Pak. J. Agri. Sci., Vol. 52(2), 339-347; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

AMMI BIPLOT ANALYSIS FOR COMPARATIVE EVALUATION OF MAIZE (*Zea may* L.) GENOTYPES UNDER DIFFERENT SALINE ENVIRONMENTS

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Salinity is amongst the leading abiotic stresses which adversely affects the crop productivity. It brings multiple disorders in normal plant functionality and suppresses yield up to drastic level. Under subjection to salinity, maize crop interacts differently with different environmental conditions and shows different responses. Plants interact positively, negatively and sometimes no interaction with environment for various plant traits. Genotype × Environment Interaction (GEI) of maize genotypes with three salinity levels was studied for some selected morpho-physiological traits i.e. root shoot ratio, cholorphyll *a*, cholorphyll *b*, β-carotenoids, ascorbic acid, root density and leaf temperature using AMMI biplot analysis. OH41, OH28, OH8, USSR40 and WM13RA for root shoot ratio (RSR) and A556 for β-carotenoids (BCart) were proved stable genotypes whereas; all other genotype interacted with stressed conditions either positively or negatively. Ascorbic acid and β-carotenoid contents were proved good indicators of salt tolerance in maize plant. AMMI biplot analysis proved to be an effective technique for study of GEI. Stable genotypes can be regarded for further breeding program.
Keywords: Genotype×Environment Interaction, AMMI biplot, ascorbate, chlorophyll contents, reactive oxygen species

INTRODUCTION

Salinity is a major drastic environmental stress amongst abiotic stresses. Influence of salinity is prominent on agricultural crops especially in arid and semiarid areas due to restricted rainfall, excessive evapo-transpiration and prevalence of heat stress. Globally more than 8% area is salt affected (Singh, 2009). In Pakistan 6.67mha area is affected with salinity stress (GOP, 2010). Salinity is critical problem in Pakistan due to extensive use of irrigation water by flooding which raises underground water table (Alam *et al*., 2000). Higher level of salinity adversely affects the photosynthesis, carbohydrate metabolism, nitrogen fixation and respiration (Chen *et al*., 2008). Along with high accumulation of salts or ions, salinity is the cause of many other stresses as: (a) water uptake by plants is reduced due to imbalance of osmotic gradient between soil and plants (Brodribb and Hill, 2000), (b) micronutrient deficiencies and imbalance, (c) ion toxicity, (d) disproportionate production of reactive oxygen species (ROS) which results in enhanced oxidative damage to plants. ROS includes hydrogen peroxide (H_2O_2), superoxide (O_2^-), singlet oxygen ($^1O_2$), alkoxyl radical (RO) and hydroxyl group (OH^-) which accumulates in plants and damage the cell membranes (Hernandez *et al*., 2001). Electrons are transferred to the oxygen from PS1(photosystem-I) due to inhibited CO_2 fixation, reduction in $NADP^+$ oxidation and over reduction of ferrodoxin, to produce superoxide radicle (O_2^-) following Mehler reaction (Amirjani, 2010). Higher concentration of ROS causes oxidative damage along-with uncharacteristic cell membrane permeability and fluidity due to increased lipid breakdown, amino acid modification at certain sites, peptide chain fragmentation, inactivation of enzymes, increased susceptibility to proteolysis and change in electric charges (Vinocur and Altman, 2005; Sharma *et al*., 2012). ROS further enforce oxidation of deoxyribose, breakdown of DNA strands, alteration in nitrogenous bases and removal of nucleotides (Sharma *et al*., 2012).

Maize is amongst the top three cereals and expected to become leading in near future. So, it is important to study the effects of salinity on maize for identification of proper germplasm to be used in breeding programs as exploited in wheat (Ali *et al*., 2002), soybean (Kamal *et al*., 2003) and cotton (Aslam *et al.,* 2013). Performance of genotypes is relatively altered with change in environment and this response to environment by genotype is termed as Genotype × Environment Interaction (GEI). Greater value of GEI hinders the normal performance of genotype (Comstock and Moll, 1963). Screening of already present or newly developed genotypes for salinity tolerance and selection of superior genotypes by studying the interaction of genotypes with saline environments will be a solution to resolve the issue of salinity stress on short term basis and will pave way for further breeding programs. This study was conducted to select most stable genotypes for various important traits through estimation of GEI of maize genotypes under different saline environments and normal condition. Secondary focus of this study is to find out most interactive/discriminative salinity tolerance incorporating physiological indicators in maize. GEI of maize genotypes

was studied at seedling stage in solution culture because superior performance at seedling establishment stage will ultimately results in good performance during late vegetative and reproductive growth stages (Willenborg *et al*., 2005).

MATERIALS AND METHODS

Research experiment was conducted in greenhouse of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Punjab, Pakistan (latitude = 31°- 30' N, longitude = 73°- 10' E and altitude = 184.4 m). Total 30 maize genotypes were collected from different maize research stations. Seed were sown in germination trays filled with sieved and washed sand. After 15 days of sowing, uniform maize seedlings were transplanted to solution culture. Solution culture consisted of sheet of polystyrene floating on ½ strength Hoagland's nutrient solution medium with proper aeration system (Hoagland and Arnon, 1950). Strength of Hoagland nutrient solution was improved up to full after two days. Seedlings were allowed to stabilize for three days in hydroponic medium. After stabilization of seedlings in solution culture, stress was imposed by using NaCl through 3/4 applications as 0 dS/m (T1, Control), 8 dS/m (T2) and 16 dS/m (T3) of NaCl.

Experiment was carried out by following triplicated completely randomized design in factorial arrangement. pH of the hydroponic solution was maintained at 6.0±0.5 on daily basis by using NaOH and HCl. After 35 days of transplanting, seedlings were harvested for the determination of selected plant traits i.e. root shoot ratio (RSR), Chlorophyll.A (C_HA; mg/100ml), Chlorophyll.B (C_HB; mg/100ml) and β-Carotenoids (BCart; mg/100ml) contents, ascorbic acid (AA; µg/ml), root density (RD; mm cm^{-2}) and leaf temperature (LT; °C). Chlorophyll contents and β-carotenoids were determined by using procedure devised by Nagata and Yamashita (1992). Ascorbic acid contents were measured by using procedure devised by Kampfenkel *et al*. (1995).

***Statistical Analysis*:** Data were analyzed for significance of treatment differences among genotypes using analysis of variance devised by Steel and Torie (1980). Tukey's HSD mean comparison was used as post-hoc test for genotypic mean comparison under three different saline environments. AMMI (*Additive* main effects and *multiplicative interaction*) biplot analysis was used for studying GEI as it was recommended by Zobel *et al*. (1998) to study the GEI in soybean (*Glycine max* L. Merr).

RESULTS

Analysis of variance (Table 1) shows significant differences among genotypes, treatments and genotype × treatment interaction (GEI). Mean values of forty maize genotypes for RSR, C_HA, C_HB, BCart, AA, RD and LT under three different salinity treatments are presented in Table 2. In the last two rows of mean values (Table 2) standard error for mean comparison and critical value for mean comparison based on Tukey's HSD test were given for genotypic mean comparison. Mean comparison showed that OH-33-1 had highest mean value for RSR under T1, for C_HA under T2 and T3, and for C_HB under T2 and T3. OH28 had lowest mean value for C_HA under T2, for C_HB under T2 and T3 (Table 2). Genotypic means showed that performance of genotypes were not consistently superior for all traits and not consistently poor for all traits which highlighted the use of proper interactive approach which can give the performance output of genotypes based on interactiveness of three saline environments for specific trait. So, we preferred the use of AMMI biplot for interactive evaluation of genotypes under saline environments.

Distance from origin (0, 0) in AMMI biplot shows the interaction of genotype over environments / environment over genotypes. AMMI biplot analysis for RSR under three salinity levels (T1, T2 and T3) explained that PC1 and PC2 collectively had highest value for interaction (86%). AMMI biplot between PC1 and PC2 represented most precise and reliable results than other PCs due to contribution of highest interaction percentage (Fig. 1.1). OH41, OH28, OH8, USSR40 and WM13RA genotypes were present near the origin which reflected that these genotypes were not sensitive to environmental interaction. Environmental vector with longer spoke length had stronger interactive force. Graph for RSR showed that T1 had longer spoke length and stronger interactive force followed by T2 and T3 (Fig. 1.1). Interaction of environment and genotype can be determined by plotting project for genotype marker on environment vector. If genotype projection falls on environmental vector then concerned genotype has positive interaction with

Table 1. Mean square analysis of maize genotypes under different salinity levels

Source of Variation	Df	RSR	C_H.A (mg/ 100ml)	C_H.B (mg/100ml)	BCart (mg/100ml)	AA (µg/ml)	RD (mm cm^{-2})	LT (°C)
Treatment (T)	2	0.093*	20.130**	26.708**	0.648**	22.370**	21.260**	54.324**
Genotype (G)	29	0.266**	2.212**	2.939**	0.167**	0.401**	1.764**	13.869**
T x G	58	0.180**	1.340**	1.806**	0.108**	0.218**	1.548**	8.880**
Residual	180	0.027	0.027	0.040	0.007	0.018	0.059	0.570

Abbreviations: RSR=Root shoot ratio; C_H.A=Cholorphyll.A; C_H.B=Cholorphyll.B; BCart=β-carotenoids; AA=Ascorbic acid; RD=Root density; LT=Leaf temperature.

Table 2. Mean values for studied traits of maize genotypes under three different salinity treatments

Genotypes	Root Shoot Ratio			Chlorophyll, a			Chlorophyll, b			Beta-Carotenoids			Ascorbic Acid			Root Density			Leaf Temperature		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
M14	1.64	1.27	1.19	2.07	1.55	1.34	2.49	1.76	1.35	0.45	0.72	0.49	1.50	1.91	0.95	2.45	2.40	1.46	21.29	23.50	24.50
A50-2	1.33	1.83	1.84	2.65	0.85	0.66	2.85	1.02	0.66	0.50	0.52	0.39	1.56	1.70	0.72	2.63	2.50	1.70	20.16	22.70	23.44
A239	1.16	1.97	1.82	1.00	0.61	0.35	1.19	0.73	0.37	0.19	0.54	0.39	1.46	2.16	1.55	1.25	1.70	1.20	19.67	20.82	21.44
A427-2	1.12	1.00	1.12	1.49	0.44	0.22	1.94	0.57	0.22	0.49	0.32	0.15	2.27	2.09	1.45	2.03	1.80	1.90	23.03	21.53	26.55
A495	1.15	1.38	1.05	1.96	1.85	1.56	2.20	2.03	1.55	0.33	0.64	0.48	1.70	1.74	0.79	2.12	1.35	1.00	22.20	24.14	23.50
A509	1.23	1.49	1.17	2.79	1.33	1.20	3.14	1.06	1.20	0.40	0.70	0.48	1.43	2.12	1.84	1.76	2.00	1.20	24.72	24.35	22.05
A521-1	1.04	1.02	1.16	1.35	0.27	1.01	1.51	0.46	1.00	0.81	0.19	0.12	2.02	2.61	1.39	2.78	1.50	1.04	24.02	22.12	24.42
A545	1.44	1.22	1.23	2.41	0.28	0.97	2.59	0.40	0.97	0.55	0.32	0.12	1.31	1.56	0.88	2.39	2.18	0.70	25.50	23.45	24.15
A556	1.63	1.28	1.33	0.97	0.70	0.51	1.25	0.89	0.51	0.46	0.43	0.28	2.03	1.79	0.73	5.06	2.26	0.98	22.68	23.00	21.16
A638	1.20	1.54	1.40	2.92	0.77	0.56	3.58	0.90	0.55	0.09	0.42	0.25	2.66	1.96	0.92	3.70	1.47	1.00	21.89	22.50	22.51
AES204	1.48	1.22	1.06	2.62	0.28	0.16	2.84	0.30	0.16	0.49	0.21	0.09	1.85	2.11	1.44	2.53	1.20	1.43	21.71	22.71	24.44
Antigua-2	1.23	1.06	1.29	2.41	0.37	0.20	2.44	1.42	0.20	0.55	0.27	0.10	2.06	1.93	0.92	2.24	0.95	2.07	23.73	23.50	22.40
OH8	1.24	1.16	1.50	2.04	0.31	0.88	1.77	0.56	1.07	0.60	0.02	0.04	2.12	1.74	0.76	1.34	1.28	0.60	27.18	23.20	21.36
OH28	1.29	1.33	1.34	2.22	0.18	0.14	2.32	0.20	0.14	0.55	0.17	0.09	1.97	2.03	1.03	5.01	1.11	1.16	23.26	23.15	19.97
OH33-1	2.12	1.27	1.20	1.18	2.82	2.46	1.41	4.35	2.45	0.55	0.09	0.07	1.51	2.02	1.00	2.05	1.40	1.06	25.25	23.20	20.67
OH41	1.32	1.44	1.01	0.93	1.01	0.70	0.84	1.12	0.68	0.28	0.43	0.21	1.91	1.92	0.94	2.02	1.50	1.53	23.37	24.45	21.81
OH54-3A	1.07	1.27	0.97	1.20	1.29	1.09	1.25	1.55	1.10	0.30	0.44	0.22	1.80	1.82	0.85	2.44	1.30	1.26	26.42	25.76	21.38
W64SP	0.88	1.28	1.29	2.51	2.06	1.75	2.76	2.27	1.74	0.47	0.54	0.34	1.60	1.95	0.95	2.86	1.80	1.33	23.22	25.36	24.43
W64TMS	1.86	1.09	1.39	0.84	1.07	0.84	0.85	1.23	0.84	0.20	0.33	0.12	1.83	2.12	1.17	4.38	1.73	1.75	24.16	27.34	22.10
WM13RA	1.32	1.28	1.30	2.40	0.46	0.20	2.69	0.63	0.20	0.37	0.45	0.27	1.46	1.93	0.94	1.27	1.30	1.96	23.19	26.52	20.60
WF#9	1.32	0.76	1.36	1.30	2.27	1.95	1.42	1.40	1.94	0.57	0.72	0.26	1.48	2.03	1.02	2.13	2.05	1.71	25.30	28.53	23.55
WFTMS	1.02	1.15	1.14	1.30	0.46	0.25	1.53	0.66	0.25	0.42	0.28	0.10	2.03	2.74	1.64	2.02	1.80	1.19	25.63	24.45	22.25
W187R	1.40	1.17	1.09	0.89	0.65	0.38	0.77	0.88	0.38	0.44	0.49	0.30	1.69	2.00	0.93	1.67	1.83	1.13	25.35	25.60	21.60
W10	1.63	1.44	0.85	1.80	0.22	0.09	1.73	0.25	0.09	0.54	0.17	0.13	2.42	1.99	0.96	2.12	1.44	0.76	24.06	26.50	22.66
WA3748	1.15	1.48	1.12	1.27	0.47	0.23	1.34	0.68	0.23	0.43	0.34	0.19	2.15	2.00	0.96	2.51	0.59	1.40	24.00	27.64	22.22
W82-3	0.78	1.52	1.23	1.48	1.15	1.93	1.85	1.28	1.92	0.80	0.25	1.29	2.06	2.02	1.00	0.36	0.70	1.31	24.44	23.91	26.15
K55 TMS	1.19	0.99	1.11	2.83	1.50	1.35	3.28	1.73	1.34	0.30	0.59	0.28	1.83	2.14	1.17	1.02	1.80	1.20	27.61	27.56	25.28
G.P.F.9	1.20	1.50	1.22	2.58	0.28	0.19	3.28	0.57	0.19	0.20	0.05	0.04	1.99	2.11	1.35	2.07	1.20	2.44	23.52	27.80	23.83
USSR40	1.21	1.32	1.21	1.34	2.82	2.40	1.69	4.07	2.43	0.14	0.11	0.14	2.11	1.98	0.97	1.28	1.65	2.16	23.85	24.73	22.52
USSR41	0.41	1.13	0.95	0.40	1.81	1.64	0.47	2.03	1.65	0.20	0.58	0.33	1.56	2.98	1.62	0.83	1.03	0.90	27.00	24.22	25.24
*St. Error	0.15	0.11	0.13	0.14	0.10	0.15	0.11	0.20	0.16	0.06	0.09	0.03	0.11	0.12	0.09	0.32	0.08	0.10	0.99	0.31	0.25
**Cr.Val.	0.59	0.46	0.53	0.53	0.40	0.62	0.43	0.80	0.63	0.23	0.38	0.14	0.43	0.49	0.35	1.24	0.34	0.40	3.88	1.23	0.98

* St. Error; Standard Error for Mean Comparison, ** Cr. Val; Critical Value for Mean Comparison

concerned environment.

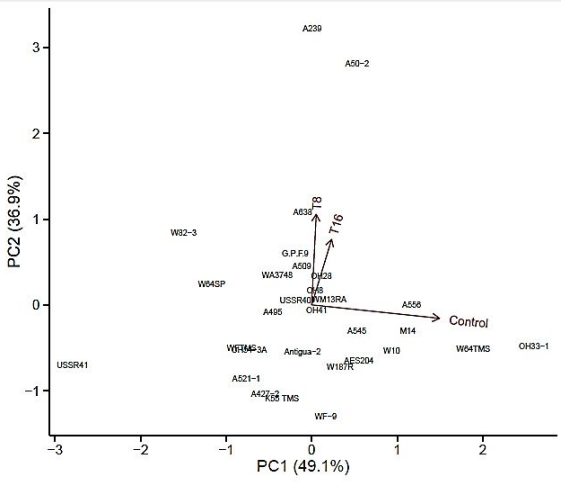

Figure 1.1. AMMI Biplot for RSR

Projection for OH33-1, W64TMS, M14, A556, and W10 genotypes fell on control environmental vector which proved strong positive interaction at T1 (Fig. 1.1). Genotypes present on the opposite side of the environment vector showed negative interaction. A521-1, WFTMS, W64SP, W82-3, and USSR41 had negative interaction with T1 for RSR (Fig. 1.1). T2 and T3 environments were less interactive than T1 for RSR. A239, A50-2, A638, W82-3 and G.P.F.9 performed better under T2 while A50-2, A239, OH8, A638, and W64TMS expressed high interaction under T3. Genotypes A239, A50-2 and A638 consistently showed high interaction under T2 and T3 (Fig. 1.1). T2 and T3 interacted with genotypes in a similar way relative to T1 which interacted differently than T2 and T3, as the angle between T2 and T3 vectors is narrow as compared to T1 which is comparatively broader (Fig. 1.1). Smaller angle between environment/interaction vectors represents similar interactive responses while larger angle shows different interactive responses. Genotypes which are present closer

together have similar interactive responses. OH54-3A and W64TMS; A427-2 and K55 TMS genotypes were present close to each other in biplot which represented that these had similar interactive responses with environments while all other genotypes were scattered which was indicative of differential interactive responses of genotypes with environments (Fig. 1.1).

Analysis for C_HA under T1, T2 and T3 among PC1 and PC2 showed highest interaction (67.2%+29.7% = 96.9%). No genotype was present near origin means that all genotypes were sensitive to salinity stress regarding C_HA contents (Fig. 2.1). Spoke length of T2 vector was longest among all treatment vectors therefore proved as most interactive treatment for C_H.A contents. A638, K55 TMS, A509, A50-2 and AES204 genotypes had strong positive interaction with T1 vector for C_H.A contents while A556, OH41, W187R, W64TMS and USSR41 had negative interaction. OH33-1, USSR40, WF-9, W64SP and A495 had strong positive interaction whereas G.P.F.9, AES204, A521-1,

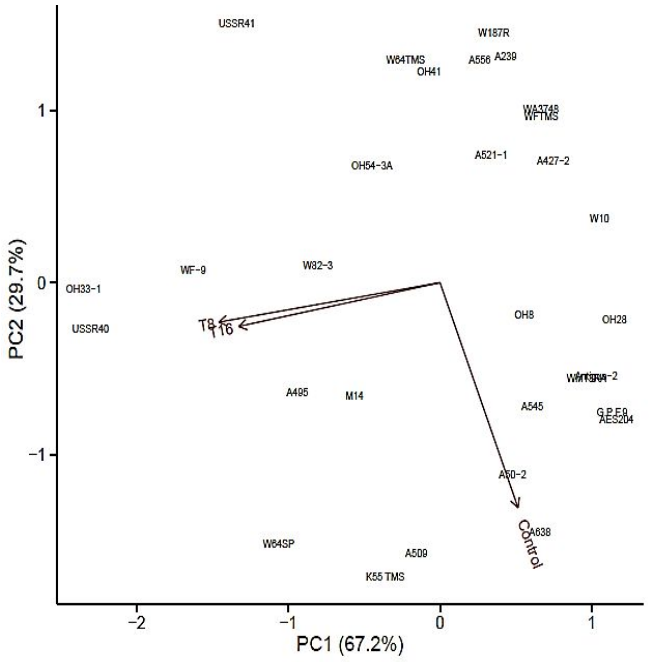

Figure 2.1. AMMI Biplot for Ch.A

W10 and OH28 had strong negative interaction with T2 for C_H.A contents. OH33-1, USSR40, WF-9, W82-3 and W64SP showed strong positive interaction whereas Antigua-2, G.P.F.9, AES204, OH28, and W10 had strong negative interaction with T3 for C_H.A contents among all genotypes. C_H.A contents of genotypes had almost similar interaction with T2 and T3. Interactive effect of control condition was entirely different from T2 and T3 for C_H.A contents due to difference of angles among environment vectors (Fig. 2.1). WA3748 and WFTMS; AES204 and G.P.F.9; Antigua-2 and W64SP genotypes shared a common place in biplot which indicated that these genotypes had similar interactive responses with environments whereas all other genotypes showed differential interactive responses for C_H.A (Fig. 2.1). Biplot for C_H.B among thirty genotypes under three different saline environments was plotted among PC1 and PC2 which contributed 94.5% of total interaction (Fig. 3.1).

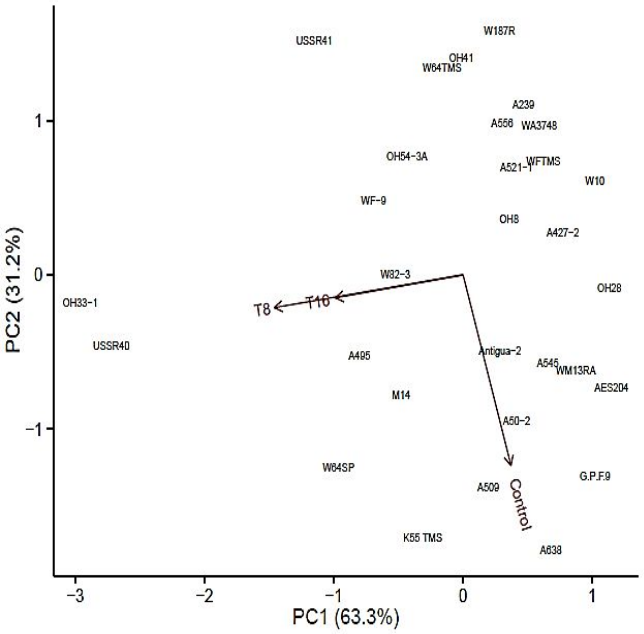

Figure 3.1. AMMI Biplot for Ch.B

None of the genotype was present at origin of graph reflected that all genotypes were sensitive for C_H.B in different saline environments (Fig. 3.1). T2 was the most interactive environment due to longest spoke length followed by T1 and T3 (Fig. 3.1). A638, K55 TMS, G.P.F.9, A509 and A50-2 at T1; OH33-1, USSR40, W64SP, A495 and USSR41 at T2; OH33-1, USSR40, WF-9, W82-3 and W64SP at T3 environments had strong positive interaction. A239, W64TMS, OH41, W187R and USSR41 at T1; A521-1, A545, AES204, W10 and OH28 at T2; Antigua-2, G.P.F.9, AES204, OH28 and W10 at T3 had strong negative interaction (Fig. 3.1). OH33-1 and USSR40 showed strong positive interaction while OH28 and W10 had strong negative interaction at T2 and T3 for C_H.A and C_H.B (Fig. 2.1 & 3.1). T2 and T3 interact in similar way with genotypes but interactive strength of T2 is more than T3 due to differences in spoke length. Greater angle of T1 vector with T2 and T3 vectors indicated that normal condition has entirely different interaction with genotypes than T2 and T3 (Fig. 3.1). There was no overlapping between locations of genotypes for C_H.B means all genotypes behave differently for T1, T2 and T3 environments (Fig. 3.1).

Biplot for BCart under three salinity environments plotted between PC1 and PC2 showed 86.4% of total interaction (Fig. 4.1). A556 showed its stability over three environments whereas other genotypes showed interaction with environments either positive or negative for β-carotenoid (Fig. 4.1). T3 was most interactive condition for BCart contents of genotypes followed by T2 and T1 on the basis of spoke length differences. All the three environments interacted differentially with genotypes for BCart contents (Fig. 4.1). A521-1, W82-3, OH8 and WF-9 in T1; WF-9, M14, A509 and A545 in T2; W82-3, M14, A509, and A545 in T3 had positive interaction with corresponding environments. USSR41, A239, USSR40 and A638 in T1; USSR40, OH33-1, G.P.F.9 and OH8 in T2; OH28, OH33-1, G.P.F.9 and OH8 in T3 had strong negative interaction with corresponding environments (Fig. 4.1). Dispersive and non-overlapping graph depicted that all genotypes under different environments interact differentially for BCart contents (Fig. 4.1).

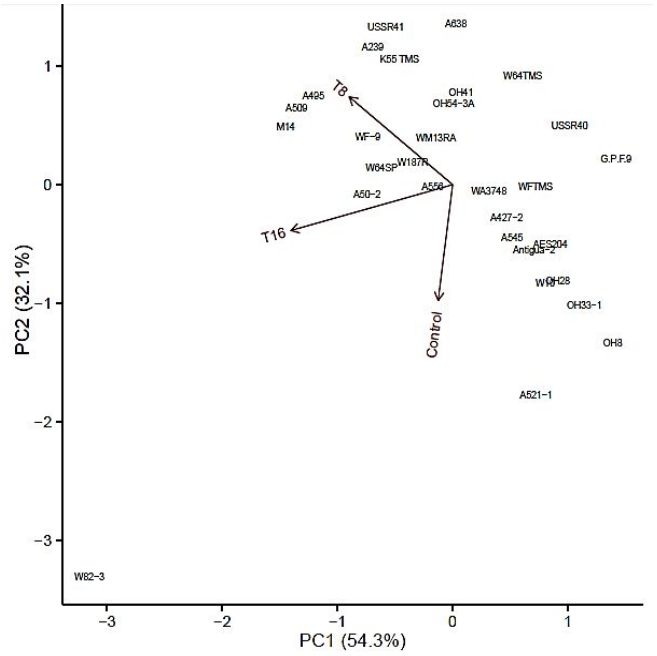

Figure 4.1. AMMI Biplot for BCart

Contribution of interaction representing PC1 and PC2 was 93.2% for AA at salinity levels T1, T2 and T3 (Fig. 5.1). Vector of T1 was most interactive than T2 and T3. Interactive strength of T2 and T3 environments was similar but different from T1 (Fig. 5.1). Genotypes A638, W10, A427-2, WA3748 and OH8 in T1; USSR41, WFTMS, A521-1, A239 and K55 TMS in T2; A509, WFTMS, USSR41, A239 and A427-2 in T3 had strong positive interaction with respective environments. Whereas WF-9, WM13RA, A239, A509 and A545 in case of T1; A556, A495, OH8, A50-2 and A545 in T2; OH54-3A, A495, OH8, A556 and A50-2 in T3 had strong negative interaction with respective environments (Fig. 5.1). K55 TMS and W64TMS showed exactly similar interaction responses with environments for AA contents.

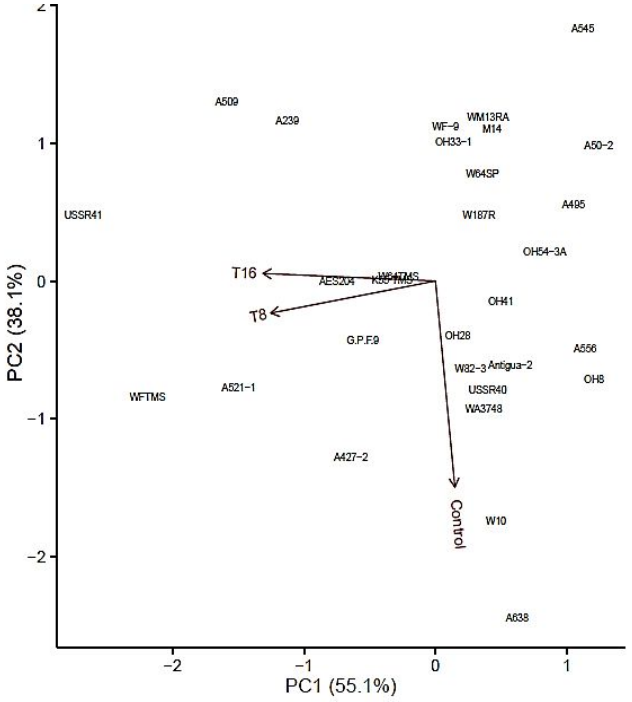

Figure 5.1. AMMI Biplot for AA

In Figure 6.1, biplot analysis exhibited 87.9% interaction, between PC1 and PC2, for root density (RD) under T1, T2 and T3. T3 and T1 vectors were least and most interactive respectively whereas T2 showed intermediate interaction on the basis of their respective spoke lengths for RD. T2 and T3 vectors are present in entirely opposite direction explaining that genotypes with strong positive interaction for T2 had strong negative interaction with T3 environment and vice versa (Fig. 6.1). A556 showed strong positive and strong negative interaction with T1 and T3 respectively. On the other hand Antigua-2 had strong positive and strong negative interaction with T3 and T2 respectively. Strong positive interaction was observed for A556, OH28, W64TMS and A638 at T1; for A50-2, M14, A556 and A545 at T2; for G.P.F.9, USSR40 and WM13RA at T3. Strong negative interaction was observed for A239, K55 TMS, USSR41 and W82-3 at T1; for USSR41, W82-3 and WA3748 at T2; for A556, USSR41, W10, A545, and OH8 at T3 (Fig. 6.1). Strong positive interaction showed the positive responsiveness of genotypes to concerned environment whereas, negative interaction showed negative responses or inhibitory effects of concerned environment on genotypes.

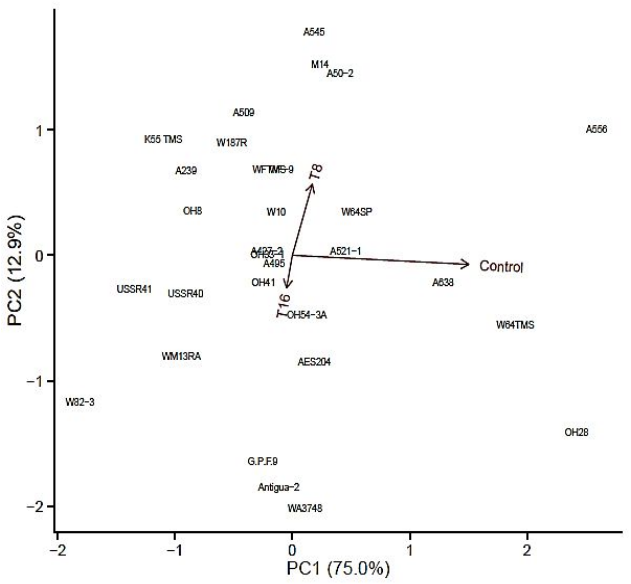

Figure 6.1. AMMI Biplot for RD.

AMMI biplot for leaf temperature (LT) under three environments was shown in Figure 7.1. T3 had longer spoke length and more interactive strength than T1 and T2 environment vectors. T3 was distantly separated with larger angle from T1 and T2 vectors while interactive strength not differed greatly between T2 and T1 for LT (Fig. 7.1). K55 TMS, OH8, USSR41, OH54-3A and WFTMS in T1; WF-9, G.P.F.9, WA3748, K55 TMS and W64TMS in T2; A427-2, W82-3, K55 TMS, USSR41 and M14 in T3 have strong positive interaction.

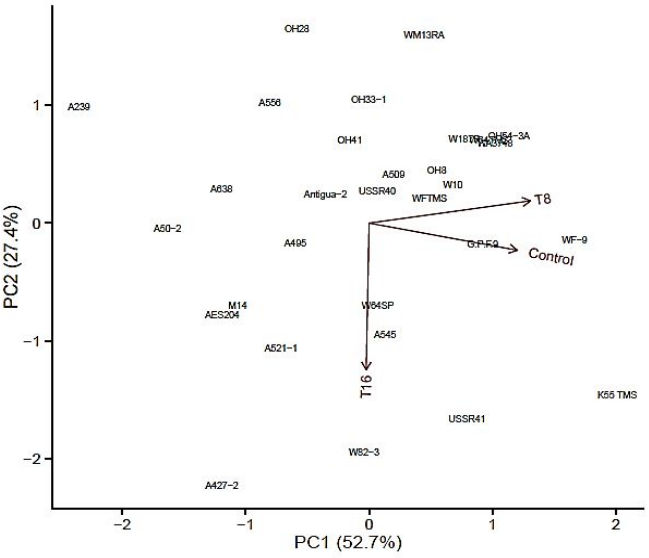

Figure 7.1. AMMI Biplot for LT

A638, AES204, M14, A50-2 and A239 in T1; A50-2, A638, A521-1, A427-2, and A239 in T2; OH8, A556, OH33-1, WM13RA and OH28 in T3 had strong negative interaction with respective environments (Fig. 7.1). OH54-3A, W187R and WA3748 overlap for biplot location which represent that these genotypes responded in similar way to environment (Fig. 7.1).

DISCUSSION

To study genotype × environment interaction (GEI) several methods have been extensively used by researchers such as univariate methods i.e. Plaisted and Peterson's mean variance component for pair-wise GE Interactions, Francis and Kannenberg's coefficient of variability, Shukla's stability variance, Perkins and Jinks's regression coefficient (Rao *et al*., 2011). When more number of accessions are needed to be tested at multiple locations, environments, years, and seasons, this poses the problem of clear cut view of genotypic responses (Yan *et al*., 2001). Biplot analysis solved the above mentioned problems and confers two dimensional graphic displays which depict the interrelationship among genotypes, environments and genotype-environment interaction. Biplot analysis is of two types: (I) Additive main and multiplicative interaction (AMMI) and, (II) Genotype & Genotype × Environment (GGE). AMMI and GGE biplots integrates certain characteristics on the basis of joint regression and type B genetic correlation but also have some differences which help in their manipulation. GGE biplot is referred to environment centered principle component analysis (PCA) while AMMI biplot analysis is based on double centered principle component analysis (Rao *et al*., 2011).

AMMI is an effective analysis for GEI estimation and genotypic selection under versatile environments (Aina *et al*., 2007). In this study AMMI biplot analysis was used to study the stability in performance of genotypes at different saline environments (0, 8, and 16 ds/m). Various researchers used AMMI biplot analysis effectively in maize (Crossa *et al*., 1990), wheat (Crossa *et al*., 1991), rice (Muthuramu *et al*., 2011), soybean (Zobel *et al*., 1998), and pearl millet (Shinde *et al*., 2002). There is an advantage of using AMMI analysis because it is capable of splitting G (genotype) from GE (genotype × environment) which is not feasible in case of GGE biplot (Gauch *et al*., 2008). AMMI biplot analysis is simple, easy, provides information about genotypic behavior, phenotypic stability, environment with optimum performance and degree of divergence among accessions (Miranda *et al*., 2009).

Under variable environmental conditions, change in performance of cultivars is associated with genotype × environment interaction. The ranking of genotypes on the basis of performance keeps on changing when grown in different environmental conditions; this causes confusion about the superiority of genotype. This may be solved

through the use of AMMI biplot analysis as genotypes showing non-sensitive behavior for most of traits were considered to have broader adaptability or more stability to the changing environments (0, 8 and 16 ds/m). Non-sensitive behavior represents non-significant change with the change in environmental conditions. Salinity stress was also previously reported to be harmful to variable extent for growth and development of crop plants (Aslam *et al.,* 2013; Aslam *et al.,* 2015).

OH33-1, A239 and A50-2 showed positive strong interaction with T1, T2 and T3 respectively for RSR (Fig. 1.1). This might be due to reduction in osmotic potential under salinity stress which inhibits plant growth by retarding shoot and leaf growth whereas root continues to elongate in search of water from lower layers of soil (Hsiao and Xu, 2000). The promotion in root and inhibition in shoot growth leads to increase in root shoot ratio. Genotypes with higher RSR proved more tolerant so can be used as selection criteria.

A638 showed positive interaction with T1 and OH33-1 with both T2 and T3 for C_HA and C_HB (Fig. 2.1 & 3.1). Maize genotypes expressed variable responses to T1, T2 and T3 regarding C_HA and C_HB, which describes significant adverse effect of salinity on chlorophyll contents (Table 2.1 & 3.1). Chlorophyll and carotenoid contents were reported to be affected by salt stress imposition, depending on severity, duration and type of crop species (Misra *et al*., 1997). Chlorophyll contents of moderately stressed rice plants were reported to be higher than sown normally. This could be associated with reduced leaf area, lower transpiration, and sodium uptake in leaves. Net photosynthesis could be higher due to higher nitrogen in per unit leaf area and Na^+ should be in the limit of tolerance otherwise, will have antagonistic effects (Amirjani, 2010).

A521-1, WF-9 and W82-3 showed positive interaction for BCart whereas A638, USSR41 and A509 had positive interaction for AA contents with Tl, T2 and T3 respectively (Fig. 4.1 & 5.1). Significant variable responses of genotypes regarding BCart and AA contents at different saline environments reflect presence of alleles with differences in responses to changing environment. Carotenoids are important non-enzymatic lipophilic antioxidants and have capability to scavenge several ROS molecules (Young, 1991). Carotenoids absorb energy from light and transfer it to chlorophyll. Signlet oxygen is scavenged by carotenoids and provides the protection to the photosynthetic machinery. Higher carotenoids contents associated with salt tolerance because these prevent the oxidative damage through scavenging of ROS (Gomathi and Rakkiyapan, 2011). Non enzymatic antioxidant defense system consists of main buffers which maintain cellular redox status and prevent the cells from oxidative damage caused by ROS. Inability to synthesize significant antioxidant contents has strong positive association with susceptibility (Semchuk *et al*., 2009). It is reported that Ascorbate (AA) contents of the cell changed due to salinity stress (Hernandez *et al*., 2001) and its enhanced biosynthesis play role in buffering the oxidative stress. Higher accumulation of AA biosynthesizing enzymes is associated with abiotic stress tolerance in plants (Chaves *et al*., 2002). AA serves as dominant antioxidant because it easily bestows the electrons to the enzymatic and non-enzymatic reactions and sustains redox status. AA has low molecular weight and highly abundant antioxidant (Sharma *et al*., 2012). It is present in redox state under stress free physiological conditions and involve in dissipation of excitation energy (Smirnoff, 2000). AA directly reacts with hydrogen peroxide (H_2O_2) and peroxides (O_2^-), protects macromolecules from oxidative stress and provides protection to membrane (Noctor and Foyer, 1998). Ascorbate peroxidase (APX) is an antioxidative enzyme which restricts ROS and reduces H_2O_2 into H_2O by using two molecules of ascorbate (Caverzan et al., 2012). Higher contents of AA may be associated with higher activity of APX which confers tolerance against prevailing saline conditions. Strong positive correlation was reported between salt tolerance and antioxidant activity (Athara *et al*., 2008). AA protects plant metabolism and chlorophyll contents from oxidative damage and plays active role in the maintenance of photosynthetic apparatus (Chen and Murata, 2002). AA participates in biosynthesis of ethylene and abscisic acid which are responsible for signaling and regulation of salinity responsive genes, the product of which confers constitutive salt tolerance in plants (Barth *et al*., 2006). A556, A50-2 and G.P.F.9 had positive interaction with T1, T2 and T3 respectively for RD. RD presents root health and serves as important selection criterion for salt tolerance. RD was reported to be decreased proportionally due to saline conditions in growth medium (soil/water) and was observed to be positively correlated with leaf surface area and root cellular turgor potential (Pascale *et al*., 2003).

It is concluded that environment interacts with various traits of genotypes differently and alters their performances. AMMI model of biplot is very important tool for exploitation of interaction. Maize genotypes showed positive interaction for certain environments which indicated that their performance was better for that typical environment whereas, genotypes with negative interaction indicated the poor performance for typical environment. Stable performance of genotypes indicated that their responses were not affected by environment. Differences in the genotypic responses are due to differences in their genetic makeup which regulate the plant physiology and morphology to allow them to respond in certain way.

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