Screening barley varieties tolerant to drought stress based on tolerant indices


a Department of Horticultural Science, Faculty of Agriculture, University of Kurdistan (UOK), Sanandaj, Iran.
b Horticulture and Crop Research Department, Kermanshah Agricultural and Natural Resources Research and Education Center (AREEO), Kermanshah, Iran.
c School of Plant Sciences, College of Agriculture & Life Sciences, The University of Arizona, Tucson, AZ 85721, USA ORCID: https://orcid.org/0000-0002-7662-2258
d Department of Plant Biotechnology, School of Agriculture, Shiraz University, Shiraz, Iran
e Food Industry Group, Faculty of Pharmacy - Medical Science Branch, Islamic Azad University, Shiraz, Iran
f Department of plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran

*Corresponding Author: pessarak@email.arizona.edu ORCID: https://orcid.org/0000-0002-7662-2258
*Second Corresponding Author: saedmoocheshi@gmail.com ORCID: https://orcid.org/0000-0001-9102-9391

Running Title: Stress indices for screening tolerant barley varieties
Abstract
With regards to the importance of drought stress and genotype screening under stress conditions, the current study was conducted to evaluate barley varieties in response to drought stress and find the tolerant ones, along with the determination of the influential ratio of each yield component on grain yield under both conditions. Accordingly, 25 barley varieties were evaluated under two water regimes including 100% (normal condition) and 50% (drought stress) of field capacity. Cluster analysis grouped the genotypes into four and three clusters under normal and stressful conditions, respectively, indicating that drought might limit the phenotypic variability among the varieties. Based on feature selection, spike weight, leaf area, and grain number per spike under normal condition, and the number of fertile spikelets and grain number per spike under water deficit conditions were the most influential traits on grain yield which verifies the impact of drought on the relationship among the agronomic traits. The overall results of biplot showed that varieties Danesiah, Eram, and Yoosef, which had higher grain yield than average for both conditions, were suitable varieties in order to being screened for both normal and drought stress conditions. Finally, according to the results of correlation of tolerance indices with grain yield, stress tolerance index (STI), harmonic mean productivity (HMP), and mean productivity (MP) which showed high correlations with grain yield under both conditions can be introduced as proper indices for screening tolerance genotypes of barley.

Keywords: stress susceptibility, drought tolerance, stress indices, biplot, cluster analysis.

Introduction
Barley (Hordeum vulgare L.) is the fourth most important crop in the world after rice, wheat, and maize in terms of cultivated area (Rollins et al., 2013). However, its yield potential and its annual productivity are significantly lower than the other aforementioned cereals (Saed-Moucheshi, 2018) due to the water shortage that occurs in areas of its cultivation. In a vast majority of these areas, barley plants are typically cultivated as a dry-land or rainfed plant. Annual precipitation in rainfed conditions is the main factor indicating the survival and productivity of the plants (Saed-Moucheshi et al., 2018).

Low precipitation leads to water shortage and results in drought stress in plants. Response to drought stress varies widely among the related species and even between different varieties and genotypes within one specific variety and genotype (Foyer et al., 1991; Sreenivasulu et al., 2000). Therefore, selecting plants carrying suitable genes with higher
adaptability to low water and screening tolerant genotypes is a necessary stage in any breeding program (Abdel-Ghani et al., 2015).

Achieving genetically higher yield under water-limited conditions has been recognized as a highly difficult challenge for plant breeders, as compared to the progress in grain yield in favorable environments (Richards et al., 2002). Therefore, different methods are suggested to provide a way for finding the best genotypes that are more tolerant to drought stress. Accordingly, drought stress indices based on yield loss under drought conditions have been used for screening drought-tolerant genotypes (Mitra, 2001). Each one of these indices basically measures a different perspective in relationship with drought resistance, tolerance, and susceptibility of the genotypes (Fernandez, 1992). Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress conditions compared with the normal condition (Blum, 1988). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI) for screening genotypes. Fernandez (1992) defined a new advanced index (stress tolerance index, STI), which can be used to identify genotypes producing higher yield under both stress and non-stress conditions. He also introduced harmonic mean productivity (HMP), geometric mean productivity (GMP), and modified stress tolerance index (MSTI) for determining tolerant genotypes. Guttieri et al. (2001) by using the SSI criterion in spring wheat cultivars suggested that SSI more than 1 indicated above-average susceptibility to drought stress. Golabadi et al. (2006) and Mardeh et al. (2006) suggested that selection for drought tolerance in wheat could be based on higher MP, GMP, and STI under stressed and non-stressed environments. Talebi et al. (2009) reported significant and positive correlations for Yp with MP, GMP, and STI showing that these indices are more effective in identifying the high-yielding cultivars under different environmental conditions.

Agronomic traits such as grain yield and its components are the major selection criteria for evaluating drought tolerance under field conditions (Lopes et al., 2012). Selection criteria are important in cases where the heritability of yield is low and direct selection based on yield might not be very successful. Consequently, the present study was carried out to find the best selection criteria for identifying drought-tolerant barley genotypes for cultivation in the drought-prone areas of Iran. Moreover, different stress indices were evaluated to identify the best indices applicable for finding the favorable genotypes by using advanced statistical techniques.
Materials and methods

This study was carried out in a research field station located at the Agricultural and Natural Resources Research Center of Fars, Shiraz, Iran in 2016. A factorial experiment based on the randomized complete block design (RCBD) with three replications was used to evaluate the effects of different water regimes, including 100 and 50% of field capacity (FC) on 25 barley genotypes (Table 1). The physical and chemical properties of the soil samples are presented in Table 2. At the cultivation time, 150 mg N kg\(^{-1}\) soil was used in the form of urea (46% N) fertilizer. Prior to seed sowing, the seeds were treated with ethanol 98% for about 20 s and washed three times with distilled water. The water regimes were applied after the flowering and maturity stages of the plant.

Measurements

Peduncle length, spike length, leaf area, grain number per spike, 1000-grain weight, biological yield, grain yield, harvest index, awn length numbers of the fertile spikelet, spike weight, and relative water content (RWC) were measured in the current study. In addition, press evolution (PEV) (Blum, 1988), stress susceptibility index (SSI) (Fischer and Maurer, 1978), tolerance (TOL) (Rosielle and Hamblin, 1981), mean productivity (MP) (Rosielle and Hamblin, 1981), yield index (YI) (Lin et al., 1986), yield stability index (YSI) (Blum, 1988), stress tolerance index (STI) (Fernandez, 1992), and harmonic mean Productivity (HMP) (Fernandez, 1992) were calculated as stress indices based on the yield of stressful and normal conditions using the following formulas:

$\text{PEV} = 1 - \frac{Y_s}{Y_p}$  \hspace{1cm} \text{Press evolution (Blum, 1988)}

$\text{SSI} = \frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{Y_s}{Y_p}}$  \hspace{1cm} \text{Stress Susceptibility Index (Fischer and Maurer, 1978)}

$\text{MP} = \frac{Y_s + Y_p}{2}$  \hspace{1cm} \text{Mean Productivity (Rosielle and Hamblin, 1981)}

$\text{TOL} = Y_p - Y_s$  \hspace{1cm} \text{Tolerance Index (McCaig and Clarke, 1982)}

$\text{YI} = \frac{Y_s}{Y_p}$  \hspace{1cm} \text{Yield Index (YI) (Lin et al., 1986)}

$\text{YSI} = \frac{Y_s}{Y_p}$  \hspace{1cm} \text{Yield Stability Index (Bouslama and Schapaugh Jr., 1984)}

$\text{STI} = \frac{Y_p \times Y_s}{Y_p^2}$  \hspace{1cm} \text{Stress Tolerance Index (Fernandez, 1992)}

$\text{YR} = 1 - \left( \frac{Y_s}{Y_p} \right)$  \hspace{1cm} \text{Yield Reduction Ratio (Choukan et al., 2006)}
Golden Mean Index (Moradi-Dezfuli et al., 2008)

\[ GM = \frac{Y_p \times Y_s}{Y_p - Y_s} \]

Harmonic Mean (Jafari et al., 2012)

\[ HMP = \frac{2 \times Y_p \times Y_s}{Y_p + Y_s} \]

In these formulas, \( Y_p \), \( Y_s \), \( \bar{Y}_p \), and \( \bar{Y}_s \) are the final yield of each genotype under non-stress condition, the yield of each genotype under stress condition, the average yield of all genotypes under non-stress condition, and average of all genotypes under stress condition, respectively.

**Statistical analyses**

The data were subjected to analysis of variance (ANOVA) based on factorial experiment arranged in completely randomized design by SAS software (V. 9.3) using PROC GLM. The Least Significant Difference (LSD \( \alpha = 0.05 \)) test was used for the multiple mean comparisons. Normality Test was then carried out on the residuals of the ANOVA model. Cluster analysis for the used varieties was performed by using Euclidian distance and Wad’s method in Minitab software (v.16). Stepwise selection procedure and correlation coefficient technique were also applied to screen the most important variables affecting grain yield based on PROC REG and PROC CORR of SAS software. The Principal Component Analysis was implemented by IBM SPSS (v. 22) for extracting the biplot graph.

**Results**

ANOVA results showed that the effects of variety and stress were significant for all the measured traits (data not provided). Mean comparisons for different varieties under both normal irrigation and stressful condition were carried out for all measured traits and the results are presented in Table 3 and Table 4. The varieties showed high variations for the measured traits. Verities 15 and 17 had high values for most of the traits, while varieties 22 and 25 showed low values.

Taking all measured traits as criteria for grouping used varieties, cluster analysis was performed. Figure 1 and Figure 2, respectively, represent the clustering results of the varieties for normal irrigation and water deficit conditions. Cluster analysis for the normal irrigation condition grouped varieties into four main groups. Varieties number 15, 13, 10, 14, and 5 were included in the first group, varieties 17, 7, 25, 20, and 4 laid in the second group, varieties 24, 23, 19, 12, 8, 9, 6, 18, and 2 fell in the third group, and finally, varieties 16, 22, 21, 11, 3, and 1 were in the fourth group (Figure 1). Under drought stress condition, three main clusters were
resulted. Variety number 25 was the only member of the first group. The second group was containing varieties number 20, 17, 11, 14, 5, 24, 19, and 2, while the third group took varieties number 8, 7, 16, 23, 21, 9, 10, 12, 6, 18, 4, 15, 13, 22, 2, and 1 as its members (Figure 2).

In order to determine the association between the measured traits and their impact on the grain yield, a correlation coefficient using the Pearson method was carried out and its results are presented in Table 5. The highest correlation coefficient (0.86) was recorded for grain yield and biological yield. In addition, the correlation between spike weight and the number of fertile spikelets, spike weight and grain number per spike, grain number per spike and number of the fertile spikelets, leaf area and biological yield, as well as spike length and biological yield were positively significant. In the same way, the correlation coefficient between grain yield and harvest index, grain number per spike, the number of fertile spikelets, spike weight, and also grain number per spike were positively significant. Other correlation coefficients were not significant.

Table 6 and Table 7 are representing the selection technique applied to screen the most important traits on the grain yield separately for the normal irrigation and stressful conditions, respectively. Apart from the biological yield and the harvest index, all other measured traits were used in the initial model. Stepwise regression method at the first stage selected spike length, then leaf area, and finally grain number per spike as the traits significantly influenced grain yield under normal irrigation condition. The calculated coefficients for the grain number per spike (0.04) and spike weight (0.26) were positive, but the leaf area coefficient was negative. Alternatively, under stressful condition, the number of the fertile spikelets and the grain number per spike were included in the final model (Table 7). Considering regression coefficients of both selected traits in stressful condition, the grain number per spike, and the number of the fertile spikelets showed positive effects on the grain yield.

Some stress indices containing PEV, SSI, TOL, MP, YI, YSI, STI, and HMP were computed based on the grain yield and the results are presented in Table 8. Variety number 15 showed the highest values for the grain yield under normal condition, PEV, SSI, and TOL, and it showed the highest value for YSI. Maximum values of HMP, YI, STI, and grain yield under stressful condition were observed in variety number 22. Variety number 16 had the highest MP value, on the contrary, varieties number 1 and 12 commonly showed the highest value for YSI. Variety number 11 had minimum values for grain yield under normal condition along with the lowest PEV, TOL, MP, STI, and HMP. Similarly, the lowest values of the grain yield under stressful condition, SSI, and YI resulted in varieties number 8, and 1.
In order to distinguish the suitable varieties for both normal and stressful conditions by taking the results of all tolerance indices into the consideration, the principal component analysis was performed and the biplot was prepared accordingly (Figure 3). The summary results of the indices’ loadings based on the first two components are presented in Table 9. Due to the high values that tolerance indices showed in the first component (dimension), the first component can be called the tolerance component. On the contrary, the second component could be considered as the susceptible component because of the high negative coefficient of susceptibility indices that showed for this component. Regarding this elicitation, varieties number 22, 24, and 14 are suitable varieties for both conditions (area B in Figure 3) and they can be planted in the area having an alternative environment condition. On the other hand, the varieties number 8, 12, 16, 11, and 9 had low values for both components, so that these varieties are not suitable for neither condition (area C in Figure 3).

With the aim of clarifying the association among the estimated tolerance indices, the correlation method was used (Table 10). STI, HMP, and MP showed a high positive significant correlation with grain yield under both conditions. PEV, SSI, and TOL had a negative correlation with the grain yield under stressful condition. Eventually, it would be better to take HMP, MP, and STI as more proper indices in comparison to other indices.

Discussion
Since the mean comparison for all measured traits showed significant differences among varieties under different normal irrigation and water deficit condition, all of these traits were used for cluster analysis of varieties. Clustering analysis resulted in clearly different grouping patterns for normal irrigation and drought stress conditions, indicating the significant effects of drought on the measured traits. There were four groups under normal condition, while three groups under drought stress condition; this result might indicate that drought stress causes to decrease the phenotypic variability among the varieties and limiting the potential of the variable. In accordance with our results, the cluster analysis of wheat genotype (Saed-Moucheshi et al., 2013b), triticale genotypes (Riasat et al., 2019), and alfalfa (Riasat et al., 2020), resulted in a lower number of clusters in the stressful condition.

In order to determine the association between the measured traits and their impact on the grain yield, a correlation coefficient using the Pearson technique was carried out. Most of the measured traits showed a high and positive correlation with the grain yield under both normal and stress conditions. Due to the importance of indirect selection and selection criteria in distinguishing and screening proper genotypes in breeding programs, feature selection was
used to identify the significant traits influencing the grain yield of barley varieties. Based on the logic that the grain yield is confounded with biological yield and harvest index and both in their measurements, these two traits were excluded from the initial feature selection model. Spike length, then leaf area, and grain number per spike under normal condition, and the number of fertile spikelets together with grain number per spikes under drought stress condition were included in the final model. The results clearly show the impact of drought on the relationship of agronomic traits and also the importance of different selection criteria for each of the conditions. Tabarzad et al. (2017) and Saed-Moucheshi et al. (2013b) showed similar results regarding the different selection criteria for drought stress condition and normal irrigation condition. In addition, there are some other studies (Feghhenabi et al., 2020; Narimani et al., 2020) that have achieved different models in their results related to making the comparison between salinity and normal conditions at different growth stages.

Based on the results of principal component analysis, the elicited biplot in a two-dimensional plat was divided into four different areas. Among these areas, the B quarter showed higher values for both the first and second components. Since the first component showed a high correlation with tolerance-related indices and the second showed a high negative correlation with susceptibility-related indices, this quarter (B in Figure 3) would contain the varieties with the ability to be screened for both normal and stressful conditions. Accordingly, the genotypes in the B quarter are the best choice for breeding programs considering the water deficit condition, especially genotypes number 12, 22, and 25. In addition, according to Aliakbari et al. (2013) and Shahrokhi et al. (2020) determining the best indices in each separate experiment would help to use them more appropriately for finding suitable genotypes. Bonea (2020) reported that the indices having a higher correlation with the grain yield under both stressful and normal conditions be more proper for determining suitable varieties. In the current study, STI, HMP, and MP showed significant and positive correlations with the grain yield under both conditions, while PEV, SSI, and TOL had a negative correlation with the grain yield under stressful condition. Eventually, these results are recommending HMP, MP, and STI as more proper indices for screening the barley genotypes.

Since drought is a serious problem reducing crop productivity, the improvement of drought tolerance in crops such as barley is a major objective for most crop breeding programs (Fayaz and Arzani, 2011; Saed-Moucheshi, 2018). In order to determine tolerant and susceptible genotypes in response to different environmental conditions, some useful multivariate techniques have been properly applied. Clustering analysis, regression methods, and principal component analysis are considered as appreciated techniques in plant breeding
and screening programs (Aliakbari et al., 2013; Saed-Moucheshi et al., 2013a; Saed-Moucheshi et al., 2021). On the other hand, there numerous univariate techniques for identifying tolerant genotypes that work differently from each other. Also, different studies pointed out relatively different univariate techniques to be suitable for finding the best genotype for different environmental conditions. For example, susceptibility indices such as SSI, TOL, and PEV and also tolerant indices such as STI appeared to be very useful for extracting suitable genotypes in the study of Saed-Moucheshi et al. (2013a) on wheat genotypes, while Kumari et al. (2020) introduced STI and GMP as the most effective ones. Moreover, considering the relationship between stress indices, Talebi et al. (2009) reported that the positive correlations between TOL and irrigated yield (Yp) and the negative correlation between TOL and yield under stress (Ys) suggested that selection based on TOL will result in reduced yield under well-watered conditions. Similar results were shown by Clark et al. (1992), Mardeh et al. (2006), and Jamaati-e-Somarin and Zabihi-e-Mahmoodabad (2012). As a result, combining the multivariate techniques with tolerance indices is a highly applicable method for using all the estimated indices in genotype screening programs. In addition to our study, similar analysis has been used for introducing tolerant and susceptible genotypes by Fernandez (1992) in mung bean, Farshadfar and Sutka (2003) in maize, Golabadi et al. (2006) and Talebi et al. (2009) in durum wheat, and Jamaati-e-Somarin and Zabihi-e-Mahmoodabad (2012) in lentil.

Conclusions
Cluster analysis based on all traits grouped the varieties into four groups under normal irrigation condition, but three groups under stressful condition, indicating the differences among the varieties under these conditions. This result indicated that drought stress would limit the variability among varieties-based agronomic traits. Feature selection models showed spike weight, leaf area, and grain number spike to be the most effective features under normal condition, while the number of fertile spikelet and grain numbers per spike were the features with the highest influence on barley grain yield under drought stress condition. The difference between the importance of the traits under normal and stressful conditions is showing the impact of drought and water deficit on the physiological relationship among agronomic traits. Varieties Yoosef, Danesiah, and Eram and were taken as the suitable varieties for both conditions based on the results of biplot and principal component analysis. Besides the overall results of the biplot, these varieties had higher grain yield than the average yield under both conditions. Finally, STI, HMP, and MP because of their high correlation with grain yield under
both conditions in this study can be considered as the most proper indices for selecting tolerant and suitable barley genotypes.

References


