ASSESSMENT OF MULTIPLE TOLERANCE INDICES FOR SALINITY STRESS IN BARLEY (*Hordeum voulgar L.*) GENOTYPES AT EARLY GROWTH STAGE

HOSSEIN ASKARI[1*], SEIYED KAMAL KAZEMITABAR[1],HAMID NAJAFI ZARRINI[1] AND MOHAMMAD HOSSEIN SABERI[2]

[1]*Department of plant breeding Sari University of Agricultural Sciences and Natural Resources, Sari, Iran.*
[3]*Seed and plant Improvement Research Department, South Khorasan Agricultural and Natural Resources Research and Eduction Center, AREEO,Birjand, Iran*
Corresponding author s email: hmss_askari@yahoo.com

Abstract

   Salinity is one of the major factors reducing plant growth and productivity worldwide, so different strategies have been adopted to overcome this problem of low productivity. Growing of salt tolerant crops is a good option to obtain economical yields from saline areas for which quick method to screen salt tolerant plants, particularly in early stages of their growth is important. For this purpose, a green-house experiment using some tolerance indices i.e., mean productivity, geometric mean productivity, stress tolerance index, stress stability index, tolerance index and yield reduction ratio, was performed for assessment of salinity tolerance of barley genotypes and their ability as a better predictor of biomass production under stress and non-stress conditions. According to results, salinity significantly reduced the biomass production of genotypes. The majority of used indices indicated MBS8712, 5Shori, WB7910 and 4 Shori were the best barley genotypes showing the highest stress resistance. This result was similar to those obtained from the analysis of variance and statistical comparison of means of genotypes biomass production. There were positive and strong correlations of genotypes performance in normal and stress condition with STI, MP and GMP in levels of salinity. Thus, this correlation seems to be a useful indicator of tolerant genotypes at this stage especially for these genotypes.

Key words: *Barley, greenhouse, tolerance indices and salinity*

INTRODUCTION

More than 800 million hectares of the land are salt-affected in the world that equating to more than 6% of the world's total land area [FAO]. As a result, the development of salinity-tolerant crops is an important option for maintaining crop production in saline soil (Sbei et al., 2014). Abiotic stresses such as drought and salinity are responsible for significant yield losses in barley on a worldwide scale, and yet under severe stress conditions, barley remains to be an important crop used as feed for animals, malt and human food (Katerji et al., 2006).

Barley is ranked as the fourth cereal crop after maize, wheat and rice in the world (Kilic et al., 2010). Plant species differ in their salt tolerance depending on their genetic makeup ranging from high to low levels of salts in the soil. Several selection criteria have been proposed for selecting genotypes based on their performance in stress and non-stress environments (Fischer and Maurer, 1978; Rosielle and Hamblin, 1981; Fernandez, 1992). Some researchers recommended selection under favourable environments, with a point of view that high yield potential is expected to sustain high yields under stress environments (Betran et al.,

2003). Many scientists have chosen a compromise solution and believe in selection under both stress and non-stress conditions (Fischer and Mourer, 1978; Fernandez, 1992; Nouri et al., 2011; Ullah et al., 2014). Fischer and Mourer (1978) proposed a stress susceptibility index (SSI) and showed that it is not independent of yield potential. Rosielle and Hamblin (1981) introduced a stress tolerance index (TOL) based on the differences in yields measured under non-stress (Yp) and stress (Ys) conditions. Rosielle and Hamblin (1981) defined mean productivity index (MP) as the average of Yp and Ys. But MP has an upward bias when there were larger differences between Yp and Ys. The geometric mean productivity (GMP), which is less sensitive to extreme values, is a better indicator than MP for separating superior genotypes in both stress and non-stress environments (Rosielle and Hamblin, 1981). Fernandez defined a new stress tolerance index (STI), which can be used to identify genotypes which produce high yields under both stress and non-stress conditions. Giancarla et al. (2012) in evaluating the ability of drought tolerance indices to identify tolerant barley genotypes under laboratory conditions stated these indices might be screened for indirect selection of drought tolerance in the initial stage of the crop growth. Some earlier researchers (Zare, 2012; Ajalli and Salehi, 2013) indicated the importance of STI, MP and GMP to identify high yielding barley advanced lines both under non-stressed and stress conditions. So this study was carried out to evaluate the tolerance barley genotypes at vegetative growth stage, based on biomass production and applying the models that were initially proposed for evaluation stress tolerance at the adult stage, which allows a quick and easy-to-measure screening tool for genotypic differences in salinity tolerance.

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 days 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. Tolerance indices were calculated with this difference that biomass production was replaced with yield. Tolerance indices of SSI (Fischer and Maurer, 1978), STI and GMP (Fernandez, 1992), MP and GMP (Rosielle and Hamblin, 1981), Yr (Nazari and Pakniyat, 2010) were calculated. For selection based on a combination of indices, some researchers have used principal component analysis (PCA) and cluster analysis. PCA is one of the most successful techniques for reducing the multiple dimensions of the observed variables to a smaller intrinsic dimensionality of independent variables (Johnson and Wichern,2007). The cluster analysis based on Euclidean distance was performed on the basis of tolerance indices by using the Ward method. 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. There was a significant difference between biomass productions in barley genotypes. Also, there was a significant difference amongst the genotype × salt stress interaction for

this character. 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. Biomass production was decreased with increased in salt concentration in all of the barley genotypes. G, H and B genotypes showed the maximum biomass production under control condition whereas with the highest levels of salinity this was the case for B, E and D

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

* 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 ($\alpha = 0.01$)

Saltgenotypes	S_1	S_2	S_3	S_4	S_5	
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)

A large value of TOL and SSI characterize quite more sensitivity to stress. Thus, the genotypes having a smaller value of TOL and SSI are more stable. While selection based on these two indices favours genotypes with low yield potential under control condition and high yield under stress conditions whereas a higher MP, GMP, and STI is indicating more tolerance to stress (Subhani et al., 2015; Ullah et al., 2014). Data regarding salt tolerance of different genotypes under investigation (Table 3) showed that the G and H genotypes had maximum values of TOL and SSI in the highest level of salinity, which indicates that these genotypes are more sensitive to salt condition. It is clear from Table 3 that A and I had the lowest TOL and SSI, which have lowest biomass production in control condition compared to other genotypes. As far as stress tolerance indices are concerned, the B, G and H have highest values of MP, GMP and STI so these lines have high stress tolerance and yield potential.

Tolerance index	Compare levels	Genotype								
		A	B	C	D	E	F	G	H	I
TOL	S_1 Vs. S_2	80.8	63.3	117.7	124.5	74.1	93.5	166	227.9	67.7
	S_1 Vs. S_3	122.1	147.1	196.4	193.4	108	209.6	176.2	300.5	63.6
	S_1 Vs. S_4	136.5	168.8	224.5	243.8	178.5	261.9	463.1	411.7	127.6
	S_1 Vs. S_5	193.5	235.5	287.9	256.9	205.8	291	473.7	445.8	144.9
MP	S1 Vs. S2	406.5	552.9	429.8	481.1	488.6	501.6	595.3	544.5	**259.1**
	S1 Vs. S3	385.9	511	390.5	446.6	471.6	443.5	590.4	508.2	**261.1**
	S1 Vs. S4	378.6	500	376.4	421.4	436.4	417.4	446.8	452.6	**229.1**
	S1 Vs. S5	350.2	466.8	344.7	414.9	422.7	402.8	441.5	435.6	**220.5**
GMP	S1 Vs. S2	404.5	552	425.8	477.1	487.2	499.4	589.5	532.5	**256.8**
	S1 Vs. S3	381	505.6	378	436	468.5	431	583.6	485.5	**259.2**
	S1 Vs. S4	372.5	492.9	359.3	403.4	427.1	396.3	382.1	403	**220**
	S1 Vs. S5	336.5	451.7	313.2	394.5	410	375.6	372.5	374.2	**208.2**
Yr	S1 Vs. S2	0.18	0.11	0.24	0.23	0.14	0.17	0.24	0.35	**0.23**
	S1 Vs. S3	0.27	0.25	0.4	0.36	0.21	0.38	0.26	0.46	**0.22**
	S1 Vs. S4	0.31	0.29	0.46	0.45	0.34	0.48	0.68	0.63	**0.44**
	S1 Vs. S5	0.43	0.4	0.59	0.47	0.39	0.53	0.7	0.68	**0.49**
SSI	S1 Vs. S2	0.85	0.51	1.13	1.08	0.66	0.8	1.15	1.62	**1.09**
	S1 Vs. S3	0.86	0.79	1.26	1.12	0.65	1.2	0.82	1.43	**0.68**
	S1 Vs. S4	0.66	0.62	0.99	0.97	0.73	1.03	1.47	1.34	**0.94**
	S1 Vs. S5	0.81	0.76	1.11	0.89	0.74	1	1.31	1.27	**0.93**
STI	S1 Vs. S2	0.58	1.09	0.65	0.81	0.85	0.89	1.24	1.01	**0.24**
	S1 Vs. S3	0.52	0.91	0.51	0.68	0.78	0.66	1.21	0.84	**0.24**
	S1 Vs. S4	0.49	0.87	0.46	0.58	0.65	0.56	0.52	0.58	**0.17**
	S1 Vs. S5	0.4	0.73	0.35	0.55	0.6	0.5	0.49	0.5	**0.15**

Table 3. Stress tolerance indices values for studied genotypes

Correlation coefficient tests of stress tolerance indices with biomass production under stress (Ys) and control (Yp) showed almost the same results in different levels of salinity (Figure 1). From the correlation matrix, it is observed that Yp and Ys had significant positive correlation in low and intermediate levels of salinity but with increasing in salinity concentration this correlation became week, indicating that high potential yield under optimal conditions does not necessarily result in improved yield in a salinity environment. Therefore, indirect selection for such conditions based on the results of optimum conditions will not be efficient. Yp significantly and positively correlated with TOL but this correlation was not significant with SSI whereas Ys correlated negatively or week with SSI and TOL. These results suggest that selection based on low values of TOL and SSI will result in reduced performance under non-stressed conditions. Biomass production under stressed conditions (Ys) and non-stressed conditions (Yp) were significantly and positively correlated with MP, GMP and STI, which indicated that they were better predictors of Ys and Yp than TOL and SSI. The indices, MP, GMP and STI were able to identify high yielding barley genotypes in both stressed and non-stressed conditions and these findings are consistent with the findings of Fernandez (1992) and Nouri et al. (2011).

Selection based on a combination of indices may provide a useful criterion for improving stress resistance of barley, but studies of correlation are useful to find out the degree of overall association only between any two measured characteristics. Thus, a better approach such as biplot analysis is needed to identify the superior varieties for both stress and non-stress environments. Varieties subjected to biplot analysis were compared for measuring association between all the traits at once. It is depicted from the principal component analysis that first two components (PCA1 & PCA2) comprised of more than 99 percent of the total variation for all comparison levels (Figure 2).
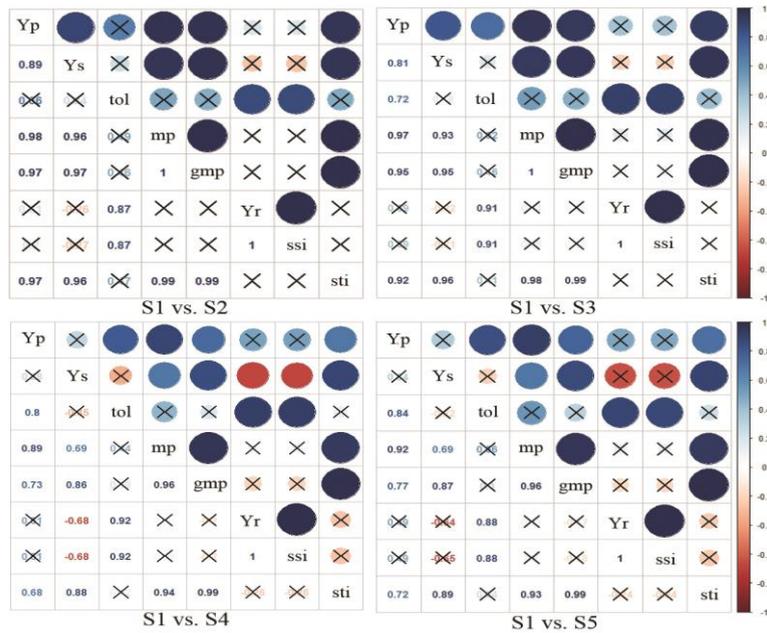
Figure 1. Correlation coefficient tests of stress tolerance indices with biomass production under stress (Ys) and control (Yp)(α = 0.05)
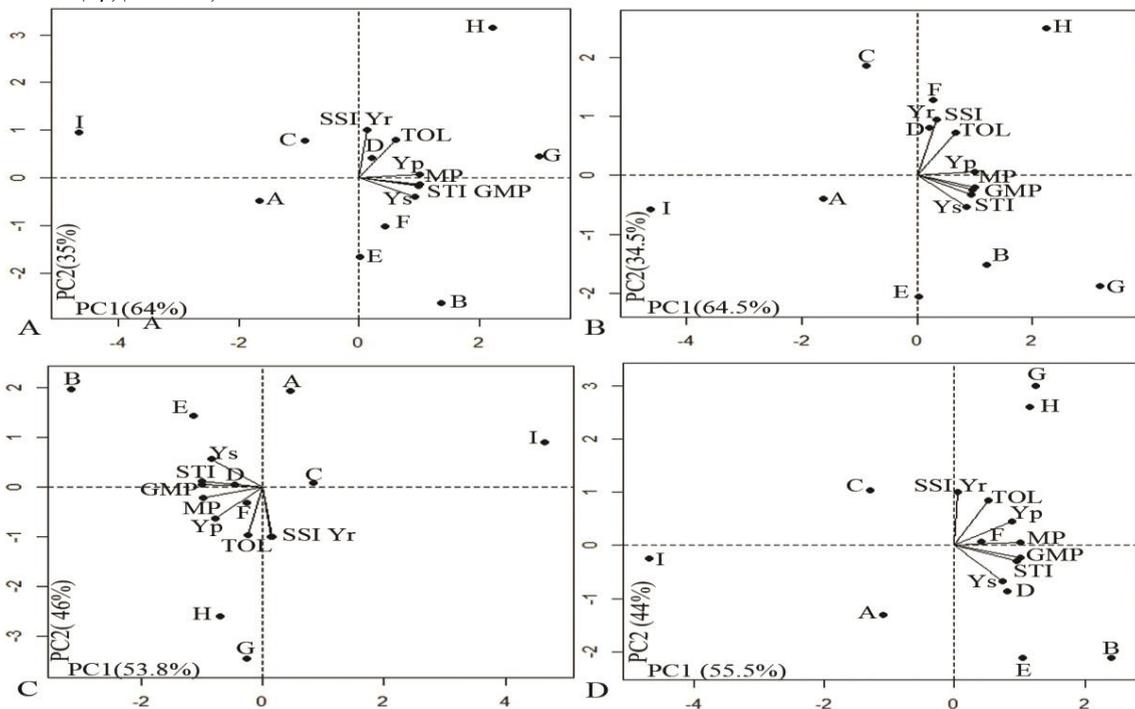

Figure 2. Distribution of genotypes on the plot based on tolerance indices and biomass production under stress (Ys) and control (Yp), A: S1 vs. S2, B: S1 vs. S3, C: S1 vs. S4, D: S1 vs. S5

Principal component analysis (PCA) revealed that first PC was related to Yp, Ys, MP, GMP and STI whereas second PC related to TOL, Yr and SSI. Thus, the first component can be termed as the yield potential and salt tolerance. Considering the high value of this component, genotypes that have high values of these indices will be high yielding in stress and non-stress conditions. The second component can be named as stress tolerant dimension and it separates the stress tolerant genotypes from non-stress tolerant, the genotypes which performedwell under stressed conditions and have low to moderate yield under control condition.

Germplasm improvement and genetic diversity is the key to reliable and sustainable production of the food crops. For effective evaluation and utilization of germplasm, measure of extent of available genetic diversity is of utmost importance (Zubair et al., 2007). The use of multivariate statistical algorithms is an important strategy for classification of germplasm and analysis of genetic relationships among breeding material (Mohammadi& Prasanna, 2003). In order to maintain, evaluate and utilize germplasm effectively, it is important to investigate the extent of genetic diversity available. The cluster analysis sequestrates genotypes into clusters, which exhibit high homogeneity within a cluster and high heterogeneity between clusters (Jaynes et al., 2003). In cluster analysis the number of clusters was determined by the cluster sum of squares in "Elbow criterion" plot. The cluster analysis showed that the genotypes identified based on indices can be divided into four groups in S2, S3 and S5 levels of salinity and three groups at fourth concentration of NaCl (Figure 3). The results obtained through cluster analysis were confirmed by Principle component analysis, which shows that the models described the clustering data very well. In this study, cluster analysis determined that genotypes with high STI, MP and GMP values (Table 3), could be considered the most tolerant and desirable genotypes for both growth conditions. In the sensitive group the genotypes with high SSI values, demonstrated their susceptibility to salt and aptness only under normal conditions.
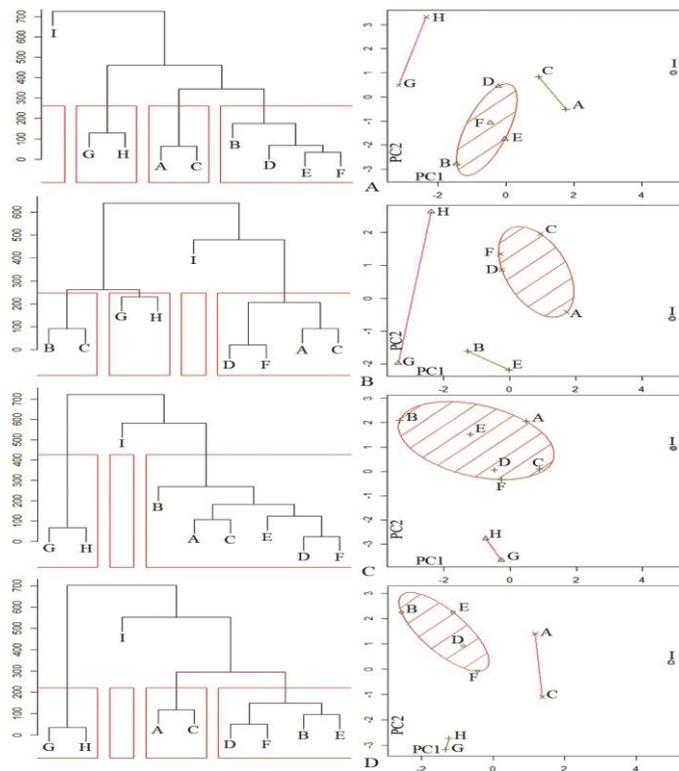

Figure 3.Dendrogram of classified genotypes using cluster analysis A: S1 vs. S2, B: S1 vs. S3, C: S1 vs. S4, D: S1 vs. S5

DISCUSSION

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 factors affecting plant growth under salinity may be evaluated in three groups: i) water stress, ii) ion toxicity, iii) problems in nutrient uptake and translocation to green plant parts, and, as a result, disorders in cells due to disruption of ionic balances (Bagci et al., 2003). Comparison of dry weight of root and shoot under various stress situations is obviously essential to define their physiological potentiality as well as to study the reasons for their better yield owing to tolerance. Also, selection of genotypes with high dry weight of root and shoot was the important objective among the breeder for the improvement of yield and other traits in breeding programs (Taghipour and Salehi, 2008).

For enhancing salt tolerance in crop plants, however, it is very essential to find sufficient variation and to devise such screening techniques which are reliable to recognize tolerant genotypes. Correlation analysis between genotypes performance and tolerance indices can be a good criterion for screening the best genotypes and indices used. A suitable index must have a significant correlation with genotype performance under both conditions (Ashraf et al., 2015).The positive correlations of MP, GMP and STI with Yp and Ys affirm their reliability for predicting yield under normal and stress conditions than TOL and SSI. The negative associations of TOL and SSI with Ys, and the positive association between both indices confirm that low values of these indices are desirable. Among the nine genotypes screened, the entries B, D, E and F were identified to be grown under both-saline and non-saline conditions. The PCA and cluster analysis showed the same results. The mentioned genotypes were in similar place on biplots especially B, D and E. Also they were clustered into a group by cluster analysis.

CONCLUSIONS

The results from this study are very useful for the planning of further barley breeding programs.

According to results, salinity significantly reduced the performance of some genotypes while some were tolerant to stress indicating that sufficient genetic variability was present for salinity tolerance among the studied genotypes. Based on correlation and multivariate statistical analysis it can be concluded that MP, GMP and STI were the best indicators of biomass production under both stressed and non-stressed conditions because these indices had positive and significant correlations with Ys and Yp. Suitable genotypes can be selected both under stressed and non-stressed conditions using MP, GMP and STI indices as a means to combine information on performance. Yet, among all genotypes MBS8712 (B), 5Shori (E), WB7910 (F) and 4 Shori (D) showed the best performance in the study.

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