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GENETIC DIVERSITY AND CHARACTERIZATION OF SALT STRESS TOLERANCE TRAITS IN MAIZE (*ZEA MAYS* L.) UNDER NORMAL AND SALINE CONDITIONS

KHALIL AHMAD[1], MUHAMMAD ASLAM[2*], MUHAMMAD HAMZAH SALEEM[3], MUHAMMAD IJAZ[1], SAMI UL-ALLAH[1], AMARA HASSAN[4] MOHAMED A. EL-SHEIKH[5] MUHAMMAD ADNAN[6] AND SHAFAQAT ALI[7,8*]

[1]*College of Agriculture, Bahauddin Zakariya University, Bahadur sub-Campus, Layyah, Pakistan*
[2]*Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan*
[3]*MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*
[4]*Department of Botany, Government College University, Allama Iqbal Road, 38000 Faisalabad, Pakistan*
[5]*Botany & Microbiology Department, College of Science, King Saud University, P.O. Box 2455, 11452 Riyadh, Saudi Arabia*
[6]*Department of Plant Soil and Microbial Sciences, Michigan State University United States of America*
[7]*Department of Environmental Sciences and Engineering, Government College University Allama Iqbal Road, 38000 Faisalabad, Pakistan*
[8]*Department of Biological Sciences and Technology, China Medical University (CMU), Taichung City 40402, Taiwan*
**Corresponding author's email: aslampbg@uaf.edu.pk; shafaqataligill@gcuf.edu.pk;*

Abstract

To study (genotype × environment) interaction (GEI) of available maize germplasm against different saline environments present study was conducted under four saline environments $S_{0.89\ dSm}^{-1}$ (T_1; Control), $S_{5.2\ dSm}^{-1}$ (T_2), $S_{6.7\ dSm}^{-1}$ (T_3) and $S_{11\ dsm}^{-1}$ (T_4) in natural saline environments of saline soil research institute, Pindi Bhattian on the basis of standards like grain yield per plant, 100 grain weight, stomata conductance, total soluble sugars, chlorophyll-*a,* chlorophyll-*b,* relative water con tents, no of grain per cob, water potential, protein contents, transpiration rate, plant height, photosynthetic rate, leaf fresh weight and leaf area. Sowing was performed with split plot arrangement by following randomize complete block design. Based on performance, UAF-0020 and UAC-0036 were selected as a most tolerant even in highly saline environment $S_{11\ dsm}^{-1}$ on the basis of protein contents, grain yield per plant and number of grains per cob, chlorophyll-*b*, chlorophyll-*a*, using biplot on the basis of principal component analysis (PCA). Based on photosynthetic rate, 100 grain weight and protein contents the most susceptible genotype recorded were UAF-0028 and UAF-0033 even in low salinity $S_{5.2\ dsm}^{-1}$. Under all the variable saline environments the ramming genotypes performed in same manner either in positive or negative fashion. Protein contents, number of grains per cob, chlorophyll-*b,* chlorophyll-*a,* rate of photosynthesis, grain yield per plant, Plant height and 100 grain weight were considered the best standard for selection. To study GEI Principal Component Analysis based biplot is proved as an effective procedure. Reported salinity tolerant genotypes could be used for further salinity tolerance breeding programs.

Key words: Screening, Genetic diversity, Maize, Salinity; Morphology.

Introduction

Maize (*Zea mays* L.) is one of the important as well as produce highest grain. It was originated in 9000 years ago in Central Mexico where was grown as a wild grass (Ahmad & Jhon, 2005; Noman *et al.*, 2015; Nawaz *et al.*, 2021). Globally, maize is used as raw material for industrial product as well as consumed as staple food. All plant parts of maize are useful for food as well as for non-food products (Ahanger *et al.*, 2020; Kaya *et al.*, 2015; Nawaz *et al.*, 2020). Maize seed contains starch 72%, protein 10%, sugar 3%, oil 4.8%, ash 17% and fiber 8.5% (Noman *et al.*, 2015). In Pakistan maize fulfils 60% demand of poultry industry (Pandolfi *et al.*, 2016). Globally, increasing population trend narrates that population will reach to 9.7 billion till the year 2050 (population prospects of United Nations 2015-2016); which is a serious threat to crop production and food security. To fulfill the anticipated loads, it is the need of hour to double crop production till 2050 (Saleem *et al.*, 2020). But in contrast, due to biotic and abiotic stresses there is reduced food production and these adverse effects are serious threat to nation (Ali *et al.*, 2021; Hassan *et al.*, 2021; Hussain *et al.*, 2021).

Salt stress is serious hazard among various abiotic stresses to economy of agriculture, particularly in semiarid and arid areas (Ahmad *et al.*, 2012, 2019; Ali *et al.*, 2020; Alam *et al.*, 2021; Kaya *et al.*, 2020). In 2400 BC, salts affected land were found in Iraq. The early civilizations were affected by salinity badly (Parida & Das, 2005). Globally, 831 M ha land is affected by sodality and salinity. Mostly these areas are arid. In these semiarid humid and coastal areas mostly, there is humid and sub humid climates along rivers and estuaries (Yun *et al.*, 2018; Hameed *et al.*, 2021; Mumtaz *et al.*, 2021; Waseem *et al.*, 2021). Many salts like NaCl, $CaS0_4$, KCI, Na_2S04, $MgCI_2$, $MgS0_4$ and $NaC0_3$ cause soil salinity in varied conditions (Afzal *et al.*, 2020; Javed *et al.*, 2020; Kaleem & Hameed, 2021). Sodium and chloride contribute significantly towards soil salinity which play important role in reducing crop production by reducing osmotic potential and specific ion toxicity (Mumtaz *et al.*, 2019; Mohamed *et al.*, 2020; Hassan *et al.*, 2021). In two stages, the maize plant growth is reduced (Carpýcý *et al.*, 2009). There is reduction take place in external water potential at first stage due salts availability in soil near roots (Anjum *et al.*, 2011; Yaseen *et al.*, 2020; Saleem *et al.*, 2021). Older leave senesces at second stage. Salt sensitive maize plants accumulate greater ionic concentration when contrasted with salt tolerant ones prompting continuous plant death (Parihar *et al.*, 2015; Yun *et al.*, 2018). Salinity destroys plants by three different ways like increasing ion toxicity (sodium and chloride), reducing water potential and obstruction with fundamental nutrient supply (Parida & Das, 2005; Baghel *et al.*, 2019). Decline in turgor pressure due to reduction in water potential causes death of plant. Leaves senescence due to high salts causes decrease in photosynthetic zone ultimately reduces photosynthetic rate in plants which disturbs carbon balance necessary for plant growth (Abdel-Hamid & Mohamed, 2014; Ali *et al.*, 2020; Saleem *et al.*, 2020). In overflowed region, decreased oxygen in roots badly influences respiration of roots eventually plant growth (Ashraf & Orooj, 2006). Furthermore, accessibility of iron, nitrate, sulfate and manganese to plant are decreased (Deng *et al.*, 2021; Walayat *et al.*, 2021) which upsets particular particle passage (Saleem *et al.*, 2020). Salinity and such anaerobic conditions together affect severely the crop development (Zafar *et al.*, 2015; Yasmin *et al.*, 2020).

There are different ways to overcome salinity issue. Out of various, cultivation of salt tolerant plants has received extensive significance because of its being an efficient manner of making use of the salt affected soils (Hussain *et al.*, 2016; Jing *et al.*, 2018; Nawaz *et al.*, 2020). Crop germplasm screening is requirement to find genotypes having tolerance against salinity for any breeding programmed. The purpose of present research was to judge the degree of variability and adaptability of maize germplasm against different salinity conditions to find salinity tolerant and sensitive genotypes and best selection traits against salinity stress.

Materials and Methods

Experimental conditions and treatments: This experiment was conducted under natural saline field conditions in Saline Soil Research Institute (SSRI), Pindi Bhatian, Punjab, Pakistan. Forty maize genotypes namely UAC-0013 to UAC-0052 were screened at different salinity concentrations i.e. $S_{0.89\ dSm}{}^{-1}$(T_1; Control), $S_{5.2\ dSm}{}^{-1}$(T_2), $S_{6.7\ dSm}{}^{-1}$(T_3) and $S_{11\ dsm-1}$ (T_4). Thirty plants per replication were grown in each treatment on ridges by maintaining 50 cm row × row and 20 cm plant × plant distance. Sowing was done following triplicated split plot design. To raise crop suggested agronomic and plant protection measures were accomplished. Data were computed for the following morphological and physiological traits; Plant height, leaf area, leaf fresh weight, photosynthetic rate, transpiration rate, water potential, relative water contents, chlorophyll-*a*, chlorophyll-*b*, protein substances, total soluble sugars, stomata conductance, no of grain per cob, 100 grain weight and grain yield per plant.

Determination of chlorophyll contents: Chlorophyll contents (Chl *a, b*) were measured by following (Nagata & Yamashita, 1992). From 10 labeled plants per enter the plant leaf samples were collected. In 80% acetone one-gram fresh plant leave was pulverized using pestle and mortar and the resolution was centrifuged at 3000 rpm for 10 minutes. By using pipette, the supernatant was taken sensibly. Total 3ml supernatant was used to measure the absorbance at 663nm, 645nm, 505nm and 453nm wavelengths using spectrophotometer (Spectronic 21 D. Milton Roy).

The Chlorophyll *a* and *b* substances were determined in (mg/g f.wt) by following (Nagata & Yamashita, 1992). Calculations were made by using the following formulas:

$$\text{Chlorophyll A (mg/100ml)} = 0.999A663-0.0989A645$$
$$\text{Chlorophyll B (mg/100ml)} = 0.328A663+1.77A645$$

Determination of physiological attributes: Leaf relative water content was determined by following methodology devised by (Jones & Turner, 1978). Entirely prolonged leaves of identical size from every replicate were weighed instantly. All leave samples were saturated for 10 hours at room temperature weight was recorded. Then leaves were oven dried at 70°C for 48 hours and weight was recorded as dry weight. Relative H_2O content of leaf was then calculated in (%) using the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh wt. of leaf} - \text{Dry wt. of leaf} \times 100}{\text{Turgid wt. of leaf} - \text{Dry wt. of leaf}}$$

Fully matured leaf from top was detached at dawn for the measurement of water potential using a Scholander pressure cavity of Arimad-2-Japan (Scholander *et al.*, 1965). Leaf water potential was taken in (-Mpa). Completely soluble proteins were determined using methodology devised by (Ku *et al.*, 1979). Total soluble sugars were calculated using method of Laboratory (Steel & approach, 1997).

Analysis of variance with randomized complete block design under triplicated split plot design was used to calculate consequence difference in conducts for each trait studies. Analyzed the recorded data by principal component based biplot analysis. It is a data reduction method to elaborate the relationship in more than one character and to divide total variance of these original characters into uncontrolled new variables. Bi-plot analysis based principal component analysis indicated presence of genetic variability among studied genotypes under both normal and stress conditions which divided into four components. Genotype farther away from origin in positive region was good performer while genotype scattered towards negative quadrant was poor performer relative to origin of graph.

Statistical analysis

(Steel & Torrie, 1980) developed analysis of variance to calculate consequence of treatment differences in genotypes. Principal component based biplot analysis was used to analyzed the variations among genotypes against diverse saline environments for each character.

Table 1. Mean sum of squares with respective levels of significance for all of studied traits in maize at different salinity levels in field condition.

SOV	DF	PH	LA	LFW	*A*	*E*	Ψw	RWC	Chl *a*	Chl *b*	PROT	TSS	G*s*	GPC	100GW	GYPP
Replication	2	20.5	238	0.03	3.2	0.03	0.01	12.9	0.002	0.004	0.06	0.03	71	68	6.5	4
Treatment	3	70055.8*	296513*	116.4*	7236.3*	20*	2.28*	34745.5*	1.8*	1.4*	176*	18.1*	245532*	630901*	10132.3*	114048*
ER×Trt	6	100	842	0.15	26	0.15	0.01	60	0.02	0.01	0.14	0.2	202	379	22.4	15
Genotype	39	2516.7*	6873*	3*	135.1*	0.5*	0.08*	725*	0.03*	0.02*	2.15*	0.3*	5978*	12243*	117.7*	1778*
Trt×G	117	762*	2877*	1.1*	49.4*	0.3*	0.03*	291*	0.01*	0.01*	0.8*	0.1009*	2437*	5082*	54.2*	776*
ER×Trt×G	312	7110	28158	5.5	675	5.6	0	1729	1.05	0.4	5.04	5.9	7504	15781	668.2	481
Total	479															

* denotes highly significant differences ($p<0.05$)

Abbreviations: PH; plant height, LA; leaf area, LFW; leaf fresh weight, *A*; photosynthetic rate, *E*; transpiration rate, Ψw; water potential, RWC; relative water contents, Chl *a*; chlorophyll-*a* contents, Chl b^-; chlorophyll-*b* contents, Prot; protein contents, TSS; total soluble salts, G*s*; stomata conductance, GPC; No. of grains per cob, 100GW; 100 grain weight, GYPP; grain yield per plant.

Results

Morphological and physiological traits: Treatment, genotypes and genotype into treatment interaction at variable saline treatments were noted as significant ($p<0.05$) for morphological and physiological traits (Table 1). In current study, Principal component analysis transformed different morphological and physiological traits into fifteen components. Among these fifteen principal components, only two components PC_1 and PC_2 were used to develop biplot graph in all salinity environments as these components were recorded with more than one eigen value (Table 2) otherwise in control environment $S_{0.89\ dsm}{}^{-1}$ and least saline environment $S_{5.2\ dsm}{}^{-1}$, 1st seven components PC_{1-7} and 1st three components PC_{1-3} harbored eigen value more than one respectively. Eigen value is used as cut off value which is decisive for retaining the principal component for further study. Contribution of all the traits in saline environment $S_{0.89\ dsm}{}^{-1}$ was positive except grain yield per plant in PC_1, while in PC_2, water potential, transpiration rate, leaf area, photosynthetic rate, leaf fresh weight, chlorophyll-*b*, protein contents, total soluble sugars, grain yield per plant and stomata contents were contributing positively. In case of salinity $S_{5.2\ dsm}{}^{-}1$, $S_{6.7\ dsm}{}^{-1}$ and $S_{11\ dsm}{}^{-1}$ except water potential, all traits were contributing positively in PC_1 while in chlorophyll-*b*, chlorophyll-*a*, total soluble sugars, relative water contents, water potential, 100 grain weight and transpiration rate in PC_2 of $S_{5.2\ dsm}{}^{-1}$. Positive contribution was seen by chlorophyll-*a*, 100 grain weight, plant height, no of grain per cob, photosynthetic rate, relative water contents, transpiration rate, stomata conductance, water potential and total soluble sugar in PC_2 of saline environment $S_{6.7\ dsm}{}^{-1}$ while in PC_2 of $S_{11\ dsm}{}^{-1}$, 100-grain weight, chlorophyll-*b*, chlorophyll-*a* leaf area, grain yield per plant, leaf fresh weight and photosynthetic rate showed positive contribution.

Consequently, performance of UAC-0020 and UAC-0036 was well and reported as tolerant genotypes in highly saline environment $S_{11\ dsm}{}^{-1}$ for approximately all traits while UAC-0028 and UAC-0033 performed poor even at less saline environment $S_{4\ dsm}{}^{-1}$. PCA biplot analysis remained best to select better and poor parents. Photosynthetic rate, 100-grain weight, Plant height, grain yield/ plant and number of grains per cob were proved as best traits for selection criteria.

Biplot graph was developed between PC1 and PC2 in controlled condition $S_{0.89\ dsm}{}^{-1}$ to elaborate the variation in all the genotypes for different morphological and physiological traits (Fig. 1). These components contributed 32.74% in variation collectively while individually PC1 contributed 20.31% and PC2 contributed 12.43% to explain the performance of genotypes. Plant height and spoke lengths of grain yield/ plant were little bit high as compare to rest of traits which was indication that these traits has high discriminating power to explain the response of genotypes. Huge angle between these vectors explain their different response towards genotypes individually. Protein contents, water potential, relative water contents and no of grain per cob also harbored high discriminating power and individual response for the elaboration of performance of genotype.

Interactions between different treatments: 64.37% interaction of PC1 and PC2 was reported in biplot developed under salinity $S_{5.2\ dsm}{}^{-1}$ to reveal variation in 40 genotypes. Individually, PC_1 and PC_2 showed 56.08 and 8.30% interaction respectively. All the genotypes placed on different location with respect to their means on graph but high mean genotypes UAC-0036, UAC-0020, UAC-0024 and placed in positive direction towards the heads of vector between photosynthetic rate and total soluble sugars with high variability termed as tolerant (Fig. 2). Low mean genotypes UAC-0041, UAC-0033 and UAC-0028 scattered in negative quadrant towards the tail traits vectors revealed comparative poor adaptability termed as susceptible (Fig. 2). Transpiration rate, 100 grain weight, chlorophyll-*a* and leaf area remained more discriminating and highly responsive towards genotypes in saline environment $S_{5.2\ dsm}{}^{-1}$ as these traits were longer with huge angle among Biplot analysis for saline environment $S_{6.7\ dsm}{}^{-1}$ showed 64.31% interaction for variation to explain the behavior of genotypes on graph for different morphological and physiological traits. 57.43% and 6.89% interaction (Fig. 3). Leaf area, grain yield per plant and leaf fresh weight remained best indicator for specifying tolerant genotypes UAC-0036, UAC-0024 and UAC-0020 as these genotypes slided in positive side of graph towards heads of traits vector with high mean of concerned traits (Fig. 3). UAC-0048, UAC-0041, UAC-0033 and UAC-0028 fall in negative region of graph having reduced variability grouped as susceptible genotypes (Fig. 3) while other genotypes positioned differently with respect to variability in positive or negative region of graph. Water potential and protein contents were making an angle of 180 which was indication of their huge individual response towards genotypes in saline environment $S_{6.7\ dsm}{}^{-1}$.

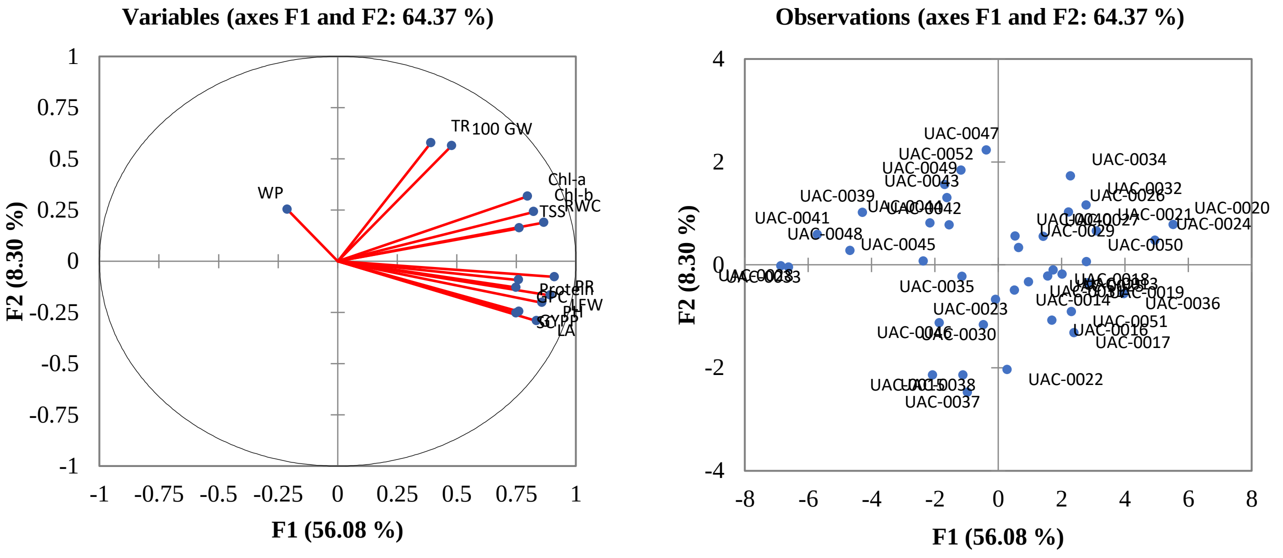

Fig. 1. PCA Biplot for normal treatment $S_{0.89\ dsm}$.

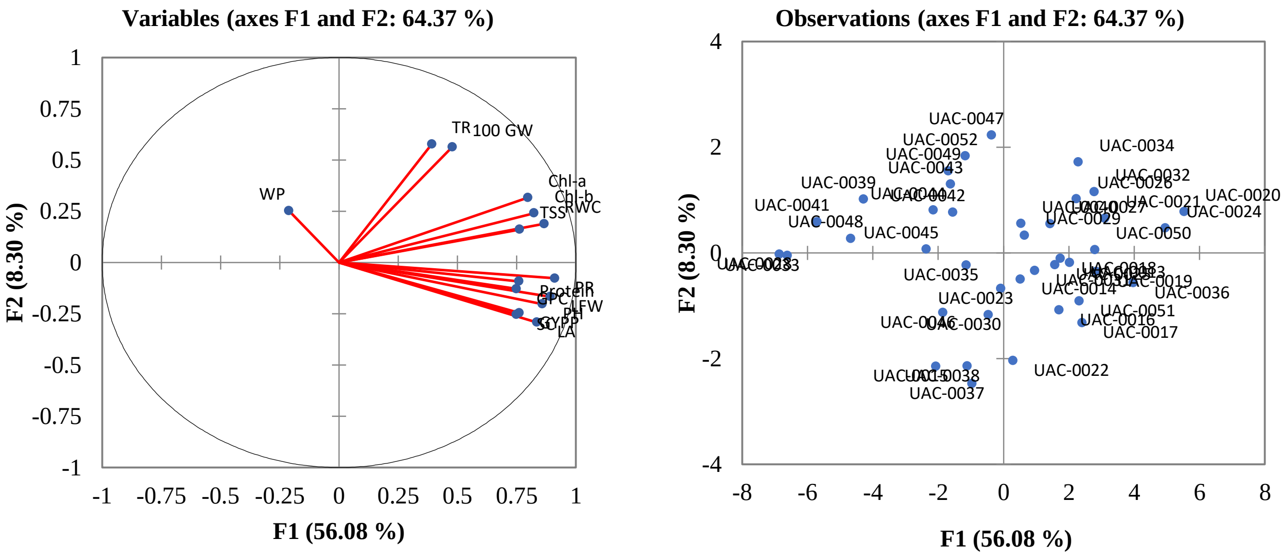

Fig. 2. PCA Biplot for stress treatment $S_{5.2\ dsm}{}^{-1}$.

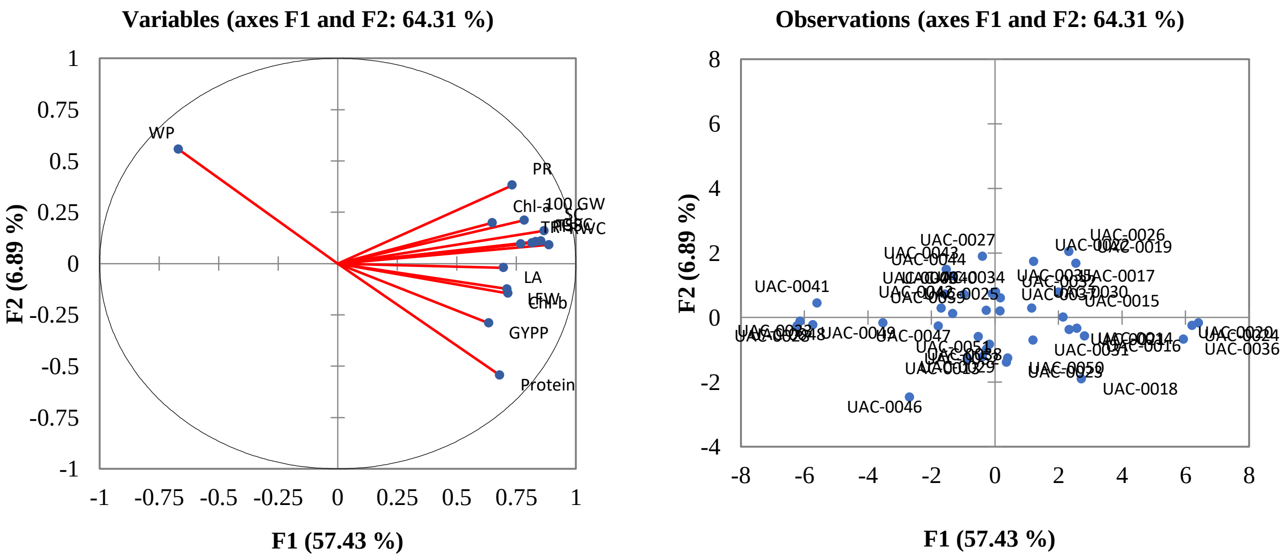

Fig. 3. PCA Biplot for stress treatment $S_{6.7\ dsm}{}^{-1}$.

Table 2. PC values, Eigenvalues, Percent Variance and Cumulative Percent of variance for different saline environments on the basis of all studied traits in field conditions.

Traits/Environments	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC(S)	Eigen values	% Variance	Cumulative % of variance
	PH										
S0.89 dsm-	0.09	-0.7	-0.10	0.27	-0.06	0.22	0.07	PC1	3.0469	20.3126	20.3126
S5.2 dsm-	0.86	-0.2	-0.03	0.11	-	-	-	PC1	8.4116	56.0772	56.0772
S6.7 dsm-	0.82	0.10	-0.26	-	-	-	-	PC1	8.6138	57.4251	57.4251
S11 dsm-	0.82	-0.2	-	-	-	-	-	PC1	8.6947	57.9648	57.9648
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	LA										
S0.89 dsm-	0.38	0.03	-0.30	-0.54	0.31	0.19	-0.24	PC2	1.8644	12.4296	32.7422
S5.2 dsm-	0.84	-0.3	-0.06	0.05	-	-	-	PC2	1.2444	8.2960	64.3732
S6.7 dsm-	0.70	-0.1	0.20	-	-	-	-	PC2	1.0332	6.8882	64.3133
S11 dsm-	0.78	0.03	-	-	-	-	-	PC2	1.1351	7.5676	65.5324
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	LFW										
S0.89 dsm-	0.68	0.07	-0.32	0.05	0.15	0.08	0.01	PC3	1.5867	10.5779	43.3201
S5.2 dsm-	0.89	-0.1	-0.05	-0.04	-	-	-	PC3	1.0516	7.0108	71.3841
S6.7 dsm-	0.71	-0.1	-0.41	-	-	-	-	PC3	0.92	6.1485	70.4618
S11 dsm-	0.81	0.14	-	-	-	-	-	PC3	0.8941	5.9609	71.4933
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	PR										
S0.89 dsm-	0.11	0.27	0.61	0.06	0.28	0.11	0.54	PC4	1.3620	9.0801	52.4002
S5.2 dsm-	0.91	-0.1	0.11	0.11	-	-	-	PC4	0.93	6.2526	77.6367
S6.7 dsm-	0.73	0.38	-0.09	-	-	-	-	PC4	0.7842	5.2283	75.6901
S11 dsm-	0.68	0.35	-	-	-	-	-	PC4	0.7660	5.1064	76.5996
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	TR										
S0.89 dsm-	0.54	0.23	0.39	-0.22	-0.44	0.23	0.00	PC5	1.0644	7.0958	59.4959
S5.2 dsm-	0.39	0.58	-0.13	-0.44	-	-	-	PC5	0.7285	4.8565	82.4932
S6.7 dsm-	0.77	0.10	0.15	-	-	-	-	PC5	0.6892	4.5945	80.2845
S11 dsm-	0.75	-0.1	-	-	-	-	-	PC5	0.6800	4.5332	81.1328

Table 2. (Cont'd.).

Environments/Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC(S)	Eigen values	% Variance	Cumulative % of variance
						WP					
S0.89 dsm-	0.54	0.42	0.22	0.27	0.02	-0.12	-0.30	PC6	1.0542	7.0278	66.5237
S5.2 dsm-	-0.2	0.25	0.83	0.37	-	-	-	PC6	0.5236	3.4909	85.9841
S6.7 dsm-	-0.6	0.56	0.14	-	-	-	-	PC6	0.5666	3.7772	84.0618
S11 dsm-	-0.8	-0.3	-	-	-	-	-	PC6	0.5045	3.3634	84.4962
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
						RWC					
S0.89 dsm-	0.49	-0.3	0.14	0.05	0.13	0.61	0.14	PC7	1.0015	6.6764	73.2001
S5.2 dsm-	0.87	0.19	-0.10	0.11	-	-	-	PC7	0.4429	2.9529	88.9370
S6.7 dsm-	0.89	0.09	0.13	-	-	-	-	PC7	0.4990	3.3266	87.3884
S11 dsm-	0.63	-0.4	-	-	-	-	-	PC7	0.4924	3.2825	87.7786
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
						Ch-a					
S0.89 dsm-	0.51	-0.2	0.00	-0.11	-0.42	-0.05	0.10	PC8	0.8542	5.6947	78.8948
S5.2 dsm-	0.80	0.32	0.22	-0.11	-	-	-	PC8	0.4147	2.7649	91.7019
S6.7 dsm-	0.65	0.20	0.56	-	-	-	-	PC8	0.4047	2.6981	90.0865
S11 dsm-	0.81	0.17	-	-	-	-	-	PC8	0.4620	3.0803	90.8589
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
						Ch-b					
S0.89 dsm-	0.42	0.09	-0.32	0.58	-0.27	-0.31	0.20	PC9	0.7406	4.9371	83.8318
S5.2 dsm-	0.82	0.24	0.08	-0.27	-	-	-	PC9	0.3304	2.2026	93.9045
S6.7 dsm-	0.72	-0.1	0.28	-	-	-	-	PC9	0.3853	2.5685	92.6549
S11 dsm-	0.67	0.23	-	-	-	-	-	PC9	0.2991	1.9937	92.8527
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
						PROT					
S0.89 dsm-	0.13	0.51	-0.01	0.49	-0.01	0.40	-0.08	PC10	0.6833	4.5556	88.3875
S5.2 dsm-	0.76	-0.1	-0.17	0.42	-	-	-	PC10	0.2683	1.7887	95.6933
S6.7 dsm-	0.68	-0.5	0.07	-	-	-	-	PC10	0.3050	2.0333	94.6882
S11 dsm-	0.84	-0.1	-	-	-	-	-	PC10	0.2716	1.8109	94.6636

Table 2. (Cont'd.).

Environments/Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC(S)	Eigen values	% Variance	Cumulative % of variance
	TSS										
S0.89 dsm-	0.53	0.07	0.42	-0.38	-0.11	-0.33	-0.03	PC11	0.5370	3.5797	91.9672
S5.2 dsm-	0.76	0.16	0.08	-0.01	-	-	-	PC11	0.2052	1.3681	97.0614
S6.7 dsm-	0.83	0.11	0.15	-	-	-	-	PC11	0.2267	1.5112	96.1994
S11 dsm-	0.79	-0.2	-	-	-	-	-	PC11	0.2542	1.6948	96.3584
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	SC										
S0.89 dsm-	0.65	0.06	-0.45	-0.09	-0.07	0.05	-0.13	PC12	0.4798	3.1990	95.1662
S5.2 dsm-	0.75	-0.2	-0.07	0.08	-	-	-	PC12	0.1644	1.0962	98.1576
S6.7 dsm-	0.87	0.16	-0.22	-	-	-	-	PC12	0.1873	1.2490	97.4483
S11 dsm-	0.81	-0.1	-	-	-	-	-	PC12	0.1735	1.1565	97.5150
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	GPC										
S0.89 dsm-	0.58	-0.2	-0.14	-0.03	0.37	-0.34	0.45	PC13	0.4247	2.8312	97.9974
S5.2 dsm-	0.75	-0.1	0.25	-0.14	-	-	-	PC13	0.1299	0.8658	99.0234
S6.7 dsm-	0.85	0.11	-0.07	-	-	-	-	PC13	0.1650	1.1003	98.5486
S11 dsm-	0.80	-0.3	-	-	-	-	-	PC13	0.1586	1.0570	98.5720
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	100GW										
S0.89 dsm-	0.32	-0.1	0.34	0.32	0.48	-0.17	-0.45	PC14	0.2016	1.3437	99.3411
S5.2 dsm-	0.48	0.56	-0.32	0.45	-	-	-	PC14	0.1034	0.6891	99.7125
S6.7 dsm-	0.78	0.21	-0.32	-	-	-	-	PC14	0.1316	0.8771	99.4257
S11 dsm-	0.58	0.57	-	-	-	-	-	PC14	0.1322	0.8814	99.4534
Environments/Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC(S)**	**Eigen values**	**% Variance**	**Cumulative % of variance**
	GYPP										
S0.89 dsm-	-0.1	0.71	-0.42	-0.15	0.15	0.08	0.26	PC15	0.0988	0.6589	100.0000
S5.2 dsm-	0.76	-0.2	0.23	-0.28	-	-	-	PC15	0.0431	0.2875	100.0000
S6.7 dsm-	0.63	-0.3	0.10	-	-	-	-	PC15	0.0861	0.5743	100.0000
S11 dsm-	0.81	0.11	-	-	-	-	-	PC15	0.0820	0.5466	100.0000

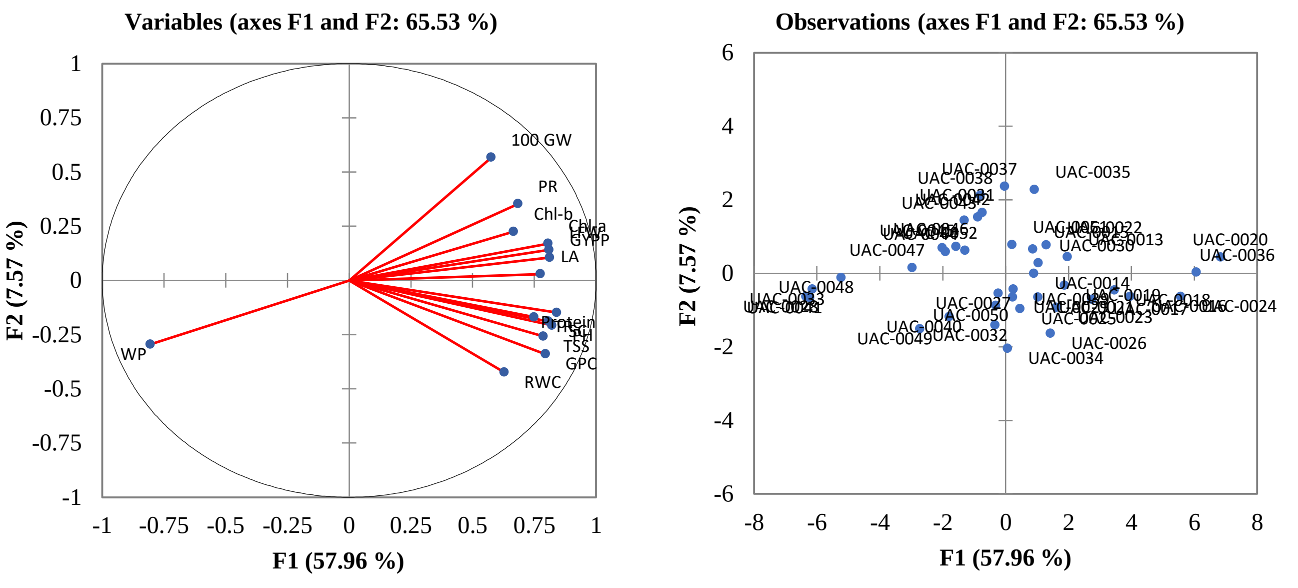

Fig. 4. PCA Biplot for stress treatment $S_{11\ dsm}^{-1}$.

Principal component analysis: Collective interaction between PC1 and PC2 towards variation shown by biplot of high saline environment $S_{11\ dsm}^{-1}$ was 65.53%. Individually, PC_1 and PC_2 contributed 57.96% and 7.57% interaction towards variation in genotypes. Genotypes UAC-0020 and UAC-0036 secured position in positive quadrant towards heads of vectors leaf area, leaf fresh weight, grain yield/ plant with high variability and good adaptability while UAC-0024 attracted by the high response of relative water contents and no of grain per cob in positive quadrant of graph away from origin termed as tolerant genotypes. Genotypes UAC-0048, UAC-0041, UAC-0033 and UAC-0028 fall in negative quadrant towards tail of traits vector showed poor adaptability in high stress treatment $S_{11\ dsm}^{-1}$ known as susceptible (Fig 4) while rest of genotypes scattered in different regions of graph with respect to response of different traits. Spoke length of water potential, 100 grain weight and relative water contents was longer showing high discriminating power for genotypes.

Discussion

The present study revealed that sensitive genotypes were badly affected while tolerant genotypes performed well during the salinity stress. Salinity $S_{5.2\ dsm}^{-1}$ produced undesirable effects on protein contents, chlorophyll-*a* and chlorophyll-*b* of susceptible genotypes (UAC-0028; UAC-0048). However, the performance of tolerant (UAC-0024; UAC-0020) genotypes were better in chlorophyll-*b*, chlorophyll-*a* and protein contents under salinity $S_{6.7\ dsm}^{-1}$. Similar results about chlorophyll-*b* and chlorophyll-*a* were reported by (Doğan *et al.*, 2012) that salinity decreases the contents of chlorophyll-*b*, chlorophyll-*a* and this reduction mainly depends on plant species regarding salinity tolerance capacity. (Mumtaz *et al.*, 2021) noted that salinity caused accumulation of ROS (reactive oxygen species) in cells by which membrane, nucleic acids, lipids and proteins are destroyed.

Harmful effects of salt stress have also been detected on proline contents, sugar contents and relative water contents in sensitive genotypes. Current results had similarity with the findings of (Yun *et al.*, 2018). All these studies concluded from their studies that salinity stress triggered significant decrease in plenty of plant parameters like potassium concentration, relative water contents, chlorophyll contents, nitrate reductase activity in pea and other plants. Experimental results of (Mumtaz *et al.*, 2019) explained that physiological parameters of maize crop were affected by salinity stress which caused a prominent decrease in leaf area, shoot length, relative water contents, fresh and dry weight.

Osmoregulation is most frequent process occurs in salt tolerant species that has capability to control the salinity stress (Mumtaz *et al.*, 2019). Photosynthesis is considered as a growth controlling vital factor and it yields organic osmotic, which have main role in osmoregulation process (Kamran *et al.*, 2020; Rana *et al.*, 2020; Saleem *et al.*, 2020). Osmoregulation has key role in adaptation to salinity stress (Kaleem and Hameed, 2021; Mumtaz *et al.*, 2021; Waseem *et al.*, 2021) and drought (Ghafar *et al.*, 2021). (Kaleem & Hameed, 2021) stated that photosynthesis rate is less inhibited in salt tolerant genotypes. It was also reported that growth related all activities under saline condition functioning properly with the production of proteins, free proline and total soluble sugars (Perveen & Nazir, 2018). A plenty of species gather proline and glycine betaine in reaction to salt stress and their gathering may help in controlling salt stress (Khodarahmpour, 2011). Similar results were shown by current study as tolerant genotypes (UAC-0020; UAC-0024) were noted with increased production of proline, proteins and sugar contents even under high salinity S_{11dsm}^{-1}. Water potential decreased without decline in cell turgor in osmotic adjustment which is due to increase in solute contents (Pandolfi *et al.*, 2016).

Reduction in leaf water potential is most apparent effect of salt stress on growth of maize which varies among maize salinity tolerance species or genotypes.

(Yun *et al.*, 2018) reported that plant shortage of water occurs before ion imbalance and toxicity. These findings showed similarity with the results of present research that salt tolerant UAC-0024 and UAC0036 genotypes shown high water potential and salt susceptible genotypes (UAC-0028; UAC-0041) displayed reduced water potential under salinity $S_{6.7\ dsm}{}^{-1}$. In current investigation, maximum leaf area and plant height were noted in salt tolerant genotypes (UAC-0020; UAC-0024) while minimum in salt susceptible genotypes (UAC-0028: UAC-0033) when maximum salt stress medium ($S_{11\ dsm}{}^{-1}$) was applied. (Kaleem and Hameed, 2021) have also reported such type of findings that salinity stress reduced leaf area and plant height. The drastic impacts of salinity nutrients deficiencies, ion cytotoxicity and reduced external water potential (Khayatnezhad & Gholamin, 2011).

Low photosynthesis, ion imbalance and toxicity in plants occur due to salinity and photosynthesis directly associated to water potential, stomata conductance, chlorophyll contents and transpiration. (Perveen & Nazir, 2018) stated that low or moderate salinity is responsible for decrease growth, which linked with reduced photosynthetic area instead of a reduced photosynthesis per unit leaf area. It was also reported that at maximum salinity level, water imbalance decreased the stomata conductance while toxic ions produced non-stomata factors (Baghel *et al.*, 2019). As result of these reactions, reduction occurred in leaf photosynthesis (Tajdoost *et al.*, 2007). The similar results of present investigation stated that salt tolerant genotypes exhibited higher photosynthetic rate and stomata conductance while salt susceptible genotypes displayed lower photosynthetic rate and stomata conductance.

Comparing drought stress with salt stress, transpiration ratio might be though the better criterion (Waseem *et al.*, 2021). Because, one of the stress induced by different salts is osmotic stress and leaf transpiration rate in salinity tolerant cultivars can be improved to increase their salt resistance under salinity stress (Agami, 2014). Many studies showed that transpiration rate is the managing factor in the salt ion accumulation in plant shoot. In current research, susceptible genotypes UAC-0028 and UAC-0033 were reported with low transpiration while tolerant UAC-0020 and UAC-0024 genotypes were noted with high transpiration rate under salinity $S_{6.7\ dsm}{}^{-1}$.

Susceptible UAC-0028, UAC-0048 genotypes were reported with reduced yield related traits while tolerant UAC-0020, UAC-0024 genotypes were observed with increased yield. (Kaya *et al.*, 2013) favored the current results that at high salt stress condition, decreased grain weight was noted; due to low photosynthetic efficiency under saline environment.

Conclusions

According to our results we concluded that different traits interact with environments differently. Plant height, 100 grain weight, grain yield/ plant, photosynthetic rate and no of grain per cob were reported as best salinity tolerant indicators. Performance of UAC-0020 and UAC-0036 genotypes was good, even in maximum salinity conditions $S_{11\ dsm}{}^{-1}$ while UAC-0033 and UAC-0028 was fewer performers even in reduced salinity level $S_{5.2\ dsm}{}^{-1}$. Biplot analysis is verified as best procedure for manipulation of GEI. Screening of present genotypes stated meaningful to deliver raw material for salinity tolerant breeding programs.

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