

Original Research

Evaluation of drought tolerance in barley doubled haploid lines using drought tolerance indices

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ABSTRACT:

Forty five barley doubled haploid lines (DH) resulted from integrating Morex and Steotoe cultivars, as the parent cultivars, along with three native cultivars are compared based on the Randomized Complete Block design (RCB) with three replications under both normal irrigation and drought stress at Agricultural Research Station of Marand during 2011-12. Based on the stress tolerance indices of SS1 and TOL2, the lines 5, 37, 34, 13 and 19, and based on the indexes of STI3, GMP4 and MP5, the lines 15, 6, 16, 22, 30 and 29 are identified as the drought stress tolerant lines. The cluster analysis through Ward method has clustered the lines in both normal irrigation and drought stress conditions into two clusters. Under the normal irrigation condition, the lines of second cluster have better yield than the lines of first cluster. Furthermore, under the drought stress, the second cluster lines have higher yield and drought tolerance than the first cluster lines.

Keywords:

Cluster analysis, drought tolerance indices, doubled haploid lines.

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INTRODUCTION

Nowadays, the environmental stress is one of the factors which reduce the yield and production of agricultural crops in most of the regions throughout the world, and confronting and mitigating the effects of environmental stress are considered as the useful solution to increase the yield of crops in these regions. Among the abiotic environmental stresses, the drought stress is the most important risk to successful production of crops in Iran and other regions of the world (Golparvar *et al.*, 2002). The barley with scientific name of *Hordeum vulgare* is the fourth most important cereal in the world after wheat, rice and maize (Martin *et al.*, 1976). The barley is a plant with a wide range of dispersal and climate adaptation (Pakniat *et al.*, 2003). The stress refers to any environmental factor which is unfavorable for the growth of living organism. In general, the stress means too much pressure of opposing forces leading the failure in activities of natural systems (Hassanpour *et al.*, 2004). Furthermore, the stress refers to the impacts of any natural factor which adversely and potentially affects the life and performance of living organisms (Yamaushi *et al.*, 2002). The stability of yield under stress conditions is among the main indices of selecting the drought tolerant genotypes in numerous breeding programs (Ghodsi *et al.*, 1998). Different criteria are utilized for selecting the plant based on the crop

yield in both drought stress and non-stress environments. Fischer and Maureur (1978) introduced the Stress Susceptibility Index (SSI) according to which the more the SSI is decreased, the more the drought tolerance is increased. The SSI-based selection leads to the selection of genotypes with low yield in normal conditions, but high yield in stress environment. Rosielle and Hamblin (1981) introduced the Tolerance Index (TOL) as the difference between the yield of stress and non-stress environments and also the Mean Productivity (MP) as the mean yield in both stress and non-stress environments. The high levels of "TOL" indicates the susceptibility of genotypes to stress, thus the selection of genotypes is done based on the low values of TOL. The "MP" Index also tends to the selection of genotypes with higher potential yield and lower stress tolerance. Fernandez (1992) proposed the Stress Tolerance Index (STI) to be utilized for identifying the genotypes with high yield under stress and non-stress conditions. The high levels of "STI" for a genotype indicated the higher drought tolerance and potential yield of genotype. The Stress Intensity (SI) is also calculated through STI. Fernandez (1992) also introduced another index as the Geometric Mean Productivity (GMP) index. Furthermore, the Relative Drought Index (RDI) is introduced by Fischer and Maureur (1978) and the Yield Stability Index (YSI) by Bousama and Schapaugh (1984). Moreo-

Table 1. Chemical and physical decomposition of soil in testing site

S. No	Measurement parameters	Depth (0-30 cm)	Depth (30-60 cm)
1	Saturation percentage (% Sp)	43	40
2	Electrical conductivity (EC) (ds/m)	1.34	2.26
3	Acidity pH	8.09	8.05
4	Calcium carbonate (%T.N.V)	4.75	6.5
5	Organic carbon (%O.C)	1.3	1.06
6	Total nitrogen (%N)	0.13	0.1
7	Available phosphorus- p (ppm)	14.62	6.69
8	Potassium - k(ppm)	444	314
9	Sand %	18	18
10	Silt %	53	52
11	Clay%	29	30
12	Soil texture (soil-text)	Silty clay loam (sicl)	Silty clay loam (sicl)

Table 2. Combined analysis of variance of studied traits under normal irrigation and drought stress in barley lines

Sources of variation	Degrees of freedom	Plant height	Peduncle length	Spike length	Number of grains per spike	1000-seed weight	hectoliter weight	Straw yield	Grain yield	Harvest index
Irrigation conditions	1	2782.3±111*	1425.82±99**	0.014±0.001 ² _{ns}	193.7±17 ^{ns}	540.89±42**	238.51±38*	159455.87±1113**	1004013.72±2314**	0.06**
Replications under line conditions	4	182.22±32	7.45±1.1	0.007±0.001	47.93±8.2	28.28±3.2	20.71±3.4	1061.65±78	19310.13±231	0.003±0.0004
Line	44	106.73±21**	28.23±3.4**	0.004±0.0003 ² _{**}	75.78±8.8**	21.75±3.2**	43.61±5.6*	4916.88±233**	13293.5±898**	0.003±0.0007**
Line× conditions	44	80.43±8.5**	16.52±2.3**	0.003±0.0006**	41.66±5.5**	7.98±2.3**	13.86±2.1*	2092.92±321**	3899.94±246**	0.001±0.0001**
Error	176	24±3.4	3.5±.98	0.001±0.0003	11.36±1.2	2.27±0.45	4.46±1.1	496.41±45	1535.21±109	0.001±0.0004 ₅
Coefficient of variation (percent)		4.71±0.89	5.92±0.98	3.38±0.68	6.11±0.99	3.73±0.55	3.49±0.45	7.08±1.89	8.49±1.09	4.84±0.86

ns, * and ** represent the insignificance, and significance at the probable levels of 1% and 5%, respectively

ver, Harmonic introduced the HARM index and the more the mean of this index is increased, the more the drought tolerance indicator is enhanced in genotype (Farshadfar, 2001). The Yield Index (YI) is introduced by Gavuzzi *et al.* (1997) and the Yield Reduction (YR) index by Golestani and Assad (1998) for evaluation of both stress and non-stress conditions.

MATERIALS AND METHODS

This study is conducted with the aim at comparing the barley double haploid lines in terms of morphological and agronomic traits under both normal and drought stress conditions in the experimental field of Marand located 22 km from Marand City at the longitude of 46' and 45° and latitude of 26' and 38° and altitude of 1100 meters in the crop year of 2011-12. Based on the climatic divisions, this region has a semi-arid climate. The average rainfall is equal to 430 mm per annum and the average temperature is equal to 26°C during the growing season. To determine the micro and macronutrients, the sampling is done at the depths of 0 to 30 cm and 30 to 60 cm and then the micro and macronutrients are measured after preparing the composite sample and the soil texture is determined (Table 1).The herbal materials applied in this study are 45 barley genotypes including 40 doubled haploid lines, three control native cultivars of Nomar, Wb-7910 and Treubi and two parent cultivars of Morex (resistant to bacterial leaf diseases with low yield) and Steptoe (susceptible to bacterial leaf diseases with high yield) and three native cultivars of Nomar, Treubi and wb-7910. The experiment is done in the form of Randomized Complete Block design (RCB) with three replications undergone-stress and drought stress conditions at agricultural research station of Mahabad during 2011-12. The first irrigation is done a day after cultivation and other irrigations based on the evaporation from Class A pan after 90 mm of evaporation for normal cultivation and 180 mm evaporation for drought stress. The traits including the plant height,

Table 3-Values of yield and drought stress tolerance indices in studied lines of barley

Number of genotypes	SSI	GMP	MP	Ys	Yp	STI
1	0.7±0.1	432.76	434.5±19	395.667±18	473.33±18	0.690±0.1
2	0.88±0.1	426.4±14	429.24±19	379.9±15	78.4±9	0.670±0.1
3	1.02±0.12	497±14	501.59±18	433.9±19	569.2±20	0.90±0.1
4	0.85±0.13	378.32±14	380.62±19	338.8±18	422.3±11	0.52±0.11
5	0.16±0.06	438.2±14	438.28±19	429.8±15	446.6±12	0.7±0.1
6	0.68±0.1	522.81±14	524.76±20	479.5±15	569.9±18	1±0.1
7	0.77±0.1	420.16±14	422.24±19	380.3±18	464.1±15	0.65±0.08
8	0.61±0.1	447.39±16	448.73±19	414.1±15	483±37	0.73±0.1
9	0.97±0.12	429.47±13	433.05±20	377.4±11	488±62	0.68±0.1
10	0.57±0.1	471.6±14	472.87±20	439.4±18	506±34	0.81±0.1
11	1.31±0.19	501.4±20	509.81±22	417.6±18	601±23	0.92±0.14
12	0.77±0.11	417.5±20	419.57±20	377.9±18	461±77	0.64±0.1
13	0.48±0.1	480.79±20	481.63±18	453.1±18	510±11	0.85±0.14
14	1.3±0.1	487.43±20	495.42±20	406.8±15	584±12	0.87±0.13
15	1.43±0.2	517.32±20	528±21	422.3±16	633±17	0.98±0.1
16	0.83±0.1	529.7±20	532.75±25	475.8±18	589±67	1.03±0.17
17	1.39±0.2	400.02±20	407.77±22	328.6±19	486±39	0.59±0.1
18	0.6±0.1	490.27±22	494.2±20	432±18	556±39	0.88±0.12
19	1.29±0.23	463.98±20	471.42±18	388±18	554±17	0.79±0.14
20	0.9±0.1	427.1±20	430.04±15	379 ±18	480±27	0.67±0.1
21	0.88±0.12	484.9±20	488.16±18	432.3±15	544±13	0.86±0.1
22	1.02±0.14	527.7±23	532.55±18	460.8±15	604±22	1.02±0.1
23	1.28±0.17	494.02±20	501.8±18	413.6±18	590±23	0.89±0.1
24	1.24±0.16	485.3±22	492.4±18	409.1±20	575±37	0.86
25	1.24±0.1	458.9±25	465.6±19	386.6±21	544±67	0.77±0.1
26	1.02±0.1	395.7±20	399.4±18	345.6±18	453±12	0.57±0.1
27	1.13±0.1	389.8±20	394.4±15	334.2±19	454± 67	0.56±0.1
28	1.17±0.1	388.5±21	393.4±18	331.4±18	455±55	0.55±0.1
29	0.53±0.1	511±20	512.1±18	478.5±16	545±42	0.96±0.1
30	0.98±0.1	534.2±20	538.7±19	469.2±18	608±37	1.05±0.1
31	1.22±0.1	461.7±19	468.3±18	390.5±18	545±9	0.78±0.11
32	1.3±0.1	411. ±20	417.8±18	342.9±15	492± 77	0.62±0.1
33	1.46±0.1	489.43±20	500.1±17	397. ±18	603±17	0.88±0.13
34	0.5±0.1	387.17±20	387.9±15	363.9±17	411±91	0.55±0.1
35	1.22±0.11	504.9±20	512±18	427±23	597±44	0.93±0.1
36	1.32±0.17	426.2±19	433.4±18	354.5±19	51±38	0.67±0.12
37	0.29±0.08	360.9±23	361.1±13	348.2±18	374±32	0.48±0.1
38	0.59±0.1	446.1±20	447.3±11	414±18	480±87	0.73±0.1
39	1.21±0.13	488.5±22	495.2±18	413.7±19	576±84	0.87±0.1
40	0.75±0.1	429.7±19	431.7±13	390.4±22	470±77	0.68±0.1
Morex	0.58±0.09	462.0±19	463.2±12	429.8±11	49±67	0.78±0.1
Steotoe	1.77±0.16	471.11	488.0±14	360.5±14	615±5	0.81±0.1
Nomar	1.35±0.15	401.7±19	408.9±13	332.7±22	485.097	0.59±0.1
Wb-7910	1.09±0.11	504.1±17	509.6±19	435.2±18	584±23	0.93±0.12
Treubi	0.95±0.1	464.4±18	468.0±18	409.7±19	520±37	0.79±0.12

Table 4. Correlation coefficients between the grain yield and drought tolerance indices

			TOL	MP	GNP	SSI	STI
	1	0.496**	0.898**	0.486**	0.995**	0.378**	0.698**
Y _s	0.589**	1	0.868**	0.440*	0.97**	0.996**	0.442
TOL	0.742**	-0.104	1	0.915**	0.827**	0.396**	0.698**
MP	0.932**	0.843**	0.448**	1	0.912**	0.845**	0.348**
GMP	0.905**	0.877**	0.386**	0.998**	1	0.504**	-0.214*
SSI	0.574**	-0.314*	0.967**	0.242	0.178	1	0.278
STI	0.901**	0.878**	0.380*	0.996**	0.998**	0.171	1

* and ** are significant at the levels of 5% and 1% respectively

Y_p: Grain yield under normal irrigation

Y_s: Grain yield under drought stress

spike and peduncle lengths in cm and also the number of grains per spike, 1000-seed weight, and grain yield in grams per square meter, hectoliter weight in Kg, and harvest index are presented in percentage. The assumptions of analysis of variance are investigated for all traits and confirmed for all except for the spike length on which the logarithmic conversion is done. After combined analysis of variance, the cluster analysis is performed for clustering the lines based on the data standardized by Ward method. The discrimination function analysis is utilized for determining the appropriate location of cutting dendrogram. SPSS, MSTAT-C and EXCEL software are applied for data analysis and drawing the charts (Fischer and Maureur, 1978, Fernandez, 1992).

RESULTS AND DISCUSSION

The combined analysis of variance of the studied traits in both normal irrigation and drought stress conditions are presented in Table 2. There is a significant difference between two experimental conditions in terms of all studied traits except for the number of grains per spike and the spike length. Furthermore, there is a highly significant difference ($P \leq 0.01$) between lines in terms of all studied traits. Moreover, the mutual effect of line \times irrigation lands has been significant for all studied traits at the significance level of 1% indicating that the behavior of lines has not been similar in terms

of these traits under different irrigation conditions (normal irrigation and drought stress). The mutual effect of line irrigation levels \times irrigation lands is significant at probable level of 1% for all studied traits in this research. These results were in part correlated with the results of Atia *et al.* (1996), El-Seidy (1997), Kherialla *et al.* (1997) and El- Kolley and El- Hami (2000).

Evaluation of lines based on the stress tolerance indices

The studied Barley grain yield under normal irrigation (Y_p) and drought stress (Y_s) and also the values of drought tolerance indices, TOL, MP, SSI, STI and GMP, for each line are presented in Table 3. Based on the Stress Susceptibility Index (SSI), in which the numerical numbers indicated the higher tolerance of cultivar to stress, the lines 5, 3, 7, 13, 34 and 29 are identified as the stress tolerant lines. The evaluation of lines by SSI classifies the experimental materials only based on the susceptibility and tolerance to stress. In other words, the use of this index can determine the lines susceptible and tolerant to stress without regard to potential of their yields (Chen *et al.*, 2004). In terms of STI and GMP, in which the higher values indicated the tolerance of cultivars, the lines 30, 35, 11, 16, 22, 6, 15 and 29 are determined as the stress tolerant lines, respectively (Table 3). In addition to the highest levels of STI and GMP, these lines are also among the yielding lines in terms of average yield. Furthermore, these

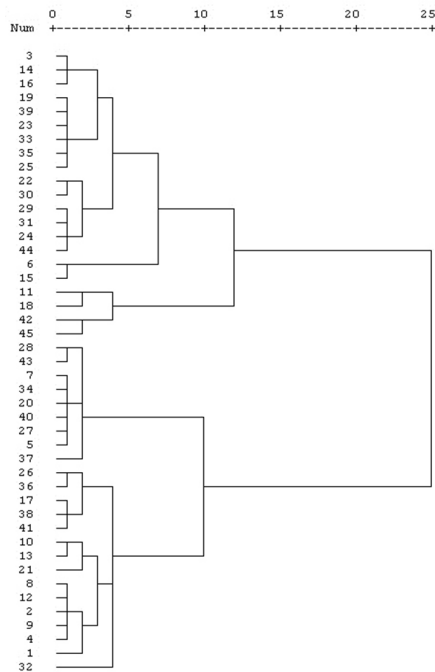


Figure 1. Dendrogram resulted from the cluster analysis of barley lines for all traits by using Ward method under normal irrigation conditions

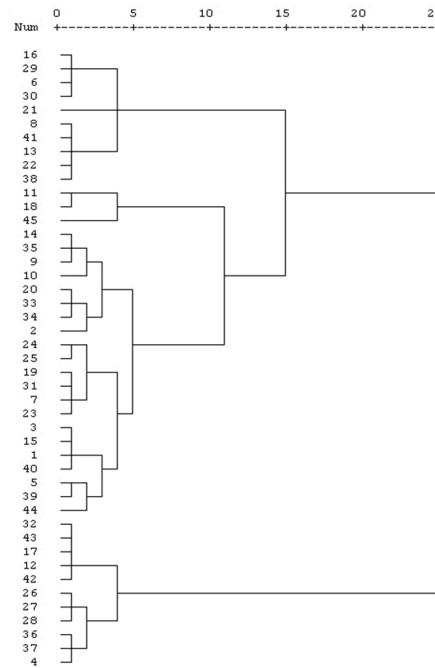


Figure 2. Dendrogram resulted from the cluster analysis of barley lines for all traits by Ward method under drought stress condition

lines have higher yield than the total mean of lines under normal irrigation. Based on the Mean Productivity (MP), the tolerant lines have higher values of this index. On this basis, the lines 30, 16, 22, 6, and 15 are identified as the most tolerant lines, respectively. The results of this index are fully consistent with the results of GMP and STI. The obtained results for STI, MP and GMP in this study are consistent with the findings by the majority of researchers (Bahadori, 1998; Rosielle and Hamblin, 1981; Vaezi and Ahmadikhah, 2010 and Tajjalli *et al.*, 2012). Ranking the line in terms of this

index indicates that the lines 5, 37, 34, 13, 10, 29, and 38 are identified as the tolerant lines. The results of Table 3 indicate that the lines selected based on the TOL Index have lower yield in non-stress conditions and this confirms the case above. In fact, the TOL index represents the variation caused by stress; in other words, the lines which have lower TOL index, show lower yield under the variation stress. It should be noted that, the low value of TOL index does not refer to the high yield of cultivar in non-stress condition because it is possible that the yield of a

Table 5. Discrimination function analysis for locating the cutting dendrogram resulted from the cluster analysis under normal irrigation

Functions	Eigenvalues	Percentage of variance	Cumulative percentage	Walks' lambda statistic	X ²
1	6.212	100	100	0.139	76.06**

Table 6. Discrimination function analysis for locating the cutting dendrogram resulted from the cluster analysis under the drought stress condition

Functions	Eigenvalues	Percentage of variance	Cumulative percentage	Wilks' lambda statistic	X ²
1	2.649	100	100	0.274	49.83**

Table 7. Mean and percentage of deviation from total mean of groups in cluster analysis under the irrigation conditions

S. No	Straw yield	Peduncle length	Hectoliter weight	Number of grains per spike	Spike length	Plant height	1000-seed weight	Harvest Index	Grain yield
1	338.92±33	37.3±16	61.4±15	56.0±17	0.89±004	100.6±21	41.7±17	0.60±003	555.5±14
2	317.2±35	36.2±8	60.5±13	53.4±11	0.89±005	101.1±19	41.3±23	0.60±005	472.3±18
3	-6.407±1	-2.741±0.09	-1.4±0.09	-4.6±12	-0.22±006	0.48±004	-1.0±003	-0.98±003	-9.6±09
4	363.7±33	38.4±12	62.42±11	59±12	-0.22±004	100.1±12	42.2±9	0.61±005	579.8±34
5	7.3±1	3.1±0.012	1.6±0.011	5.282±0.11	0.33±0.05	-0.55±0.04	1.2±0.09	0.98±0.008	10.9±0.09

Table 8. Mean and percentage of deviation from total mean of groups in cluster analysis under the drought stress conditions

S. No	Straw yield	Peduncle length	Hectoliter Weight	Number of grains per spike	Spike Length	Plant Height	1000-seed weight	Harvest Index	Grain yield
1	290.324	32.722	39.55	54.346	0.878	107.118	38.948	0.578	400.541
2	286.988	32.312	59.118	54.461	0.88	106.538	38.909	0.579	684.352
3	-1.149	-1.253	-0.725	0.212	0.228	-0.541	-0.1	0.173	-0.793
4	298.538	33.732	60.613	54.062	0.874	108.545	39.045	0.577	408.357
5	2.829	3.087	1.785	0.523	-0.456	1.332	0.249	-0.173	1.951

cultivar is low in normal conditions and leads to less drop in stress conditions and this leads to the low value of TOL index and thus this cultivar may be introduced as a tolerant cultivar (Tajjalli *et al.*, 2012). It is true for lines 5 and 37 in this test.

Correlation between indexes and grain yield

The correlation coefficients of tolerance assessment of lines with each other and with grain yield under the normal irrigation and drought stress conditions (Table 4) indicate that there is a significant positive correlation between the MP, GMP and STI and grain yield under both irrigation and drought stress conditions; on the other hand, these three indices have a very high correlation with each other. The highest significant correlation is obtained between STI and GMP with MP ($r=0.998$). According to these results, the GMP, STI and MP can be utilized for selection of lines with yield under drought stress conditions. These results are largely consistent with the researcher' findings (Dastbari *et al.*, 2008; Vaezi and Ahmadikhah, 2010; Pedram and Eyvazi, 2011; Tajjalli *et al.*, 2012). According to the conclusion above and given that the values of STI, MP and GMP for lines 6, 16, 22, 29,30 and 15 have been at the maximum level and since the mentioned lines under normal irrigation conditions have high yield (Table 4) and the evaluation indexes of drought tolerance (including the MP, STI and GMP) have had high significant correlation with yield under both irrigated and rain fed conditions, thus the use of these three criteria can be useful for assessment of drought tolerance inbreeding programs.

Clustering the lines

The cluster analysis by Ward method with standardized data under both favorable irrigation and drought stress conditions is utilized for clustering the studied Barley lines based on all measured traits. In cluster analysis, 45 barley lines and cultivars are clustered into two clusters based on all measured traits under favorable irrigation conditions and according to the discrimination

function analysis (Figure 1). 24 lines are allocated by the first cluster and 21 lines by the second cluster. In this clustering, the cultivars of Morex and Nomar are in the first cluster and the cultivars of Steptoe, WB-7910 and Treubi in the second cluster (Tables 5 and 6).

Under normal irrigation, the mean and standard deviation from the total mean of each group are listed in Table 7. The lines of Group 1 have lower values than the total mean of lines in terms of all evaluated traits except for the plant height. It can be concluded that most of lines in this cluster have poor yield in normal irrigation condition. The values of all traits of available lines in the second cluster are higher than the total mean of lines at the positive direction except for the plant height. The susceptible to drought cultivars, Steptoe, WB-7910 and Treubi, which have higher grain and straw yields than the total mean according to the results of comparing the lines in normal irrigation, are put in this group. It can be argued that the available lines of this group are at the opposite direction of the lines and cultivars of the first cluster under the normal irrigation, thus they are among the lines with high grain yield and with relatively desirable yield components in these conditions (Table 7). In general, it can be concluded that the lines available in the second cluster can be utilized as the parents with high yield potential in hybridization and for estimating the genetic parameters of grain yield inbreeding projects of grain yield under the irrigation conditions.

Clustering under the drought stress condition

The cluster analysis has clustered 45 lines and cultivars of barley into two clusters based on all measured traits under the drought stress conditions and according to the discrimination function analysis (Figure 2). The first cluster includes 32 lines (11.7%) and the second covers 13 lines (89.28%). In this clustering, the cultivars, Steptoe and Nomar, are allocated to the first group, and Morex, WB-7910 and Treubi to the second group. The mean and percentage of deviation from total

mean for each cluster are listed in Table 8. The cultivars and lines in Group 1 have the values lower than the mean of lines in terms of traits such as the grain yield, 1000-seed weight, plant height, hectoliter weight, peduncle length and straw yield. A half of lines in this cluster have lower yield than the total mean of lines under the drought stress conditions. Furthermore, 15 lines in this cluster are among the lines with high yield and with the values higher than the total mean of lines. Moreover, some of the lines in this cluster have higher and the others lower values than STI, GMP and MP. Thus, the desired clustering is not performed for the line of this cluster according to all evaluated traits and also the values of drought tolerance indices for lines in this cluster. The lines in the second cluster have relatively lower values than the total mean in terms of the traits such as the harvest index, number of grains per spike and the spike length. The mean yield of cluster lines is two times 408.357 grams per square meter. Furthermore, the mean of STI is equal to 0.78 for lines in this cluster and this indicates that most of the lines in this cluster have relatively high drought tolerance. The lines 30, 10, 35, 15, 24, and the cultivars of Morex, WB-7910, and Treubi have been in this cluster and have had the higher grain yields than the total mean of lines under the drought stress condition. Moreover, the lines 40, 25, 2, 34, and 26 in this cluster have had the grain yield lower than the total mean of lines. Under the drought stress conditions, clustering based on all studied traits could not properly divide the tested lines and cultivars into the more resistant and susceptible groups in terms of drought tolerance due to the lack of significant and strong correlation between most of the studied traits and different behavior of lines based on each trait. However, the desirable traits of lines in these clusters can be utilized in drought tolerance breeding projects. (Table 8, Figure 2).

CONCLUSION

Finally, the results of this study revealed the differences between the studied barley genotypes in terms of yield relating to stress and irrigation, as well as in terms of tolerance to drought stress due to genetic variation among genotypes. By choosing genotypes tolerant to water stress, it is possible to use them in breeding programs to obtain superior genotypes tolerating drought stress and producing acceptable yield under both favorable irrigation and water stress conditions. The results of correlation analysis and main components on estimated indices in 45 barley genotypes, MP, GMP and STI index were introduced as the most desirable indices for selection of high yielding and tolerant genotypes under drought stress conditions. Based on these indices and plotted graphs, among the 45 genotypes studied, lines 15, 6, 16, 22, 30 and 29 were called the most durable genotype and had the highest yield under both moisture conditions. In contrast, line 37 was the most sensitive genotype compared with other genotypes and had the lowest yield in the two conditions.

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