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ANALYSIS OF THE GENOTYPE BY SALT INTERACTION OF BARLEY (*HORDEUM VOULGAR L.*) GENOTYPES AT EARLY GROWTH STAGE BY GRAPHICAL MODELS

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Abstract

Soil salinity is a major abiotic stress in worldwide agriculture. This has incited a quest towards with an aim of improving the crop plants. Establishment of plants at early growth stage as one of the most important determinants of high yield is severely affected by soil salinity. Objective of this study was tolerance assessment and estimating the magnitude of genotype x salt interaction and stability for barley biomass production in the salinity conditions. For this purpose, a green-house experiment with nine genotypes in five levels of salinity using graphical models of AMMI and GGE biplot was performed. According to results, salinity significantly reduced the biomass production of genotypes and genotype x salt interaction effect was significant. The obtained result from graphical models was similar to those obtained from the analysis of variance and statistical comparison of means of genotypes biomass production. According to results, WB7910 was the most stable genotype with higher yielding on average and MBS8712 and 5Shori had a positive interaction with salinity in a wide range of stress. The level of 13.5 ds/m of salinity was determined as representativeness and discriminating among salinity levels.

Key words: Barley, Greenhouse, AMMI, GGE-biplot and Salinity

INTRODUCTION

Excess amount of salt in the soil adversely affects plant growth and development. Increased salt tolerance in crops is widely recognized as an effective way to overcome the limitations of crop production in a salinized area (Munns and James, 2003). Barley (*Hordeum vulgare* L.) is the fourth cereal crop following wheat, rice, and maize in the world (Lai and Feng, 2012). It is widely cultivated around the world because of its moderate resistance to barren soil, salinity and drought conditions and it is necessary to increase the barley gross production mostly through breeding new varieties with both high and stable yield, as well as wide adaptation (Meng et al., 2016). For improving the salt stress tolerance of crop varieties by plant breeding, it is necessary to identify donor genotypes that have proven tolerance to salt stress during all the growth stages. Effects of genotype, environment and genotype × environment

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interaction determine the phenotypic performance and its general and specific adaptation to different environments. Genotype × environments (G × E) interaction plays a major role in evaluation of genotypes under different environment (salinity stress) to identify genotypes suitable to different stresses (Munns and James, 2003). A significant G × E interaction for a quantitative trait, such as yield can seriously limit efforts in selecting superior genotypes for both new crop production and improved cultivars development (Kang, 1990) and would reduce the usefulness of subsequent analysis of means and inferences that would otherwise be valid. The analysis of variance (ANOVA) is useful for identifying the sources of variability but it provides no insight into the particular pattern of the underlying interaction. Several methods have been proposed to analysis GEI and phenotypic stability. Among multivariate methods, the additive main effects and multiplicative interaction (AMMI) analysis widely used for GEI investigation. The AMMI model is a hybrid statistical model incorporating both ANOVA (for additive component) and PCA (for multiplicative component) for analyzing two-way (genotype-by-environment) data structure (Sing et al., 2016). The model has, in recent past, been recommended for statistical analysis of yield trials, and was preferred over other customary statistical analyses, such as ordinary ANOVA, principal component analysis and linear regression analysis (Gauch, 1988). AMMI model has been widely applied in analysis of data; however, it only allows one to study the interaction between Genotype and Environment (GE). Moreover, G and GE must be considered simultaneously when making cultivar selection decisions. For this reason, instead of trying to separate G and GE, Yan et al. (2000) combined G and GE and referred to Genotype main effect (G) and Genotype by Environment interaction (GGE) model. The methodology based on this model, called the GGE biplot methodology, has been recommended and used widely by many scientists and has been successfully employed to determine relationships among genotypes, environments and G × E interaction effects (Meng et al., 2016). So this study was carried out to evaluate the tolerance barley genotypes at vegetative growth stage, based on biomass production and applying the graphical models to determine the nature and magnitude of genotype × salt interaction effect on biomass production in different salinity levels.

MATERIALS AND METHOD

Nine barely genotypes i. e. STW82153 (A), MBS8712 (B), ESBYTM8910 (C), 4Shori (D), 5Shori (E), WB7910 (F), Valfajr (G), MBS8715 (H) and Jo torsh (I) were tested in green house at 5 levels of electrical conductivities (ds/m) (S1 (control) =4.5, S2=7.5, S3=10.5, S4=13.5 and S5=16.5). Treatments were arranged in a factorial design with 3 replications on the base of a Completely Randomized Design. Relative humidity was maintained at about 60% (± 5), and the day/night temperature was 24/16°C (± 2). First, seeds of each genotype were surface sterilized with 5% sodium hypochlorite solution for 10 min and then rinsed with sterile distilled water three times. Eight seeds of the nine barley genotypes were sown in 5 kg pots filled with a 2:1:1 mixture of clay, sand and cattle manure. In order to prevent osmotic shock and ensure plant establishment salinity stress was done gradually. After 14 days plants were thinned to five per tube and salt stress evaluation was started for five weeks. Irrigation occurred every five day and involved wetting the soil to beyond field capacity. After this period the effects of salinity treatments were studied by sampling on dry weight of shoot and root as biomass production for each treatment. The dry weights were measured by drying the shoot and root at 75ºC for 48h, to give a constant weight. Biomass production was calculated by dividing the total weight by the number of plants. The biomass data were subjected to AMMI and GGE biplot analysis. All statistical procedures were carried out using the R program.

RESULTS

Analysis of data presented in Table 1 and 2 showed that salt stress had adverse effect on growth of barley genotypes. Assessment of trait showed considerable variance among barley genotypes under normal and stress conditions. On the basis of plant growth performance, the highest values of plant biomass production were recorded for G, H and B genotypes in control level, whereas with the highest levels of salinity this was the case for B, E and D. Due to

significant statistical difference of genotype × salt stress interaction, a selection of genotypes with best performance in a level of salinity based on their production in other levels of salinity will not be possible.

Table 1. Analysis of variance of biomass production

S.O.V	Treatment	Genotype (G)	Salt (S)	G×S	Error
df	44	8	4	32	90
MS	51169^{**}	77132^{**}	338451^{**}	8768^{**}	1338

ns, * and **: Not significant, and significant at the 5% and 1% levels of probability, respectively

Table 2. Statistical comparison of means for genotype biomass production by Duncan's multiple range test (α = 0.01)

Salt	S_1	S_2	S_3	S_4	S_5	
genotypes						
A	447^{efghi}	366^{hijkl}	324.7^{lmno}	310.3^{lmnopq}	253.7^{opqrst}	340.3_{d}
B	584.3^{bc}	521.3^{cde}	437.3^{efghij}	416^{ghijk}	348.7^{jklmn}	461.5_{a}
C	488.7^{defg}	371^{hijkl}	$292^{lmnopqrs}$	$264^{mnopqrs}$	200.7^{stu}	323.3_{d}
D	543.3^{cd}	418.7^{ghijk}	350^{jklm}	$299.7^{lmnopqr}$	$286.3^{lmnopqrs}$	379.6_{c}
E	525.7^{cde}	451.3^{efgh}	417.3^{ghijk}	347.3^{jklmn}	319.7^{lmnop}	412.3_{b}
F	548.3^{cd}	455^{efgh}	338.7^{klmno}	$286.3^{lmnopqrs}$	257^{nopqrs}	377.1_{c}
G	678.3^{a}	512.3^{cdef}	502^{cdefg}	215.3^{rstu}	204.7^{stu}	422.5_{b}
H	658.7^{ab}	430.3^{fghij}	358^{ijkl}	247^{opqrst}	212.7^{rstu}	381.3_{c}
I	$293^{lmnopqrs}$	225.3^{qrstu}	229^{pqrstu}	165.3^{tu}	148^{u}	212.1_{e}
	529.7_{a}	416.8_{b}	361_{c}	283.5_{d}	247.9_{e}	

Value followed by different letter (s) differs significantly. Genotypes: STW82153 (A), MBS8712 (B), ESBYTM8910 (C), 4 Shori (D), 5 Shori (E), WB7910 (F), Valfajr (G), MBS8715 (H) and Jo torsh (I)

By plotting both the genotypes and the environments on the same graph, the associations between the genotypes and the environments can be seen clearly. The greater the interaction principal component axes (IPCA) scores, either positive or negative, as it is a relative value, the more specifically adapted a genotype is to certain environments. The more IPCA scores approximate to zero, the more stable the genotype to overall environments sampled (Adugna and Labuschagne, 2002). To identify positive and negative interactions when trying to understand which genotypic and phenotypic qualities adapt a variety for a particular environment, AMMI 2 plot (Figure 1) can be useful. Looking at S5 it is clear that genotypes A, B and E have a large positive GS interaction with this salinity levels. The genotypes H and G also appear to have a positive interaction with low and moderate salinity stress levels. Some relationships between genotypes and environments may be missing from these first two dimensions because the biplot is not displaying all of the variation. To visualize the genotypes' performance in relation to stability, main effects (mean performance) was plotted against IPCA1 (Figure 1, AMMI 1) It is clear to see S1 has the highest mean yield while B is the genotype with the highest mean yield. This biplot estimates that those genotypes with points close to the X axis are more stable than those further away. Accordingly, A and G genotypes appear more unstable than the others. Checking back with AMMI 2 biplot, the A genotype had a high yield in S4 and S5 while G had an unexpectedly low yield in this same level of salinity and a higher than expected yield in S1, S2 and S3. The best genotype should

combine high yield and stable performance across range of environments. So F and D can be select as stable genotypes with higher yielding on average.

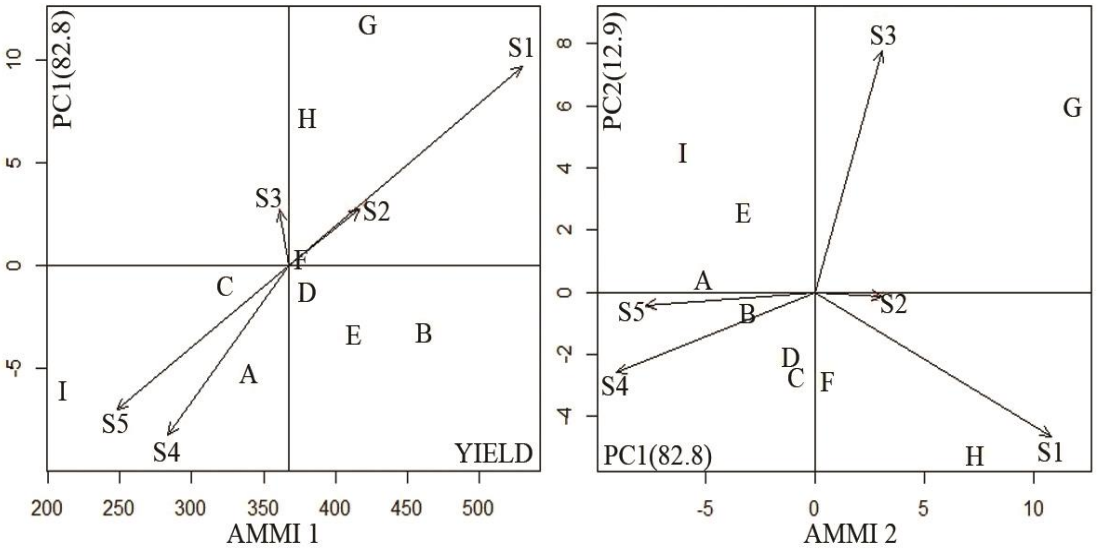

Figure 1. AMMI 1 and AMMI 2 biplots to show genotype performance in relation to stability of barley genotypes across salinity levels

To make comparisons between genotypes, as it has been showed in Figure 2 (part A), the genotypes that are close to each other tending to do similarly in the same salinity levels. So the G and H genotypes were positively correlated while with the A genotype were negatively correlated and they performed differently over the levels of stress.

The mean yield and stability of genotypes are evaluated by mean against stability plot (Figure 2, B), the single-arrowed line is the average-environment coordination (AEC) abscissa (or average-environmental axis, AEA) and points to higher mean yield across environments. Thus, B had the highest mean yield, followed by G, E, etc., whereas I had the lowest mean yield. This result is similar with the data in Table 2 and AMMI 1 plot. The AEC ordinate passes the plot origin and is perpendicular to the AEC abscissa and points to greater variability (poorer stability) in either direction. Thus G, B and H were highly unstable, whereas F and I were highly stable.

The polygon view of the GGE-biplot analysis helps one detect cross-over and non-cross-over genotype-by-environment interaction and possible mega environments in multi-location yield trials (Yan et al. 2007). A, B, G and I were vertex genotypes (Figure 2, C). They are best in the environment lying within their respective sector in the polygon view of the GGE-biplot (Yan and Tinker, 2006); thus these genotypes are considered specifically adapted. The B genotype showed best performance in S2, S4 and S5 and this case was for G in S1 and S3. Genotypes close to the origin of axes have wider adaptation (Abay and Bjornstand, 2009).

Further information about the discriminating power of environments, together with a representation of their mutual relationships, can be obtained by the environment-vector view of the GGE-biplot. In this case, a long environmental vector reflects a high capacity to discriminate the genotypes. Furthermore, the cosine of an angle between vectors of two environments approximates the correlation between them: a wide obtuse angle indicates a strong negative correlation; an acute angle indicates a positive correlation while a close-to-90° angle indicates lack of correlation (Yan and Tinker, 2006). With the longest vectors from the origin, environments S1 and

S2 were the most discriminating. Considering the angles between environmental vectors, yield results in S1, S2 and S3 were strongly correlated, similarly to those obtained in S4 and S5 that it means genotypes tend to do well or badly in the correlated salinity levels.

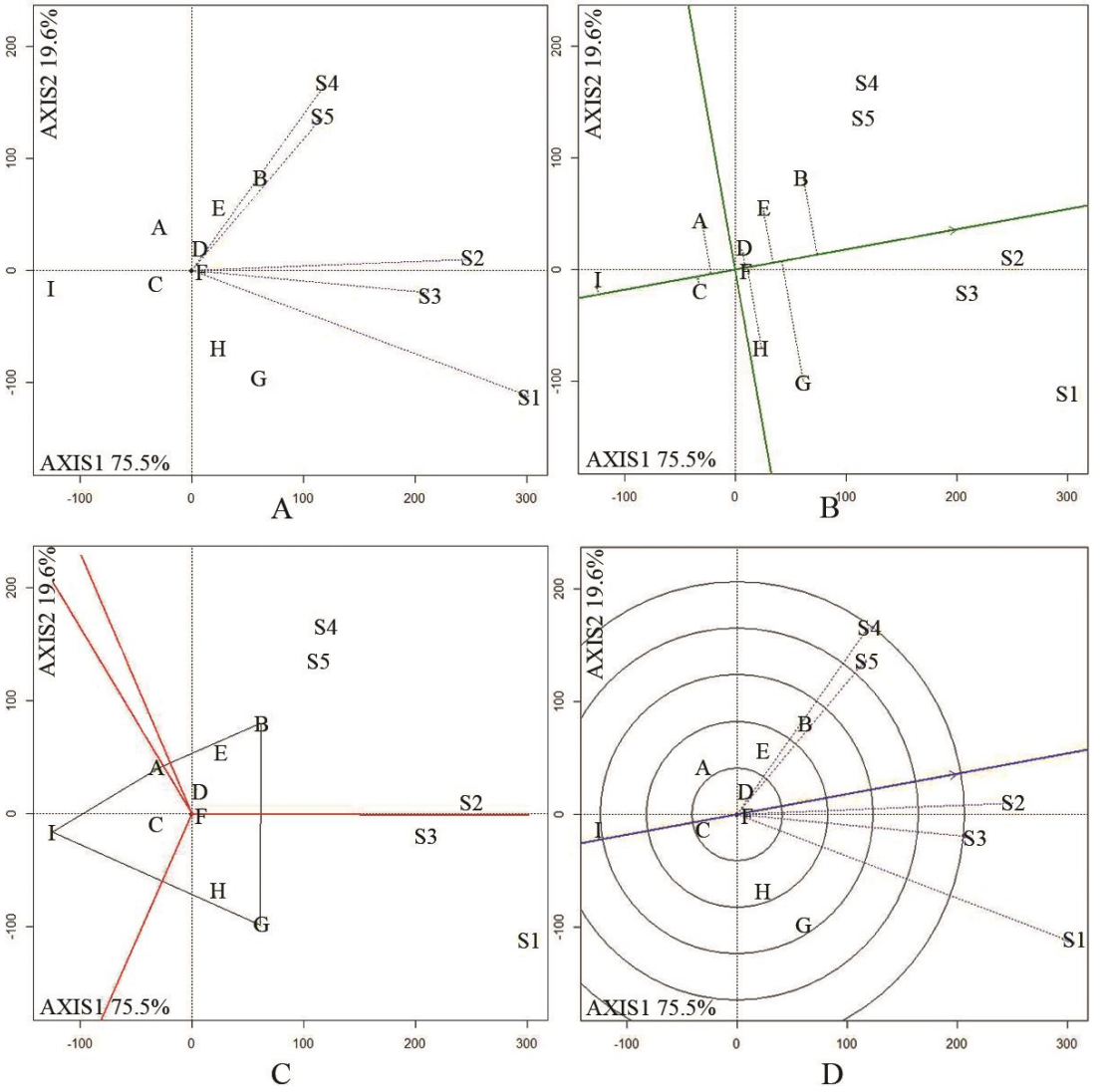

Figure 2. View of the GGE biplot based on a subset of the G × S data. A: Main biplot for data based on a ‘‘Tester-centered (G + GE)’’ table, without any scaling and it is column metric preserving. B: Mean performance and stability of the genotypes plot, C: Which-won-where plot, D: The representativeness and discriminating ability of the environments plot

DISCUSSION

Salinity reduces plant growth and yield by two mechanisms, osmotic stress and ion cytotoxicity. Salt inhibits growth by reducing the plant ability to take up water, subjecting it to a water-deficit effect. Excessive amounts of salt entering the plant tissues injure the cells, leading to further reduced growth, through the ion-excess effect (Munns and Tester, 2008). The significant genotypic variation for biomass production in control and salinity treatments suggested that the magnitude of differences was

sufficient to provide some scope for selection to improve salinity tolerance. The presence of significant G × S interaction indicated the inconsistency in performance of barley genotypes across salinity levels. Therefore, developing genotypes that would have low G × E interaction could result in improving barley productivity for the target area. In AMMI 1 biplot, genotypes showed more variation for interaction than for the main effects. This was manifested by relatively wider distribution of genotypes in the vertical than in the horizontal direction. S1 and S2 as low salinity levels showed strong correlation to each other this means that the genotypes act similar and have a little portion in GS interaction in these two levels of stress and with increasing in salinity concentration the genotypes showed different reaction. There are two possible strategies for developing genotypes with low GE interactions. The first is sub-division or stratification of heterogeneous area into smaller, more homogeneous sub-regions, with breeding programs aimed at developing genotypes for specific sub-regions. However, even with this refinement, the level of interaction can remain high, because breeding area does not reduce the interaction of genotypes with location on years (Eberhart and Russell, 1966). The second strategy for reducing GE interaction involves selecting genotypes with a better stability across a wide range of environments in order to better predict behaviour (Yaghotipoor and Farshadfar, 2007; Askari et al., 2017). An ideal genotype possesses: 1) high yield performance; 2) low sensitivity to adverse conditions and 3) is capable of responding positively when environmental conditions are improved (Ferreira and Demetrio, 2006). As results showed S1 and S2 had a mean above total average and can be grouped as favourable environments that G and H genotypes had better performance in these salinity levels but with increasing salinity concentration the E and B genotypes appeared better than others. This fact reveals the importance of assessment and selection of genotypes for both potential yield and performance under stress

CONCLUSIONS

The results of this study showed highly significant differences ($P<0.01$) for salinity (S), genotypes (G) and GS interaction, indicating the presence of genotypic variability, different responses of genotypes to salt stress conditions. Analysis of genotype by environment interaction is vital for breeders in order to design the dissemination strategies for new varieties. It is important to identify cultivars with specific and general adaptation. Precise recommendation of lines for general and specific adaptation requires clear understanding of the real pattern of genotype by environment interaction. The AMMI and GGE biplot models were as effective tools in understanding GS interactions in salinity levels of barley genotypes. Result revealed an adapted genotype to several levels of salinity (F). Thus it can be proposed for planting in a wide range of salinity environments with suitable establishment. Also, it was detecting that only the S4 can be sufficient for deciding about which genotypes are recommended for salinity conditions and this case was for S1 in low and moderate salinity levels. However, additional work on other cultivars and crops by other researchers would add clarification to these results.

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