

## Genetic diversity among barley genotypes and path analysis of several agronomic and physiological characters at normal and salinity stress conditions

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### Abstract

The current research was performed to evaluate 150 barley cultivars based on several agronomic and physiological traits. The experiment was conducted as an alpha lattice design with five incomplete blocks in two replications at normal and salinity stress ( $EC= 12 \text{ dsm}^{-1}$ ) conditions at Agriculture and Natural Resources Research and Education Center, Yazd, Iran, for two years. The combined analysis of variance showed a significant difference among genotypes in all traits except harvest index and relative water content, indicating the existence of genetic diversity among the evaluated barley genotypes. Phenotypic correlation coefficients based on the average of two cropping years showed that biological yield, days to physiological maturity, and leaf chlorophyll index under normal conditions and biological yield, harvest index, number of fertile tillers, and plant height under salinity stress had a significant positive correlation with grain yield. According to the path analysis, the days to physiological maturity and number of fertile tillers had the highest positive direct effect on grain yield in normal and salinity stress conditions, respectively, followed by leaf chlorophyll index in the normal conditions and plant height under salinity stress. Cluster analysis by Ward's method grouped the studied genotypes into three clusters in both environments based on an average of two years. The discriminant function analysis was used to determine the number of clusters and check the accuracy of the grouping in the cluster analysis. Percentage deviation from the grand mean of clusters under salinity stress showed that genotypes of the first cluster had the highest grain yield and have the shortest maturity period. In the second cluster, physiologically efficient genotypes, and in the third cluster, late maturing and low-yielding genotypes were included. Therefore, according to the results of this study, it can be concluded that under salinity stress, the genotypes of the first cluster with the highest grain yield and earliest maturity dates can be used in future breeding programs to improve the salinity tolerance.

**Keywords:** Barley; Cluster analysis; Discriminant function analysis; Path analysis; Salinity stress

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### Introduction

Environmental stresses such as salinity have always been a factor in reducing the quantity and quality of crops. These changes depend on the plant species, varieties within species, and type

and amount of stress. Since most crops are sensitive to salinity, salinity is an important threat to agriculture (Flowers and Flowers 2005). Salinity in Iran and many arid and semi-arid regions of the world is considered a common

agricultural problem and limits crops' growth and yield. Among cereals, barley (*Hordeum vulgare* L.) is the most salinity-tolerant crop (Munns and Tester 2008). Identifying salinity stress-tolerant genotypes is one of the main goals in breeding programs in regions where salinity stress is common. A better understanding of phenological, morphological, and physiological characteristics related to salinity stress can help to identify more salinity-tolerant barley genotypes.

Plant breeders often select for grain yield indirectly through other traits, especially in the segregating generations because of the lower heritability of grain yield as compared to the yield-related traits. However, determining the most effective traits on the grain yield through the correlation coefficient of those traits with the grain yield may be misleading when other traits also vary in the population under study. On the other hand, path coefficient analysis assesses the direct and indirect effects of yield contributing traits on grain yield by decomposing the correlation coefficient of a trait with the grain yield. In this method, the direct effect of a trait (i.e. standardized partial regression coefficient) is determined by fixing other traits in the model. Hosein Babaei *et al.* (2013) used path analysis in barley to study the relationship of several agronomic characteristics with the grain yield. Spike length, awn length, 1000 grain weight, and the number of fertile tillers had a significant direct effect on grain yield. Singh *et al.* (2015) evaluated 25 diverse genotypes of barley under normal and drought stress conditions and observed a high and positive direct effect on the number of grains per ear, 1000 grain weight, and the number of

effective tillers per plant on grain yield under both conditions. Madakemohekar *et al.* (2015) conducted research on 40 F1 crosses of barley and their respective parents under rainfed conditions. Path coefficient analysis revealed that among the different yield contributing characters, the number of effective tiller per plant, 1000 grain weight, and number of grains per spike influenced grain yield per plant directly and these effects on grain yield were positive and high. Kumar *et al.* (2017) separated the genotypic correlations of grain yield with yield contributing traits in 64 barley varieties using path coefficient analysis under partially reclaimed saline-sodic soil. Path analysis revealed the positive direct effect of 1000 grain weight, (0.303) and the number of fertile tillers per plant (0.246) on grain yield per plant while the direct effects of the number of grains per main spike (0.061) and plant height were low. It looks like three major components of the grain yield (i.e. 1000 grain weight, number of fertile tillers per plant, number of grains per spike) mostly contribute to this trait under normal, drought, and salinity conditions.

To increase the efficiency of breeding programs, especially to reduce the number of crosses, plant breeders usually use multivariate statistical methods such as cluster analysis and principal component analysis to group the evaluated germplasm based on the measured traits. The success of a breeding program depends on the genetic diversity within the available germplasm. Many investigators have shown considerable variability among the evaluated barley genotypes. Enyew *et al.* (2019) evaluated 48 barley landrace accessions to determine the

amount of genetic diversity among the accessions and to identify the main traits contributing to the observed variations. Based on the principal component analysis, the first three components explained 74.20% of the total variation. The first principal component contributed to 49.96% of the total variations mainly because of grain yield, biomass yield, 1000 grain weight, and plant height in their respective order. The second principal component explained 15.98% of total variation mainly by the total number of tillers per plant, the number of effective tillers per plant, and the number of spikes per plant. The third principal component contributed to 8.25% of the total variation mainly due to days to maturity, days to 50% heading, and the number of grains per plant. The accessions were also grouped by the cluster analysis into six clusters where early maturing genotypes were placed in cluster 3, late-maturing accessions in cluster 6, and high yielding and tall genotypes in cluster 4. Their results indicated the existence of large genetic variability among the studied landraces. Hailu *et al.* (2016) studied the genetic diversity among 64 genotypes of barley for yield and its contributing characters based on cluster analysis in three locations. Cluster analysis grouped the barley genotypes into five distinct clusters in the first and second locations, and into six clusters in the third location. Dyulgerova *et al.* (2016) evaluated 25 barley genotypes during two crop years using cluster analysis, and the genotypes were grouped into three clusters. There was a correspondence between the origin and cluster groups based on all measured traits. The first cluster included the genotypes with the highest grain number and grain weight per spike.

They stated a considerable genetic diversity among the evaluated genotypes and suggested exploiting this variability for the selection of superior genotypes aimed at increasing grain yield.

The present study was performed to determine the genetic diversity among 150 barley genotypes in terms of agronomic and physiological characteristics. Also, path analysis was carried out to identify the most effective traits that contribute to the grain yield of barley under normal and salt stress conditions.

### **Material and Methods**

In this study, 148 barley cultivars of northern and western Europe that were collected by the Wageningen University, Netherlands (see Kraakman *et al.* 2004 for more details) together with two Iranian cultivars as checks (Khatam, resistant to salinity cultivars, and Nosrat, not resistant to salinity) were evaluated. The experimental design was an alpha-lattice with five incomplete blocks in two replications. Each block included 30 plots. The experiment was conducted under normal and salinity stress ( $EC = 12 \text{ dsm}^{-1}$ , Pirasteh-Anosheh 2017) environments at the Agriculture and Natural Resources Research Station of Yazd ( $31^{\circ} 55' \text{ N}$ ,  $54^{\circ} 16' \text{ E}$ , 1213 m of sea level), Iran, for two years. The measured traits were as follows: grain yield, days to tillering, days to stem elongation, days to heading, grain filling period, days to physiological maturity, plant height, 1000-grain weight, biological yield, harvest index, flag leaf length, flag leaf width, flag leaf area, number of fertile tillers, spike length, grain weight per spike, number of grains

per spike, relative water content, leaf chlorophyll index, and leaf proline content.

Leaf chlorophyll index was measured from three points on the flag leaves at heading in 10 plants per plot with a manual chlorophyll meter (SPAD). Leaf proline content was measured by detaching 15 flag leaves in each plot at the heading stage. The detached leaves were immediately transferred to the laboratory and their proline content was extracted by using the method of Bates *et al.* (1973). The relative water content (RWC) of the flag leaves was calculated as follows (Fitter and Hay 1987):

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturation weight} - \text{Dry weight}} \times 100$$

The flag leaf area was calculated based on Muller (1991) as follows:

$$S = 0.75 \times L \times W$$

where S= flag leaf area, L= flag leaf length, and W= flag leaf width

The harvest index was calculated as follows:

$$\text{HI} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

The normality of data was first tested based on the Shapiro Wilk method using SPSS software. Then, the combined analysis of variance and calculation of phenotypic correlations were performed with SAS 9.1 software. Also, path analysis, cluster analysis, and discriminant function analysis were performed based on two-year data averages with Minitab16 and SAS 9.1 software. The discriminant function analysis was used to check the accuracy of the groupings performed in the cluster analysis, and the best grouping was selected with the highest confidence percentage.

## Result and Discussion

### *Analysis of variance*

The results of the combined analysis of variance in two years and two environmental conditions (normal and salinity stress) showed a significant difference among genotypes for all traits except harvest index and relative water content. Zaare and Jafari (2013) and Khalili and Mohammadian (2016) also reported significant differences among the tested genotypes under salinity conditions. The environment  $\times$  year, environment  $\times$  genotype, year  $\times$  genotype, and environment  $\times$  year  $\times$  genotype interactions were significant for most of the traits, indicating that the differences among genotypes vary according to environmental conditions and years (Supplementary Table 1).

### *Phenotypic correlations*

The Pearson correlation coefficients among the measured traits for normal and salinity stress conditions are presented in Table 1. The results showed that biological yield ( $r = 0.54^{**}$ ), days to physiological maturity ( $r = 0.51^*$ ), and leaf chlorophyll index ( $r = 0.44^*$ ) under normal conditions and biological yield ( $r = 0.88^{**}$ ), harvest index ( $r = 0.79^{**}$ ), number of fertile tillers ( $r = 0.63^{**}$ ), and plant height ( $r = 0.45^*$ ) under salinity stress had positive and significant correlation with the grain yield. Hosseinpour (2012), Ahmadi *et al.* (2014), and Abarnak *et al.* (2018) also showed that barley grain yield had a positive and significant correlation with biological yield.

According to the results of Table 1, in the normal conditions, days to stem elongation had a

Table 1. Phenotypic correlation coefficients of the studied traits based on the average of two cropping years (above-diagonal values for the normal and below-diagonal values for the salinity stress conditions)

Traits	GY	DT	DS	DH	GFP	DPM	PH	TGW	BY	HI
GY (kg/m <sup>2</sup> )	1	-0.29	-0.08	0.15	0.22	0.51*	0.2	-0.09	0.54**	0.2
DT (day)	-0.24	1	0.54**	0.32	-0.19	0.21	-0.22	0.005	-0.26	0.07
DS (day)	-0.16	0.17	1	0.64**	-0.36	0.43*	-0.35	0.02	-0.24	0.13
DH (day)	-0.33	0.32	0.32	1	-0.63	0.6**	0.006	-0.12	0.03	0.14
GFP (day)	0.12	-0.11	-0.03	-0.7	1	0.24	0.06	0.11	0.15	-0.13
DPM (day)	-0.04	0.04	0.15	-0.31	0.9**	1	0.07	-0.04	0.19	0.04
PH (cm)	0.45*	-0.13	-0.35	-0.29	0.15	0.03	1	-0.04	0.27	-0.12
TGW (g)	0.16	-0.1	-0.11	-0.16	0.08	0.01	0.35	1	0.001	0.06
BY (kg/m <sup>2</sup> )	0.88**	-0.31	-0.26	-0.25	0.1	-0.01	0.52*	0.22	1	-0.21
HI (%)	0.79**	-0.02	-0.03	-0.34	0.1	-0.07	0.03	0.04	0.47*	1
FLL (cm)	0.08	-0.1	-0.18	0.17	-0.04	0.04	0.38	0.09	0.32	-0.15
FLW (cm)	0.1	-0.13	-0.12	0.14	-0.03	0.05	0.43*	0.13	0.32	-0.14
FLA (cm <sup>2</sup> )	0.06	-0.08	-0.13	0.18	-0.04	0.06	0.36	0.04	0.31	-0.17
NFT	0.63**	-0.23	-0.05	-0.29	0.12	-0.01	0.17	0.007	0.61**	0.51*
SL(cm)	0.18	-0.002	0.15	0.002	-0.03	-0.03	-0.004	0.18	0.09	0.2
GWS (g)	0.22	-0.1	-0.22	-0.06	0.07	0.05	0.5*	0.44*	0.35	-0.02
NGS	0.18	-0.06	-0.23	-0.007	0.045	0.06	0.4	0.01	0.3	-0.02
RWC (%)	0.007	0.23	-0.01	0.16	-0.07	0.008	0.08	0.12	0.05	-0.04
LChC	-0.08	0.2	0.1	0.15	0.06	0.17	-0.03	0.11	-0.05	-0.06
LPC (μmol/g)	-0.02	0.18	0.15	0.003	0.06	0.08	-0.12	-0.06	-0.08	0.12

Table 1 continued

Traits	FLL	FLW	FLA	NFT	SL	GWS	NGS	RWC	Chl	LPC
GY (kg/m <sup>2</sup> )	0.14	0.14	0.12	0.08	0.06	0.1	0.12	0.007	0.44*	0.02
DT (day)	-0.12	-0.13	-0.1	0.01	-0.03	-0.05	-0.05	0.11	-0.007	0.01
DS (day)	-0.11	-0.12	-0.06	0.013	-0.03	0.013	-0.013	0.13	0.17	0.006
DH (day)	0.19	0.18	0.21	-0.15	-0.02	0.18	0.2	0.008	0.4	0.04
GFP (day)	-0.007	-0.01	-0.04	0.12	0.18	-0.09	-0.13	0.04	-0.09	-0.05
DPM (day)	0.23	0.22	0.22	-0.06	0.15	0.13	0.12	0.06	0.42	0.001
PH (cm)	0.57**	0.58**	0.55**	-0.19	-0.06	0.49*	0.53*	-0.1	0.14	0.02
TGW (g)	0.1	0.08	0.1	0.1	0.18	0.23	-0.08	-0.03	-0.17	-0.13
BY (kg/m <sup>2</sup> )	0.15	0.13	0.12	0.004	0.004	0.11	0.1	-0.002	0.15	0.1
HI (%)	-0.02	-0.02	-0.04	0.08	0.04	-0.03	-0.04	-0.02	0.08	-0.36
FLL (cm)	1	0.99**	0.96**	-0.1	-0.13	0.59**	0.57**	0.005	0.19	-0.03
FLW (cm)	0.95**	1	0.95**	-0.09	-0.13	0.58**	0.56**	-0.01	0.18	-0.03
FLA (cm <sup>2</sup> )	0.98**	0.93**	1	-0.08	-0.19	0.67**	0.63**	0.05	0.17	-0.01
NFT	0.07	0.1	0.06	1	0.17	-0.04	-0.08	0.08	-0.09	0.03
SL(cm)	-0.15	-0.09	-0.17	0.2	1	-0.24	-0.33	0.06	0.06	0.07
GWS (g)	0.35	0.36	0.33	-0.07	-0.17	1	0.94**	-0.02	0.02	-0.05
NGS	0.37	0.36	0.37	-0.06	-0.26	0.89**	1	-0.008	0.08	-0.01
RWC (%)	0.06	0.12	0.07	-0.1	-0.01	0.08	0.05	1	0.06	-0.03
Chl	0.05	0.07	0.06	-0.08	0.03	0.06	0.01	0.26	1	0.05
LPC (μmol/g)	-0.08	-0.05	-0.07	0.11	0.08	0.05	-0.03	-0.1	-0.04	1

\* and \*\*: significant at 5% and 1% probability levels, respectively; GY: grain yield, DT: days to tillering, DS: days to stem elongation, DH: days to heading, GFP: grain-filling period, DPM: days to physiological maturity, PH: plant height, TGW: thousand-grain weight, BY: biological yield, HI: harvest index, FLL: flag leaf length, FLW: flag leaf width, FLA: flag leaf area, NFT: number of fertile tillers, SL: spike length, GWS: grain weight per spike, NGS: number of grains per spike, RWC: relative water content, Chl: leaf chlorophyll index, LPC: leaf proline content, CV: coefficient of variation

positive and significant correlation with days to tillering, days to heading, and days to physiological maturity. Days to heading showed a positive and significant correlation with days to physiological maturity. Plant height, flag leaf length, flag leaf width, flag leaf area, grain weight per spike, and the number of grains per spike were positively and significantly correlated with each other under normal conditions. Under salinity stress (Table 1), a positive and significant correlation between the grain-filling period and days to physiological maturity, and also between 1000-grain weight and grain weight per spike was detected. Plant height was positively and significantly correlated with the biological yield, flag leaf width, and grain weight per spike. Harvest index, biological yield, and the number of fertile tillers showed a positive and significant correlation with flag leaf length, flag leaf width, and flag leaf area. Grain weight per spike had also a positive and significant correlation with the number of grains per spike.

### **Path analysis**

Path analysis was done to determine the direct and indirect effects of the measured traits on grain yield. In this study, before performing path analysis, biological yield and harvest index were removed from the model due to their high impact on the grain yield.

According to the path analysis results, the days to physiological maturity had the largest direct effect (0.43) and the highest correlation ( $r=0.51^*$ ) with the grain yield under normal

conditions (Table 2). Matin *et al.* (2019) also reported a direct and positive effect of days to maturity on grain yield. However, Hailu *et al.* (2016b) indicated that days to maturity had the highest negative direct effect on grain yield. Path analysis results under salinity stress conditions (Table 3) showed that the number of fertile tillers had the largest direct effect (0.58) on the grain yield. Also, this trait strongly correlated with the grain yield ( $r=0.63^{**}$ ), which indicates its remarkable effect on grain yield and, therefore, can be used for selection to increase the grain yield in barley. The leaf chlorophyll index in normal and plant height under salinity stress conditions showed also a large direct effect on grain yield. Their correlation coefficients with grain yield were also significant ( $r=0.44^*$  and  $r=0.45^*$ , respectively) (Tables 2 and 3). According to Table 2, days to physiological maturity also had an indirect effect on grain yield via leaf chlorophyll index under normal conditions. The indirect effect of days to physiological maturity was negligible through the plant height. Plant height also had an indirect effect on the grain yield of barley via grain weight per spike under salinity stress conditions (Table 3). Given that the correlation coefficient between the plant height and grain yield was approximately equal to its direct effect on the grain yield under both normal and saline conditions, the correlation coefficient expressed the extent of the true relationship between the two variables. Under salinity conditions, the grain weight had also a direct effect on the grain yield.

Table 2. Path coefficients analysis of direct and indirect effects of days to physiological maturity, leaf chlorophyll content, and plant height on grain yield of barley under normal conditions

Traits	Direct effect	Indirect effect via			Correlation with yield
		Days to physiological maturity	Leaf chlorophyll content	Plant height	
Days to physiological maturity	0.43	-	0.11	0.05	0.51*
Leaf chlorophyll index	0.39	0.11	-	0.60	0.44*
Plant height	0.13	0.05	0.06	-	0.20
Residual Error	0.76				

\*: significant at the 5% probability level

Table 3. Path coefficients analysis of direct and indirect effects of the number of fertile tillers, plant height, and grain weight per spike on grain yield of barley under salinity stress conditions

Traits	Direct effect	Indirect effect via			Correlation with yield
		Number of fertile tillers	Plant height	Grain weight per spike	
Number of fertile tillers	0.58	-	0.4	-0.01	0.63**
Plant height	0.41	0.05	-	0.10	0.45*
Grain weight per spike	0.17	0.04	0.09	-	0.22
Residual Error	0.64				

\* and \*\*: significant at the 5% and 1% probability levels, respectively

### Cluster analysis

The discriminant function analysis was used to check the accuracy of the groupings performed in the cluster analysis method (Table 4), and the best grouping was selected with the highest confidence percentage. The results of the discriminant function showed that the accuracy of cluster analysis in grouping genotypes under non-stress and salinity stress conditions was 94.7 % and 95.3%, respectively.

The results of cluster analysis by Ward's method based on Euclidean distance under normal and salinity stress conditions and on the data average of two years are shown in Figures 1 and 2, respectively. The number of groups in the cluster analysis was determined by the discriminant function analysis, which resulted in three clusters in both environments. Also based on discriminant function analysis the accuracy of the groupings was confirmed (Table 4); the accuracy of the grouping genotypes under normal and

salinity stress conditions was 94.7 % and 95.3%, respectively. Under normal conditions, 103 genotypes were allocated in the first cluster, 38 genotypes in the second cluster, and 9 genotypes in the third cluster (Figure 1). Under salinity stress, 99, 32, and 19 genotypes were located in the first, second, and third clusters, respectively (Figure 2). These results indicate an appreciable variability among the test barley genotypes. Hailu *et al.* (2016a) evaluated 64 barley genotypes and grouped them into five clusters. Enyew *et al.* (2019) grouped the barley genotypes under investigation into six clusters due to the existence of genetic diversity among these genotypes. In their study, cluster analysis separated the maturity groups. Early maturing genotypes were grouped in cluster 3, late-maturing genotypes in cluster 6, and high yielding and tall genotypes in cluster 4. Kumar *et al.* (2020) classified 87 barley genotypes into eight clusters using cluster analysis. Abdullah *et al.* (2018) also used Ward's method of

Table 4. Result of discriminant function analysis to check for the accuracy of the groupings performed in the cluster analysis

Environment	Put into groups	True group			Total
		1	2	3	
Normal	1	97	2	0	
	2	6	36	0	-
	3	0	0	9	
	Total	103	38	9	150
	% Correct	97	36	9	142
	% Correct	94.2	94.7	100	<b>94.7</b>
Salinity stress	1	95	2	1	
	2	2	30	0	-
	3	2	0	18	
	Total	99	32	19	150
	% Correct	95	30	18	143
	% Correct	96	93.8	94.7	<b>95.3</b>

clustering and grouped the barley genotypes under investigation into four clusters based. Also, a study on twenty-two barley genotypes was conducted by Derbew (2020) during two crop years, and the cluster analysis grouped them into five clusters with 4 to 10 genotypes each, and two genotypes remained solitary without grouping.

Percentage deviation from the total mean of clusters for the studied traits are shown in Tables 5 and 6). In the normal environment, genotypes of the first cluster had the highest positive percentage deviation from the grand mean for days to tillering, flag leaf length, flag leaf width, and flag leaf area. The codes for the genotypes of this cluster are as follows: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 22, 23, 24, 26, 27, 29, 31, 32, 33, 34, 35, 37, 38, 39, 40, 41, 42, 43, 45, 46, 47, 48, 54, 55, 58, 60, 61, 62, 63, 64, 65, 66, 67, 68, 74, 75, 76, 77, 78, 79, 80, 81, 83, 84, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 102, 103, 104, 111, 112, 113, 114, 115, 119, 120, 121, 122, 123, 124, 125, 127, 129, 130, 131, 132, 135, 138, 139, 140, 141, 144, 145, 146, 147, 148. The second cluster showed the highest positive deviation from the grand mean in biological yield,

flag leaf width, and flag leaf area. In this group, days to tillering and days to stem elongation had the highest negative values; therefore, early maturing genotypes were included in this cluster. These lines were 13, 14, 15, 19, 21, 25, 28, 30, 49, 50, 51, 53, 56, 59, 69, 70, 71, 72, 73, 82, 100, 101, 105, 106, 107, 108, 109, 116, 117, 118, 126, 128, 133, 134, 136, 137, 142, and 143. In the third cluster, grain yield, plant height, biological yield, flag leaf length, flag leaf width, flag leaf area, grain weight per spike, and number of grains per spike had the highest positive values as compared to other clusters. Therefore, this cluster contained high yielding genotypes that can be exploited in the breeding programs. The genotypes' codes were 20, 36, 44, 52, 57, 85, 110, 149, and 150.

Under the salinity-stress environment (Table 6), the genotypes of the first cluster had the highest positive deviation from the grand mean for the grain yield, biological yield, harvest index, and the number of fertile tillers. Therefore, high yielding genotypes were included in this cluster. The codes for these lines were 1, 2, 3, 6, 18, 27, 29, 33, 34, 35, 37, 38, 39, 40, 41, 42, 43, 45, 46, 47, 48, 49, 50, 51, 54, 55, 56, 57, 60, 61, 62, 63, 65,



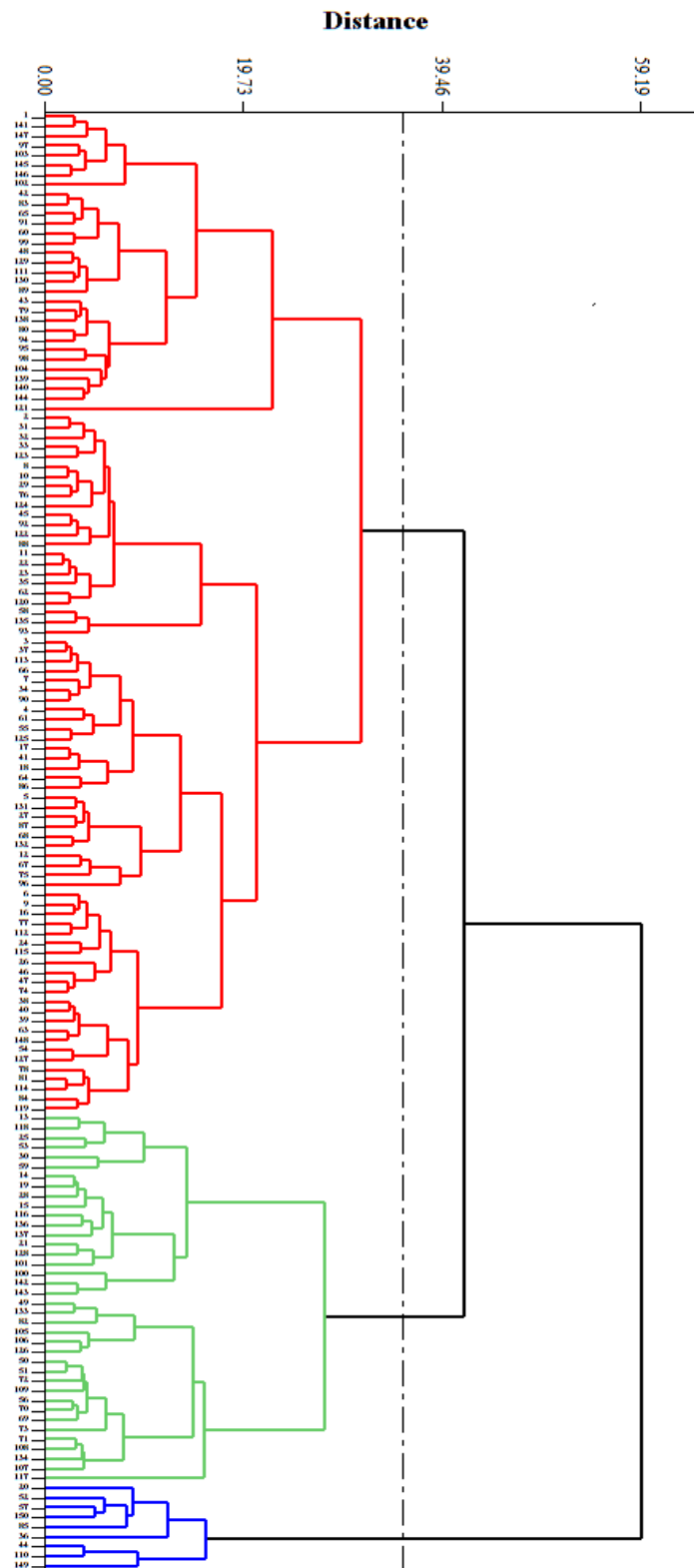


Figure 1. Dendrogram of the cluster analysis of the barley genotypes using Ward's method based on the average of the two cropping years under normal conditions

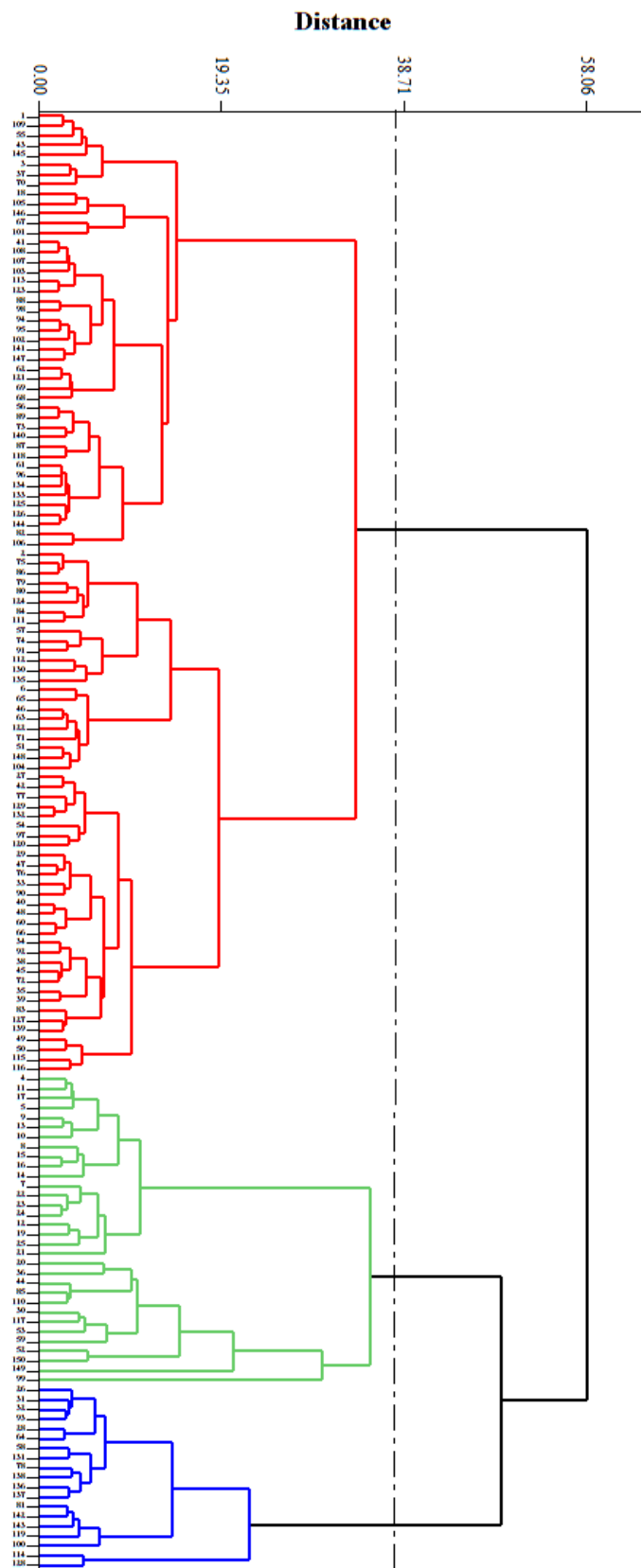


Figure 2. Dendrogram of the cluster analysis of the barley genotypes using Ward's method based on the average of the two cropping years under salinity stress conditions

Table 5. Percentage deviation from the grand mean of clusters for different traits of the barley genotypes under normal conditions

Trait	Cluster 1	Cluster 2	Cluster 3	Grand mean
GY (kg/m <sup>2</sup> )	-1	1.4	5.4	0.83
DT (day)	5.3	-10.8	-3.2	25.36
DS (day)	2.97	-7.82	-1	100.91
DH (day)	0.61	-2.6	3.9	130.28
GFP (day)	-0.85	4.3	-8.2	41.83
DPM (day)	0.26	-0.93	1	172.1
PH (cm)	-3.28	2.5	27	52.76
TGW (g)	0.3	0.72	-6.4	42.39
BY (kg/m <sup>2</sup> )	-5.6	11.7	14.3	2.39
HI (%)	4.2	-7.8	-15.3	37.36
FLL (cm)	-6.86	3.8	62.6	6.2
FLW (cm)	-9.47	5.4	85.7	0.43
FLA (cm <sup>2</sup> )	-18.5	5.03	190.6	2.31
NFT	3.02	-6.2	-8.4	97.84
SL(cm)	1.83	0.1	-21.4	7.66
GWS (g)	-5.2	-3.9	76.7	1.03
NGS	-6	-4	85.3	24.45
RWC (%)	0.54	-1.5	-0.1	80.18
Chl	0.88	-3.3	4	40.19
LPC (μmol/g)	0.13	0.4	-3.2	8.08

See Table 1 for the abbreviation of the traits used here

Table 6. Percentage deviation from the grand mean of clusters for different traits of the barley genotypes under salinity stress conditions

Trait	Cluster 1	Cluster 2	Cluster 3	Grand mean
GY (kg/m <sup>2</sup> )	12.1	-9.8	-46.3	0.48
DT (day)	-2.7	-1.1	16	26.4
DS (day)	4.2	-23.3	17.5	99.41
DH (day)	-0.6	-0.35	3.7	136.06
GFP (day)	-1	11.4	-14.2	36.38
DPM (day)	-0.7	2.1	-0.1	172.44
PH (cm)	0.11	6.8	-12	44.41
TGW (g)	0.7	1.8	-6.5	35.91
BY (kg/m <sup>2</sup> )	6.5	0.43	-34.7	1.67
HI (%)	7.3	-10.6	-20.4	28.09
FLL (cm)	-1.2	12	-14.1	6.04
FLW (cm)	0.16	10.9	-19.2	0.40
FLA (cm <sup>2</sup> )	-3.3	29.1	-32	1.94
NFT	5.4	-7.9	-15	90.23
SL(cm)	3.2	-8.4	-2.6	6.55
GWS (g)	-2	20.3	-24.4	0.67
NGS	-2.1	18.6	-20.3	18.68
RWC (%)	-0.31	-0.43	2.3	64.4
Chl	-0.32	0.41	1	42.94
LPC (μmol/g)	3.1	-7	-4.2	10.53

See Table 1 for the abbreviation of the traits used here

66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 79, 80, 82, 83, 84, 86, 87, 88, 89, 90, 91, 94, 95, 96, 97, 98, 101, 102, 103, 104, 105, 106, 107, 108, 109, 111, 112, 113, 115, 116, 118, 120, 121, 122, 123, 124, 125, 126, 127, 129, 130, 132, 133, 134, 135, 139, 140, 141, 144, 145, 146, 147, and 148. The second cluster of genotypes had the highest positive values for the grain filling period, plant

height, flag leaf length, flag leaf width, flag leaf area, grain weight per spike, and the number of grains per spike. In this cluster, days to stem elongation had the highest negative value, therefore, the physiologically efficient genotypes were included in this group. These genotypes were 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25, 30, 36, 44, 52, 53, 59,

85, 99, 110, 117, 149, and 150. In the third cluster, days to tillering and days to stem elongation had the highest values as compared to other clusters. Thus, late-maturing genotypes were included in this group. Percentage deviation from the grand mean was negative for yield and yield components in this group. Thus, this group included late matured and low-yielding genotypes. The codes for the genotypes of this cluster were 26, 28, 31, 32, 58, 64, 78, 81, 93, 100, 114, 119, 128, 131, 136, 137, 138, 142, and 143.

### Conclusion

Phenotypic correlation coefficients based on the average of two cropping years showed that biological yield, days to physiological maturity, and leaf chlorophyll index under normal conditions and biological yield, harvest index, number of fertile tillers, and plant height under salinity stress had a positive and significant correlation with the grain yield of barley. According to the results of the path analysis, the days to physiological maturity and number of fertile tillers had the largest positive direct effect on grain yield in normal and salinity stress conditions, respectively, followed by leaf chlorophyll index in normal conditions and plant

height under the salinity-stress environment. Percentage deviation from the grand mean of the clusters under salinity stress showed that genotypes of the first cluster had the highest grain yield and shortest maturity period. In the second cluster, physiologically efficient genotypes, and in the third cluster, late maturing and low-yielding genotypes were included. Therefore, according to the results of this study, it can be concluded that under salinity stress, the genotypes of the first cluster, which were early maturing and had the highest grain yield, could be used as superior lines in the breeding programs for salinity tolerance.

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### Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

### References

- Abarnak S, Zarei L, and Cheghamirza K, 2018. Evaluation of some important agronomic and physiological traits in barley cultivars in rain fed conditions. *Crop Physiology Journal* 9(36): 41-63. (In Persian with English abstract).
- Abdullah, Akbar M, Subhani MG, Ahmad J, and Anwar J, 2018. Multivariate analysis of some yield and yield related traits of barley (*Hordeum vulgare* L.) genotypes. *Academia Journal of Agricultural Research* 6(7): 189-197.
- Ahmadi A, Pourghasemi R, and Hosseinpour T, 2014. Relationship between grain yield and yield components of barley genotypes by multivariate statistical methods. *Agroecology Journal* 10(3): 1-13. (In Persian with English abstract).

- Bates LS, Waldren RP, and Teare ID, 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* 39: 205-207.
- Derbew S, 2020. Multivariate analysis of hulled barley (*Hordeum vulgare* L.) landraces of southern Ethiopia. *Cogent Food and Agriculture* 6(1): 1841357.
- Dyulgerova B, Dimova D, and Valcheva D, 2016. Genetic diversity in six-rowed winter barley (*Hordeum sativum* jess., ssp. *Vulgare* L.) genotypes. *Bulgarian Journal of Agricultural Science* 22: 114-118.
- Enyew M, Dejene T, Lakew B, and Worede F, 2019. Clustering and principal component analysis of barley (*Hordeum vulgare* L.) landraces for major morphological traits from north western Ethiopia. *International Journal of Agricultural Science and Food Technology* 5(1): 58-63.
- Fitter AH and Hay RKM, 1987. *Environmental physiology of plants*. 2nd ed. Academic Press, London, UK.
- Flowers TJ and Flowers SA, 2005. Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management* 78: 15-24.
- Hailu A, Alamerew S, Nigussie M, and Assefa E, 2016a. Study of genetic diversity in different genotypes of barley (*Hordeum vulgare* L.) based on cluster and principal component analyses. *Agricultural Science Research Journal* 6(2): 31-42.
- Hailu A, Alamerew S, Nigussie M, and Assefa E, 2016b. Correlation and path coefficient analysis of yield and yield associated traits in barley (*Hordeum vulgare* L.) germplasm. *Advances in Crop Science and Technology* 4(2): 100216.
- Hosein Babaei A, Aharizad S, Mohammadi SA, Yarnia M, and Norouzi M, 2013. Identification of effective traits on barley lines grain yield via path analysis. *Journal of Crop Breeding* 5(11): 49-59. (In Persian with English abstract).
- Hosseinpour T, 2012. Relationship among agronomic characteristics and grain yield in hull-less barley genotypes under rainfed conditions of Koohdasht. *Iranian Journal of Crop Sciences* 14(3): 263-279. (In Persian with English abstract).
- Khalili M and Mohammadian R, 2016. Identifying QTLs associated with salinity tolerance in early stages of barley germination. *Journal of Crop Biotechnology* 5(13): 41-55 (In Persian with English Abstract).
- Kraakman ATW, Niks RE, Van den Berg P, Stam P, and Van Eeuwijk FA, 2004. Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics* 168(1): 435-446.
- Kumar A, Kumar J, Bharti B, Verma PN, Jaiswal JP, Singh GP, and Vishwakarma SR, 2017. Genotypic correlation and path coefficient analysis for yield and yield contributing traits in released varieties of barley (*Hordeum vulgare* L.) under partially reclaimed saline sodic soil. *Journal of Applied and Natural Science* 9(1): 192-195.
- Kumar Y, Niwas R, Nimbale S, and Dalal MS, 2020. Hierarchical cluster analysis in barley genotypes to delineate genetic diversity. *Electronic Journal of Plant Breeding* 11(3): 742-748.
- Matin MQI, Amiruzzaman M, Billah MdM, Banu MB, Naher N, and Choudhury DA, 2019. Genetic variability and path analysis studies in barley (*Hordeum vulgare* L.). *International Journal of Applied Sciences and Biotechnology* 7(2): 243-247.
- Madakemohekar AH, Prasad LC, Lal JP, Lodhi RD, and Prasad R, 2015. Studies on genetic variability, correlation and path analysis for yield and its contributing traits in barley (*Hordeum vulgare* L.) under rainfed environment. *Indian Research Journal of Genetics and Biotechnology* 7(3): 305-310.
- Muller G, 1991. Determining leaf water surface area by means of linear measurement in wheat and triticale. *Archiv fur Zuchtungsforschung* 21(2): 121-123.
- Munns R and Tester M, 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681.
- Pirasteh-Anosheh H, Emam Y, Kazemeini SA, and Dehghany F, 2017. Effect of irrigation water salinity on soil moisture and salinity during growing season, barley yield, and its water productivity. *Iranian Journal of Soil Research (Formerly Soil and Water Sciences)* 31(2): 155-166. (In Persian with English abstract).
- Singh S, Madakemohekar AH, Prasad LC, and Prasad R, 2015. Genetic variability and correlation analysis of yield and its contributing traits in barley (*Hordeum vulgare* L.) for drought tolerance. *Indian Research Journal of Genetics and Biotechnology* 7(1): 103-108.
- Zaare M and Jafari H, 2013. Quantitative trait loci diversity for salt tolerance at the early growth stage of barley (*Hordeum vulgare* L.). *Crop Breeding Journal* 3(2): 69-77.

## تنوع ژنتیکی بین ژنوتیپ‌های جو و تجزیه ضرایب مسیر صفات زراعی و فیزیولوژیک در شرایط نرمال و تنش شوری

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### چکیده

پژوهش حاضر به منظور ارزیابی ارقام جو از نظر صفات زراعی و فیزیولوژیک انجام شد. در این پژوهش، ۱۵۰ واریته جو در قالب طرح آلفا لاتیس با پنج بلوک ناقص در دو تکرار تحت شرایط نرمال و تنش شوری ( $EC=12 \text{ dsm}^{-1}$ ) در مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی یزد کشت شدند. تجزیه مرکب نشان داد که اختلاف معنی داری بین ژنوتیپ‌های جو وجود دارد که بیانگر وجود تنوع ژنتیکی قابل توجه بین ارقام جو مورد مطالعه است. عملکرد بیولوژیک، روز تا رسیدگی فیزیولوژیک و شاخص کلروفیل برگ در شرایط نرمال و عملکرد بیولوژیک، شاخص برداشت، تعداد پنجه‌های بارور و ارتفاع بوته در شرایط تنش شوری همبستگی معنی داری با عملکرد دانه داشتند. با توجه به نتایج تجزیه ضرایب مسیر، روزهای تا رسیدگی فیزیولوژیک و تعداد پنجه بارور به ترتیب در شرایط نرمال و تنش شوری بیشترین تأثیر مستقیم مثبت را بر عملکرد دانه داشتند و پس از آن شاخص کلروفیل برگ در شرایط نرمال و ارتفاع بوته در شرایط تنش شوری قرار گرفتند. تجزیه کلاستر به روش وارد، ژنوتیپ‌های مورد مطالعه را بر اساس میانگین دو سال در هر دو محیط در سه کلاستر گروه‌بندی کرد. برای تعیین تعداد کلاسترها و بررسی صحت گروه‌بندی در تجزیه کلاستر از تجزیه تابع تشخیص استفاده شد. درصد انحراف از میانگین کل کلاسترها تحت تنش شوری نشان داد که ژنوتیپ‌های کلاستر اول بیشترین عملکرد دانه و کوتاه‌ترین دوره رسیدگی را دارند. در کلاستر دوم، ژنوتیپ‌های کارآمد فیزیولوژیک و در کلاستر سوم، ژنوتیپ‌های دیررس و کم محصول قرار گرفتند. بنابراین با توجه به نتایج این تحقیق نتیجه گرفته می‌شود که در شرایط تنش شوری می‌توان از ژنوتیپ‌های کلاستر اول که دارای بالاترین عملکرد دانه و زودترین تاریخ رسیدگی بودند، در برنامه‌های اصلاحی آبی برای بهبود تحمل به شوری استفاده کرد.

**واژه‌های کلیدی:** تجزیه تابع تشخیص؛ تجزیه ضرایب مسیر؛ تجزیه کلاستر؛ تنش شوری؛ جو

