



## RESEARCH PAPER

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## Assessing biomass stability of barley (*hordeum vulgare* L.) genotypes under salinity at early growth stage

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### Abstract

As a result, the development of salinity-tolerant crops is an important option for maintaining crop production in saline soil so this study was performed to assess tolerance, stability and selection criteria of barley genotypes under salinity stress. Nine barley genotypes (STW82153 (A), MBS8712 (B), ESBYTM8910 (C), 4Shori (D), 5Shori (E), WB7910 (F), Valfajr (G), MBS8715 (H) and Jo torsh (I)) in five salinity levels (electrical conductivities, ds/m) (S<sub>1</sub> (control) =4.5, S<sub>2</sub>=7.5, S<sub>3</sub>=10.5, S<sub>4</sub>=13.5 and S<sub>5</sub>=16.5) were evaluated for biomass production. A green-house experiment was laid out in completely randomized design with three replications. Analysis of variance indicated high differences among the genotypes and salinity levels. Biomass production decreased with increasing salinity level and MBS8712 genotype showed better performance than other genotypes. Due to significant genotype × salt effect, for assessing genotypes reaction in salt levels some stability parameters were used. Most of the stability methods indicated that the 4Shori genotype was the most phenotypically stable with above-average performance. The results revealed both potential and performance under stress with stability should be considered simultaneously to take advantage of the selection desirable genotypes for stress tolerance.

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## Introduction

An environmental factor that limits crop productivity or destroys biomass is referred to as a stress or disturbance. Salinity in soil or water is one of the major stresses and, especially in arid and semi-arid regions, can severely limit crop production. The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors. All of these cause adverse pleiotropic effects on plant growth and development at physiological and bio-chemical levels and at the molecular level (Ashraf and Haris, 2004). Barley (*Hordeum vulgare* L.) has a long history as a domesticated crop, as one of the first crops adopted for cultivation and yet under severe stress conditions, barley remains to be an important crop used as feed for animals, malt and human food (Elakhdar *et al.*, 2016). As a result, three main strategies have been recognized that plants use to cope with stress: (i) *specialization*, the genotype is adapted to the specific environment; (ii) *generalization*, the genotype has moderate suitability in most environments; and (iii) *phenotypic plasticity*, signals from the environment interact with the genotype and stimulate the production of alternative phenotypes (Fritsche and Borém, 2005). A desirable genotype produces a satisfactory yield when subjected to stress conditions but that have a high productivity under ideal growing conditions. The interaction of cultivar with environmental factors is an important consideration for plant breeders. Genotypes  $\times$  environment (GE) interaction has been defined as failure of genotypes to achieve the same relative performance in different environments (Sabaghnia *et al.*, 2013). There are two major approaches to studying GE interaction and determining the adaptation of genotypes. The first and most common approach is parametric, which relies on distributional assumptions about genotypes, environmental and GE interaction effects. The second major approach is the non-parametric or analytical clustering approach, which does not need any assumption (Mohammadi and Mahmoodi, 2008).

Although several methods for the statistical measurement of stability have been proposed, no single method can adequately explain genotype performance across environments. From the parametric measure the most widely used is the univariate stability parameters are the Wricke's ecovalence ( $W^2i$ ) (Wricke, 1962), the joint regression including coefficient regression ( $bi$ ) and variance in regression deviations ( $S^2di$ ) (Eberhart and Russell, 1966), Roemer's (1917) environmental variance ( $S^2xi$ ), Shukla's stability variance (Shukla, 1972). These stability methods have their own advantages and limitations (Anley *et al.*, 2013). Some authors preferred to use of univariate parametric stability models due to easy use and interpretation (Khalili and Pour-Abou ghadareh, 2016). A criticism of the use of simple linear regression models is based on the potential non linear pattern of genotype responses to environmental variation. The first proposal to solve this deficiency was presented by Verma *et al.* (1978). They separated the environments into two groups (Favorable and Unfavorable) and fit a simple linear regression model separately to each part. Screening for salt tolerance in the field is difficult as soil salinity is dynamic; the level of salt varies both horizontally and vertically in the soil profile and changes with time.

These environmental perturbations can be overcome by assessment genotypes under conditions where the testing environment is controlled. Therefore the present investigation was conducted to assess the genotype  $\times$  salt interaction and stability of barley genotypes under salinity stress based on biomass production by stability parameters at vegetative growth stage under greenhouse conditions.

## Materials and methods

### Experimental design 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) (S<sub>1</sub> (control) =4.5, S<sub>2</sub>=7.5,

S<sub>3</sub>=10.5, S<sub>4</sub>=13.5 and S<sub>5</sub>=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 75°C for 48h, to give a constant weight. Biomass production was calculated by dividing the total weight by the number of plants.

**Statistical analysis**

Analysis of variance (ANOVA) and statistical comparison of means for genotype biomass production were undertaken. Then Stability parameters were calculated with this difference that biomass production was replaced with yield.

Five stability parameters were performed in accordance with Eberhart and Russell's (1966) the slop value (bi) and deviation from regression (S<sup>2</sup>di), Roemer's (1917) environmental variance (S<sup>2</sup>xi), Wricke's (1962) ecovalance (W<sup>2</sup>i), and Verma model(1978) slop values according to the following formula.

$$S^2 xi = \frac{\sum(xij - \bar{x}.i)^2}{(E - 1)}$$

Where x<sub>ij</sub> is the performance of genotype i in environment j, x<sub>.i</sub> is the mean yield of genotype i and E is the number of environments.

$$w^2i = \sum_{i=1}^n (xij - \bar{x}_i. - \bar{x}_j + \bar{x}..) ^2$$

Where X<sub>ij</sub> is the observed yield response, x<sub>i.</sub> = mean yield of genotype i, x<sub>.j</sub>= mean yield of environment j and x<sub>..</sub> is the grand mean.

$$bi = 1 + \frac{\sum_i(xij - \bar{x}_i. - \bar{x}_j + \bar{x}..)(\bar{x}_j + \bar{x}..)}{\sum_j(\bar{x}_j + \bar{x}..) ^2}$$

$$S^2 di = \frac{1}{E - 2} [\sum_i (xij - \bar{x}_i. - \bar{x}_j + \bar{x}..) ^2 - (bi - 1)^2 \sum_j (\bar{x}_j + \bar{x}..) ^2]$$

That X<sub>ij</sub> is the biomass production of genotype i in environment j, x<sub>i.</sub> is the mean yield of genotype i and x<sub>.j</sub> is the mean yield of the environment j, x<sub>..</sub> is the grand mean and E is the number of environments.

The division of favorable and unfavorable environments was made based on the environmental index that represents the deviation of each environmental mean from the overall mean. Unfavorable environments are those with negative or zero indices and favorable environments have positive indices, so third level of salinity treatments was determined as middle point of tow environments. All statistical procedures were carried out using the R program by agricolae package.

**Results**

*Biomass production*

Analysis of variance results on the performance of barley genotypes under salt stress conditions were presented in Table 1. Biomass production differed significantly due to salinity levels, genotypes and their interactions. The increase in water salinity decreased the barley biomass production. At 4.5 dS m<sup>-1</sup> level, the maximum biomass was produced by G, H and B, respectively. At 16.5 dS m<sup>-1</sup>, three genotypes, B, E and D gave the highest dried weight. The I genotype showed the minimum of biomass at all levels of salinity (Table 2.).

*Stability across salinity levels*

The significant difference of G × S interactions for biomass also is indicating differential response of genotypes to environment and complicate selection

because it measures the degree to which performance in one environment fails to predict performance in the other. The results of five parametric stability statistics are given in Figure 1. A genotype with lower  $W^2i$  is regarded as stable in all environments. The genotype D was with lower Wricke's Ecovalence value. Hence, it was stable genotype followed by the genotypes C and E. The genotypes G and H were with higher Wricke's Ecovalence value and as a result these genotypes were unstable and showed the most change

with increasing in salt concentration. According to Eberhart and Russell's model, genotypes performance is generally expressed in terms of three parameters, mean yield, regression coefficient (bi) and deviation from the regression ( $S^2di$ ). So a stable genotype should have a high mean yield,  $b = 1$  and  $S^2di = 0$ . Considering the three evolution of joint regression the genotype D was stable with outstanding yield performance, having the regression slope close to one and the minimum standard deviation.

**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.

Generally according to the regression coefficient (bi) all the barley genotypes had different response to the varying stress conditions. Genotypes with regression coefficient greater than 1 would be adapted to more favourable, while those with coefficient less than 1 would be relatively better adapted to less favourable

growing conditions (Mehari *et al.*, 2014). Therefore the genotypes G and H for control condition the genotypes B and E in high levels of salinity can be selected as desirable genotypes that are consistent with table 2.

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

Salt genotypes	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	
A	447.7 <sup>efghi</sup>	366 <sup>hijkl</sup>	324.7 <sup>lmno</sup>	310.3 <sup>lmnopq</sup>	253.7 <sup>opqrst</sup>	340.3 <sup>d</sup>
B	584.3 <sup>bc</sup>	521.3 <sup>cde</sup>	437.3 <sup>efghij</sup>	416 <sup>ghijk</sup>	348.7 <sup>ijklmn</sup>	461.5 <sup>a</sup>
C	488.7 <sup>defg</sup>	371 <sup>hijkl</sup>	292 <sup>lmnopqrs</sup>	264 <sup>mnopqrs</sup>	200.7 <sup>stuv</sup>	323.3 <sup>d</sup>
D	543.3 <sup>cd</sup>	418.7 <sup>ghijk</sup>	350 <sup>ijklm</sup>	299.7 <sup>lmnopqr</sup>	286.3 <sup>lmnopqrs</sup>	379.6 <sup>c</sup>
E	525.7 <sup>cde</sup>	451.3 <sup>efgh</sup>	417.3 <sup>ghijk</sup>	347.3 <sup>ijklmn</sup>	319.7 <sup>lmnop</sup>	412.3 <sup>b</sup>
F	548.3 <sup>cd</sup>	455 <sup>efgh</sup>	338.7 <sup>klmno</sup>	286.3 <sup>lmnopqrs</sup>	257 <sup>nopqrs</sup>	377.1 <sup>c</sup>
G	678.3 <sup>a</sup>	512.3 <sup>cdef</sup>	502 <sup>cdefg</sup>	215.3 <sup>rstuv</sup>	204.7 <sup>stuv</sup>	422.5 <sup>b</sup>
H	658.7 <sup>ab</sup>	430.3 <sup>efghij</sup>	358 <sup>ijkl</sup>	247 <sup>opqrst</sup>	212.7 <sup>rstuv</sup>	381.3 <sup>c</sup>
I	293 <sup>lmnopqrs</sup>	225.3 <sup>qrstuv</sup>	229 <sup>pqrstuv</sup>	165.3 <sup>tu</sup>	148 <sup>u</sup>	212.1 <sup>e</sup>
	529.7 <sup>a</sup>	416.8 <sup>b</sup>	361 <sup>c</sup>	283.5 <sup>d</sup>	247.9 <sup>e</sup>	

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).

Desirable genotypes have concave pattern for regression linear models and D and H genotypes showed such pattern across the levels of salinity and

the means of their biomass were more than total average biomass (Fig. 2.). The stability variance ( $S^2xi$ ) revealed that the genotypes I, A and E had the

smallest variance across the environments and were stable, while the genotypes G and H had the largest ( $S^2_{xi}$ ) and were unstable.

**Discussion**

Salt stress results in a considerable decrease in the fresh and dry weights of leaves, stems, tillers, fertile tillers and roots (Turhan and Seniz, 2012). The main

purpose of the study was to identify salt tolerant in barley genotypes in relation to biomass production at early vegetative growth stages under different levels of salinity. 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.

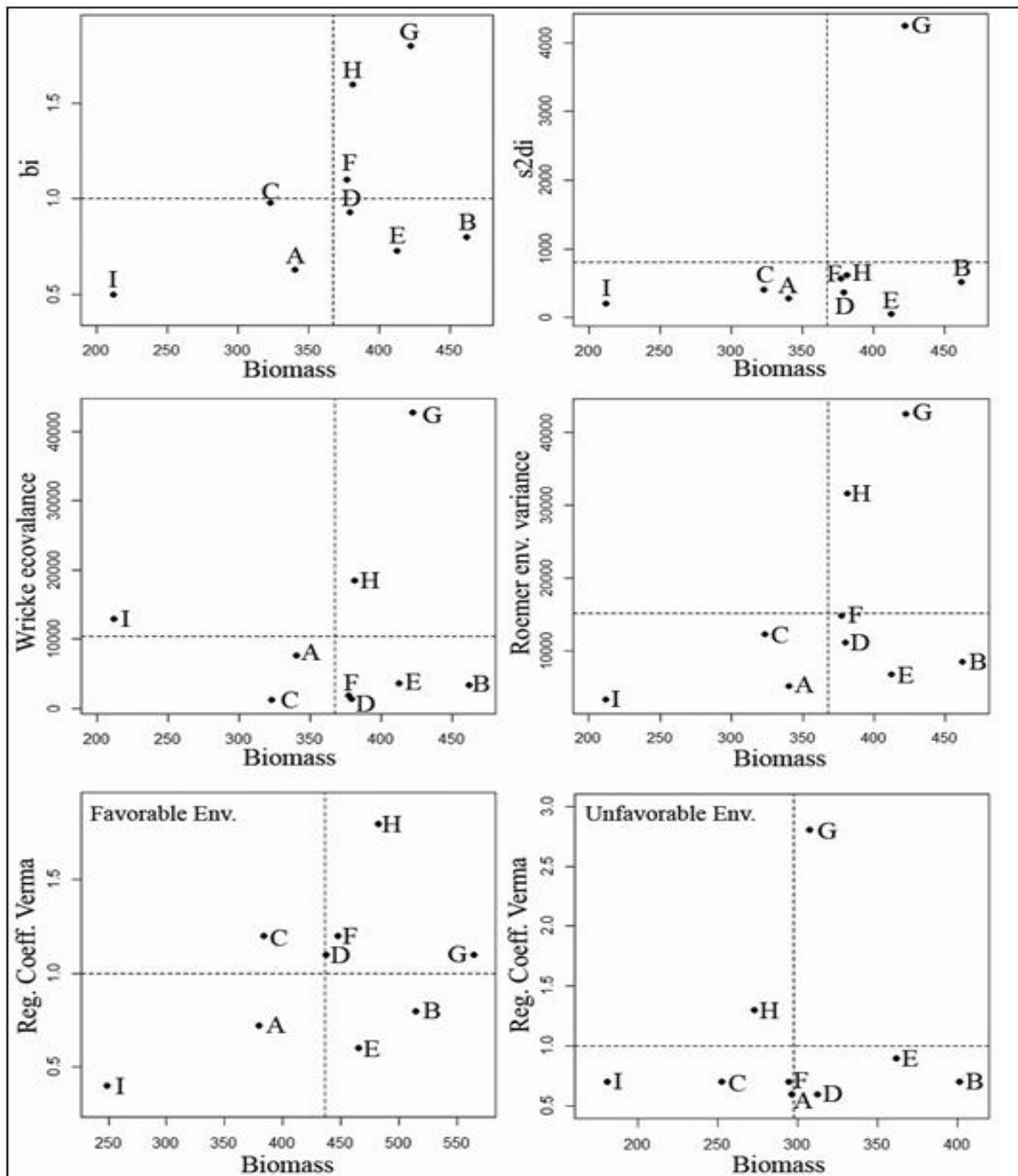


Fig. 1. Distribution of genotypes on the plot based on Stability parameters and biomass production

Two primary ideas have been used by plant breeders for improvement their materials to stress conditions. The first of these philosophies states that high input responsiveness and inherently high yielding potential, combined with stress-adaptive traits will improve performance in stress-affected environments. The breeders who advocate selection in favorable environments follow this philosophy.

The second is the belief that progress in yield and adaptation in stress-affected environments can be achieved only by selection under the prevailing conditions found in target environments. Therefore, based on achieved results, testing and selection under non-stress and stress conditions alone may not be the most effective for increasing yield under salt stress.

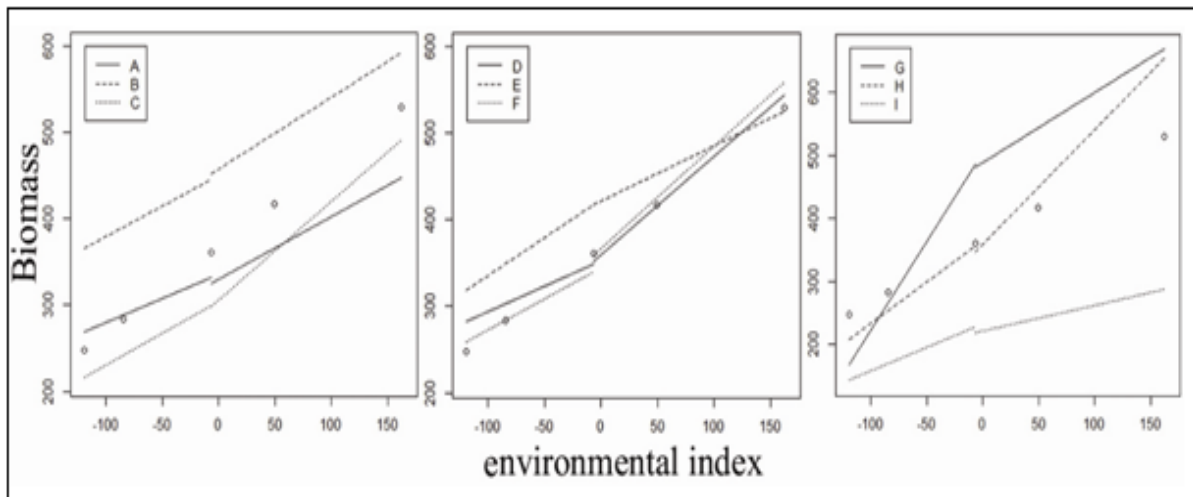


Fig. 2. Performance of barley genotypes across salinity levels based on Verma regression model.

Genotype  $\times$  environment interaction (GEI) is important source of variation in any crop and the term stability is sometimes used to characterize a genotype, which shows a relatively constant yield, independent of changing environmental conditions. On the basis of this idea, genotypes with a minimum variance for yield across different environments are considered stable. This idea of stability may be considered as a biological or static concept of stability (Becker and Leon, 1988).

This concept of stability is not acceptable to most breeders and agronomists who would prefer an agronomic or dynamic concept of stability; therefore they prefer genotypes with high mean yields and the potential to respond to agronomic inputs or better environment conditions. In the dynamic concept of stability, it is not required that the genotype response to environmental conditions should be equal for all genotypes (Becker and Leon, 1988). 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). On this fact the ideal genotype has a regression coefficient smaller than 1 for unfavourable environments and greater 1 for favourable environments.

### Conclusions

The use of appropriate biometrics techniques is necessary for identifying the most adapted, responsive and stable genotypes. In general, both yield and stability of performance should be considered simultaneously to take advantage of the useful effect of GE interaction and to make a selection of the lines with more precise and refined and results in the present study confirm this subject. Most of the stability methods indicated that the D genotype was the most phenotypically stable with above-average performance but the best results belonged to B genotype with high biomass production in all salinity levels.

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