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Physiological and biochemical response of Maize (*Zea mays* L.) to different levels of salinity

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Abstract

Globally, maize is an important cereal crop and is used as staple food in many countries. Salinity is a wide spreading problem of the world; it is inevitable now to develop the verities which can produce well in salt effected soils. Present study was planned to visualize the responses (shoot length, root length, shoot fresh weight, root fresh weight, Na^+ and K^+) of genetically distant maize accessions at variable salinity levels (Control, 5 dSm^{-1}, 10 dSm^{-1} and 15 dSm^{-1}). Experiment was conducted in solution culture following completely randomized design (CRD). Maximum growth in all aspects was observed at lowest level of salinity stress. Growth decreased in all the genotypes with the increase of salinity level. Analysis was performed in R-Software; all the treatments showed significant differences in all the evaluating standards. Analysis of variance was further divided into linear, quadratic and cubic contrasts. Best performing accessions could be used in future breeding programs with focus to develop salinity tolerant maize varieties.

Keywords: maize, salinity, seedling stage, regression analysis
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Introduction

Among different types of abiotic stresses, salinity is major stress to crop production. Salinity means accumulation of salts under the soil (Giaveno *et al.*, 2007). The salt affected area is increasing globally with the passage of time. It is estimated that land under salt stress account for nearly 15% of the total land surface (Akram *et al.*, 2010). Out of total geographical area of 80.0 million hectares of Pakistan almost 6.30 million hectares is covered by different types of salts. Saline soils lies on an estimated area of 1.89 million hectares, permeable saline-sodic soil extends to an area of 1.85 million hectare, an area of 1.02 million hectares is under impermeable saline-sodic and 0.028 million hectare is sodic in nature. The area suitable for forming is 19.3 mha, out of which, 16 mha land is present where irrigated agriculture is practiced (Alam *et al*., 2000).

Maize (*Zea mays* L.) is an important cereal. United States and China both produce approximately 60% of the world maize (Anonymous, 2011). It is consumed as staple food worldwide and used as raw material in many industrial products. In Pakistan, maize is grown on 4.8% of the total cropped area and contributes 3.5% in the value of agricultural production. Its share in total grain production is 6.4 % (Anonymous, 2011). Yield recovery at farmer's field is very low in Pakistan as compared to the other developed and developing countries of the world.

Maize (*Zea mays* L.) is a salt sensitive crop but some intraspecies variability regarding salt resistance has been reported. Desalinization has been reported an economically possible option in some high-salinity areas (Hajlaoui *et al*., 2009). Another option to combat the problem of salinity is the use of salt resistance crop (Aslam *et al*., 2013; Aslam *et al*., 2015a, b). As suggested by Qu *et al.* (2012) higher NaCl concentrations in the substrate tend to significant increase in germination inhibition. It is said that salinity leads to drought stress because of the property of dissolved solutes to retain water. At higher level of salinity, seeds follow two mechanisms to germinate. Firstly, lower water potential is tolerated by the seeds in the substrate (Collado *et al*., 2010). In second process the inhibitory effect of NaCl is tolerated as some seeds show specific tolerance to NaCl (Hichem *et al*., 2011). Germination inhibition in species is caused by the process of Na^+ shoot accumulation or Na^+ exclusion. Maize genotypes possess different mechanisms of Na^+ shoot accumulation (Zorb *et al*., 2004). Nuran and Cakirlar (2002) reported that plants were affected differently by the salt stress at early growth stages; they observed that at different salt levels seeds were able to germinate but were unable to continue their growth and development. With the increase of stress treatments there was significant decrease in fresh / dry weights of shoot, leaf area and shoots length. Leaf proline contents increase under salt stress at different osmotic potential levels in maize genotypes.

The objectives of this research work is to assess the possibility of using physiological and morphological traits as selection standards against salt stress in tropical maize (*Zea mays* L.) and to look for maize accessions which posses the capability of Na^+ exclusion.

Materials and Methods

Healthy seed of maize accessions were sown in sand filled iron trays by following completely randomized design (CRD). The sand was cleaned and washed with distilled water before filling in trays. The sand was kept moist with distilled water for germination and seedlings establishment. Seedlings were raised up to three leaf stage by the application of standard agronomic practices. The uniform seedlings were transplanted in foam-plugged holes of polystyrene sheets floating over 1/2 strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Salinity was developed in respective treatments by adding NaCl in three/four applications, starting two days after transplanting to have final salinity levels of 5, 10 and 15 dSm^{-1}. The pH of the solution was maintained. Proper aeration of the culture solution was provided for 12 hours daily by an aeration pump. The pH of the solution was monitored daily and adjusted at 6.0±0.5, when needed by using pH meter.

The substrate solutions were changed fortnightly. After four weeks, plants were harvested manually and different parameters (shoot length, root length (total root length), and shoot and root fresh) were recorded; plant leaves were stored in eppendorf tubes in the refrigerator for the determination of Na^+, K^+. In solution culture experiments, the youngest fully expanded leaves were detached at harvesting, rinsed in distilled water, blotted with tissue paper and stored in separate eppendorf tubes at freezing temperature for leaf sap extraction to determine Na^+, K^+. Frozen leaf samples were thawed and crushed using a stainless steel rod with tapered end. The sap was collected in other eppendorf tubes by Gilson pipette and centrifuged at 6500 rpm for 10 minutes. The supernatant sap was taken in new eppendorf tubes and was stored in the refrigerator for the determination of Na^+, K^+ (Gorham *et al*., 1984). The leaf sap was diluted as required by adding distilled water. The dilution factor was correlated with the original value and sodium and potassium concentrations by using Sherwood 410 Flame photometer with the help of self prepared standard solutions using reagent grade salts of NaCl and KCl. Growth parameters i-e shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were recorded using standard procedure.

Results

ANOVA for the shoot length clearly depicted the significant differences between all the treatment levels (Table 1). Verities response was significantly affected with the increase in salinity levels. Contrast analysis was performed to assess the linear, quadratic and cubic relations between all the possible contrasts. Intercept showed that "0" level of salinity have a mean of 50.733 (Table 2). The ANOVA for root length is presented in Table 1. ANOVA was performed in R-software and significant differences were seen between all the salinity levels. The data was quantitative, so orthogonal contrast was performed to see the linear, quadratic and cubic relations between all the possible contrasts (K-1) (Table 1). Highly significant contrasts were shown in case of quadratic and linear contrasts. Intercept was found to be 43.07 (Table 2). Results of shoot fresh weight are presented in Table 1. All genotypes behaved in a similar manner at all the levels of increasing salinity. With the increase in salinity in experiments, all the genotypes showed significant differences as it is evident in ANOVA. As the results were significant so the possible contrasts analysis were conducted to see the linear, quadratic and cubic relations between all the possible contrasts i.e. K-1 (Table 1). No relationship was found in case of linear contrast whereas quadratic and cubic contrasts showed significant results; the significant results in ANOVA were due to the quadratic and cubic contrasts and no effect was due to the linear contrast. The results of ANOVA regarding root fresh weight is presented in Table 1. Root fresh weight was found to be affected by the increase in salinity levels and treatment differences had significant changes on the expression of verities.

Three possible contrasts i-e linear, quadratic and cubic were performed (Table 1) and the results in R-software showed that linear relation has no effect on the results while quadratic and cubic had significant effects on the treatment differences. Results of Na^+ concentration in ANOVA performed in R-software are presented in Table 1. All the genotypes behaved in a similar manner at all levels of increasing salinity. With increase in salinity in experiments, all genotypes showed increase in Na^+ concentration significantly in each level. Treatments affect was significant in ANOVA and ANOVA was further splitted into three contrasting analysis to see the treatment relation in linear, quadratic and cubic form (Table 1). All the contrasts had significant effect on the treatments. Intercept in case of Na^+ concentration was obtained to be 27.09.

The ANOVA (Table 2) showed results of K^+ concentration of 6 maize genotypes grown under four different salinity levels. Treatments affected significantly therefore, ANOVA were further splitted into 3 contrasts i-e linear, quadratic and cubic (Table 1). All the contrasts had significant effect showing relationship in the treatment effects. The intercept was found 136.38 in case of K^+ (Table 2). Analysis of variance and contrasts analysis performed in R-Software had shown that all the treatments have significant effects on the performance of verities at all levels (Table 2).

Table 1: Analysis of variance for different seedling traits of maize

	DF	SUM SQUARES	MEAN SQUARES	F VALUE	PR(>F)
SHOOT LENGTH (CM)					
TRT	3	3824	1275	71.04	< 2e-16 ***
TRT: L	1	1	1	0.07	0.79738
TRT: Q	1	3529	3529	196.71	< 2e-16 ***
TRT: C	1	293	293	16.35	0.00012 ***
RESIDUALS	68	1435	17.94		
ROOT LENGTH (CM)					
TRT	3	6438	2146	89.33	< 2e-16 ***
TRT: L	1	110	110	4.58	0.036 *
TRT: Q	1	5776	5776	240.41	< 2e-16 ***
TRT: C	1	552	552	22.99	9.25e-06 ***
RESIDUALS	68	1634	24		
SHOOT FRESH WEIGHT (G)					
TRT	3	3422	1140.6	105.34	< 2e-16 ***
TRT: L	1	7	6.9	0.64	0.427
TRT: Q	1	2856	2856	263.76	< 2e-16 ***
TRT: C	1	559	559	51.63	6.58e-10 ***
RESIDUALS	68	736	10.8		
ROOT FRESH WEIGHT (G)					
TRT	3	257.00	85.67	37.54	1.99e-14 ***
TRT: L	1	4.71	4.71	2.06	0.15527
TRT: Q	1	235.83	235.83	103.35	2.77e-15 ***
TRT: C	1	16.46	16.46	7.21	0.00909 **
RESIDUALS	68	155.16	2.28		
SODIUM IONS (PPM)					
TRT	3	112220	3740785	199.15	<2e-16 ***
TRT: L	1	2030	2030	10.81	0.0016 **
TRT: Q	1	86229	86229	459.08	<2e-16 ***
TRT: C	1	23960	23960	127.56	<2e-16 ***
RESIDUALS	68	12773	188		
POTASIUM IONS (PPM)					
TRT	3	114365	38122	481.12	< 2e-16 ***
TRT: L	1	3004	3004	37.91	4.51e-08 ***
TRT: Q	1	101322	101322	1278.76	< 2e-16 ***
TRT: C	1	10039	10039	126.70	< 2e-16 ***
RESIDUALS	68	5388	79		

Discussion

The primary objective of current research study was to observe the effect of treatments on the performance of maize verities under saline conditions. Results of the study depicted that saline growth medium adversely effected the growth traits of maize genotypes whereas, inhibitory effect of NaCl are more pronounced at higher salinity levels. NaCl treatment had significantly reduced the plant growth. Decline in plant height was highest at salinity level of 15 dSm^{-1} as compared to the other levels of salt stress. The effect of high salinity (15 dSm^{-1}) was more pronounced than the effect of low salinity (5 dSm^{-1}). The concentration of Na^+ in the leaves was significantly increased under both salinity levels while leaf K^+ concentration was significantly reduced. Higher concentrations Na^+ disrupt the osmotic balance of cells therefore, cause the water deficit. The higher Na^+ concentration in the leaves is also toxic and cause injury (Fricke *et al.*, 2004). The response of accessions was also variable significantly within a treatment. Varietal comparison showed that the genotypes accumulating more Na^+ in their leaves exhibited poor growth performance. Genotypes produced less shoot fresh weight due to higher accumulation of Na^+ in leaves. Maize is a salt-sensitive crop (Fortmeier and Schubert, 1995), and it

Table -2: Regression analysis for different seedling traits of maize

(Intercept)	**X**	**I(x^2)**
	Shoot length (cm)	
50.73	-1.13	-0.0047
	Root length (cm)	
43.075	-0.93	-0.049
	Shoot fresh weight (g)	
21.86	-1.41	0.0123
	Root fresh weight (g)	
7.207	-0.49	-0.49
	Sodium ion	
27.096	3.72	0.212
	Potassium ion	
136.39	-3.16	-0.26

is genotype dependent sensitivity (Cramer *et al*., 1994). According to Nuran and Cakirlar (2002) plants are affected differently by the salt stress at early growth stages as they observed that at different salt levels seeds could grow but are unable to continue their growth and development. With the increase of stress treatments it resulted in significant decrease in fresh weights of shoot and shoot length. Engineering techniques, use of amendments and mineral nutrients are recommended to improve plant survival under salt stress (Hsiao and Xu, 2000). Plant traits like dry matter production, Na^+ and K^+ concentration are reported to be useful to assess salinity tolerance in genotypes and selection of genotypes (Santa-Maria and Epstein, 2001). Chinnusamy *et al*. (2005) reported that main cause of plant growth reduction is ion toxicity than osmotic stress under salinity stress. Bastias *et al*. (2004) reported a large decrease in dry weight production (40%) of local maize cultivars under 100 and 430 mM NaCl treatment. The genotype of maize (*Zea mays* L.) amylacea reported to be tolerant than Pioneer hybrid 3578 (Cramer *et al*., 1994), line G2 and SRO73 (Abd-El Baki *et al*., 2000), which have been reported as most salt tolerant maize cultivars. Maize genotypes with lower Na^+ concentration in the shoots are relatively more salt tolerant (Fortmeier and Schubert, 1995). Na^+ and K^+ concentration, shoot fresh weight and root fresh weight are considered as appropriate selection criteria for salinity tolerance. Different physiological parameters as, exclusion or compartmentalization of sodium, potassium selectivity, osmotic adjustment and accumulation of organic solutes are also related to salt tolerance (Barrett-Lennard *et al*., 1999). Jamil *et al*. (2005) reported adverse effects of salinity stress (0, 4.7, 9.4 and 14.1 dSm^{-1}) on seed germination, shoot fresh weight, shoot length, and root fresh weight of canola, cauliflower and cabbage.

Decline in plant growth and dry-matter production under salinity stress were reported in numerous legume crops (Tejera *et al*., 2006). Qadir and Schubert (2002) reported that crop plants are variable in their rates of Na^+ absorption but also in the pattern of Na^+ translocation to their shoots. Shoot fresh weight production in maize genotypes was negatively correlated (r=0.74) with Na^+ concentration under NaCl stress. Higher Na^+ concentration in leaves causes toxicity and leads to salt injury (Saqib *et al*., 2005). These results were in accordance with Munns *et al*. (2006) who mentioned that the salt tolerance in wheat was correlated with lower Na^+ concentration in shoots.

Conclusion

Salinity has devastating effect on all plant growth parameters including shoot length, root length, shoot fresh weight and root fresh weight as decrease in performance was observed at increased level of salinity. All the genotypes showed increase in Na^+ content in case of increase in salinity level while k^+ concentration was found to be decreasing in case of increased level in salinity.

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