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Evaluation of drought tolerance indices for the selection of Iranian barley (*Hordeum vulgare*) cultivars

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Drought is an important factor limiting crop production in arid and semi-arid conditions. Drought indices which provide a measure of drought based on yield loss under drought condition in comparison to normal condition was used for screening drought-tolerant genotypes. This study was conducted to determine drought tolerant genotypes with high yield in stress and non-stress conditions. Ten barley genotypes were tested in a randomized complete block design with three replications at the Research Centre of Islamic Azad University of Firoozabad, Iran during 2010-2011 growing season. Eight drought tolerant indices mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), tolerance index (TOL), stress susceptibility index (SSI), harmonic mean (HARM), yield index (YI) and yield stability index (YSI) were estimated for each genotype based on yield under stress (Ys) and non-stress (Yp) conditions. There were significant differences for all criteria among the genotypes. The correlation coefficients indicated that MP, STI, GMP and HARM were the best criteria for selection of high yielding genotypes under stress and non-stress conditions. Principal components analysis showed two components which explained 99.66% variation. Based on the results of principal component analysis, biplot and cumulative grain yield diagrams and cluster analysis, Nosrat cultivar was the most tolerant genotype and showed considerable potential to improve drought tolerance in barley breeding programs. Kavir and Nimruz cultivars were identified as high drought susceptibility and low yield stability genotypes.

Key words: Mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), tolerance index (TOL), stress susceptibility index (SSI), harmonic mean (HARM), yield index (YI), yield stability index (YSI).

INTRODUCTION

Drought continues to be a major constraint on the productivity of cereal crops and water deficit will increase in most arid and semi-arid regions under future climate-change scenarios (IPCC, 2007; Wassmann et al., 2009). Simultaneously, drought resistance in crops is probably the most difficult trait to understand (Ashraf, 2010; Bruce et al., 2002). Hence, in the absence of thorough

information related to the genetic mechanism of drought tolerance, grain yield under dry conditions is most often used to quantify the level of drought resistance of a genotype rather than a direct selection criterion, which can accurately measure the level of crop drought resistance (Farshadfar and Sutka, 2002). Thus, drought indices which provide a measure of drought based on yield loss under drought condition in comparison to normal condition have been used for screening drought-tolerant genotypes (Mitra, 2001). Tolerance index (TOL) and mean productivity (MP) (Rosielle and Hambline, 1981), stress susceptibility index (SSI) (Fischer and Mau-rer, 1978), geometric mean productivity (GMP) (Kristin et al., 1997; Fernandez, 1992), stress tolerance index (STI)

(Fernandez, 1992) and harmonic mean (HARM) (Kristin et al., 1997) have all been employed under various conditions.

A study was conducted from 2003 to 2004 to evaluate quantitative drought resistance criteria of barley (*Hordeum vulgare*) genotypes in stressed and non stressed conditions. The results showed that genotypes were significantly different for their yield under stress and non-stress conditions. This study was conducted to assess the selection criteria for identifying drought tolerant genotypes and high-yielding genotypes in drought stress and non-stress conditions (Giancarla et al., 2010). Khokhar et al. (2012) evaluated twelve barley genotypes based on different selection methods under drought and irrigated conditions. Drought stress reduced the yield of some genotypes while others were tolerant to drought, suggesting genetic variability in this material for drought tolerance. The results of a correlation matrix revealed highly significant associations between grain yield (Yp) and mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP) and yield index (YI) under irrigated conditions while, the mean productivity (MP), yield stability index (YSI), stress tolerance index (STI), geometric mean productivity (GMP) and yield index (YI) had a high response under stressed condition. Based on a principal component analysis, GMP, MP and STI were considered to be the best parameters for the selection of drought-tolerant genotypes. Nazari and Pakniat (2010) indicated that STI, MP and GMP are the best criteria for the selection of high yielding barley genotypes both under stress and non-stress conditions. Results of calculated gain from indirect selection indicated that selection under moisture stress would be efficient in yield improvement compared to non-stress condition. Genotypes were significantly different for their yield under stress and non-stress conditions.

Ahmadizadeh et al. (2012) showed that MP, STI, GMP and HARM were the most suitable indices to screen durum wheat genotypes in drought stress condition. Khalili et al. (2004) showed that based on geometric mean productivity (GMP) and STI indices, corn hybrids with high yield in both stress and non-stress environments can be selected. Abdi et al. (2012) showed that mean productivity (MP), geometrical mean productivity (GMP) and stress tolerance index (STI) were suitable resistance indices for the identification of bread wheat genotypes to drought stress. Talebi et al. (2009) reported that MP, GMP and STI were more effective in identifying high yielding cultivars under different moisture conditions. Akcura et al. (2011) revealed that SSI was suggested as useful indicator for wheat breeding where the stress was severe while MP, GMP, TOL, HARM and STI were suggested if the stress was less severe.

In other research, three triticale cultivars with different yield performance were grown in separate experiments under the rain-fed and irrigated conditions at Eskisehir,

Turkey, in 2006 to 2007 growing season. The cultivars showed significant differences in spike length, spike weight, number of kernels per spike and grain yield traits. None of the indicators could clearly identify cultivars with high yield under both stress and non-stress conditions. It is concluded that the effectiveness of selection indicated depends on the stress severity (Kutlu and Kinaci, 2010).

The objectives of this study were to (i) identify drought tolerant barley varieties under different conditions in southern Iran, (ii) determine the efficiency of tolerance indices to classify barley varieties into sensitive and tolerant and (iii) interpret interrelationships among the tolerance indices by biplot analysis.

MATERIALS AND METHODS

Ten barley genotypes Gorgan4 (G1), Nosrat (G2), Reyhan (G3), Makoii (G4), Valfajr (G5), Zarjou (G6), Gorgan (G7), Kavir (G8), Esterain (G9) and Nimruz (G10) were chosen for this study based on their reputed differences in yield performance under stress and non-stress conditions. Two separate experiments were carried out at the research station of Islamic Azad University of Firoozabad, Iran (28.35°N, 52.40°E and 1327 m above sea level). In each experiment, the ten genotypes were planted in a randomized complete block design with three replications. Every one of the experiments was in the same condition and the only existing difference among them was stopping the irrigation in one of the experiments at blooming stage till complete maturity stage. Seeds were hand-sown 3 to 5 cm deep on November 28 in 2009-2010 growing season.

Each plot consisted of 4 rows with 2.5 m long and spacing of 5 cm between plants within row and 50 cm between rows (200 plant plot^{-1}). The experiments were conducted as fertilizer broadcast were 100 kg ha^{-1} of ammonium phosphate, 50 kg ha^{-1} of potassium sulphate and 50 kg ha^{-1} N applied prior to planting plus an additional 100 kg ha^{-1} N at the beginning of stem elongation. Hand-weeding was carried out four times during the growth period. Grain yield was measured at physiological maturity and yield was adjusted to 10% seed moisture content.

Eight selection indices mean productivity (MP), stress tolerance index (STI), geometric mean productivity (GMP), tolerance index (TOL), stress susceptibility index (SSI), harmonic mean (HARM), yield index (YI) and yield stability index (YSI) were estimated for each genotype based on grain yield under stress (Ys) and non-stress (Yp) conditions. Quantitative drought resistance indices were calculated using the following formulas:

1) $MP = \frac{Ys+Yp}{2}$ (Rosielle and Hambline, 1981)

2) $STI = \frac{Ys \times Yp}{Y_p^2}$ (Fernandez, 1992)

3) $GMP = \sqrt{Ys \times Yp}$ (Fernandez, 1992)

4) $TOL = Yp-Ys$ (Rosielle and Hambline, 1981)

5) $SSI = \frac{1-(Ys/Yp)}{1-(\overline{Ys}/\overline{Yp})}$ (Fischer and Maurer, 1978)

6) $HARM = \frac{2(Yp \times Ts)}{(Yp+YS)}$ (Kristin et al., 1997)

7) $YI = \frac{Ys}{Yp}$ (Lin et al., 1986; Gavuzzi et al., 1997)

8) $YSI = \frac{Ys}{Yp}$ (Bouslama and Schapaugh, 1984)

Reduction percentage was calculated as follows:

% Reduction = $\frac{Yp-Ys}{Yp} \times 100$ (Choukan et al., 2006)

Where, Yp is the yield under non-stress condition; Ys the yield under stress; $\bar{Yp}$ and $\bar{Ys}$ is the mean yields of all genotypes under non-stress and stress condition, respectively and $1 - (\bar{Ys}/\bar{Yp})$ is the stress intensity (SI).

The data were tested for skewness, kurtosis, homogeneity of variance and normality by MINITAB (1998) statistical software. Then, analysis of variance based on Random Complete Block Design (RCBD), phenotypic correlation, comparison of quantitative traits means based on Duncan's new multiple range test (DNMRT) and principal component analysis were performed in SAS (2001). Cluster analysis of genotypes for Yp, Ys and drought tolerance indices was carried out using Ward's method and Euclidian distance by Minitab software. Biplot and cumulative grain yield diagrams were drawn with StatGraphics Plus 2.1 and Excel 2007 softwares, respectively.

RESULTS

Analysis of variance of Ys, Yp and drought tolerance indices showed that there were high significant differences (p<0.01) among the barley genotypes (Table 1) indicating the presence of high genetic variability. Ahmadizadeh et al. (2012), Shahryari and Mollasadeghi (2011) and Nazari and Pakniat (2010) also reported significant differences for all criteria among the genotypes. Nosrat and Reyhan cultivars had the highest grain yield in non-stress and stress conditions, respectively (Table 2). The average grain yield under non-stress condition was 501.066 gm^{-2} while in stress condition it was 258.168 gm^{-2}, with a decrease of 46.940%. Stress intensity (SI) has been given in stress susceptibility index (SSI) formula that it can be at most 1. In this experiment, stress intensity was calculated SI=0.485. Ahmadizadeh et al. (2012) also reported similar results for durum wheat genotypes.

According to SSI, Reyhan cultivar followed by Zarjou and Gorgan cultivars had the lowest values, which were considered as genotypes with low drought susceptibility and high yield stability in stress and non-stress conditions, whereas Esterain cultivar followed by Valfajr, Makoii, Kavir, Gorgan4 and Nosrat cultivars with SSI values higher than unit can be identified as high drought susceptibility and low yield stability genotypes (Table 2). The lowest TOL was observed for Reyhan cultivar followed by Nimruz, Zarjou and Gorgan cultivars, but the highest TOL belonged to the Esterain cultivar followed by Nosrat and Valfajr cultivars (Table 2). Based on STI, Nosrat cultivar followed by Reyhan cultivar with the high-

est values were considered to be tolerant genotypes, whereas the Makoii cultivar followed by Esterain cultivar with the lowest STI were intolerant (Table 2). These results indicated that the genotypes with high STI usually have high difference in yield in two different conditions. In general, similar ranks for the genotypes were observed by GMP and MP indices as well as STI, which suggested that these three indices were equal for selecting genotypes (Table 2).

Mohammadi et al. (2010) showed YSI to be a more useful index to discriminate drought-resistant from drought-susceptible genotypes. Therefore, breeders should select this index for selection of stress-tolerant geno-types. Based on YSI values, the highest and lowest YSI index belong to Reyhan and Esterain cultivars, respectively. In addition, these cultivars had the lowest and highest TOL and SSI (Table 2). Based on YSI, TOL and STI indices, Reyhan and Esterain cultivars could be considered relatively drought tolerant and sensitive, respectively (Table 2). YI can be used as a selection criterion, although it only ranks cultivars on the basis of Ys. Based on YI, Reyhan cultivar had the highest YI and Ys (Table 2).

To determine the most desirable drought tolerance criteria, the correlation coefficient between Yp, Ys and other indices of drought tolerance were calculated (Table 3). Grain yield under drought stress was not significantly correlated with grain yield under non-stress condition (r=0.39), suggesting that high potential yield under optimum conditions does not necessarily result in improved yield under stress condition (Table 3). Thus, indirect selection for a drought-prone environment based on the results of optimum conditions will not be efficient. These results are in agreement with those of Bonea and Urechean (2011), Talebi et al. (2009) and Yazdchi (2008) that maize, wheat and barley genotypes, respectively with low yield potential was more productive under stress conditions. Correlation coefficient of Ys and Yp with TOL (0.72 and -0.36, respectively), SSI (0.27 and -0.77, respectively), YI (0.39 and 1, respectively) and YSI (-0.26 and 0.78, respectively) showed that MP, STI, GMP and HARM indices were better predictor of Yp and Ys than TOL, SSI, YI and YSI. These results agree with the finding of Khokhar et al. (2012) and Yazdchi (2008) in barley and Talebi et al. (2009) in durum wheat.

TOL index was significantly correlated with grain yield in non-stress condition and had less negative correlation with grain yield under stress condition, having in mind that small value of TOL is desirable. These results agree with the results of Shirani Rad and Abbasian (2011) in winter rapeseed cultivars that TOL was strongly correlated with yield under non-stress condition and had negative and non-significant correlation with yield under stress condition. Selection for this parameter would tend to favor low yielding genotypes. TOL and SSI indices had significantly positive correlation with each other (r=0.86).
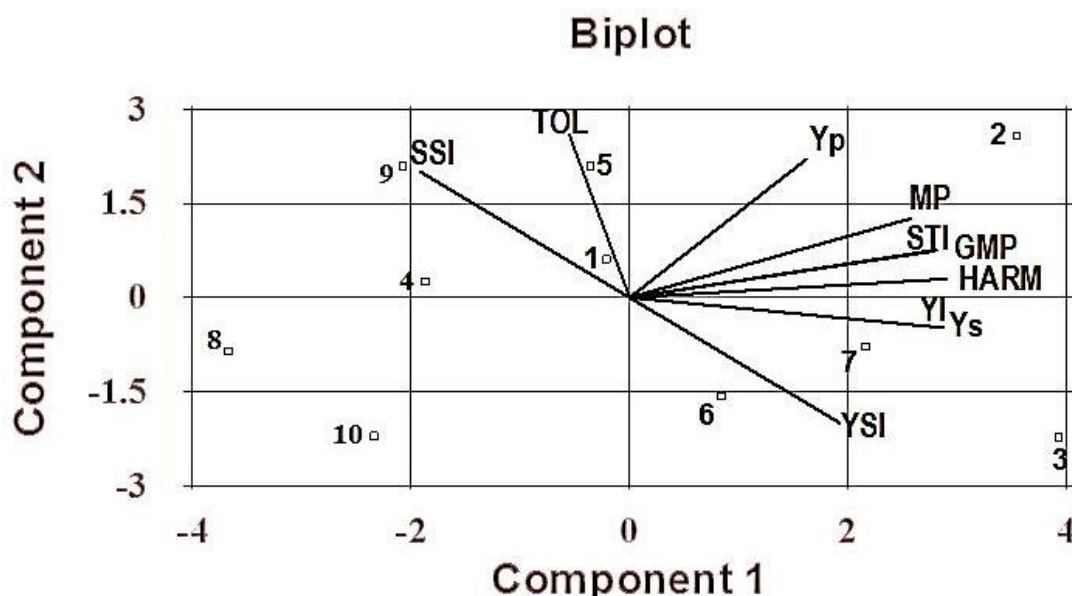
Biplot

Figure 1. Biplot of drought tolerance indices based on the first two principal components axes (PC1 and PC2) for 10 barley genotypes in non-stress and stress conditions. Yp: grain yield under non-stress condition, Ys: grain yield under drought stress condition, SSI: stress susceptibility index, TOL: stress tolerance, MP: mean productivity, STI: stress tolerance index, GMP: geometric mean productivity, HARM: harmonic mean, YI: yield index, YSI: yield stability index. Genotypes' names: 1. Gorgan4, 2. Nosrat, 3. Reyhan, 4. Makoii, 5. Valfajr, 6. Zarjou, 7. Gorgan, 8. Kavir, 9. Esterain, 10. Nimruz.

Therefore, these indices can be considered to reflect the same information. YSI had significantly negative correlation with SSI and TOL (r= -0.99 and r= -0.86, respectively). Thus, a big value of this index is desirable and selection for this parameter would also tend to favor low yielding genotypes. Ahmadizadeh et al. (2012), Bonea and Urechean (2011) and Giancarla et al. (2010) also reported that TOL was significantly positively correlated with SSI.

HARM index was significantly positively correlated with MP and GMP indices. If there were significant correlation between MP, GMP and HARM, HARM can be considered to reflect a little better the performance under stress than MP and GMP like GMP, the correlation of HARM with grain yield under stress being better (r=0.96 and r=0.64 in stress and non-stress conditions, respectively), but the correlation of MP with grain yield under non-stress is better (r=0.88 and r=0.78 in non-stress and stress conditions, respectively). STI was perfectly correlated with MP, GMP and HARM. Therefore, STI index contains the same information; like HARM it was significant and positive correlated with both Yp and Ys, that its correlation with grain yield under stress condition was better than grain yield under non-stress condition (r=0.89 and r = 0.75, respectively). Siahsar et al. (2010) also reported similar results in lentil lines. Selection based on a combination of indices may provide a useful criterion for improving drought resistance of barley, but studies of

correlation coefficients are useful in finding out the degree of overall linear association solely between any two considered attributes. Thus, a better approach such as biplot analysis is needed to identify the superior genotypes for both stress and non-stress conditions. Genotypes subjected to biplot analysis, are compared for assessing relationships between all the attributes at once. Principal component analysis revealed that the first two PCAs accounted for about 99.66% of total variation (Table 4).

The first PCA, which accounted for 67.14% of variation among all variables, was positively correlated with YI, Ys, HARM, GMP, STI and MP (Table 4). Thus, the first dimension can be named as the yield potential and drought tolerance. Considering the high and positive value of this component, genotypes that have high values of these indices will be high yielding under stress and non-stress conditions. As a result, Reyhan and Nosrat cultivars with high PC1 were more suitable for stress and non-stress conditions (Figure 1). The second PCA accounted for 32.52% of all variation and was highly positively correlated with Ys, but negatively with TOL and SSI (Table 4). It is thus a stress-tolerant dimension that is capable of separating stress-tolerant from non-stress tolerant genotypes.

In consequence, selection of genotypes that have high PCA1 and PCA2 are suitable for both stress and non-stress conditions (Kaya et al., 2006). Therefore, Nosrat
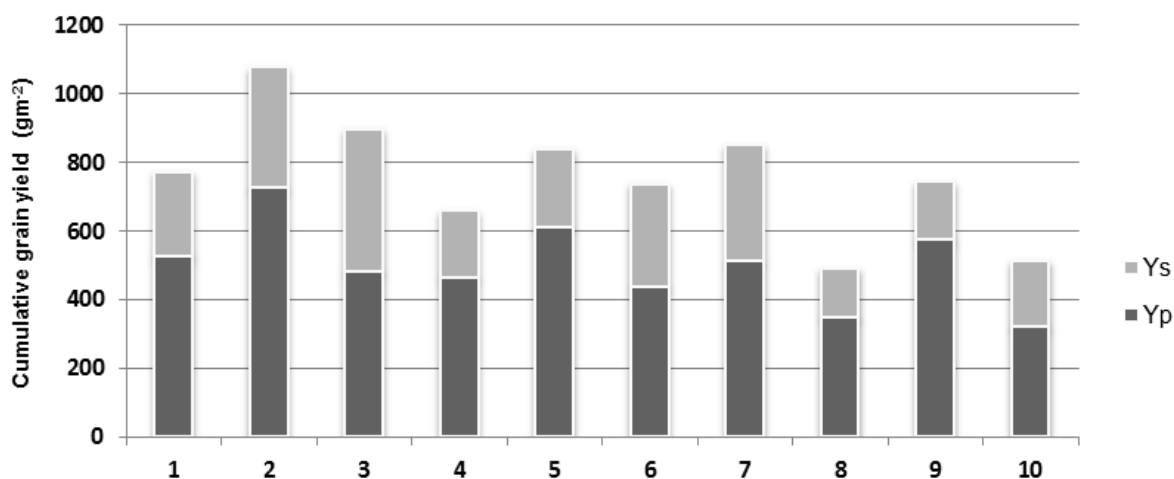

Figure 2. Cumulative grain yield of barley genotypes under both drought stress and non-stress conditions. Genotypes' names: 1. Gorgan4, 2. Nosrat, 3. Reyhan, 4. Makoii, 5. Valfajr, 6. Zarjou, 7. Gorgan, 8. Kavir, 9. Esterain, 10. Nimruz.

cultivar with higher PCA1 and PCA2 is a superior genotype under both stress and non-stress conditions (Figure 1). Cumulative grain yield diagram also illustrated that Nosrat cultivar was the best genotype under both stress and non-stress conditions (Figure 2). Reyhan cultivar with high PCA2 was more suitable for stress than for non-stress condition (Figures 1 and 2). Kavir and Nimruz cultivars with lower PCA1 and PCA2 were the worst genotypes under both stress and non-stress conditions (Figures 1 and 2). Therefore, they can be identified as high drought susceptibility and low yield stability genotypes. A similar result was reported by Ahmadizadeh et al. (2012) in durum wheat.

Cluster analysis has been widely used for description of genetic diversity and grouping based on similar characteristics (Souri et al., 2005). Separate cluster analysis (using Ward's method) based on Yp, Ys and other drought stress indices were performed for barley genotypes. In the dendrogram (Figure 3), genotypes are presented on the horizontal axis and the Euclidean distances on the vertical. The discriminate function analysis allowed the highest differences among groups when genotypes were categorized into four groups (Figure 3). Based on the results, Nosrat cultivar, which had the highest PCA1 and PCA2, was located in the first cluster. Reyhan, Zarjou and Gorgan cultivars, which had high PCA1 and low PCA2, were placed in the second cluster that was partitioned into two small clusters. Therefore, cluster analysis supported the results of principal component analysis because Reyhan, Zarjou and Gorgan cultivars were in this group. Esterain, Valfajr, Makoii and Gorgan4 cultivars with low PCA1 and high PCA2 were placed in the third cluster that was partitioned into two small clusters and confirmed the results of principal component analysis. Kavir and Nimruz cultivars, which had low PCA1

and PCA2, were placed in the fourth cluster that confirmed principal component results (Figure 3).

DISCUSSION

The greater the TOL value, the larger the yield reduction under stress condition and the higher the drought sensitivity. Therefore, based on TOL and SSI, Esterain cultivar was selected as a sensitive one. A high STI value indicates higher stress tolerance and high yield potential (Khodarahmpour et al., 2011). According to the study of Fernandez's theory (1992), a suitable index or criterion is an index that is able to identify genotypes with a steady superiority that have a high correlation with yield in both stress and non-stress environments. MP, STI, GMP and HARM had the most significant correlation with yield at both non-stress and stress conditions. Therefore, the best indices to select barley genotypes were MP, STI, GMP and HARM (Table 3).

Based on these indices at non-stress and stress conditions, the most tolerant genotypes was Nosrat. Following Nosrat, Reyhan cultivar was the most tolerant genotype. The most sensitive genotypes based on these indices were Kavir and Nimruz cultivars, respectively. In fact, the tolerance of different cultivars was because of their physiological ability to control water loss during stress conditions. These results agree with the results of Ahmadizadeh et al. (2012) in durum wheat and Bonea and Urechean (2011) in maize that MP, STI, GMP and HARM were the most suitable indices to screen genotypes in drought stress condition.

Generally, the best indices to select barley genotypes were MP, STI, GMP and HARM. Based on the results of principal component analysis, biplot and cumulative grain yield diagrams and cluster analysis, Nosrat cultivar was
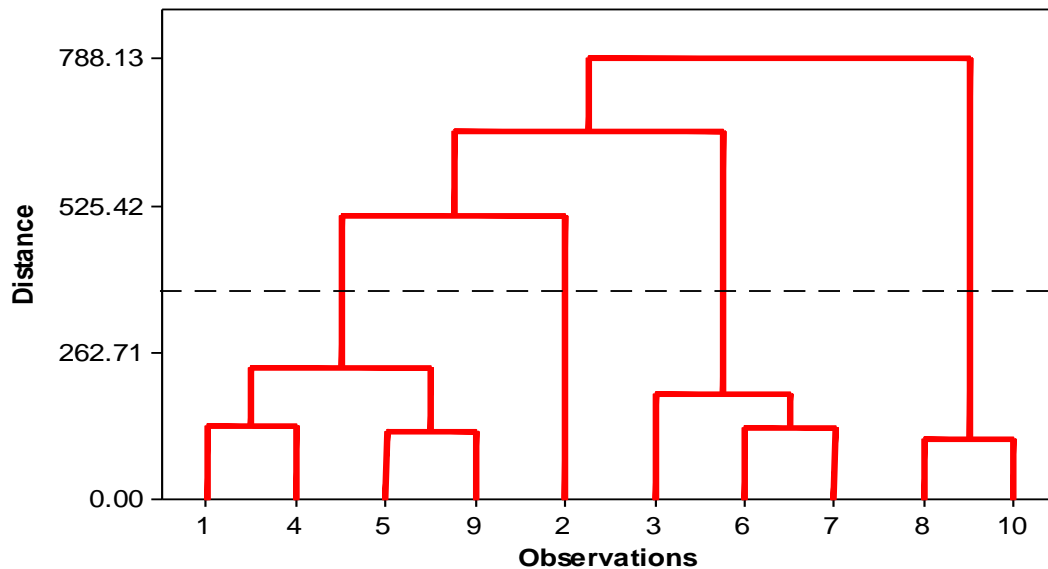
Figure 3. Dendrogram of cluster analysis of 10 barley genotypes using Ward's method based on Yp, Ys and drought tolerance indices. Genotypes' names: 1. Gorgan4, 2. Nosrat, 3. Reyhan, 4. Makoii, 5. Valfajr, 6. Zarjou, 7. Gorgan, 8. Kavir, 9. Esterain, 10. Nimruz.

identified as the most tolerant genotype and showed considerable potential to improve drought tolerance in barley breeding programs. Reyhan cultivar with high PCA2 was more suitable for stress than for non-stress condition. Kavir and Nimruz cultivars were identified as high drought susceptible and low yield stability geno-types.

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