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ASSESSMENT OF MAIZE CULTIVARS FOR SALT TOLERANCE BASED ON PHYSIOLOGICAL INDICES

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Abstract

Salinity is the main threat causing huge losses in crop production all over the world. To fulfill future food demands of increasing population around the globe, production of salt tolerant genotypes is essential. Reliable and fast screening methods would be helpful in the developing high yielding and salt tolerant maize cultivars. In the study, an effort to screen maize cultivars regarding salt tolerance was performed. Thirteen maize cultivars were assessed for salt tolerance under laboratory conditions using germination stress tolerance index (GSI), shoot length stress tolerance index (SLSI), root length stress tolerance index (RLSI), fresh weight stress tolerance index (FWSI), dry weight stress tolerance index (DWSI). The data so obtained was subjected to various multivariate practices comprising correlation and cluster analysis to evaluate the assortment for salt tolerance in maize cultivars. Highly significant and positive correlations were found between GSI, SLSI, RLSI, FWSI and DWSI. Cluster analysis classified the cultivars into divergent groups. Cultivars Sultan and Pearl exhibited adequate degree of salt tolerance, whereas, Sahiwal 2002, NMRI yellow, showed medium level of salt tolerance while Akbar and FH 963KH 15 were classified as sensitive cultivars. On the basis of multivariate analysis of the examined traits, physiological indices could be used as markers for the selection of cultivars for salt tolerance in maize.

Key words: Physiological indices, Screening tools, Mutivariate analysis, NaCl stress, Maize.

Introduction

Soil salinization is a crucial factor limiting the production of crops, biodiversity and nutritional security all over the world (Zafar *et al*., 2015). In Pakistan 6.3 million hectares are salt affected, out of which most of the soil is saline sodic (Qureshi, 2016). Seed germination as well as seedling establishment are the most important and sensitive phases during the growth period of plants and can be used as the most viable criteria for determining the salt tolerance potential of genotypes (Khodarahmpour *et al*., 2012). An important step in breeding genotypes with salt tolerance potential is to use the considerable genetic variation among them to tolerate salt stress (Khan *et al*., 2022).

High salt contents in soil makes it difficult for plant roots to extract water, resulting in physiological drought and ion toxicity due to uptake of specific ions (Huang *et al*., 2021). High Na^+ contents in plant tissues hampers the plant metabolism due to the production of reactive oxygen species (O_2, H_2O_2 and OH), (Singh *et al*., 2021; Zafar *et al*., 2018), disruption of cellular homeostatis, oxidative damage to proteins and nucleic acids ultimately leading to cell death (Gaschler & Stockwell, 2017). The salinized areas are increasing annually due to improper method of drainage and irrigation. Salinity decrease agricultural production and disturbs ecological balance of the area. Salinity increases osmotic stress in plants and decrease photosynthetic activity (Marium *et al*., 2019). One of the most detrimental effect of salinity stress is ionic imbalance due to accumulation of Na+ and Cl^- ions. High Na^+ inhibit K^+ uptake resulting in lower productivity of crops causing cell death (Misra *et al*., 2021).

Maize (*Zea mays* L.) is one of the most essential grain crop with C_4 metabolism. Soil salinity is a major threat to its production worldwide, however the growth pattern varies with development stages of crop under salt stress (Farooq *et al*., 2015). Screening of maize cultivars is necessary to recognize the salt resistant cultivars. Breeders look for cultivars with improved tolerance to osmotic and specific ion stress. Variation among cultivars at germination stage has been observed among a number of plant species (Alvi *et al*., 2022). The studied cultivars can further be exploited by growing in saline areas of land. The existing study aims to assess maize cultivars using NaCl salt stress in order to analyze the genetic variation in their behavior to salinity. The cultivars are evaluated using stress tolerance indices under varying degree of salt stress.

Materials and Methods

The present screening study was performed at Nuclear Institute for Agriculture and Biology Faisalabad, Pakistan. The trial was conducted under lab conditions in growth chamber using control (0), 40, 60 and 80 mM NaCl levels, to investigate the physiological indices of maize cultivars. Seeds of 13 maize cultivars i-e Sultan, Sahiwal 2002, NMRI yellow, Sadaf, Pearl, Akbar, FH 1275 KH 15, FH 1231 KH 15, FH 1276 KH 15, FH 1042 KH 15, FH 1227 KH 15, FH 963 KH 15, FH 1212 KH 15 were sown in glasses containing sand. The grains were surface sterilized using 10% sodium hypochlorite solution followed by washing three times with distilled water. The grains were allowed to germinate in growth chamber (Sanyo-Gallenkamp, United Kingdom) and the temperature at 28˚C ± 2˚C was maintained. The germination was observed on daily basis and after fifteen days of germination, all the plants were harvested and washed with tap water. Length of shoot and root was determined. The fresh and dry weight of seedlings were measured. All the plants were dried in an oven at 70°C for 48 hours for the calculation of dry weight (Ashraf *et al*., 2008).

Stress tolerance indices: Germination stress tolerance index (GSI) was determined by calculating the promptness index using formula:

$$PI = nd_1\,(1.00) + nd_2\,(0.75) + nd_3\,(0.50) + nd_4\,(0.25)$$

where n represents the number of seeds d_1, d_2, d_3, d_4 revealed the seeds germinated on each day respectively, indices were calculated using the given formula:

$$GSI = \frac{\text{PI of seeds under stress}}{\text{PI of control seeds}} \times 100$$

$$SLSI = \frac{\text{Shoot length of plants under stress}}{\text{Shoot length of control plants}} \times 100$$

$$RLSI = \frac{\text{Root length of plants under stress}}{\text{Root length of control plants}} \times 100$$

$$FWSI = \frac{\text{Fresh weight of plants under stress}}{\text{Fresh weight of control plants}} \times 100$$

$$DWSI = \frac{\text{Dry weight of plants under stress}}{\text{Dry weight of control plants}} \times 100$$

Statistical analysis

The data was evaluated by means of analysis of variance practice (Steel *et al.*, 1997) and comparison of means was performed using least significant difference technique at 5% probability. The correlation was performed using statistix software and cluster analysis by Minitab-6.

Results

Statistical analysis indicated that salinity significantly reduced all the recorded traits. Salt stress under all the levels significantly reduced the seed germination stress tolerance index (GSI). As the salinity level increased to 80 mM NaCl, the maximum reduction of seed germination was recorded. Regarding seed germination stress tolerance index (GSI), the highest mean at 40 mM NaCl was recorded in Pearl, while the lowest GSI was recorded in Akbar (Table 1). At 60 mM level of NaCl stress, the maximum GSI was recorded in Sahiwal-2002, whereas Akbar showed minimum values. Similarly, at 80 mM NaCl the maximum GSI was recorded in Pearl and lowest in Akbar.

For shoot length stress tolerance index, FH 1227 KH 15 cultivar exhibited highest values at 40 mM NaCl salinity level followed by Pearl and FH 1212 KH 15, while Akbar revealed minimum values (Table 2). As salinity level enhanced to 60 mM NaCl, Pearl and Sahiwal 2002 cultivars exhibited greater values of SLSI, while, FH 1276 KH 15 and Akbar exhibited minimum values. However, at 80 mM level of salt stress, maximum SLSI was observed by Pearl and Sultan and minimum values were revealed by Akbar and FH 1212 KH 15.

Similar trend was observed for root length stress tolerance index RLSI. Root length of the maize cultivars were considerably affected by NaCl salt stress. The maximum value of RLSI at 40 mM NaCl was recorded in Sahiwal 2002 and FH 1227 KH 15 while minimum value was recorded in Akbar. At 60 mM NaCl increased RLSI was recorded in Sultan followed by Pearl while cultivar FH 1276 KH 15 followed by Akbar exhibited lowest values. Same results were obtained at 80 mM NaCl where Pearl followed by Sultan showed highest values while FH 1231 KH 15 followed by Sadaf revealved minimum values (Table 3).

Salt stress showed a negative effect on the fresh weight stress tolerance index FWSI (Table 4) of the maize cultivars. The FWSI differed significantly among cultivars. The maximum FWSI was observed in Pearl followed by Sultan at 40 mM and 80 NaCl. The lowest FWSI at 40 mM was exhibited by the cultivar FH 963 KH 15 however, at 60 mM salt stress the lowest values were exhibited by Akbar.

Under 80 mM NaCl the highest FWSI was recorded by Pearl followed by Sultan while lowest FWSI was observed by Akbar followed by FH 963 KH 15.

Salinity significantly reduced seedling dry weight stress tolerance index (DWSI) (Table 5) in all the maize cultivars. At all levels of salt stress maximum value was observed by Pearl and Sultan, while minimum value was showed by FH 963 KH 15 and Akbar. Overall, cultivar means indicated that Pearl and Sultan ranked first and second and Akbar at 13^{th} position.

Table 1. Germination stress tolerance index of various maize cultivars.

Cultivars	NaCl treatments in Mm				Ranking
	40	60	80	Mean	
Sultan	97.222	94.444	83.333	91.666ab	3
Sahiwal 2002	100	100	77.777	92.592ab	2
NMRI yellow	88.571	85.714	80	84.761bcd	7
Sadaf	91.176	88.235	85.294	88.235abc	6
Pearl	100	94.444	91.666	95.370a	1
Akbar	81.25	68.75	59.375	69.791e	13
FH 1275 KH 15	88.571	80	51.428	73.333e	12
FH 1231 KH 15	91.428	74.285	65.714	77.142de	10
FH 1276 KH 15	97.142	85.714	68.571	83.809bcd	8
FH 1042 KH 15	94.285	91.428	80	88.571abc	5
FH 1227 KH 15	94.285	88.571	8.714	89.523abc	4
FH 963 KH 15	84.848	72.727	69.696	75.757de	11
FH 1212 KH 15	85.714	80	77.142	80.952cde	9

Note: Means sharing similar letter did not differ significantly ($p>0.05$) in row and column

Table 2. Shoot length stress tolerance index of various maize cultivars.

Cultivars	NaCI treatment in Mm				Ranking
	40	60	80	Mean	
Sultan	79.446	74.152	71.095	74.898abcd	4
Sahiwal 2002	83.233	81.131	68.198	77.52lab	2
NMRIyellow	85.102	67.612	52.782	68.498cde	7
Sadaf	84.919	78.788	58.938	74.215abcd	5
Pearl	89.109	81.314	71.531	80.651a	1
Akbar	71.722	56.174	43.044	56.979f	13
FH 1275 KH 15	79.040	65.871	50.530	65.147ef	11
FH 1231 KH 15	81.185	62.255	51.350	64.960ef	12
FH 1276 KH 15	85.479	58.758	55.628	66.622de	10
FH 1042 KH 15	82.865	73.611	50.354	68.944bcde	6
FH 1227 KH 15	90.928	78.751	58.389	76.023abc	3
FH 963 KH 15	85.537	61.359	55.555	67.48cde	9
FH 1212 KH 15	86.324	69.978	48.717	68.341cde	8

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column.

Table 3. Root length stress tolerance index of various maize cultivars.

Cultivars	NaCI treatments in Mm				Ranking
	40	60	80	Mean	
Sultan	81.803	78.281	63.714	74.599a	2
Sahiwal 2002	88.888	57.407	55.926	67.407ab	4
NMRI yellow	83.011	67.977	50.306	67.101ab	5
Sadaf	82.222	68.292	41.136	63.883bc	7
Pearl	82.222	68.292	41.136	75.086a	1
Akbar	59.366	48.185	45.453	51.001d	13
FH 1275 KH 15	78.235	59.638	46.265	61.379bc	9
FH 123 KH 15	71.62	58.030	41.072	56.907cd	10
FH 1276 KH 15	69.430	51.355	46.046	55.611cd	11
FH 1042 KH 15	80.359	67.767	52.123	66.750ab	6
FH 1227 KH 15	86.007	69.714	53.363	69.695ab	3
FH 963 KH 15	66.669	55.547	43.555	55.257cd	12
FH 1212 KH 15	69.034	64.124	57.954	63.704bc	8

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 4. Fresh Biomass stress tolerance index of various maize cultivars.

Cultivars	NaCI treatments in Mm				Ranking
	40	60	80	Mean	
Sultan	91.043	87.858	82.436	87.113ab	2
Sahiwal 2002	90.703	84.583	72.387	82.557abc	3
NMRI yellow	84.671	75.045	70.781	76.832cde	5
Sadaf	82.049	72.593	51.821	68.821ef	7
Pearl	92.935	90.747	83.772	89.151a	1
Akbar	68.487	48.163	43.053	53.234g	13
FH 1275 KH 15	78.22	72.124	52.727	67.691f	8
FH 1231 KH 15	81.66	62.099	48.358	64.042f	11
FH 1276 KH 15	76.132	68.302	48.041	64.158f	10
FH 1042 KH 15	87.22	67.258	60.259	71.502def	6
FH 1227 KH 15	83.485	77.587	74.052	78.375bcd	4
FH 963 KH 15	68.31	53.469	38.366	53.3819g	12
FH 1212 KH 15	81.604	68.757	52.272	67.545f	9

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 5. Dry Biomass stress tolerance index of various maize cultivars.

Cultivars	NaCI treatments in Mm				Ranking
	40	60	80	Mean	
Sultan	91.234	86.097	77.374	84.902a	2
Sahiwal 2002	83.822	80.848	54.682	73.117bc	4
NMRI yellow	80.400	68.466	59.228	69.365bcd	7
Sadaf	80.928	76.842	57.676	71.815bc	5
Pearl	92.764	89.34	83.087	88.397a	1
Akbar	43.076	35.793	27.841	35.570f	13
FH 1275 KH 15	64.481	62.448	54.926	60.618de	10
FH 1231 KH 15	71.537	63.475	45.258	60.090e	11
FH 1276 KH 15	76.234	63.74	58.323	66.099bcde	8
FH 1042 KH 15	83.086	74.822	50.772	69.560bcd	6
FH 1227 KH 15	83.747	77.097	64.014	74.953b	3
FH 963 KH 15	43.420	38.504	28.411	36.778f	12
FH 1212 KH 15	71.454	63.891	59.598	64.981cde	9

Note: Means sharing similar letter did not differ significantly (p>0.05) in row and column

Table 6. Co-relation between different screening techniques.

Techniques	GSI	SLSI	RLSI	FWSI	DWSI
SGI					
SLSI	0.9204**				
RLSI	0.8607**	0.8567**			
FWSI	0.8749**	0.8468**	0.9546**		
DWSI	0.8888**	0.8239**	0.8986**	0.9348**	

** = Significant (p<0.01); GSI = Germination stress tolerance index; SLSI = Shoot length stress tolerance index; RLSI = Root length stress tolerance index; FWSI = Fresh weight stress tolerance index; DWSI = Dry weight stress tolerance index

Table 7. Mean square values of data for plant germination, shoot length, root length, fresh weight, dry weight stress tolerance indices.

Techniques	DF	GSI	SLSI	RLSI	FWSI	DWSI
G	12	182.655	122.67	167.13	390.01	723.03
T	2	879.044	2339.41	2149.15	1600.92	1068.73
Error	24	37.50	27.88	31.60	27.31	29.55

Abbreviations: GSI, Germination stress tolerance index; SLSI, Shoot length stress tolerance index; RLSI, Root length stress tolerance index; FWSI = Fresh weight stress tolerance index; DWSI = Dry weight stress tolerance indices

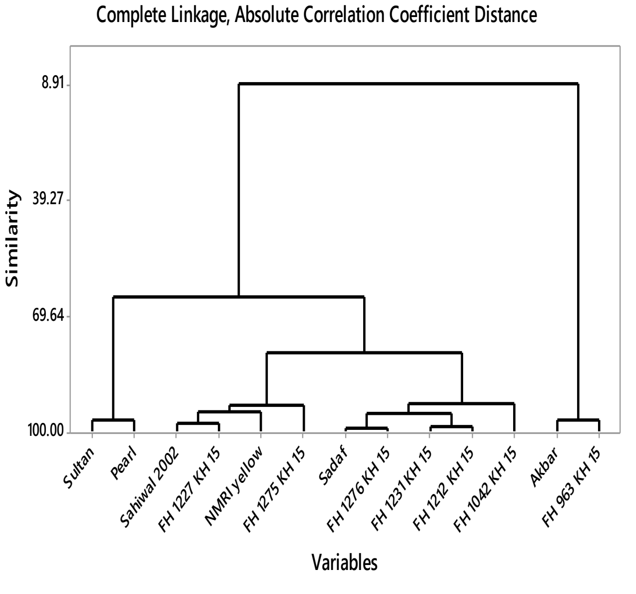

Fig. 1. Dandrogram showing cluster analysis for salt tolerance among various maize cultivars using physiological indices.

The correlation analysis showed significantly positive relationship among GSI, SLSI, FSI and DSI indicating that cultivars with higher values of tolerance indices were tolerant to salt stress (Table 6).

The cluster analysis split the cultivars regarding salt tolerance into four clusters based on the physiological indices (Fig. 1). The mean square values for maize cultivars at germination, shoot length, root length, fresh and dry biomass are given below (Table 7).

Discussion

The present study categorized the salt tolerant maize cultivars at seedling stage under different levels of NaCl salt stress. The data regarding physiological indices GSI, SLSI, FWSI, DWSI screened the maize cultivars for salt tolerance. The maize cultivars Pearl and Sultan showed better results and were classified as salt tolerant whereas, FH 963 KH 15 and Akbar were ranked among sensitive cultivars. It is documented in literature that under saline conditions the cultivars with superior germination exhibited substantial morphological characteristics having high yield

(Marium et al., 2019). The salt tolerant cultivars can be categorized through GSI. The salt stress affects the seed vigour and storage conditions during germination. Therefore, seed germination is reduced due to adverse effect of elevated levels of salt stress. The outcomes of the study are in accordance with Aziz *et al*., (2021). The screened maize cultivars possess genetic potential for salt tolerance. Increased salt stress can reduce the radicle and plumule growth due to osmotic stress which badly influence cellular division and differentiation (Wang *et al*., 2020). The seedling development, biomass and the tolerance indices are adversely influenced by salt stress, due to toxicity of specific ions, disturbance in nutrient uptake (Akhtar *et al*., 2012) and osmotic stress decreasing biosynthesis of enzymes (Ashraf *et al*., 2008). The decrease in growth of seedlings might be due to osmotic stress under salt stress or water may enter into transpiration stream and damage cells of transpiring leaves through ion toxicity (Munns, 2005).

The fresh and dry weight stress tolerance index showed that cultivars Pearl and Sultan accumulated maximum biomass at all NaCl stress levels while Akbar exhibited minimum values and ranked among salt-sensitive genotypes. Several researchers Noreen & Ashraf (2009) and Alvi *et al*., (2022) showed that shoot length and biomass decrease in pea and maize with increase in level of salinity. Salt stress decreased the photosynthetic rate, assimilation of carbon dioxide and significantly decline the absorbance of soil nutrients and in conclusion plant growth is getting decreased (Ghazizade *et al*., 2012).

It is reported that salinity inhibit plant growth (Zafar *et al*., 2020). In maize genotypes, Na^+ ions are accumulated in leaves and reduce dry biomass. It is documented by Ashraf *et al*., (2008) that Na^+ uptake is reduced in salt tolerant genotypes.

Seed germination is considered as most significant physiological process that is indication for a genotype to cope with environmental stress (Peralta *et al*., 2001). Applications of salinity, reduced the uptake of water from the soil resulting in lowering of water potential. Salt stress is also involved in distortion of many enzymatic activities that play important role in seed germination. Salinity is also employed in protein dehydration, contraction of seeds, alter chemical reactions and turned the seed in non-viable condition (Bewley & Black, 2012). Our results are also related with the results of Shahid *et al*., (2021), in which seed germination is directly associated with uptake of water and is interrupted with salinity stress. Under salinity stress, reduction in seed germination is also reported by Perveen *et al*., (2021) in brinjal. Present findings are in accordance with the opinion of Kaleri (2010) and Zafar *et al*., (2015) in canola and wheat, respectively.

Increasing concentration of NaCl significantly reduced the shoot length of maize hybrids. Radić *et al*., (2007) exhibited the same results that increasing concentration of NaCl negatively affected shoot length of maize plants. The results are in compliance with Khodarahmpour *et al*., (2012) findings that growth of shoot length is reduced at highest level of salt stress.

Root is the main organ that provides all essential nutrients to plant, it has direct link with the medium and has functional information about any stress. Pessarakli & Kopec (2009) determined that shoot length is reduced by enhancing salt concentrations. The same results were also observed by Ganie & Ahammed (2021) in rice that shoot length is reduced by extreme deposition of salt concentration in cell wall, so wall become rigid and efficiency of turgor pressure in cell wall decreased rapidly.

The correlation analysis showed a positive association between seedling germination, shoot length, root length and biomass. It is clear that physiological indices can be used to evaluate germplasm for salt tolerance potential. A positive correlation between GSI and stress tolerance indices is reported by Shah *et al*., (2020) in chickpea.

The information related to correlation is important as it helps in selection of genotypes with desirable characters in breeding programs (Ali *et al*., 2009).

Scientists have also used cluster analysis to group the genotypes on the basis of similarities within a group (Zafar *et al*., 2021). The cluster-1 included two cultivars and were categorized as salt tolerant. Cluster-II comprised of nine maize cultivars and were classified as medium tolerant. However, cluster-III consisted of two cultivars and showed less similarly with other cultivars for the characters and did not performed well. They were considered as sensitive ones. Literature focuses on the use of cluster analysis to screen the germplasm for salt tolerance (Noorifarjam *et al*., 2013). The cultivars categorized could be used in future breeding experiments for salt tolerance.

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