

RESEARCH PAPER

Study of morpho-physiological traits in maize (*Zea mays* L.) genotypes with environmental stress indices

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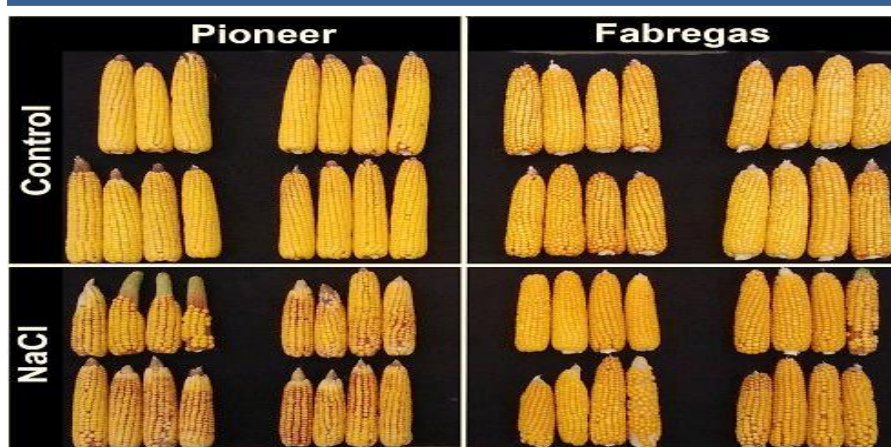
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Highlights

- Correlation analysis showed a positive association between grain yields under normal and stress conditions.
- With increasing salinity, significant reduction in leaf relative water content was observed.
- Proline content increased with increasing soil salinity in all genotypes.

Graphical Abstract



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Abstract

To evaluate some agrophysiological traits to identify salt tolerance in maize, eight different maize genotypes differing in yield performance were evaluated using an RCBD design in two different environments: one with normal soil and the other with saline soil. During the experiment, chlorophyll a, chlorophyll b, leaf relative water content (LRWC), proline content, stress tolerance (TOL), stress tolerance index (STI), stress susceptibility index (SSI), and yield stability index (YSI) were evaluated. The results of the experiment showed that there were significant differences between locations (normal and saline) and genotypes for most traits. Comparison of traits at different salinity levels showed that there were significant differences between genotypes for most traits. Proline content increased with increasing soil salinity. Na^+ accumulation in leaves increased sharply with increasing salinity, with the greatest accumulation observed in S.C704. The highest amount of chlorophyll a under normal conditions was observed at S.C704. Positive correlations were observed between chlorophyll a and chlorophyll b under normal and stress conditions. Genotypes SC301, SC704 and SC540 showed the highest levels of TOL and SSI. Genotypes KSC647 and SC604 showed the highest value for YSI (0.627 and 0.578, respectively).



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1. Introduction

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. 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 (Salah et al., 2011). 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). Salt stress affects crops under extreme saline conditions by severely impairing plants' 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 (Gill et al., 2014). The deleterious effects of salinity on plant growth are associated with (1) high osmotic potential of soil solution but low water potential (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Tuna et al., 2008). Different methods of water management are used to cope with water shortage (Sepaskhah and Yousofi-Falakdehi, 2010). 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 (Pagter et al., 2009; Siringam et al., 2011).

salinity stress indices are used for performance evaluation, examination and estimation of maize genotypes under salinity stress condition. It helps to distinguish salinity stress-tolerant and susceptible maize genotypes (Sharma et al., 2013). There are various types of stress indices among them stress tolerance (TOL), mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), stress susceptibility index (SSI) and yield stability index (YSI) that are used as selection criteria for salinity stress tolerance genotypes. TOL index leads to the selection of genotypes with low yield potential in non-stress condition and high yield potential under stress condition (Rosielle and Hamblin, 1981). In the some research reported that selection based on mean productivity (MP) will improve yield both on stress and non-stress conditions (Nouri et al., 2011; Kamrani, 2015). STI and GMP are used for the selection of genotypes with high yield under both stress and non-stress conditions whereas SSI identifies genotypes with good yield under stress condition (Kamrani, 2015). GMP is better index than MP in distinguishing salinity stress-sensitive and resistant genotypes (Ramirez-vallejo and Kelly, 1998). In other study was found high YSI for genotypes with low grain yield in both stress and non-stress conditions, and low YSI for genotypes with high and moderate grain yield (Khan et al., 2014). The most important component of genotypic changes for the plant's response to environmental changes is the plant's phenotypic variance, which actually shows how effective the environment is in the plant's genetics. Phenotypic changes in the plant affect the potential of the plant and cause changes in qualitative characteristics, genotypic and phenotypic value, breeding value, dominance changes, additive variance and heritability of the plant. In stress tolerance indices, yield stability parameters are investigated based on environmental stress and genotype stability.

2. Materials and Methods

In order to study the effects of saltiness on growth and yield characteristics of maize plant, its different items were investigated in 2013. Seeds of 8 maize genotypes including S.C604, S.C540, KSC689, KSC647, S.C704, K3545/64, Osk60, and S.C301 were cultivated in two pieces of land in Astara, one with normal soil and the other with salty soil (6-8dsm⁻¹). Experiment was carried out in the form of randomized complete block design in three replications. During the experiment, Na⁺ accumulation in leaves, chlorophyll a, chlorophyll b, leaf relative water content (LRWC), proline content, biomass per plant and grain yield per plot were measured.

2.1. Sodium concentrations

Sodium concentrations were determined by using Eppendorf Elex 6361 model flame photometry described by (Ashraf and Wu, 1994).

2.2. Relative chlorophyll content

During the experiment, before dealing amount of 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), filtered and make up to a final volume of 5 mL. Then the solution for 10 minutes away in 3000 (rpm) centrifuged. Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula given below:

$$\text{Chlorophyll a (mg/g FW)} = [12.7 \times (A_{663}) - 2.69 \times (A_{645})] \times 0.5 \quad (1)$$

$$\text{Chlorophyll b (mg/g FW)} = [22.9 \times (A_{645}) - 4.69 \times (A_{663})] \times 0.5 \quad (2)$$

2.3. Leaf relative water content (LRWC)

Leaf relative water content (LRWC) was calculated on the basis of Yamasaki and Dillenburg method (Yamasaki and Dillenburg, 1999). Two leaves were randomly chosen from middle parts of the plants in each replication. At first, leaf was separated from the stems and their fresh mass (FM) were calculated. In order to measure the Turgid mass (TM), they were placed into the distilled water in closed containers for 24 hours under the air condition of 22° C, for the purpose of being reached to their greatest amount of saturation mass and then, they were weighed. Then leaves were placed inside the electrical oven for 48 hours under the air condition of 80° C and the dry mass of the leafs (DM) were obtained. All of the measurements were done by scales with 0.001g accuracy and were placed into the following formula and into the following formula:

$$\text{LRWC (\%)} = [(FM-DM) / (TM -DM)] \times 100 \quad (3)$$

Where: F.M., Fresh Mass; D.M., Dry Mass; T.M., Turgid Mass.

Free proline accumulation was determined using the method (Bates et al., 1973). 0.04 gr dry weight of leaf was homogenized with 3% sulfosalicylic acid and after 72h that proline was released; the homogenate was centrifuged at 3000 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).

2.4. Stress tolerance index (STI)

Stress tolerance includes performance potential in non-stress environments, performance in stress environments and SI stress intensity. STI estimation is based on the geometric mean of productivity and is due to the fact that it has a single rank correlation (one) with the geometric mean of productivity (Fernandez, 1992).

$$\text{STI} = (Y_p) (Y_s) / (\bar{Y}_p)^2 \quad (4)$$

2.5. Stress susceptibility index (SSI)

were calculated using the following relationships (Fischer and Maurer, 1978). Stress tolerance attributes were calculated by the following formula:

$$\text{SSI} = 1 - (Y_s / Y_p) / \text{SI} \quad (5)$$

$$\text{SI} = 1 - (\bar{Y}_s / \bar{Y}_p) \quad (6)$$

where Y_s and Y_p are the yields of genotypes evaluated under stress and non-stress conditions and \bar{Y}_s and \bar{Y}_p are the mean yields over all genotypes evaluated under stress and non-stress conditions.

Yield Stability Index (YSI) = Y_s / Y_p (Bousslama and Schapaugh, 1984).

Statistical analysis of the data was done on the basis of randomized complete block design in 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.

3. Results and Discussion

Results from the experiment showed that, regarding the most of the characteristics, there were significant differences among genotypes and that, compared to normal conditions; saltiness had caused reduction in their values. Between locations (normal and saline) in most traits, significant differences were seen (Table 1). Between varieties in all traits, significant differences were seen. Comparison traits in different salinities showed that in most traits, there are significant differences between genotypes. The highest amount of chlorophyll a, in normal condition was observed in S.C704 with 1.873 mg/g fresh weight of leaves, which was no significant difference with K3545/6. Lowest chlorophyll a, in condition of salt, was measured in SC604. There were no significant differences between the varieties in this attribute in salinity condition. Maximum chlorophyll b in K3545/6 was measured in normal conditions which was no significant difference with SC704 and OSSK502 at 5% level. Lowest chlorophyll b, in salty condition, was measured in SC604. There were no significant differences between the varieties in this attribute in salinity condition. Similar results were also reported in other study (Iqbal et al., 2006; Khan et al., 2009; Almodares et al., 2008). Maximum LRWC in K3545/6 was measured in normal condition which was no significant difference with other genotypes in normal condition at 5% level. Maimaiti et al., (2014) in their study indicated that leaf relative water content decreases with increasing salt concentration. Lowest LRWC, in condition of salt, was measured in KSC647 (Maimaiti et al., 2014).

Table 1. Analysis of variance for maize varieties.

Mean Square								DF	Source
Grain yield	Biomass per plant	Dry weight per plant	LRWC	Proline	Na ⁺	Chl b	Chl a		
2.445	613027.25	288.995*	261.2	405.720**	334.5**	0.694	0.407*	1	Location
0.044	40406.41	30.174	281.095	0.001	0.007	0.122	0.058	4	R(L)
0.053ns	83199.2**	11.228ns	23.393ns	0.506**	1.838**	0.06*	0.034ns	7	Genotype
0.034ns	13073.2ns	11.161ns	12.438ns	1.668**	1.567**	0.041ns	0.024ns	7	L*G
0.029	7390.097	7.915	25.51	0.028	0.03	0.027	0.021	28	Error
15.88	18.14	22.84	8.23	5.97	2.87	14.25	13.92	CV%	

Ns: Non-significant, *: significant at 5% , **: significant at 1%.

In the study of gibberellic acid and salinity on plant growth parameters and antioxidants of maize showed that with increasing salt concentration, significant reduction in dry weight, chlorophyll content and leaf relative water content was observed (Tuna et al., 2008). The highest yield per plant, in S.C704 obtained in normal condition. The lowest grain yield, of soil salinity conditions were seen in SC301. 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 genotypes (Table 2). Maize cultivar S.C704 and KSC689 showed higher accumulation of proline than others but was not seen significant difference between them. The least proline content was seen in SC301.

The results of biomass indicated that applied NaCl inhibited the growth of maize plant and led to a decrease in biomass (Table 2). The greatest biomass was recorded with KSC689, K3545/6, and S.C704, while no significant difference between them was noticed. In the some study, indicated that plant biomass reduced with increasing salt concentration (Maimaiti et al., 2014; Murat et al., 2010). Soil Salinity reduced plant dry weight. The maximum of plant dry weight was seen in normal condition in K3545/6, OSSK502, and S.C704. There was no significant difference between them. Agami (2014) show that with increasing salinity to 100 and 200 mM leaf

number, leaf area, chlorophyll a, chlorophyll b, carotenoid and RWC was decreased (Agami, 2014). Li et al., (2013) presented that overexpression of LcSAIN2 (*Leymus chinensis* saltinduced 2) 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).

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

Condition	Genotypes	Chl a Mg/g FW	Chl b Mg/g FW	Na ⁺ Mg/g DW	Proline Mmol/g FW	LRWC (%)	Dry weight per plant (g)	Biomass per plant (g)	Grain yield per plant (Kg)
Normal	1- KSC689	1.140 bcd	1.207 bcde	0.3300 hij	1.033fgh	63.02 abc	172.66 bcdef	774.35 a	1.832 ab
	2- KSC647	1.510 abc	1.63 bcd	0.3310 hij	1.010gh	59.88 abc	170.7 bcdef	297.0 d	1.516 abc
	3-OSSK502	1.22 bcd	1.810 abc	0.2890 ij	1.323ef	65.79 ab	283.46 ab	580.8 bc	1.408 bc
	4- K3545/6	1.657 ab	2.61 a	0.2957 hij	1.30efg	68.82 a	406.22 a	734.9 a	1.47 bc
	5-S.C704	1.873 a	1.95 ab	0.3497 h	1.150efgh	57.64 abc	250.9 abc	695.0 ab	2.383 a
	6- SC604	1.137 bcd	1.37 bcde	0.2730 j	1.430e	63.89 abc	176.2 bcdef	551.0 bc	1.264 bcd
	7- SC301	1.08 bcd	1.613 bcd	0.3320 hi	0.953h	63.07 abc	207.7 abcd	540.0 bc	1.945 ab
	8- SC540	1.053 bcd	1.137 cde	0.3163 hij	0.987h	60.76 abc	185.6 bcde	522.3 c	1.772 ab
Salty	1- KSC689	0.95 cd	1.107 cde	4.094 b	4.847ab	59.47 abc	102.23 def	542.06 bc	0.859 cde
	2- KSC647	0.907 cd	1.013 de	3.277 d	4.660bc	55.61 c	127.34 cdef	283.2 d	0.952 cde
	3-OSSK502	1.033 cd	1.12 cde	2.840 e	3.91d	59.70 abc	84.04 def	271.23 d	0.607 e
	4- K3545/6	0.977 cd	1.13 cde	2.753 f	4.443c	59.69 abc	91.783 def	425.8 cd	0.689 de
	5-S.C704	0.993 cd	1.16 cde	4.215 a	5.067a	60.34 abc	171.68 bcdef	431.3 cd	1.036 cde
	6- SC604	0.717 d	0.863 e	2.611 g	3.743d	59.60 abc	72.84 f	307.15 d	0.731 de
	7- SC301	1.013 cd	1.28 bcde	3.769 c	4.663bc	56.45 bc	115.15 cdef	332.16 d	0.491 e
	8- SC540	0.887 cd	1.017 de	3.314 d	4.710bc	61.62 abc	71.47 ef	294.3 d	0.519 e

* Different letters indicate significant differences at the level of 5%.

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. Soaking the seeds in proline increased the Chl a, Chl b, and Carotenoid content in the presence or absence of the NaCl stress. Similar findings were obtained in *Brassica campestris* (Khan et al., 2009). 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 (Ameer et al., 2010; Pyngrupe et al., 2013). Wani et al., (2012) showed that 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 genotypes of mustard greens (*Brassica juncea* L.) (Wani et al., 2012). 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., (2009) suggested that proline mitigates cellular damage and clearly improves survival rate of cells under NaCl stress (Banu et al., 2009).

Pearson's correlation for normal and salinity condition was calculated (Table 3). Between chlorophyll a and chlorophyll b significant positive correlations were observed in normal and stress condition. In normal conditions, intense positive correlation between the accumulations of sodium in leaves with yield were obtained. Between chlorophyll b and Grain yield significant positive correlations were observed in salinity condition. In normal condition, between proline with plant dry weight and leaf relative water content was found a significant positive correlation but the Pearson's correlation between the amounts of sodium in leaf and proline was significant negative. Sleimi et al., (2015) showed that a significant positive correlation between

proline and Na⁺ content in roots of *Plantago maritima* grown on nutritive solution supplemented with different NaCl concentrations (Sleimi et al., 2015). A significant positive correlation between plant biomass and plant dry weight was achieved in normal and stress condition.

Table 3. Pearson's Correlation between traits in normal and salinity condition.

Traits		Chlorophyll b	Dry weight	Na ⁺	LRWC	Proline	Biomass per plant	Grain yield per plant
Chlorophyll a	Normal	0.425*	0.13	0.169	0.163	0.108	0.079	0.159
	Salinity	0.957**	0.132	0.141	0.155	0.056	0.509*	0.268
Chlorophyll b	N	1	0.067	-0.089	0.193	0.285*	0.115	0.141
	S	1	0.183	0.168	0.147	0.096	0.442**	0.282*
Dry weight	N		1	-0.187	-0.186	0.273*	0.409**	0.283*
	S		1	.439**	0.127	0.378**	0.443*	0.284
Na ⁺	N			1	0.060	-0.65**	0.224	0.545**
	S			1	-0.005	0.793**	0.427**	0.199
LRWC	N				1	0.261*	-0.049	-0.117
	S				1	0.026	0.273*	0.266*
Proline	N					1	0.183	-0.301*
	S					1	0.359**	0.173
Biomass per plant	N						1	0.355
	S						1	0.664**

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

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

Salinity stress tolerance indices were calculated based on grain yield under normal and stress conditions (Table 4). The genotypes SC301, SC704, and SC540 showed the highest values of TOL (1454, 1347, and 1253, respectively). These genotypes had high yield potential under normal condition and lower yield potential under heat stress condition indicating higher susceptibility under stress condition. The lowest values of TOL belonged to genotypes SC604 (533), KSC647 (564), and K3545/6 (781). But these genotypes had low yield potential under both conditions. Therefore, grain yield is also considered along with TOL in the determination of stress tolerance genotypes. Selection based on TOL favors genotypes with high grain yield under stress condition and low grain yield under normal condition (Fernandez, 1992). SSI value higher than one indicates above-average susceptibility, while SSI less than one indicates below-average susceptibility (Guttieri et al., 2001).

Table 4. Grain yield of maize genotypes (g/plot) under normal (Y_p), salt stress conditions (Y_s), and salinity stress tolerance indices.

SSI	STI	Gmp	Tol	YSI	Mp (g/plot)	Ys (g/plot)	Yp (g/plot)	genotype
0.936651	0.54533	1254.467	973	0.468886	1345.5	859	1832	KSC689
0.6561	0.500123	1201.346	564	0.627968	1234	952	1516	KSC647
1.003276	0.296164	924.4761	801	0.431108	1007.5	607	1408	OSSK502
0.936967	0.350976	1006.395	781	0.468707	1079.5	689	1470	K3545/6
0.99686	0.85551	1571.238	1347	0.434746	1709.5	1036	2383	S.C704
0.743653	0.320188	961.2409	533	0.578323	997.5	731	1264	SC604
1.318364	0.330935	977.2385	1454	0.252442	1218	491	1945	SC301
1.247033	0.318693	958.9932	1253	0.292889	1145.5	519	1772	SC540

Note: Y_p = Grain yield of genotypes under normal condition, Y_s = Grain yield of genotypes under salinity stress condition, MP = Mean productivity, TOL = Tolerance index, GMP = Geometric mean productivity, YSI = Yield stability index, SSI = Stress susceptibility index, STI = Stress tolerance index.

According to YSI, genotypes KSC647 and SC604 were identified as tolerant genotypes under salinity stress condition (Fig. 1). In the other research reported that YSI indices can identify the genotypes with high yield potential under stress condition than under normal condition (Kamrani et al., 2018). S.C704, KSC689, KSC647, and SC540 genotypes had shown the highest values for MP and GMP. These genotypes have higher stability and production potential than other genotypes under salinity stress condition (Poudel et al., 2021; Kamrani et al., 2018). also found similar results. The highest values of STI indices were observed for genotypes S.C704 and KSC689. Correlation analysis showed a positive association between grain yields under normal and stress conditions. Which mean that genotypes with high grain yield potential under normal condition will have high grain yield under stress condition (Puri et al., 2015).

YSI and SSI showed a positive association with Ys (0.675 and 0.850). Therefore, the selection of maize genotypes with higher values of YSI and STI and lower values of SSI helps to identify salinity stress-tolerant genotypes (Nouri et al., 2011). MP, TOL, and GMP had shown positive and highly significant association with grain yield under normal condition (0.913, 0.845, and 0.738, respectively). Positive correlation of MP, GMP and TOL were also observed with grain yield under stress condition indicating these indices were suitable selection criteria under both normal and saline stress conditions; In the other study also found a positive and high significant association of MP and GMP with grain yield under both normal and saline stress conditions (Kamrani et al., 2018; Poudel et al., 2021). Therefore, MP, GMP and TOL were suitable cri teria for the selection under both normal and saline stress-tolerant genotypes whereas, YSI and STI were found suitable selection criteria under saline stress condition (Table 5).

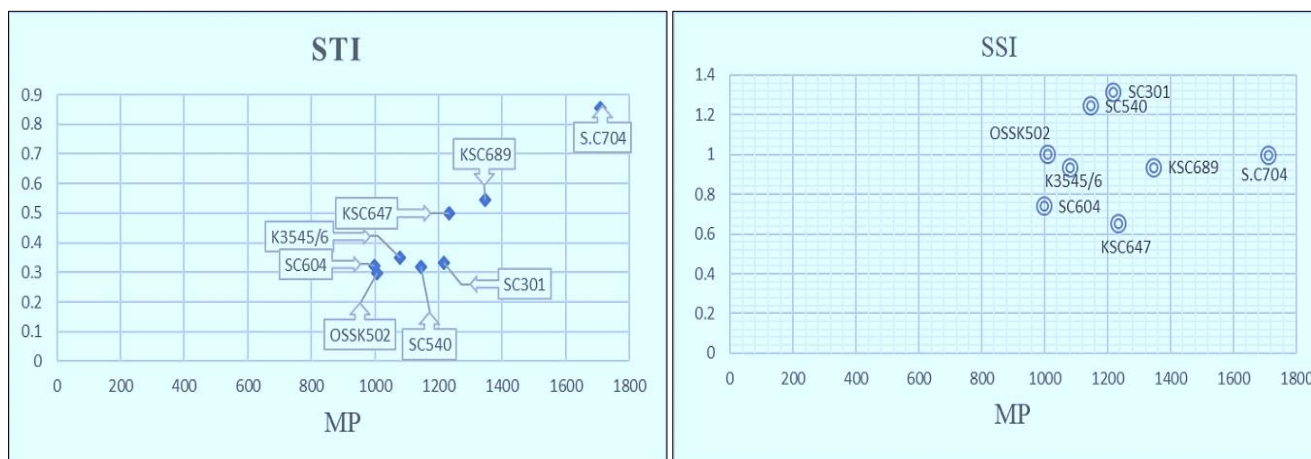


Fig. 1. Diagram of different understudy characteristics in eight genotypes of the maize under the normal and salty conditions.

Table 5. Correlation coefficient between grain yield of maize genotypes and salinity stress indices under normal and salinity stress conditions.

	Yp	ys	MP	Tol	Gmp	Ysi	STi
Ys	0.311	1					
MP	0.913**	0.672	1				
Tol	0.845**	-0.246	0.552	1			
Gmp	0.738*	0.869**	0.949**	0.264	1		
Ysi	-0.484	0.675	-0.086	-0.874**	0.223	1	
STi	0.484	-0.675	0.086	0.874**	-0.223	-1.000**	1
SSi	0.756*	0.850**	0.954**	0.293	0.997**	0.192	-0.192

*, **: significant at 0.05 and 0.01 level of significance.

4. Conclusion

In this research, Studies of agronomic and morphological traits of genotypes under salinity stress showed that the effect of salinity is significant in most of the traits. Considering that these types of traits are more influenced by the environment, so it should be repeated in many years and places. Significant differences were seen between the genotypes in normal and stressed conditions in most of the traits, which indicates high genetic diversity in terms of these traits and the possibility of selection and crossbreeding between them. S.C 704 was able to show the highest yield in both normal and saline environments. there were significant ($P < 0.05$) differences among genotypes for proline content. Data showed that genotypes under salinity conditions had the highest value of proline compared to the normal conditions. Proline of the genotypes increased under saline stress conditions. According to YSI, genotypes KSC647 and SC604 were identified as tolerant genotypes under salinity stress condition. Also, in some study reported that YSI indices can identify the genotypes with high yield potential under stress condition than under normal condition.

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