.5 (C1) 68.50^a 33.75ª 4.83ª 4.20^a 40.70^a 27.67^a 14.01ª 1 (C2) 69.78^a 28.51ª 5.88ª 4.56^a 40.81ª 29.27ª 15.76^a 2 (C3) 64.23ª 28.51ª 5.78ª 4.00^{a} 38.75ª 28.68ª 14.56ª The interactions between two oat varieties and NaCl concentration 9.00^b T1C0 42.70° 45.40^a 6.70^a 6.00^a 46.80^a 36.77^a T1C1 52.23bc 37.63^{ab} 4.30^b 36.33ab 30.53abc 11.23^{ab} 4.66^a 39.23^{ab} T1C2 4.76^b 32.43abc 55.46^b 28.20bc 4.66ª 14.86^a T1C3 49.90^{bc} 34.33abc 4.66^a 4.00^b 34.30^b 34.20^{ab} 13.13^{ab} T2C0 76.43ª 34.33abc 4.13^a 3.90^b 41.43^{ab} 25.00^{bc} 13.66^{ab} T2C1 84.76^a 29.86^{bc} 5.00^a 4.10^{b} 45.06^{ab} 24.80^{bc} 16.80^a T2C2 84.10^a 29.87^{bc} 7.10^a 4.36^b 42.40^{ab} 26.10^{bc} 16.66^a 6.90ª 43.20^{ab} 23.17° 16.00^a T2C3 78.56 22.90 4.00^b *Means followed by the same letters within columns are not significantly different at P≤0.05 according to the Duncan test

TABLE II

partially with the study of Kumar, *et al.*, [9] and Amira and Abdul [24].

Results in Table V showed that significant differences were found between two oat varieties on chlorophyll content, whereas irrigation water with salinity (2%) had a significant effect on the chlorophyll a, b, and total chlorophyll as compared with control. The interaction between different varieties and salinity concentrations had significant effect on the chlorophyll a, b, and total chlorophyll characteristic. The highest values (5.50, 4.63, and 10.06) were obtained from ICARDA TALL varieties,

TABLE III Effect of Irrigation With Saline Water and Two Oat Varieties and Their Interactions on Spike Length and Number

Treatments	Length of spike (cm)	Number of seed spike/plan
Varieties of oat		
POSSUM (T1)	13.87 ^b	25.19 ^a
ICARDA TALL (T2)	16.76ª	23.20 ^a
Salinity		
concentration (%)		
Tap water (C0)	15.78ª	24.71ª
0.5 (C1)	15.58ª	24.62ª
1 (C2)	15.83ª	25.05ª
2 (C3)	14.05 ^a	22.40ª
The interactions between		
two oat varieties and		
NaCl concentration		
T1C0	18.33ª	28.53ª
T1C1	13.37 ^b	24.23ª
T1C2	12.43 ^b	26.10 ^a
T1C3	11.33 ^b	21.90ª
T2C0	13.23 ^b	20.90ª
T2C1	17.80ª	25.00 ^a
T2C2	19. 23ª	24.00ª
T2C3	16.77ª	22.90ª

*Means followed by the same letters within columns are not significantly different at $P \le 0.05$ according to the Duncan test.

TABLE IV Effect of Irrigation With Saline Water and Two Oat Varieties on Shoot and Root Dry Matter

Treatments	Shoot dry matter (%)	Root dry matter (%)
Varieties of oat		
POSSUM (T ₁)	41.94 ^b	45.94ª
ICARDA TALL (T ₂)	51.85ª	50.33ª
Salinity concentration %		
Tap water (C0)	54.57ª	39.80ª
0.5 (C1)	42.48 ^a	45.42ª
1 (C2)	45.48 ^a	47.10 ^a
2 (C3)	49.26 ^a	58.18ª
The interactions between two oat		
varieties and NaCl concentration		
T1C0	45.00 ^{ab}	53.90ª
T1C1	37.63 ^b	28.90ª
T1C2	45.03 ^{ab}	55.90ª
T1C3	42.13 ^{ab}	50.37ª
T2C0	57.76 ^a	35.10 ^a
T2C1	47.33 ^{ab}	61.93ª
T2C2	45.93 ^{ab}	38.30ª
T2C3	56.40 ^{ab}	66.00ª

^{*}Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test

and irrigation water with 2% salinity, whereas the lowest values (3.10, 1.10, and 4.20) were obtained from POSSUM varieties with no salinity.

The result showed that the salt treatment increased significantly chlorophyll a, b, total chlorophyll, and total

carotenoids increased with increasing salinity concentration, the results agree with the study of Amira and Abdul [24] and Saida *et al.* [25]. Plants can implement to cope with the challenge of salt stress. Plants tolerant to NaCl implement a series of adaptations to acclimate to salinity, including morphological, physiological, and biochemical changes. These changes include increases in the root/canopy ratio and the chlorophyll content [26].

The results in Table VI and Figs. 1 and 2 showed the stomata structure in *A. sativa* plants where both adaxial and abaxial epidermis has stomata. The anatomical study showed that there were no significant differences between varieties on this parameter. Except for stomata length in lower (abaxial) surface there was significant increases in ICARDA TALL varieties, its height value (7.08 μ), whereas the lowest value was recorded in POSSUM varieties (6.38 μ). However, no significant effects were observed by irrigation with saline water. The interaction between the different varieties of oat and irrigation with saline water had a significant effect on each of stomata number on the upper surface, stomata length, and width of upper surface, respectively.

Salt stress induces the synthesis of abscisic acid which closes stomata when transported to guard cells. As a result of the stomatal closure, photosynthesis declines and



Fig. 1. Upper (adaxial) and Lower (abaxial) *Avena sativa* leaves surfaces stomata $\times 400$ (T₁C₀ U and L) stomata in POSSUM plants and no irrigation water with salinity, (T1C1 U and L) stomata in POSSUM plants and irrigation water with salinity 0.5%, (T₁C₂U and L) stomata in POSSUM plants and irrigation water with salinity1%, (T₁C₃ U and L) stomata in POSSUM plants and irrigation water with salinity 2%.



Fig. 2. Upper (adaxial) and Lower (abaxial) *Avena sativa* leaves surfaces stomata $400 \times (T_2C_0 U \text{ and } L)$ stomata in ICARDA TALL plants and no irrigation water with salinity, $(T_2C_1 U \text{ and } L)$ stomata in ICARDA TALL plants and irrigation water with salinity 0.5%, $(T_2C_2U \text{ and } L)$ stomata in ICARDA TALL plants and irrigation water with salinity1%, $(T_2C_3 U \text{ and } L)$ stomata in ICARDA TALL plants and irrigation water with salinity2%.

TABLE V
EFFECT OF IRRIGATION WITH SALINE WATER AND TWO OAT VARIETIES ON LEAF CONTENT OF CHLOROPHYLL A, B, TOTAL CHLOROPHYLL AND TOTAL CAROTENE

Treatments	mg/100 g fresh weight					
	Chlorophyll a	Chlorophyll b	Total chlorophyll	Total carotenoids		
Varieties of oat						
$POSSUM(T_1)$	4.13ª	4.51ª	8.63ª	4.33ª		
ICARDA TALL (T ₂)	4.35ª	3.35 ^b	7.69 ^a	4.06 ^a		
Salinity concentration %						
Tap water (C0)	4.30 ^{ab}	2.97 ^b	7.27 ^b	4.13 ^{ab}		
0.5 (C1)	3.68 ^b	3.51 ^b	7.21 ^b	3.87 ^b		
1 (C2)	4.05 ^b	3.83 ^{ab}	7.86 ^{ab}	4.05 ^{ab}		
2 (C3)	4.98 ^a	4.88ª	9.83ª	4.73ª		
The interactions between two oat varieties and NaCl concentration						
T1C0	3.10 ^d	1.10 ^e	4.20 ^d	4.03 ^{ab}		
T1C1	3.43 ^{bcd}	3.93 ^{abcd}	7.36 ^{abcd}	3.80 ^{ab}		
T1C2	4.83 ^{ab}	5.60 ^a	10.40ª	4.87ª		
T1C3	4.46 ^{bcd}	5.13 ^{ab}	9.60 ^{ab}	4.63ª		
T2C0	4.70 ^{abc}	3.60 ^{bed}	8.30 ^{ab}	4.23 ^{ab}		
T2C1	3.93 ^{bcd}	3.10 ^{cd}	7.06 ^{bcd}	3.93 ^{ab}		
T2C2	3.26 ^{cd}	2.06 ^{de}	5.33 ^{cd}	3.23 ^b		
T2C3	5.50ª	4.63 ^{abc}	10.06 ^{ab}	4.83ª		

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test

TABLE VI
EFFECT OF IRRIGATION WITH SALINE WATER AND TWO OAT VARIETIES AND THEIR INTERACTIONS ON SOME STOMATA CHARACTERISTICS

Treatments	Stomata number/mm ²		Stomata length (µ)		Stomata width (µ)	
	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface	Upper leaf surface	Lower leaf surface
Varieties of oat						
POSSUM (T ₁)	190.50ª	210.41ª	6.71ª	6.38 ^b	4.39ª	4.00 ^a
ICARDA TALL (T ₂)	185.61ª	194.93ª	6.73ª	7.08 ^a	4.49 ^a	4.19 ^a
Salinity concentration %						
Tap water (C0)	203.53ª	233.13ª	6.80ª	7.07 ^a	4.10 ^b	4.07 ^a
0.5 (C1)	179.30ª	205.97 ^{ab}	6.41ª	6.63 ^a	4.18 ^b	4.20 ^a
1 (C2)	179.85ª	188.32 ^b	6.77 ^a	6.66 ^a	4.66 ^a	3.76 ^a
2 (C3)	193.88ª	190.85 ^b	6.91ª	6.78 ^a	4.71ª	4.36 ^a
The interactions between two oat varieties						
and NaCl concentration						
T1C0	237.50ª	236.70 ^a	7.10 ^a	6.50ª	4.10 ^c	4.10 ^a
T1C1	179.17 ^b	222.20ª	6.70 ^{ab}	6.33ª	4.16 ^{bc}	4.20ª
T1C2	182.23 ^{ab}	199.70 ^a	6.26 ^{ab}	6.10 ^a	4.43 ^{abc}	3.70 ^a
T1C3	194.43 ^{ab}	200.57ª	7.03 ^{ab}	6.66ª	4.66 ^{abc}	4.06 ^a
T2C0	192.20 ^{ab}	231.93ª	6.70 ^{ab}	7.26ª	4.10 ^c	4.06 ^a
T2C1	179.17 ^b	189.73ª	6.13 ^b	6.93ª	4.20 ^{bc}	4.20ª
T2C2	177.47 ^b	176.93ª	7.26 ^a	7.23ª	4.90 ^a	3.83ª
T2C3	193.33 ^{ab}	181.13ª	6.80 ^{ab}	6.90ª	4.76 ^{ab}	4.66ª

*Means followed by the same letters within columns are not significantly different at $P \leq 0.05$ according to the Duncan test

photoinhibition and oxidative stress occur. An immediate effect of osmotic stress on plant growth is its inhibition of cell expansion either directly or indirectly through abscisic acid [27].

Salt stress significantly reduced the growth of the oat grass genotypes during the seedling stage. The stomatal inhibition of photosynthesis, caused by direct effects of NaCl on the photosynthetic apparatus independent of the stomatal closure, might be responsible for the reduction in photosynthetic rate. Stomata characteristics such as the number, length, and width are affected by the genetic constitution, season, leaf position, and leaf surface (upper or lower). These results partially agreed with the study of Zan *et al.* [28].

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