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PHYSIOLOGICAL RESPONSES OF EIGHT MAIZE VARIETIES TO SALINITY UNDER THE FIELD AND GREENHOUSE CONDITIONS

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Abstract

An experiment was conducted in two fields (normal and saline) to study the effect of salinity on eight varieties of maize (*Zea mays* L.) for three years. Another experiment was carried out in three salinity levels, zero (control), 50 and 100 mM NaCl in greenhouse at the factorial design in a year. During the experiment characters such as chlorophyll a, chlorophyll b and proline content were measured. Comparison of means in different soil of salinity showed that, there were significant differences in genotypes in most traits. Maximum amount of chlorophyll a was observed in S.C704 in control condition and there was no significant difference from B73. In field condition maximum and minimum amount of proline were observed in S.C704 in saline condition and K3615/1 in normal condition, respectively. In greenhouse, maximum amount of chlorophyll a was observed in K3615/1 variety.

Introduction

Salinity is one of the major environmental threats for agriculture and affects approximately 7% of the world's total land area (Ben-Salah *et al.* 2011). In salt-affected soil, there are many salt contaminants, especially NaCl which readily dissolves in water to yield the toxic ions, sodium ion (Na^+) and chloride ion (Cl^-). Also, the water available in the salt-contaminated soil is restricted, inducing osmotic stress. Increasing Na^+ and Cl^- content in plant tissue can increase oxidative stress, which causes deterioration in chloroplast ultrastructure and even loss in chlorophyll (Siringam *et al.* 2011).

After wheat and rice, maize (*Zea mays* L.) 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 in which salinity tolerance may exist. Maize is considered as moderately salt sensitive (Carpici *et al.* 2009). This crop is cultivated on more than 142 million hectares of land worldwide and it is estimated to produce around 913 million tons of grain in the period 2012/2013 (IGC. 2012), accounting for one third of the total global grain production (Heng *et al.* 2009). In the current global climate change scenario one expected threat is the increase in soil salinization (FAO 2011). Thus soil salinization is a major global issue because of its adverse impact on agricultural productivity, sustainability and as a threat for food supply. Plants need to have special mechanisms for adjusting internal osmotic conditions and changing of osmotic pressure in the root environment. In salt stressed plants osmotic potential of vacuole decreased by proline accumulation. 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 (Selvakumar *et al.* 2014). One of the mechanisms adopted by plants to tolerate salt stress is the accumulation of compatible solutes that help in maintaining osmotic homeostasis (Gill *et al.* 2014). Mechanisms of salt tolerance, not yet completely clear, can be explained to some extent by stress adaptation effectors that mediate ion

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However, attempts to improve yield under stress conditions by plant improvement have been largely unsuccessful, primarily due to the multigenic origin of the adaptive responses. Therefore, a well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to develop salt tolerant crop varieties. In recent decades exogenous protectant such as osmoprotectants (proline, glycinebetaine, trehalose, etc.), plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, etc.), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), signaling molecules (nitric oxide, hydrogen peroxide, etc.), polyamines (spermidine, spermine, putrescine), trace elements (selenium, silicon, etc.) have been found effective in mitigating the salt induced damage in plant (Ahmad *et al.* 2010, 2012, Azzedine *et al.* 2011, Rawia *et al.* 2011, Nounjan *et al.* 2012, Iqbal *et al.* 2012, Yusuf *et al.* 2012).

The present experiment was aimed to study the effect of salinity on leaf pigments, proline content and leaf relative water content.

Materials and Methods

Effects of salt stress on leaf relative water content (LRWC), chlorophyll a, chlorophyll b and proline content in eight maize varieties were investigated in field condition. The experiments were laid out in randomized complete block design with three replications in three consequitive years (2007-2009) and in greenhouse condition in different salinity (0, 50 and 100 mM). Eight varieties namely, K3615/1, S.C704, B73, S.C302, Waxy, K3546/6, K3653/2 and Zaqatala were cultivated in two pieces of land in Astara: one under normal soil and the other under saline soil. Before starting the experiment amount of leaf relative water content (LRWC), proline, chlorophyll a and chlorophyll b content were measured in the laboratory. Leaf chlorophyll were determined in acetone extracts according to Arnon (1949) using a spectrophotometer (Schimadzu, Kyoto, Japan). Photosynthetic pigments (chlorophyll a and b) were measured in fresh leaf samples, a week before the harvest. LRWC was calculated by Yamasaki and Dillenburg method (1999). Free proline accumulation was determined using the method of Bates *et al*. (1973). Statistical analysis of the data was done on the basis of randomized complete block design with MSTAT-C and SPSS17 software. The average of attendances was calculated on the basis of Duncan method at 5% probability level.

Results and Discussion

Results from the experiment in field condition showed that, there were significant differences among varieties in the most of the characteristics, compared to normal conditions; salinity 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 conditions (normal and saline) in all traits, significant differences were seen. Significant differences were seen among varieties in all traits. The interaction between years and varieties, years and varieties and conditions for all traits was not significant. The interaction between varieties and conditions for all traits showed significant differences at 1% level.

Results from the experiment in greenhouse condition showed that, there were significant differences in the most of the characteristics, among varieties compared to normal conditions; salinity had caused reduction in their values. Results from the analysis of variance showed that there were significant differences between salinity except proline (Table 2). Except chlorophyll b, significant differences were seen among varieties in all traits.

Table 1. Analysis of variance for maize varieties in field condition.

Source of variation	DF	Chl. a	Chl. b	Total chl.	Ratio of chl. a/b	LRWC	Proline
Year	2	0.0001ns	0.0001ns	0.008ns	0.001ns	0.001ns	0.0005ns
Location	1	1.025**	1.025**	26.643**	0.024ns	17.851ns	405.720**
YL	2	0.001ns	0.001ns	0.018ns	0.001ns	0.0001ns	0.0001ns
R (LY)	12	0.061	0.061	1.589	0.093	280.255	0.001
Variety	7	0.101**	0.101**	3.295**	0.087**	76.171**	0.506**
YA	14	0.000ns	0.000ns	0.003ns	0.001ns	0.0001ns	0.0001ns
LA	7	0.074 **	0.074 **	2.005**	0.083**	41.653**	1.668**
YLA	14	0.000ns	0.000ns	0.002ns	0.001ns	0.0002ns	0.001ns
Error	84	0.023	0.023	0.396	0.024	24.681	0.028
	CV%	5.9%	8.10	17.74	25.58	15.49	14.53

Table 2. Analysis of variance for maize varieties in greenhouse condition.

Source of variation	DF	Chl. a	Chl. b	LRWC	Proline
Replication	2	0.001ns	0.001ns	471.911ns	31.179ns
Salinity	2	0.007**	0.002*	3054.333**	2.356ns
Variety	7	0.001*	0.0001ns	3186.795**	95.916**
Salt* variety	14	0.0001ns	0.001ns	865.230**	70.857**
Error	46	0.0001	0.001	293.617	22.279
	CV%	25.20	23.89	1.26	0.35

ns. Non-significant, * significant at 5% **, significant at 1%.

In field condition, there were significant differences among genotypes in most traits. In normal condition the highest amount of chlorophyll a was observed in S.C704 with 1.837 mg/g fresh weight of leaves (Table 3), which showed no significant difference with B73. Lowest chlorophyll a, in saline condition, was measured in Waxy. Maximum chlorophyll b was measured in B73 in normal conditions which showed significant difference with all varieties at 5% level. In saline condition lowest chlorophyll b, was observed in Waxy. In normal condition the highest ratio of chlorophyll, was observed in K3545/6, which showed no significant difference with Zaqatala, S.C302, S.C704 and Waxy. In saline condition lowest chlorophyll b, was observed in Waxy (Table 3).

In greenhouse condition, the highest amount of chlorophyll a was observed in K3615.1 varieties showing significant differences among all the varieties (Table 4). Under greenhouse condition no significant differences were seen in the control (0 mM) between varieties in terms of chlorophyll b (Table 5). With increasing salinity, chlorophyll b decreased. Lowest chlorophyll b, in 50 and 100 mM, were measured in K3653/2 varieties (Table 5). This situation was also observed for chlorophyll a. Proline increased with increasing salinity in most varieties. It has been stated that genotypes with a high proline accumulation and chlorophyll content, high K/Na ratio and low Na^+ and Cl^- accumulation are more tolerant to salt (Mane *et al.* 2011).

Table 3. Comparison of the average of understudy characteristics in eight varieties of the maize in field condition and combined analysis.

Condition	Variety	Chl. a mg/g FW	Chl. b mg/g FW	Total chl. mg/g FW	Ratio of chl. a/b	LRWC (%)	Prolin µmol/g FW
Normal	1-Zaqatala	1.107 d	1.091 def	2.196 ef	1.003 ab	61.02 bc	1.033 fgh
	2-S.C302	1.474 bc	1.519 cd	2.996 cd	0.9644 abc	57.88 c	1.010 gh
	3-K3653/2	1.192 cd	1.996 b	3.188 bc	0.6767 e	63.78 ab	1.323 ef
	4-B73	1.616 ab	2.492 a	4.108 a	0.7667 de	67.15 a	1.30 efg
	5-S.C704	1.837 a	1.840 bc	3.677 ab	1.003 ab	62.57 abc	1.150 efgh
	6-Waxy	1.114 cd	1.279 def	2.393 de	0.9089 abcd	61.89 abc	1.430 e
	7-K3615/1	1.024 de	1.494 cde	2.519 de	0.7667 de	61.07 bc	0.953 h
	8-K3545/6	1.038 de	1.016 ef	2.056 ef	1.032 a	58.43 bc	0.987 h
Salinity	1-Zaqatala	0.9267 de	1.098 def	2.024 ef	0.8678 abcd	61.47 bc	4.847 ab
	2-S.C302	0.8956 de	1.036 def	1.931 ef	0.8611 abcd	57.27 c	4.660 bc
	3-K3653/2	1.030 de	1.193 def	2.226 ef	0.8822 abcd	61.70 bc	3.91 d
	4-B73	0.9778 de	1.142 def	2.118 ef	0.8778 abcd	61.68 bc	4.443 c
	5-S.C704	0.9989 de	1.174 def	2.171 ef	0.8567 bcd	62.36 abc	5.067 a
	6-Waxy	0.7378 e	0.8556 f	1.588 f	0.8856 abcd	61.60 bc	3.743 d
	7-K3615/1	1.016 de	1.278 def	2.293 e	0.8122 cde	58.44 bc	4.663 bc
	8-K3545/6	0.8867 de	1.012 ef	1.898 ef	0.8711 abcd	63.62 ab	4.710 bc

Within each column, same letter indicates no significant difference between treatments ($p < 0.05$).

Table 4. Comparing the average of understudy characteristics in eight varieties of the maize in greenhouse condition.

Variety	Chl. a mg/g FW	Chl.b mg/g FW	LRWC (%)	Proline µmol/g FW
Zaqatala	0.2756 bc	0.2178 a	79.55 a	468.6 ab
S.C704	0.2300 c d	0.2833 a	80.00 a	388.4 ab
B73	0.3078 bc	0.1844 a	84.08 a	320.1 bc
K3653\2	0.1578 d	0.1000 a	26.14 b	200.7 c
S.C302	0.2967 bc	0.1944 a	74.10 a	415.1 ab
K3615\1	0.4700 a	0.2778 a	73.93 a	295.0 bc
K3545\6	0.32 bc	0.1956 a	74.11 a	498.0 a
Waxy	0.3700 b	0.2689 a	81.83 a	327.0 abc

Within each column, same letter indicates no significant difference between treatments ($p < 0.05$).

In field and normal condition maximum LRWC was observed in B73 which showed no significant difference with K3653/2, S.C704 and Waxy at 5% level. In saline condition lowest

LRWC, was measured in S.C302. Maize variety S.C704 and Zaqatala showed higher accumulation of proline than others but no significant difference was found between them. The least proline content was seen in B73 that did not have any significant difference with S.C302, K3615/r and K3545.6.

Table 5. Comparison of the average of understudy characteristics in eight varieties of the maize in greenhouse condition in different salinity.

Salinity	Variety	Chl. a mg/g FW	Chl. b mg/g FW	LRWC (%)	Proline μmol/g FW
Control	Zaqatala	0.4900 abc	0.1933 ab	77.51 ab	9abcd
	S.C704	0.4867 abc	0.3100 ab	82.21 ab	378.5abc
	B73	0.4633 abc	0.1833 ab	85.4 ab	237.3cd
	K3653/2	0.4533 abcd	0.2800 ab	78.43 ab	602.0a
	S.C302	0.4900 abc	0.1667 ab	82.72 ab	236.4cd
	K3615/1	0.5933 a	0.3733 ab	81.92 ab	268.1bcd
	K3545/6	0.5100 abc	0.3800 ab	82.06 ab	482.9abc
	Waxy	0.4367 abcde	0.4167 ab	77.30 ab	268.5bcd
50 mM	Zaqatala	0.2400 bcdef	0.3233 ab	84.36 ab	574.0ab
	S.C704	0.1133 def	0.5167 a	86.07 a	325.1abc
	B73	0.2233 bcdef	0.2233 ab	87.55 a	325.6abc
	K3653/2	0.01000 f	0.010 b	0.0000 c	0.0000d
	S.C302	0.2300 bcdef	0.3833 ab	84.01 ab	471.8abc
	K3615/1	0.5333 ab	0.2867 ab	82.7 ab	304.4abcd
	K3545/6	0.2633 bcdef	0.1867 ab	88.97 a	426.8abc
	Waxy	0.3933 abcde	0.0933 ab	86.82 a	337.1abc
100 mM	Zaqatala	0.09667 ef	0.1367 ab	76.79 ab	533.1abc
	S.C704	0.09000 ef	0.0233 b	71.72 ab	461.7abc
	B73	0.2367 bcdef	0.1467 ab	79.28 ab	397.3abc
	K3653/2	0.01000 f	0.0100 b	0.0000 c	0.0000d
	S.C302	0.1700 cdef	0.0333 b	55.56 ab	537.0abc
	K3615/1	0.2833abcdef	0.1733 ab	57.10 ab	312.6abc
	K3545/6	0.1633 cdef	0.0200 b	51.31 b	584.4ab
	Waxy	0.2800abcdef	0.2967 ab	81.39 ab	375.4abc

Within each column, same letter indicates no significant difference between treatments ($p < 0.05$).

Agami (2014) 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.* (2013) reported that overexpression of *Leymus chinensis* salt induced 2 (LcSAIN2) in *Arabidopsis* enhanced salt tolerance of transgenic plants by accumulating osmolytes, such as free proline 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 (Li *et al.* 2013).

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 a NaCl stress was also reported in maize, wheat, canola, etc. (Ali *et al.* 2007). Soaking the seeds in abscisic acid or proline increased the Chl. a, Chl. b, and carotenoid content in the presence or absence of the NaCl stress. Similar findings were recorded by Khan *et al.* (2010) in *Brassica campestris.*

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 resistance mechanisms (Pyngrope *et al.* 2013). Wani *et al.* (2012) showed that exogenous application of proline (pre-sowing seed soaking in 20 mM proline, for 8 hrs) 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.

References

Agami RA 2014. Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. Biologia Plantarum. 58(2): 341-347.

Ahmad P, Jaleel CA, Salem MA, Nabi G and Sharma S 2010. Roles of Enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Crit Rev. Biotechnol. **30**(3): 161-175

Ahmad P, Hakeem KR, Kumar A, Ashraf M and Akram NA 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). Afr. J. Biotechnol. **11**: 2694-2703.

Ali Q, Ashraf M and Athar HUR 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pak. J. Bot. **39**: 1133-1144,

Arnon DI 1949. Copper enzymes in isolated chloroplast. Polyphenol-oxidase in *Beta vulgaris* L. Plant Physiol. **24**: 1-5.

Azzedine F, Gherroucha H and Baka M 2011. Improvement of salt tolerance in durum wheat by ascorbic acid application. J. Stress Physiol. Biochem. **7**: 27-37.

Bates LS, Waldron RP and Teare ID 1973. Rapid determination of free proline for water stress studies. Plant Soil **39**: 205-208.

Ben-Salah I, Slatni T, Gruber M, Messedi D and Gandour M 2011. Relationship between symbiotic nitrogen fixation, sucrose synthesis and anti-oxidant activities in source leaves of two Medicago ciliaris lines cultivated under salt stress. Environ. Exp. Bot. **70**: 166-173.

Carpici EB, Celik N and Bayram G 2009. Effects of Salt Stress on Germination of Some Maize (*Zea mays* L.) Cultivars. Afr. J. Biotechnol. **8**(19): 4918-4922.

FAO 2011. Proceedings of the Global Forum on Salinization and Climate Change (GFSCC2010), R.P. Thomas (Ed). World Soil Resources Reports, Rome, Italy.

Gill SS, Anjum NA and Gill R 2014. Target osmoprotectants for abiotic stress tolerance in crop plants glycine betaine and proline. *In:* Anjum NA, Gill SS, Gill R (Eds), Plant Adaptation to Environmental

Heng LK, Hsiao T, Evett S, Howell T and Steduto P 2009. Validating the FAO Aqua Crop Model for irrigated and water deficient field maize. Agron. J. **101**: 488-498.

IGC 2012. International Grains Council, Grain Market Report, *In:* IGC Grains conference 2012, London - 7 June, 2012.

Iqbal N, Masood A and Khan NA 2012. Phytohormones in salinity tolerance: ethylene and gibberellins cross talk. In: Khan NA, Nazar R, Iqbal N, Anjum NA (Eds) Phytohormones and abiotic stress tolerance in plants. Springer, Berlin. pp. 77-98

Khan A, Iqbal I, Shah A, Ahmad A and Ibrahim M 2010. Alleviation of adverse effects of salt stress in brassica (*Brassica campestris*) by pre-sowing seed treatment with ascorbic acid. J. Agr. Environ. Sci. **7**: 557-560.

Li X, Gao Q, Liang Y, Ma T, Cheng L, Qi D, Liu H, Xu X, Chen S and Liu G 2013. A novel salt-induced gene from sheepgrass, LcSAIN2, enhances salt tolerance in transgenic *Arabidopsis*. Plant Physiol Biochem. **64**: 52-59

Mane AV, Deshpande TV, Wagh VB, Karadge BA and Samant JS 2011. A critical review on physiological changes associated with reference to salinity. International Journal of Environmental Sciences **6**: 1192-1216.

Nounjan N, Nghia PT and Theerakulpisut P 2012. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. J Plant Physiol. **169**: 596-604

Pyngrope S, Bhoomika K and Dubej RS 2013. Reactive oxygen species, ascorbate–glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedlings subjected to progressing levels of water deficit. Protoplasma **250**: 585-600

Rawia Eid A, Taha LS and Ibrahiem SMM 2011. Alleviation of adverse effects of salinity on growth, and chemical constituents of marig old plants by using glutathione and ascorbate. J. Appl. Sci. Res. **7**: 714-721

Selvakumar G, Kim K, Hu S and Sa T 2014. Effect of salinity on plants and the role of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria in alleviation of salt stress. *In:* Parvaiz Ahmad P, Wani RM (Eds), Physiological Mechanisms and Adaptation Strategies in Plants Under Changing Environment. Springer, New York. pp. 115-144.

Siringam K, Juntawong N, Cha-um S and Kirdmanee C 2011. Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. indica) roots under isoosmotic conditions. Afr. J. Biotech. **10**(8): 1340-1346.

Wani AS, Irfan M, Hayat S and Ahmad A 2012. Response of two mustard (*Brassica juncewa* L.) cultivars differing in photosynthetic capacity subjected to proline. Protoplasma. **249**: 75-87

Yamasaki S and Dillenburg LC 1999. Measurements of leaf relative water content in *Araucaria angustifolia*. R. Bras. Fisiol. Veg. **11**: 69-75.

Yusuf M, Fariduddin Q, Varshney P and Ahmad A 2012. Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (*Brassica juncea*) through an improved antioxidant system. Environ. Sci. Pollut Res. **19**: 8-18.

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