Pol. J. Environ. Stud. Vol. 24, No. 6 (2015), 2537-2542

DOI: *10.15244/pjoes/59400*

Original Research

Morpho-Physiological Characterization in Eight Varieties of Maize (*Zea mays* L.) under Soil Salinity

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Received: October 16, 2014
Accepted: September 13, 2015

Abstract

Considering Iran and Azerbaijan to be origin countries in the Astara region, and in order to study the effects of salt stress on morphological and physiological characteristics, changes to eight maize cultivars were experimented on in three replications on the basis of randomized complete block design over three years. Cultivars included K3615/1, S.C704, B73, S.C302, Waxy, K3546/6, K3653/2, and Zaqatala-68, and they were cultivated in two pieces of land in Astara: one with normal soil and the other with salty soil. During the experiment, Na^+ accumulation in leaves, chlorophyll *a*, chlorophyll *b*, leaf relative water content (LRWC), proline content, biomass per plant, harvest index, and grain yield per hectare were measured. Results from the experiment showed that, between locations (normal and saline) in most traits, significant differences were seen. And between varieties in all traits, significant differences were seen. The interaction between years and varieties for all traits was not significant. Comparison traits in different salinities showed that in most traits there are significant differences between genotypes. Proline content increased with increasing soil salinity. With increasing salinity, Na^+ accumulation in leaves severely increased and the biggest accumulation was observed in S.C704. Maximum LRWC in B73 was measured in normal conditions. The highest amount of chlorophyll *a*, in normal conditions, was observed in S.C704 with 1.873 mg/g fresh weight of leaves, which was not significantly different from B73. Between chlorophyll *a* and chlorophyll *b*, Na^+ and LRWC positive correlations were observed in non-stress conditions. Between chlorophyll *a* and chlorophyll *b*, yield per plant, and yield grain, significant positive correlations were observed in salty conditions.

Keywords: salinity, proline, maize, chlorophyll, yield

Introduction

Abiotic stresses such as drought, salinity, extreme temperatures, chemical toxicity, and oxidative stress are serious threats to agriculture and the natural status of the environment. Salinity is one of the major environmental threats for agriculture and affects approximately 7% of the world's total land area [1]. Salinity stands for hyper salt accumulation in soils beyond the tolerance limits for most plants, and approximately 20% of the world's total irrigated agricultural land suffers from poor yield due to high salt content [2]. Salt stress affects crops under extreme saline conditions by severely impairing plant metabolism due to osmotic stress and loss of turgor. One of the mechanisms adopted by plants to tolerate salt stress is the accumulation of compatible solutes that help maintain osmotic homeostasis [3].

The deleterious effects of salinity on plant growth are associated with:

1) high osmotic potential of soil solution but low water potential (water stress)

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2) nutritional imbalance
3) specific ion effect (salt stress)
4) a combination of these factors [4].

Different methods of water management are used to cope with water shortages [5]. In salt-affected soil, there are many salt contaminants, especially NaCl, which readily dissolves in water to yield toxic, sodium (Na^+), and chloride (Cl^-) ions. Also, the water available in the salt-contaminated soil is restricted, inducing osmotic stress [6, 7].

Many studies have shown that the height [8-10], growth index [11], and fresh and dry weights of the shoot and root systems [12-14] are affected negatively by changes in salinity concentration, type of salt present, or type of plant species. Numerous studies have shown the affection of leaf area negatively by using different concentrations of NaCl [15, 16].

Maize (*Zea mays* L.) is one of the most important crops both for human and animal consumption. This crop is cultivated on more than 142 million ha of land worldwide and it was estimated to have produced some 913 million tons of grain in the period 2012-13 [17], accounting for one third of total global grain production [18].

Materials and Methods

Considering Iran and Azerbaijan to be origin countries, and in order to study the effects of saltiness on growth and yield characteristics of maize, its different items were investigated in 2007-09. Seeds of eight maize cultivars, including K3615/1, S.C704, B73, S.C302, Waxy, K3546/6, K3653/2, and Zaqatala-68, were cultivated in two pieces of land in Astra – one with normal soil and the other with salty soil (6-8 dsm^{-1}). The experiment was carried out in the form of randomized complete block design in three replications. The experiment measured Na^+ accumulation in leaves, chlorophyll *a*, chlorophyll *b*, leaf relative water content (LRWC), proline content, biomass per plant, harvest index, and grain yield per hectare. Sodium concentrations were determined using Eppendorf Elex 6361 model flame photometry as described by Miller [19].

During the experiment, before dealing with the leaf relative water content (LRWC), chlorophyll *a* and chlorophyll *b* content were measured in the laboratory. Photosynthetic pigments (chlorophyll *a* and *b*) were measured in fresh leaf samples a week before the harvest. One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples (0.5 g) were homogenized with acetone (80% *v*/*v*) and filtered and make up to a final volume of 5 mL. Then the solution was centrifuged for 10 minutes at 3,000 (rpm). Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula given below:

$$\text{Chlorophyll } a \text{ (mg/g FW)} = [12.7\times(A663) - 2.69\times(A645)]\times0.5$$

$$\text{Chlorophyll } b \text{ (mg/g FW)} = [22.9\times(A645) - 4.69\times(A663)]\times0.5$$

Leaf relative water content (LRWC) was calculated on the basis of the Yamasaki and Dillenburg method [20]. Two leaves were randomly chosen from middle parts of the plants in each replication. At first, leaves were separated from the stems and their fresh mass (FM) were calculated. In order to measure the Turgid mass (TM), they were placed in the distilled water in closed containers for 24 hours at 22ºC for the purpose of reaching their greatest amount of saturation mass, and then they were weighed. Then leaves were placed inside an electrical oven for 48 hours at 80ºC and the dry mass of the leaves (DM) was obtained. All of the measurements were done by scales with 0.001 g accuracy and were placed into the following formula:

$$\text{LRWC (\%)} = [(FM - DM)\ (TM - DM)]\times100$$

...where FM is fresh mass, DM is dry mass, and TM is turgid mass.

Free proline accumulation was determined using the method of Bates et al. [21]. 0.04 g dry leaf weight was homogenized with 3% sulfosalicylic acid, and after 72 h that proline was released; the homogenate was centrifuged at 3,000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 hour, and then absorbance at 520 nm was determined by UV-visible spectrophotometer (Shimadzu UV-120, Japan).

Statistical analysis of the data was done on the basis of randomized complete block design in three years and two locations (normal and salty field) with MSTATC and SPSS17 software. The average of attendances was calculated on the basis of Duncan method at 5% probability level.

Result and Discussion

Analysis of Variance

Results from the experiment showed that, regarding most of the characteristics, there were significant differences among cultivars and that, compared to normal conditions, saltiness had caused reduction in their values. Results from the analysis of variance showed that there were no significant differences between different years (Table 1). Between locations (normal and saline) in most traits, significant differences were seen. Between varieties in all traits, significant differences were seen. The interaction between years and varieties, and years, varieties, and locations for all traits was not significant. The interaction between varieties and locations for all traits showed significant differences at 1% level.

Comparison of Mean

Comparison traits in different salinities showed that in most traits, there are significant differences between genotypes. The highest amount of chlorophyll *a* in normal conditions was observed in S.C704 with 1.873 mg/g fresh weight of leaves, which was not significantly different from B73.

Table 1. Analysis of variance for maize varieties.

Source	DF	Mean Square							
		Chl *a*	Chl *b*	Na^+	Proline	LRWC	Dry weight per plant	Biomass per plant	Grain yield
Year	2	0.0001 ns	0.0001 ns	0.0003 ns	0.0005 ns	0.001 ns	0.0001 ns	0.0002 ns	0.0001 ns
Location	1	1.025**	1.449**	334.506**	405.720**	17.851 ns	818.946**	1,969,973.642**	16,889.972**
YL	2	0.001 ns	0.001 ns	0.0001 ns	0.0001 ns	0.0001 ns	0.002 ns	0.0001 ns	0.0002 ns
R(LY)	12	0.061	0.148	0.007	0.001	280.255	29.690	35643.497	108.598
Variety	7	0.101**	0.222**	1.838**	0.506**	76.171**	33.566**	286,634.775**	403.136**
YA	14	0.000 ns	0.0001 ns	0.0002 ns	0.0001 ns	0.0001 ns	0.0002 ns	0.0003 ns	0.0001 ns
LA	7	0.074 **	0.136**	1.567**	1.668**	41.653**	33.506**	41,104.152**	259.220**
YLA	14	0.000 ns	0.000 ns	0.0002 ns	0.001 ns	0.0002 ns	0.0002 ns	0.0001 ns	0.0003 ns
Error	84	0.023	0.031	0.003	0.028	24.681	7.906	7,620.643	72.088
CV%		14.53	15.49	2.87	5.97	8.10	22.70	18.27	16.03

ns – non-significant, *significant at 5%, **significant at 1%

The lowest chlorophyll *a*, in conditions of salt, was measured in waxy cultivar. There were no significant differences between the varieties in this attribute in saline conditions.

Maximum chlorophyll *b* in B73 was measured in normal conditions, which was significantly different for all varieties at 5% level. Lowest chlorophyll *b*, in salty conditions, was measured in waxy. There were no significant differences between the varieties in this attribute in saline conditions. Similar results were also reported by Iqbal et al. [22], Khan et al. [23], and Almodares et al. [24]. Maximum LRWC in B73 was measured in normal conditions, for which there were no significant differences with K3653.2, S.C704, and waxy at the 5% level.

Maimaiti et al. [25] indicated that leaf relative water content decreases with increasing salt concentration. This reduction was not significant until 200 mM, and in 300 mM of salinity were significant differences. The lowest LRWC, in conditions of salt, was measured in S.C302. Tuna et al. [26] in their study of gibberellic acid and salinity on plant growth parameters and antioxidants of maize showed that with increasing salt concentrations, significant reductions in dry weight, chlorophyll content, and leaf relative water content were observed. Molazem et al. [27] in studying the effect of salt stress on the antioxidant enzyme activities on the maize leaves in different salinity levels showed that with increasing salinity, significant reductions in leaf relative water content were observed.

The highest yield per plant, in S.C704 in normal conditions, was significantly different from all varieties in normal and saline conditions. The lowest grain yield, of soil salinity conditions were seen in K3615.1. With the increase of salt in the soil, salt accumulation in leaves increased. The most sodium accumulation in the leaves was seen in S.C704, that with all varieties were significantly different. Yield was reduced by salinity in all cultivars. The highest yields per hectare was obtained in Zaqatala, S.C704, and K3653.2 (Table 2). Maize cultivar S.C704 and Zaqatala showed higher accumulation of proline than others, but was not seen to have a significant difference between them. The least proline content was seen in B73, which didn't have any significant difference with S.C302, K3615/1, and K3545.6. The results of biomass indicated that applied NaCl inhibited the growth of maize and led to a decrease in biomass (Table 2). The greatest biomass was recorded with Zaqatala, B73, and S.C704, while no significant difference between them was noticed. Maimaiti et al. [25] in their study indicated that plant biomass was reduced with increasing salt concentrations. Soil salinity reduced plant dry weight. The maximum of plant dry weight was seen in normal conditions in B73 and K3653.2. There was no significant difference between them.

Agami [28] showed that with increasing salinity to 100 and 200 mM, leaf number, leaf area, chlorophyll *a*, chlorophyll *b*, carotenoid, and RWC decreased. Li et al. [29] showed that the overexpression of LcSAIN2 (*Leymus chinensis* salt-induced 2) in *Arabidopsis* enhanced salt tolerance of transgenic plants by accumulating osmolytes such as free praline, and improving the expression levels of some stress-responsive transcription factors and key genes. LcSAIN2 might play an important positive modulation role in salt stress tolerance and be a candidate gene utilized for enhancing stress tolerance in wheat and other crops [29].

The reduction in growth traits in plants subjected to NaCl stress is often associated with a decrease in photosynthetic pigments, and a reduction in Chl content due to NaCl stress was also reported in maize, wheat, canola, etc. [30]. Soaking the seeds in AA or proline increased the Chl *a*, Chl *b*, and Car content in the presence or absence of the NaCl stress. Similar findings were obtained by Khan et al. [31] in *Brassica campestris*.

Table 2. Comparing the average of understudy characteristics in eight maize cultivars.

Condition	Cultivars	Chl *a*	Chl *b*	Na^{+}	Proline	LRWC	Dry weight per plant	Biomass per plant	Grain yield
		$mg \cdot g^{-1}$ FW		$mg \cdot g^{-1}$ DW	$Mmol \cdot g^{-1}$ FW	%	g		$Kg \cdot ha^{-1}$
Normal	1-Zaqatala	1.107^{d}	1.091^{def}	0.3300^{hij}	1.033^{fgh}	61.02^{bc}	172.7^{cdef}	833.5^{a}	$8,799.167^{a}$
	2-S.C302	1.474^{bc}	1.519^{cd}	0.3010^{hij}	1.010^{gh}	57.88^{c}	170.7^{def}	297.0^{e}	$5,289.3^{def}$
	3-K3653.2	1.192^{cd}	1.996^{b}	0.2890^{ij}	1.323^{ef}	63.78^{ab}	284.3^{ab}	580.8^{c}	$7,613.17^{abc}$
	4-B73	1.616^{ab}	2.492^{a}	0.2957^{hij}	1.30^{efg}	67.15^{a}	406.0^{a}	738.0^{b}	$6,486.33^{bcde}$
	5-S.C704	1.837^{a}	1.840^{bc}	0.3497^{h}	1.150^{efgh}	62.57^{abc}	250.9^{bc}	695.0^{b}	$8,213.667^{ab}$
	6-Waxy	1.114^{cd}	1.279^{def}	0.2730^{j}	1.430^{e}	61.89^{abc}	175.8^{cdef}	550.5^{c}	$5,494.167^{def}$
	7-K3615.1	1.024^{de}	1.494^{cde}	0.3320^{hi}	0.953^{h}	61.07^{bc}	207.8^{bcd}	540.0^{c}	$6,300.833^{bcde}$
	8-K3545.6	1.038^{de}	1.016^{ef}	0.3163^{hij}	0.987^{h}	58.43^{bc}	185.6^{cde}	522.3^{c}	$6,682.167^{bcd}$
Salty	1-Zaqatala	0.9267^{de}	1.098^{def}	4.094^{b}	4.847^{ab}	61.47^{bc}	104.9^{fgh}	550.0^{c}	$5,603.500^{cdef}$
	2-S.C302	0.8956^{de}	1.036^{def}	3.277^{d}	4.660^{bc}	57.27^{c}	130.0^{defg}	280.8^{e}	$4,600.03^{def}$
	3-K3653.2	1.030^{de}	1.193^{def}	2.840^{e}	3.91^{d}	61.70^{bc}	86.21^{gh}	270.0^{e}	$4,799.333^{def}$
	4-B73	0.9778^{de}	1.142^{def}	2.753^{f}	4.443^{c}	61.68^{bc}	94.11^{gh}	423.8^{d}	$5,195.40^{def}$
	5-S.C704	0.9989^{de}	1.174^{def}	4.215^{a}	5.067a	62.36^{abc}	175.3^{cdef}	432.8^{d}	$5,990.16^{cde}$
	6-Waxy	0.7378^{e}	0.8556^{f}	2.611^{g}	3.743^{d}	61.60^{bc}	75.05^{h}	301.8^{e}	$3,702.67^{f}$
	7-K3615.1	1.016^{de}	1.278^{def}	3.769^{c}	4.663^{bc}	58.44^{bc}	118.0^{efgh}	332.2^{e}	$5,103.6^{def}$
	8-K3545.6	0.8867^{de}	1.012^{ef}	3.314^{d}	4.710^{bc}	63.62^{ab}	74.12^{gh}	294.3^{e}	$4,502.0^{ef}$

Different letters indicate significant differences at the level of 5%

Table 3. Pearson's correlation between traits in normal and saline conditions.

Traits	Condition	Chlorophyll *b*	Dry weight	Na^{+}	LRWC	Proline	Biomass per plant	Grain yield
Chlorophyll *a*	Normal	0.344**	0.129	0.169	0.171	0.108	0.113	0.146
	Salinity	0.957**	0.132	0.141	0.155	0.056	0.409**	0.255*
Chlorophyll *b*	Normal	1	0.058	-0.089	0.231	0.285*	0.066	-0.215
	Salinity	1	0.199	0.168	0.147	0.096	0.442**	0.282*
Dry weight	Normal		1	-0.187	-0.163	0.273*	0.409**	0.283*
	Salinity		1	0.439**	0.127	0.378**	0.398**	0.383**
Na^{+}	Normal			1	0.060	-0.655**	0.224	0.545**
	Salinity			1	-0.005	0.793**	0.427**	0.199
LRWC	Normal				1	0.261*	0.0001	-0.115
	Salinity				1	0.026	0.273*	0.266*
Proline	Normal					1	0.183	-0.301*
	Salinity					1	0.359**	0.173
Biomass per plant	Normal						1	0.341**
	Salinity						1	0.476**

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

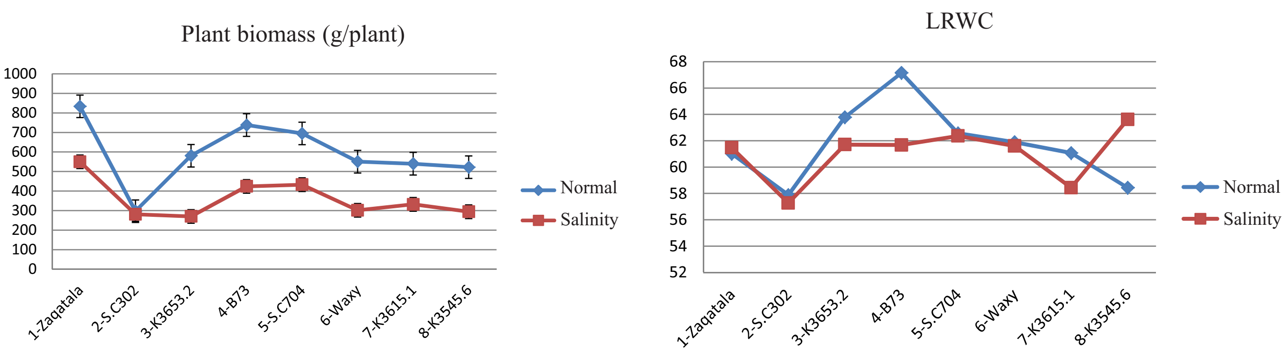

Fig. 1. Diagram of different understudy characteristics in eight maize cultivars under normal and salty conditions.

Proline accumulation is one of the most frequently reported modifications induced by salinization and drought in plants, and it is often considered to be involved in stress-resistant mechanisms [32]. Wani et al. [33] showed that the exogenous application of proline (pre-sowing seed soaking in 20 mM proline, for 8 h) significantly increased, e.g., plant growth and photosynthetic rate in high and low photosynthesizing cultivars of mustard greens (*Brassica juncea* L.) (Varuna and RH-30). They showed that exogenous proline and betaine induce the accumulation of proline and betaine in BY-2 cells under salt stress and mitigate the inhibition of cell growth under salt stress. Banu et al. [34] suggested that proline mitigates cellular damage and clearly improves survival rates of cells under NaCl stress.

Pearson's correlation for normal and saline conditions was calculated (Table 3). Between chlorophyll *a* and chlorophyll *b* significant positive correlations were observed in non-stress conditions. In normal conditions, intense positive correlation between the accumulations of sodium in leaves with yield per hectare were obtained. Between chlorophyll *a* and chlorophyll *b* and grain yield, significant positive correlations were observed in normal conditions. Between LRWC and grain yield, significant positive correlations were observed in salty conditions (Fig. 1). In normal conditions – between proline with plant dry weight and leaf relative water content – we found a significant positive correlation, but Pearson's correlation between the amounts of sodium in leaf and proline was significantly negative. Sleimi et al. [35] showed a significant positive correlation between proline and Na^+ content in roots of *Plantago maritima* grown on a nutritive solution supplemented with different NaCl concentrations. A significant positive correlation between plant biomass and plant dry weight was achieved without stress conditions (0.409**). In conditions of soil salinity, a positive correlation between plant biomass was achieved with all traits.

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