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Effect of sodium chloride on some morpho-physiological traits in *Zea mays* L.

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ABSTRACT

To evaluate the effect of salinity originated from sodium chloride on the morphological and physiological characteristics of corn, a factorial experiment was conducted based on randomized complete block design with seven replications in greenhouse condition. Treatments were four levels of soil salinity (0, 5, 10, 15 and 20 ds/m) and two types of irrigation water (0 and 2 ds/m of sodium chloride). Results of ANOVA showed the significant effect of soil salinity on chlorophyll *a* and total chlorophyll, and significant interaction effect between soil salinity and sodium chloride concentration of irrigation water on chlorophyll *b*, proline and total soluble carbohydrates. The highest content of leaf chlorophyll *b* (0.67 mg/l) was obtained from control treatment (without salinity of soil and water). The lowest concentration of leaf chlorophyll *b* (0.29 mg/l) belonged to plants irrigated with water (0 ds/m) in 15 ds/m saline soil. The highest (37.65 mg/l) and lowest (24.53 mg/l) total soluble carbohydrates content were obtained from control and 15 ds/m of soil salinity, respectively. While, the minimum proline content (20.39 mg/l) belonged to control and the maximum proline (0.030mg/l) belonged to 20 ds/m soil salinity. Despite ascending trends in proline along with higher salinity, these arises was sever in saline soil.

KEYWORDS

Chlorophyll;
Corn;
Irrigation water;
Proline;
Saline soil.

INTRODUCTION

Maize (*Zea mays* L.) is the most abundant cereal grain produced in the world and is a staple food for large groups of people in Latin America, North America, Asia, and Africa[20]. After wheat and rice, maize is the third most important cereal crop grown all over the world in a wide range of climatic condition. Maize, being highly cross pollinated, has become highly polymorphic through the course of natural and domesticated evolution and thus contains enormous variability[18]. It provides around 42 million tons of protein a year, which represents approximately 15% of the world annual production of food crop protein[11]. Salinity is one of the major environmental threats to agriculture and affects approximately 7% of the world's total land area[23]. Soil salinity has plagued its agriculture for a long time due to its dry climate, flat terrain and inadequate drainage systems[25]. The first effect of salts is reducing the ability of plants to absorb water (osmotic effect), which leads to slower growth; second, salts may enter the transpiration and injure leaf cells, further reducing growth[16]. High NaCl salinity leads to a decrease in plant and leaf growth and onset of senescence in most crop plants, therefore, to a reduction in total photosynthetic capacity. These effects limit the ability to generate further biomass or to maintain defense mechanism[26]. The high concentration of Na^+ and Cl^- in soil solution is generally the main cause of the saline stress[6] and the consequent slower growth is an adaptive feature for plant survival. The negative effect of salinity on plant growth has been also attributed to physiological parameters, such as the inhibition of enzyme activities; particularly those involved versus oxidative stress[23]. Osmotic adjustment is also a mechanism to avoid salinity. Proline and quaternary ammonium compounds are key osmolytes, which help plants to maintain cell turgor[8,19]. A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in defense against salinity stress. However, data do not always indicate a positive correlation between osmolytes accumulation and an ability to adapt to stress[3,13,14]. Thus, the main objective of this research is the study of maize plants morphological and physiological responses to salinity of soil and water.

MATERIALS AND METHODS

Plant materials and experimental design

The experiment was arranged according to a factorial based on randomized complete block design with seven replications for morphological traits and three replications for physiological characteristics of maize (*Zea mays* L. *cv*. SC704) in the greenhouse conditions at Urmia University, Iran, from August to November 2011. The seed were sown in pot at depth of 2 cm. Water salinity were started 14 days after sowing. Treatments were soil salinity including (0, 5, 10, 15 and 20 ds/m of NaCl) and two types of irrigation water quality (0 and 2 ds/m of sodium chloride).

Measurements

Morphological traits were measured at physiological maturity stage for seed harvesting. To determine the dry weight of shoots and leaves, plants were harvested and then samples were dried at 72°C for 48 hours.

Leaf Relative water content (LRWC) was determined on upper most fully expanded leaves as LRWC (%) = [(FW-DW)/(TW-DW)] ×100. Where: fresh weight (FW), dry weight (DW) was obtained after drying the samples at 72°C for at least 48 hours. Turgid weight (TW) was determined by subjecting leaves to rehydration for four hours in darkness.

In order to determine the leaf chlorophyll content and carotenoid, 0.25 g of complete leaves were ground in cool water in darkness and adjusted to volume 25 ml by distilled water. Then 0.5 ml of this solute was mixed with 4.5 ml acetone 80% and centrifuged 3000 rpm for 10 min. The upper zone of this solution was taken for spectrophotometery at 645, 663 and 470 nm wavelengths. To estimate the leaf chlorophyll *a*, *b*, total chlorophyll and carotenoid content by spectrophotometery, the following equations were used[5,22]:

Chlorophyll *a* (mg/l) = [(0.0127 × OD663) + (0.00269 × OD645)] ×1000

Chlorophyll *b* (mg/l) = [(0.0229 × OD645) + (0.00468 × OD663)] ×1000

Total chlorophyll (mg/l) = [(0.0202 × OD645) + (0.0082 × OD663)] ×1000

Carotenoid (mg/l) = [(OD470) – (0.114 × OD663) – (0.638 × OD645)]×1000

OD645, OD663 and OD470 present the absorption in 645, 663 and 470 nm wavelengths, respectively.

To determine the amount of leaf proline and total soluble carbohydrates, 0.5 g of complete leaves were ground in 5 ml ethanol 95%. Its upper zone was washed with ethanol 70% twice, centrifuged at 3500 rpm for 10 min[9] and measured by spectrophotometer at 515 nm wavelength for proline[17] and 625 nm for total soluble carbohydrate[9].

Statistical analysis

Analysis of data was carried out through SAS soft-ware version 9.13. The graphs were designed by using Mi-crosoft Office Excel software. Mean comparisons were carried out using Student-Neuman Keul's test (SNK).

RESULTS AND DISCUSSION

The results of analysis of variance (ANOVA) on physiological traits showed the significant effect of soil salinity on chlorophyll *a* and total chlorophyll at 1% probability level. The interaction effect between soil and water salinity on chlorophyll *b* and proline were significant at 1% probability level, and on total soluble carbohydrates was significant at 5% probability level (TABLE 1).

TABLE 1 : Analysis of variance of physiological characteristics of *Zea mays* L. under soil and water salinity.

Source of variation	df.	Mean of square (MS)						
		Leaf relative water content (LRWC)	Chlorophyll			Carotenoid	Proline	Total soluble carbohydrates
			a	*b*	Total			
Replication	2	0.00080^{ns}	0.00752^{ns}	0.00603^{ns}	0.007174^{ns}	0.0027^{ns}	0.000041**	0.68^{ns}
Water quality(A)	1	0.00008^{ns}	0.00075^{ns}	0.00002^{ns}	0.000001^{ns}	0.0403^{ns}	0.004165**	490.29**
Soil salinity(B)	4	0.00245^{ns}	0.04663**	0.07146**	0.086876**	0.0046^{ns}	0.002853**	110.67*
A×B	3	0.00174^{ns}	0.00662^{ns}	0.02863**	0.025315^{ns}	0.0068^{ns}	0.000402**	140.46*
Error	16	0.00316	0.00723	0.00464	0.0103630	0.0143	0.000022	32.36
Coefficient of variation (%)		2.97	25.05	16.67	22.13	4.92	14.99	16.20

ns, *, and **, non-significant, significant at *P* d” 0.05 and *P* d” 0.01, respectively. df, Degree of freedom

The results of analysis of variance (ANOVA) of morphological traits showed the significant effect of soil salinity on the numbers of leaf per plant at 1% probability level. However, there was significant interaction effect between soil and water salinity on the stem diameter, stem weight, leaf length and leaf weight at the 1%, and on the leaf width and stem height at 5% probability level (TABLE 2).

The maximum value of chlorophyll *b* (0.67 mg/l) was obtained from control treatment (0 ds/m soil salinity and 0 ds/m of water salinity) and the minimum value (0.285 mg/l) belonged to 15 ds/m of soil salinity and 0 ds/m of water salinity. This minimum chlorophyll (chlorophyll *b*) was the same with leaf chlorophyll obtained from 20 ds/m of soil salinity as equal as all treatments of 2 ds/m water salinity (TABLE 3).

The maximum concentration of proline (0.030 mg/l) was obtained from 20 ds/m of saline soil irrigated by 2 ds/m water. The leaf proline content was reduced along with downturn of salinity in soil and water, so the minimum leaf proline content (0.008 mg/l) was obtained from control treatment (0 ds/m of soil and water) (TABLE 3).

The highest concentration of total soluble carbohydrates (39.12 mg/l) was occurred in maize plants treated with 5 ds/m of soil salinity irrigated by saline water (2 ds/m). Total soluble carbohydrates were in high and same content for all treatments except 20 ds/m soil and 2 ds/m of water salinity in that the lowest concentration

TABLE 2 : Analysis of variance of morphological traits of *Zea mays* L. under soil and water salinity.

Source of variation	Mean of square (MS)							
	df	Leaf number	Stem height	Stem diameter	Stem weight	Leaf length	Leaf width	Leaf weight
Replication	6	1.83^{ns}	30.40^{ns}	0.1875^{ns}	27.51^{ns}	34.7197^{ns}	0.4944^{ns}	0.0129^{ns}
Water quality(A)	1	7.54^{ns}	1011.49**	0.0484^{ns}	115.54*	238.4794**	1.1113^{ns}	0.0530^{ns}
Soil salinity(B)	4	17.90**	2332.00**	0.2655^{ns}	471.40**	318.0540**	1.2311^{ns}	0.0292^{ns}
A×B	3	4.89^{ns}	437.69*	1.4182**	127.46**	139.0253**	1.4307*	0.1140**
Error	45	1.89	107.26	0.2645	20.60	19.3630	0.4996	0.0209
Coefficient of variation (%)		14.07	21.63	31.5920	37.65	13.0507	17.2709	31.2936

ns, *, and **, non-significant, significant at *P* d” 0.05 and *P* d” 0.01, respectively. df. Degree of freedom

TABLE 3 : Means comparison of Chlorophyll *b*, Proline and Total soluble carbohydrates of maize.

Water quality (ds/m)	Soil salinity (ds/m)	Chlorophyll *b* (mg/l)	Proline (mg/l)	Total soluble carbohydrates (mg/l)
0	0	0.6706a	0.008f	37.65a
	5	0.5512b	0.010ef	34.06ab
	10	0.4242c	0.014ef	34.20ab
	15	0.2846d	0.017de	20.39ab
	20	0.3257cd	0.018de	34.01ab
2	0	0.3486cd	0.021cd	32.88ab
	5	0.3851cd	0.020cd	39.12a
	10	0.3294cd	0.024bc	38.22a
	15	0.3186cd	0.028ab	35.47ab
	20	0.3129cd	0.030a	24.53b

The mean with the same letters in each column are not significantly different at $P \leq 5\%$.

(20.39 mg/l) was observed (TABLE 3).

Means comparison indicated that increasing soil salinity levels caused to decrease the content of leaf chlorophyll. The maximum amount of chlorophyll *a* (0.49 mg/l) and total chlorophyll (0.68 mg/l) were obtained from control treatment. The minimum amount of chlorophyll *a* (0.25 mg/l) and total chlorophyll (0.35 mg/l) were obtained from 10 ds/m of saline soil treatment as same as other saline soils (TABLE 4). The maximum leaf number (11 leaves per plant) was observed in control treatment, and the minimum leaf number (8 leaves per plant) belonged to plants sown in 10 ds/m salinity of soil. All levels of soil salinity produced the same leaf numbers and physiological characters like chlorophyll (TABLE s 3 and 4).

The widest leaf (5.08 cm) belonged to plants irrigated by saline water in 15 ds/m of soil salinity. But decreasing in salinity of soil and water caused to narrow leaves in corn, so the minimum leaf width (3.60 cm) belonged to 20 ds/m of soil salinity and saline water, followed down with lower salinity (TABLE 5).

The longest leaf (47.10 cm) was observed in control treatment. And severity of salinity, both soil and water, led to reducing trends in leaf length. So, the shortest leaf (26.72 cm) belonged to 5 ds/m soil salinity irrigated by saline water. More concentrations of salt in soil (more than 5 ds/m) produced the leaves in same length in minimum amount (TABLE 5).

Soil salinity higher than 10 ds/m produced the maximum stem diameter (1.98 mm for 15 ds/m). Stem diameter become larger along with reducing the salinity, but in very low concentration, especially in control, stem diameter grow up to maximum amount (TABLE 5).

The tallest plant (73.10 cm) was obtained from control treatment. But the gradual increase in salt concentrations of soil and water caused to stunted plants (TABLE 5).

Like stem height, the largest amount of stem weight (24.68 g) and leaf weight (0.675 g) belonged to control treatment, and a descending trend was observed in stem weight by severe salinity of soil and water (TABLE 5).

Salt stress is known to be one of the most important abiotic stresses and seriously affects crop productivity and survival. The deleterious effects of excessive salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), which re-

TABLE 4 : Means comparison of Chlorophyll *a*, Total chlorophyll and the leaf numbers of maize.

Soil salinity (ds/m)	Chlorophyll *a* (mg/l)	Total chlorophyll (mg/l)	Leaf number
0	0.49195a	0.68690a	11.2262a
5	0.29027b	0.37820b	8.7500c
10	0.25007b	0.35223b	8.2857c
15	0.34010b	0.44730b	9.3809bc
20	0.30990b	0.40993b	9.3787bc

The mean with the same letters in each column are not significantly different at $P \leq 5\%$.

FULL PAPER

TABLE 5 : Means comparison of some morphological characteristics of maize under soil and water salinity.

Water quality (ds/m)	Soil salinity (ds/m)	Leaf width (cm)	Leaf length (cm)	Stem diameter (mm)	Stem height (cm)	Stem weight (g)	Leaf weight (g)
0	0	3.850bc	47.10a	1.783ab	73.10a	24.68a	0.675a
	5	4.198bc	36.45b	1.879ab	50.86b	17.37b	0.511b
	10	4.176bc	32.43bc	1.889ab	36.36cd	16.69b	0.507b
	15	4.025bc	31.35cd	1.595abc	48.14b	5.693d	0.448bcd
	20	3.605c	28.93cd	1.340bc	42.00bc	3.353d	0.335d
2	0	4.219bc	36.75b	1.229c	64.74a	14.12bc	0.401bcd
	5	3.691c	26.72d	1.157c	25.433d	11.49c	0.343bcd
	10	4.546ab	30.26cd	1.955a	44.12bc	6.353d	0.482bcd
	15	5.080a	32.55bc	1.986a	45.14bc	6.412d	0.461bcd
	20	3.917bc	29.55cd	1.925a	43.00bc	6.173d	0.495bcd

The mean with the same letters in each column are not significantly different at $P \leq 5\%$.

duces the availability of water to plants, (2) nutritional imbalance, (3) effect on specific ions (salt stress), and (4) a combination of all the three factors[2,12]. All of these cause adverse effects on plant growth and development at physiological and biochemical levels[4,10,15]. The plant photosystem is easily damaged by stress, and chlorophyll often is measured as an indication of photosystem integrity when plants are exposed to extreme environmental conditions. Several physiological studies demonstrated that non-toxic compatible solutes, such as amino acide, glycine betaine and sugars, can accumulate under salt stress conditions without any negative influence on the cell physiology[23]. Proline accumulates in larger amounts than other amino acids in salt-stress plants[1]. Proline is a very important indicator because it is osmotically very active and regulates the accumulation of useable nitrogen (N), contributes to membrane salinity and mitigates the effect of NaCl on cell membrane disruption[3].

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