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Evaluation of Effective Morphophysiological Characteristics on Grain Yield of Barley Under Salinity Stress Conditions

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Abstract

Salinity stress is one of the most important abiotic stresses, especially in arid and semi-arid regions. The current research was performed to identify traits associated with barley yield under normal and salinity stress conditions. In this research, 150 barley cultivars were planted in alpha lattice design with five incomplete blocks in two replications at non-stress and salinity stress (EC=12 dsm-1) conditions at Agriculture and Natural Resources Research and Education Center, Yazd, Iran. Correlation analysis showed that the biological yield and harvest index had the highest significant positive correlation with grain yield in experimental environments. The result of stepwise regression indicated that biological yield, harvest index, relative water content, leaf chlorophyll content, thousand-grain weight, and flag leaf length were the most important influential traits under normal conditions that explained 97.93% of the total variance of grain yield. While biological yield, harvest index, days to tillering, grain filling period, and days from stemming to heading entered in the model under salinity stress, which explained 97.46% of the total variance. According to the path analysis results, the biological yield and harvest index had the most direct and positive effect on grain yield in non-stress and salinity stress conditions. The factor analysis determined seven factors for non-stress and eight factors for salinity stress conditions that explained 75.2 and 80.5% of the data's total variance. Overall, this study showed that biological yield and harvest index were the main traits related to grain yield that can be used in barley breeding programs in selecting high-yielding cultivars and lines.

Keywords: Barley, Correlation, Factor analysis, Path coefficient analysis, Stepwise regression analysis

Introduction

Barley (Ahmadi and Hosseinpour 2013) is one of the oldest and most important crops in the Fertile Crescent that were domesticated about ten thousand years ago (Kilian, Özkan *et al.* 2009). This plant belongs to the family of cereals. Barley has a more large-scale climatic adaptation compared to other grains (Belete and Bediru). Barley yields higher than wheat under salinity stress and adverse conditions, so barley is always used to rehabilitate barren and saline lands. Salinity stress is one of the most important abiotic stresses, especially in arid and semi-arid regions, which has always reduced crop production. Because most crops are sensitive to salt, so salinity is a severe risk for farmers today (Flowers and Flowers 2005). Improvement of quantitative traits such as tolerance to salinity is the main goal in agriculture and plant breeding. Determining the relationships between traits and discovering hidden factors, and studying genetic diversity under salinity stress to determine affecting traits grain yield can be an efficient step in barley breeding programs to solve this problem.

In-plant breeding programs, the selection is based on many agronomic traits that

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may influence positively or negatively correlated. Therefore, statistical methods that reduce useful traits on grain yield without removing useful information are valuable to researchers (Guertin and Bailey 1970). In one study, (Ataei 2006) performed a correlation analysis between traits. The results showed that the barley grain yield had a positive and significant correlation with harvest index and biological yield. Furthermore, it not observed a significant correlation between grain yield and grain weight. (Hosseinpour 2012, Mohammadi, Sharifi *et al.* 2012, Ahmadi, Pourghasemi *et al.* 2014) also reported that the correlation between grain yield and biological yield was more than the correlation between grain yield and other traits. However, although there is a positive relationship between yield and some of its components, negative correlations between some of these components mean that selecting all of them cannot be useful in increasing grain yield (Rharrabti, Elhani *et al.* 2000). Also, an increase in one performance component may decrease some other components (Belete and Bediru , Del Blanco, Rajaram *et al.* 2001), so using multivariate methods is a good suggestion. Factor analysis is a powerful multivariate method used to reduce data, explain the contribution of traits to the total variance, identify hidden characteristics affecting performance, and group traits based on the internal relationships between them. In this method, variables that are strongly correlated with each other are placed in several independent factors. (Jabbary and Zolfaghary 2014), in the study on barley, introduced eight factors and five factors under normal and stress conditions, respectively. They named the productivity factor and the origin of making photosynthetic materials for similar traits.

Another multivariate method is path coefficient analysis, which can be provided by creating causal models to properly analyse the correlation between variables. Path coefficient analysis is used to assess the importance of useful traits on grain yield. This method reveals the relationships between traits and their direct and indirect effects on grain yield. (HOSEIN, AHARIZAD *et al.* 2013, Saroei, Cheghamirza *et al.* 2017) reported a direct and positive effect of biological yield on grain yield in their research using the path coefficient analysis method. Also, in a study using this method, the harvest index’s direct effect on grain yield was confirmed (Zaefizadeh, Ghasemi *et al.* 2011). This study aimed to estimate the correlation between traits and yield and identify traits affecting yield in barley genotypes using factor analysis, stepwise regression analysis, and path coefficients under salinity and normal stress conditions. We hope it can provide researchers with helpful information on barley breeding.

Materials and Methods

This experiment used 148 common barley cultivars of northern and western Europe collected by the Wageningen University of Netherlands. Two control cultivars named Khatam (resistant to salinity) and Nosrat (non-resistant to salinity) from Iranian cultivars were used. The present study was performed in an alpha-lattice design with five incomplete blocks in two replications. Each block includes 30 plots in non-stress and salinity stress (EC=12 dsm^{-1}) environments at the Agriculture and Natural Resources Research Station of Yazd (31° 55’ N, 54° 16’ E, 1213 m of sea level), Iran. The plot size was 0.45 × 2 meters dimensions consisting of three 2-meter lines, 15 cm apart. The studied traits include phenological, morphological, and physiological traits as follows: Grain yield (GY), Days to tillering (DT), Days to stemming (DS), Days to heading (DH), Days from stemming to heading (DSH), Grain filling period (GFP), Days to physiological maturity (DPM), Plant height (PH), Thousand-grain weight (TGW), Biological yield (BY), Harvest index (HI), Flag leaf length (FLL), Flag leaf width (FLW), Flag leaf area (FLA), Number of fertile tillers (NFT), Number of total tillers (NTT), Spike length (SL), Grain weight per spike (GWS), Number of grains per spike (NGS), Relative water content (RWC), Leaf chlorophyll content (LChC) and Leaf Proline content (LPC).

Leaf chlorophyll content was measured from three flag leaf points at the heading stage in 10 samples of each plot with a manual chlorophyll meter (SPAD). Then the average of each replicate was used for statistical analysis. Leaf Proline content was measured from 15 flag leaves in each plot at the heading stage and immediately transferred to the laboratory. Proline was extracted by using the method of (Bates, Waldren *et al.* 1973). The relative water content calculated after the completion of the flag leaf from Equation (1) (Fitter and Hay 2012):

$$RWC=(\text{Fresh weight} - \text{Dry weight}/(\text{Saturation weight} - \text{Dry weight})\times 100 \quad (1)$$

The flag leaf area calculated based on the proposed formula by Muller (1991) as follows:

$$S=0.75\times L\times W \quad (2)$$

S: Flag leaf area, L: Flag leaf length, and W: Flag leaf width

Also, the harvest index is calculated as follows:

$$HI=(\text{Grain yield}/ \text{Biological yield})\times 100 \quad (3)$$

The data normality test was first performed based on the Kolmogorov-Smirnov method using SPSS software. Then combined analysis of variance was performed with SAS 9.1 software. Simple phenotypic correlation between traits under normal conditions and salinity stress calculated. Also, salinity tolerance and studied traits were evaluated in stress and non-stress environments using multivariate, including factor analysis, stepwise regression analysis, and path coefficient analysis with SAS 9.1 and Path analysis software.

Results and Discussion

Analysis of Variance

Table 1: Analysis of variance of the studied traits under non-stress conditions

Source of variation	Df	Mean squares										
		GY (kg/m^2)	DT (day)	DS (day)	DH (day)	DSH (day)	GFP (day)	DPM (day)	PH (cm)	TGW (g)	BY (kg/m^2)	HI (%)
Rep	1	2.65^{**}	28.83^{**}	554.88^{**}	6.16^{ns}	444.08^{**}	28.21^{ns}	60.75^{*}	1056.56^{**}	9.36^{ns}	3.55^{**}	1087.6^{**}
Block(Rep)	8	0.21^{**}	12.04^{**}	539.06^{*}	12.44^{*}	590.96^{**}	19.18^{*}	15.35^{ns}	107.03^{*}	44.77^{**}	0.93^{*}	83.2^{**}
Genotype	149	0.098^{**}	6.86^{**}	48.18^{**}	27.38^{**}	39.61^{**}	19.16^{**}	20.63^{**}	135.79^{**}	13.35^{**}	0.67^{**}	30.3^{*}
Error	141	0.064	3.72	20.29	6.17	19.8	8.86	9.43	44.53	4.93	0.37	20.97
R-Square (%)	-	67.68	68.6	80.8	72.78	79.87	70.84	71.01	77.9	82.37	68.23	67.95
CV (%)	-	21.83	6.08	6.64	2.9	9.3	7.7	1.87	11.6	9.3	17.4	12.7
Source of variation	Df	FLL (cm)	FLW (cm)	FLA (cm^2)	NFT	NTT	SL(cm)	GWS (g)	NGS	RWC (%)	LChC	LPC (µmol/g)
Rep	1	92.18^{**}	0.58^{**}	185.02^{**}	53067^{**}	81543.05^{**}	10.37^{**}	0.025^{ns}	2.8^{ns}	0.6^{ns}	282.46^{**}	530.67^{**}
Block(Rep)	8	10.49^{**}	0.044^{**}	21.66^{**}	415.97^{ns}	3282^{**}	2.16^{**}	0.08^{*}	24.19^{*}	274.5^{**}	117.88^{**}	1.26^{**}
Genotype	149	7.79^{**}	0.07^{**}	22.16^{**}	1234.59^{ns}	1519.88^{*}	1.65^{**}	0.19^{**}	86.5^{**}	54.1^{**}	35.75^{*}	2.35^{**}
Error	141	2.9	0.016	5.79	1032.13	1085.58	0.5	0.03	10.99	35.66	25.39	0.135
R-Square (%)	-	76.66	83.69	81.75	62.3	68.59	79.43	86.59	89.41	67.1	64.66	98
CV (%)	-	15.95	18	35.7	22.9	24.7	11.3	29	26.54	6.3	8.5	5

The combined analysis of variance (not presented) showed significant genotype × environment interaction for most of the traits except for flag leaf width and leaf chlorophyll content; Thus, analysis of variance was performed separately for each non-stress and salinity stress conditions (Tables 1 and 2). The results show a significant difference between genotypes for most studied traits except the number of fertile tillers under non-stress conditions and relative water content and Leaf chlorophyll content under salinity stress (Tables 1 and 2). This difference indicates the diversity between genotypes for genetic analysis of these traits.

Table 2: Analysis of variance of the studied traits under salinity stress conditions

Source of variation	Df	Mean squares										
		GY (kg/m^2)	DT (day)	DS (day)	DH (day)	DSH (day)	GFP (day)	DPM (day)	PH (cm)	TGW (g)	BY (kg/m^2)	HI (%)
Rep	1	5.43^{**}	82.16^{**}	1474.08^{**}	52.92^{**}	968.4^{**}	$0.03^{n.s}$	50.43^{*}	156.96^{*}	187.23^{**}	92.44^{**}	63.76^{*}
Block(Rep)	8	0.24^{**}	5.78^{**}	$23.85^{n.s}$	28^{**}	56.1^{**}	$22.68^{n.s}$	22.08^{**}	155.43^{**}	$7.98^{n.s}$	3.34^{**}	$25.1^{n.s}$
Genotype	149	0.082^{**}	6.16^{**}	31.81^{**}	70.23^{**}	56.28^{**}	28.11^{**}	32.58^{**}	75.38^{**}	23.35^{**}	0.61^{**}	42.74^{**}
Error	141	0.05	2.13	11.96	6.29	18.85	12.16	7.15	31.32	10.21	0.32	14.2
R-Square (%)	-	77.72	79.16	92.37	78.67	71.83	83.44	74.1	72.15	73.37	82.18	76.81
CV (%)	-	29.9	4.56	4.22	4.7	15.4	12.45	2.57	10.07	12.4	21.9	18.35
Source of variation	Df	FLL (cm)	FLW (cm)	FLA (cm^2)	NFT	NTT	SL(cm)	GWS (g)	NGS	RWC (%)	LChC	LPC (μmol/g)
Rep	1	144.42^{**}	2.91^{**}	386.81^{**}	5720.3^{**}	7234.33^{**}	17.4^{**}	$0.095^{n.s}$	145.6^{**}	$175.03^{n.s}$	$1.63^{n.s}$	$0.64^{n.s}$
Block(Rep)	8	8.99^{**}	0.13^{**}	21.82^{**}	17230.4^{**}	1031.02^{**}	1.59^{**}	0.17^{**}	104.83^{**}	328.57^{*}	132.54^{**}	1.32^{*}
Genotype	149	8.05^{**}	0.09^{**}	17.02^{**}	4142.9^{**}	6546.5^{**}	1.6^{**}	0.084^{**}	55.48^{**}	$172.01^{n.s}$	$42.92^{n.s}$	5.6^{**}
Error	141	2.34	0.039	6.4	234.8	256	0.73	0.03	19.11	136.91	35.8	0.55
R-Square (%)	-	81.07	76.14	77.44	95.8	96	72.35	77.14	77.44	59.56	59.63	92
CV (%)	-	15.9	24.8	37.06	9.6	10.5	14.5	29.7	28.07	11.31	9.3	8.1

n.s, * and **: Not-significant and significant at 5% and 1% probability levels, respectively. **GY:** Grain yield, **DT:** Days to tillering, **DS:** Days to stemming, **DH:** Days to heading, **DSH**: Days from stemming 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, **NTT:** Number of total tillers, **SL:** Spike length, **GWS:** Grain weight per spike, **NGS:** Number of grains per spike, **RWC:** Relative water content, **LChC:** Leaf chlorophyll content, **LPC:** Leaf Proline content, **CV:** Coefficient of Variation.

Correlation Analysis

Examining the correlation between different traits makes it possible to act more accurately in removing ineffective traits. In this study, simple phenotypic correlation coefficients were calculated using the Pearson correlation coefficient to investigate the relationship between grain yield and other measured traits. Correlation analysis of traits for normal conditions and salinity stress is presented in Table 3. The results showed that biological yield ($r = 0.79^{**}$) and harvest index ($r = 0.64^{**}$) under normal conditions and grain filling period ($r = 0.44^{*}$), biological yield ($r = 0.79^{**}$) and harvest index ($r = 0.7^{**}$) under salinity stress had the highest positive and significant correlation with grain yield.

Therefore, phenotypic correlation coefficients of traits in both experimental environments showed a positive and significant correlation between grain yield with biological yield and harvest index. (Ataei 2006) also showed that barley grain yield had a positive and significant correlation with harvest index and biological yield. (Hosseinpour 2012, Ahmadi, Pourghasemi *et al.* 2014) also reported that the correlation between grain yield and biological yield was more than the correlation between grain yield and other traits.

Table 3: Phenotypic correlation coefficients of the studied traits (above the diameter for non-stress conditions and below the diameter for salinity stress)

Traits	GY	DT	DS	DH	DSH	GFP	DPM	PH	TGW	BY	HI

GY (kg/m^2)	**1**	**-0.1**	**-0.03**	**-0.06**	**-0.02**	**0.35**	**0.27**	**0.1**	**0.1**	**0.79****	**0.64****
DT (day)	**-0.42**	**1**	**0.03**	**0.19**	**0.12**	**0.01**	**0.24**	**0.21**	**0.08**	**-0.06**	**-0.09**
DS (day)	**-0.14**	**0.21**	**1**	**0.51***	**-0.71**	**-0.2**	**0.41**	**-0.16**	**-0.32**	**-0.1**	**0.06**
DH (day)	**-0.37**	**0.2**	**0.5***	**1**	**0.25**	**-0.6**	**0.61****	**-0.04**	**-0.41**	**-0.05**	**-0.05**
DSH (day)	**-0.31**	**0.07**	**-0.18**	**0.77****	**1**	**-0.27**	**0.03**	**0.15**	**0.03**	**0.07**	**-0.11**
GFP (day)	**0.44***	**-0.16**	**-0.23**	**-0.75**	**-0.68**	**1**	**0.27**	**0.19**	**0.23**	**0.29**	**0.19**
DPM (day)	**-0.13**	**0.15**	**0.52***	**0.78****	**0.5***	**-0.16**	**1**	**0.14**	**-0.26**	**0.23**	**0.13**
PH (cm)	**0.2**	**-0.27**	**-0.28**	**0.16**	**0.39**	**-0.11**	**0.14**	**1**	**0.048**	**0.4**	**-0.36**
TGW (g)	**0.25**	**-0.17**	**-0.17**	**-0.12**	**-0.01**	**0.21**	**0.01**	**0.33**	**1**	**0.02**	**0.19**
BY (kg/m^2)	**0.79****	**0.51***	**-0.19**	**-0.07**	**0.06**	**0.14**	**0.02**	**0.49***	**0.19**	**1**	**0.06**
HI (%)	**0.7****	**-0.06**	**-0.04**	**-0.52**	**-0.56**	**0.59****	**-0.21**	**-0.22**	**0.23**	**0.14**	**1**
FLL (cm)	**0.18**	**-0.28**	**0.005**	**0.14**	**0.15**	**-0.04**	**0.16**	**0.37**	**0.22**	**0.4**	**-0.15**
FLW (cm)	**0.02**	**-0.12**	**0.06**	**0.32**	**0.32**	**-0.14**	**0.35**	**0.42***	**0.22**	**0.29**	**-0.29**
FLA (cm^2)	**0.09**	**-0.19**	**0.03**	**0.27**	**0.29**	**-0.11**	**0.3**	**0.44***	**0.25**	**0.36**	**-0.25**
NFT	**0.09**	**0.21**	**0.1**	**0.07**	**-0.01**	**0.02**	**0.12**	**-0.3**	**-0.11**	**0.01**	**0.18**
NTT	**0.05**	**0.22**	**0.14**	**0.11**	**0.03**	**-0.03**	**0.14**	**-0.32**	**-0.13**	**-0.03**	**0.15**
SL(cm)	**0.2**	**-0.16**	**0.05**	**-0.28**	**-0.36**	**0.28**	**-0.15**	**-0.09**	**0.18**	**0.05**	**0.28**
GWS (g)	**0.13**	**-0.17**	**-0.34**	**-0.21**	**0.01**	**0.14**	**-0.18**	**0.38**	**0.17**	**0.19**	**0.01**
NGS	**0.06**	**-0.12**	**-0.29**	**-0.19**	**-0.01**	**0.1**	**-0.19**	**0.27**	**-0.01**	**0.13**	**-0.05**
RWC (%)	**0.02**	**0.1**	**-0.05**	**-0.06**	**-0.02**	**0.14**	**0.04**	**0.04**	**0.05**	**0.09**	**-0.04**
LChC	**-0.01**	**0.15**	**0.16**	**0.23**	**0.14**	**-0.12**	**0.23**	**-0.08**	**0.07**	**-0.05**	**0.09**
LPC (μmol/g)	**-0.04**	**0.13**	**0.05**	**0.06**	**0.03**	**-0.02**	**0.07**	**-0.12**	**-0.14**	**-0.13**	**0.07**

Continuation of Table 3: Phenotypic correlation coefficients of the studied traits (above the diameter for non-stress conditions and below the diameter for salinity stress)

Traits	FLL	FLW	FLA	NFT	NTT	SL	GWS	NGS	RWC	LChC	LPC
GY (kg/m^2)	**-0.05**	**-0.13**	**-0.08**	**0.05**	**-0.018**	**-0.07**	**0.03**	**-0.02**	**-0.042**	**-0.15**	**0.01**
DT (day)	**0.11**	**0.24**	**0.17**	**-0.09**	**-0.13**	**-0.02**	**0.196**	**0.18**	**0.14**	**0.097**	**-0.03**
DS (day)	**0.005**	**0.12**	**0.056**	**0.08**	**0.09**	**0.12**	**-0.05**	**0.04**	**-0.04**	**0.17**	**0.04**
DH (day)	**0.14**	**0.31**	**0.23**	**0.08**	**0.046**	**0.06**	**0.085**	**0.22**	**0.19**	**0.18**	**0.11**
DSH (day)	**0.11**	**0.121**	**0.124**	**-0.022**	**-0.064**	**-0.09**	**0.12**	**0.14**	**0.21**	**-0.046**	**0.048**
GFP (day)	**0.19**	**-0.014**	**0.07**	**0.18**	**0.19**	**-0.004**	**0.04**	**-0.07**	**-0.07**	**0.005**	**-0.15**
DPM (day)	**0.35**	**0.35**	**0.35**	**0.27**	**0.24**	**0.06**	**0.14**	**0.2**	**0.17**	**0.22**	**-0.012**
PH (cm)	**0.44***	**0.48***	**0.47***	**-0.13**	**-0.12**	**-0.23**	**0.55****	**0.53***	**-0.27**	**0.02**	**-0.06**
TGW (g)	**-0.03**	**-0.13**	**-0.05**	**-0.14**	**-0.16**	**-0.004**	**0.18**	**-0.07**	**0.003**	**-0.28**	**-0.06**

BY (kg/m^2)	0.07	0.09	0.08	-0.05	-0.1	-0.19	0.27	0.21	-0.14	-0.06	0.004
HI (%)	-0.22	-0.36	-0.27	0.14	0.08	0.1	-0.28	-0.32	0.16	-0.22	0.02
FLL (cm)	1	0.75**	0.88**	0.11	0.15	0.03	0.34	0.34	-0.07	0.25	-0.1
FLW (cm)	0.76**	1	0.94**	-0.02	0.0007	-0.18	0.54**	0.58**	-0.17	0.2	-0.03
FLA (cm^2)	0.89**	0.96**	1	0.02	0.051	-0.14	0.52**	0.53**	-0.11	0.2	-0.06
NFT	0.07	-0.04	-0.02	1	0.97**	0.27	-0.35	-0.34	0.09	0.13	-0.02
NTT	0.05	-0.04	-0.03	0.98**	1	0.29	-0.33	-0.31	0.05	0.15	-0.01
SL(cm)	0.08	-0.1	-0.05	-0.06	-0.06	1	-0.36	-0.39	0.12	0.15	0.04
GWS (g)	0.33	0.32	0.4	-0.095	-0.11	-0.12	1	0.93**	-0.23	-0.05	-0.02
NGS	0.25	0.24	0.29	-0.09	-0.1	-0.17	0.93**	1	-0.22	0.03	0.01
RWC (%)	-0.03	-0.03	-0.03	-0.03	-0.036	0.07	-0.05	-0.065	1	0.06	-0.06
LChC	0.12	0.09	0.09	0.06	0.06	0.09	-0.09	-0.09	-0.046	1	0.06
LPC (µmol/g)	-0.22	-0.2	-0.2	0.02	0.02	-0.01	-0.14	-0.1	0.05	-0.04	1

* **and** ****:** significant at 5% and 1% probability levels, respectively. **GY:** Grain yield, **DT:** Days to tillering, **DS:** Days to stemming, **DH:** Days to heading, **DSH**: Days from stemming 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, **NTT:** Number of total tillers, **SL:** Spike length, **GWS:** Grain weight per spike, **NGS:** Number of grains per spike, **RWC:** Relative water content, **LChC:** Leaf chlorophyll content, **LPC:** Leaf Proline content

Stepwise Regression Analysis

A linear multivariate stepwise regression method was used to determine the contribution of the cumulative effect of traits in determining grain yield. This method investigated grain yield as a dependent variable with other traits as independent variables under normal conditions. Results of stepwise regression under non-stress conditions are shown in Table 4.

Table 4: Stepwise regression analysis for grain yield (dependent variable) and other traits (independent variable) under non-stress conditions

Step	Variable entered	**Parameter estimate**	Partial R^2	**Model R^2**	F Value
1	Biological yield (x_1)	0.31	62.21	62.21	243.6**
2	Harvest index (x_2)	0.033	35.41	97.62	2179.7**
3	Relative water content (x_3)	-0.001	0.11	97.73	6.95**
4	Leaf chlorophyll content (x_4)	0.002	0.1	97.83	6.59**
5	Thousand-grain weight (x_5)	-0.002	0.05	97.88	3.3$^{n.s}$
6	Flag leaf length (x_6)	0.003	0.05	97.93	3.41$^{n.s}$

n.s, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

Biological yield (R^2= 62.21%), harvest index (R^2= 35.41%), relative water content (R^2= 0.11%), Leaf chlorophyll content (R^2= 0.1%), thousand-grain weight (R^2= 0.05%) and flag leaf length (R^2= 0.05%) entered to the model as effective traits that highly contributed to grain yield variations. These traits explained 97.93% of total variations of grain yield. The model of stepwise regression was as below:

$Y=0.008+0.31X_1+0.033X_2-0.001X_3+0.002X_4$

In this model, Y, X_1, X_2, X_3 and X_4 denote grain yield, biological yield, harvest index, relative water content and leaf chlorophyll content, respectively. The significant coefficient in the regression equation indicating these traits are to be effective in increasing yield (table 4). The above Equation showed that biological yield, harvest index, leaf chlorophyll content and flag leaf length had a positive impact and relative water content and thousand-grain weight had a negative effect on increasing grain yield. (Dadashi, Noorinia *et al.* 2010) used stepwise regression to introduce three traits: number of seeds per spike, number of fertile tillers and 1000-seed weight as traits affecting grain yield. (Ahmadi, Pourghasemi *et al.* 2014) studied barley by stepwise regression analysis. They identified biological yield, spike weight, flag leaf length, flag leaf area, and spike length as traits affecting grain yield. Also, (Nasri, Paknejad *et al.* 2013), with the stepwise regression analysis method, introduced the traits of spike weight, total dry weight and harvest index as important traits on barley grain yield.

Results of stepwise regression under salinity stress conditions are shown in Table 5. Biological yield (R^2= 62.07%), harvest index (R^2= 35.17%), days to tillering (R^2= 0.1%), grain filling period (R^2= 0.07%), and days from stemming to heading (R^2= 0.05%) entered to the model as effective traits that highly contributed to grain yield variations. These traits explained 97.46% of total variations of grain yield. The model of stepwise regression was as below:

$Y= -0.05+0.27X_1+0.03X_2-0.005X_3+0.002X_4$

In this model, Y, X_1, X_2, X_3 and X_4 denote grain yield, biological yield, harvest index, days to tillering and grain filling period, respectively. According to the results of Table 5, all entered traits into the model except day from stemming to heading had a significant effect on grain yield, which indicates that these traits are effective in grain yield. The above Equation showed that the biological yield and harvest index had a positive impact, and days to tillering, grain filling period, and days from stemming to heading a negative effect on increasing grain yield. (Afzalifar, Zahravi *et al.* 2011), with respect to stepwise regression analysis, introduced the total number of grains, biomass and plant height as traits affecting grain yield.

Table 5: Stepwise regression analysis for grain yield (dependent variable) and other traits (independent variable) under salinity stress conditions

Step	Variable entered	**Parameter estimate**	Partial R^2	**Model R^2**	F Value
1	Biological yield (x_1)	0.27	62.07	62.07	242.2^{**}
2	Harvest index (x_2)	0.03	35.17	97.24	1870.9^{**}
3	Days to tillering (x_3)	-0.005	0.1	97.34	5.61^{*}
4	Grain filling period (x_4)	-0.002	0.07	97.39	3.91^{*}
5	Days from stemming to heading (x_5)	-0.001	0.05	97.46	$2.88^{n.s}$

n.s, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

Path Analysis

Path analysis was done based on correlation coefficients to determine important traits' direct and indirect effects on grain yield.

According to the path analysis results, the biological yield had the most direct effect under both non-stress (0.75) and salinity stress (0.7) conditions (Tables 6 and 7); also, it had a strong correlation with yield ($r=0.79^{**}$) which indicates its remarkable effect on grain yield and therefore it can be used for selection with the aim of increasing grain yield. (Ahmadi, Pourghasemi *et al.* 2014, Saroei, Cheghamirza *et al.* 2017) also reported a direct and positive effect of biological yield on grain yield. After biological yield, harvest index showed the most direct effect on improving grain yield, and its total effect under normal conditions and salinity stress were $r = 0.64^{**}$ and $r = 0.7^{**}$, respectively (Tables 6 and 7). (Zaefizadeh, Ghasemi *et al.* 2011) also reported the direct effect of harvest index on grain yield. Harvest index had the most indirect effect by improving thousand-grain weight on grain yield in non-stress and salinity stress conditions (Tables 6 and 7). The indirect effect of harvest index was negligible through other traits, which could increase grain yield by assuming other variables are constant.

Given that the correlation coefficient between the two traits was approximately equal to the causal coefficient between the two traits in normal and saline conditions, the correlation coefficient expressed the extent of the actual relationship between the two variables. Thus selection through this trait can be useful. The biological yield indirectly increased grain yield by increasing thousand-grain weight under salinity stress.

Table 6: Path coefficients analysis of direct and indirect effects of the traits with grain yield under non-stress conditions

Traits	Direct effect	Indirect effect via			Correlation with yield
		Biological yield	Harvest index	Thousand-grain weight	
Biological yield (kg/m^2)	0.75	-	0.05	0.01	0.79^{**}
Harvest index (%)	0.6	0.04	-	0.11	0.64^{**}
Thousand-grain weight (g)	0.005	0.0001	0.001	-	$0.1^{n.s}$
Residual Error	0.13				

n.s, * and **: Not-significant and significant at 5% and 1% probability levels, respectively.

Table 7: Path coefficients analysis of direct and indirect effects of the traits with grain yield under salinity stress conditions

Traits	Direct effect	Indirect effect via			Correlation with yield
		Biological yield	Harvest index	Thousand-grain weight	
Biological yield (kg/m^2)	0.7	-	0.1	0.13	0.79^{**}
Harvest index (%)	0.61	0.09	-	0.14	0.7^{**}
Thousand-grain weight (g)	0.003	0.0006	0.0007	-	$0.25^{n.s}$
Residual Error	0.15				

n.s, * and **: Not-significant and significant at 5% and 1% probability

levels,

Factor Analysis

The purpose of factor analysis is to reduce data. In this method, variables that are strongly correlated with each other are placed in several independent factors. In general, factor analysis is used to reduce data, describe the total diversity of a community, explain the contribution of traits to total diversity, and grouping traits based on the interrelationships between them and the study of genetic diversity. The sign of factor coefficients within each factor indicates the relationship between these traits. The largest coefficient in any factor or set of significant traits that are morphologically distinct and important is used to name the factors. The variance of each factor in percentage indicates its importance in interpreting the data’s overall changes. The commonality rate of the trait indicates a part of the variance of a trait related to common factors.

According to factor analysis results by the Varimax method under non-stress conditions (Table 8), the first seven factors had eigenvalues greater than one, which explained 75.2% of the data’s total changes. The first factor, which had the highest variance, explained 21.4% of the total changes. This factor is mainly defined by plant height (0.62), flag leaf length (0.82), flag leaf width (0.92), flag leaf area (0.93), grain weight per spike (0.71) and the number of grains per spike (0.72). Therefore, this factor can be called “efficiency and source of making photosynthetic materials”. This factor shows that phenological traits cause the storage of available materials for the plant’s reproductive growth by affecting the vegetative growth traits related to the plant’s fixed capital. (Jabbary and Zolfaghary 2014), in a study on barley, introduced six and five factors under stress and in non-stress conditions, respectively. They named the efficiency and source of making photosynthetic materials for similar traits.

Table 8: Results of factor analysis of the studied traits by the varimax method under non-stress conditions

Traits	**Rotated Factors**							**Communality value**
	FA1	**FA2**	**FA3**	**FA4**	**FA5**	**FA6**	**FA7**	
GY (kg/m^2)	**-0.088**	**0.026**	**0.93**	**-0.06**	**0.03**	**0.02**	**-0.24**	**0.93**
DT (day)	**0.22**	**-0.23**	**-0.05**	**-0.12**	**-0.003**	**0.63**	**0.18**	**0.54**
DS (day)	**0.06**	**0.05**	**-0.02**	**0.33**	**0.92**	**0.08**	**0.04**	**0.97**
DH (day)	**0.26**	**0.06**	**-0.003**	**0.82**	**0.18**	**0.41**	**-0.03**	**0.95**
DSH (day)	**0.15**	**-0.01**	**0.02**	**0.29**	**-0.88**	**0.24**	**-0.07**	**0.95**
GFP (day)	**0.09**	**0.2**	**0.45**	**-0.74**	**0.09**	**-0.01**	**0.14**	**0.83**
DPM (day)	**0.39**	**0.27**	**0.44**	**0.25**	**0.3**	**0.48**	**0.1**	**0.82**
PH (cm)	**0.62**	**-0.23**	**0.27**	**-0.15**	**-0.2**	**-0.16**	**0.25**	**0.66**
TGW (g)	**-0.007**	**-0.16**	**-0.04**	**-0.6**	**-0.18**	**0.09**	**-0.45**	**0.63**
BY (kg/m^2)	**0.14**	**-0.14**	**0.87**	**-0.004**	**-0.12**	**-0.15**	**0.09**	**0.84**
HI (%)	**-0.35**	**0.2**	**0.44**	**-0.086**	**0.18**	**0.23**	**-0.57**	**0.76**
FLL (cm)	**0.82**	**0.24**	**-0.026**	**-0.14**	**-0.01**	**0.1**	**0.11**	**0.77**
FLW (cm)	**0.92**	**0.003**	**-0.07**	**0.09**	**0.04**	**0.05**	**0.09**	**0.87**
FLA (cm^2)	**0.93**	**0.09**	**-0.05**	**-0.02**	**0.01**	**0.07**	**0.03**	**0.89**
NFT	**0.027**	**0.92**	**0.07**	**0.03**	**-0.01**	**-0.03**	**0.01**	**0.86**
NTT	**0.06**	**0.93**	**0.01**	**0.01**	**0.02**	**-0.09**	**0.04**	**0.88**
SL(cm)	**-0.23**	**0.43**	**-0.15**	**-0.07**	**0.12**	**0.23**	**0.14**	**0.35**

GWS (g)	**0.71**	**-0.51**	**0.13**	**-0.02**	**-0.04**	**-0.09**	**-0.08**	**0.79**
NGS (g)	**0.72**	**-0.49**	**0.1**	**0.17**	**-0.002**	**-0.1**	**0.01**	**0.8**
RWC (%)	**-0.22**	**0.12**	**-0.06**	**0.06**	**-0.17**	**0.7**	**-0.05**	**0.6**
LChC	**0.1**	**0.16**	**-0.04**	**0.08**	**0.11**	**0.2**	**0.76**	**0.67**
LPC (µmol/g)	**-0.11**	**-0.02**	**0.08**	**0.36**	**-0.07**	**-0.17**	**0.1**	**0.2**
Eigen Value	**4.7**	**3.12**	**2.6**	**2.03**	**1.73**	**1.32**	**1.03**	**-**
Relative variance (%)	**21.4**	**14.2**	**11.8**	**9.2**	**7.9**	**6**	**4.7**	**-**
Cumulative variance (%)	**21.4**	**35.6**	**47.4**	**56.6**	**64.4**	**70.5**	**75.2**	**-**

FA: Factor analysis

The second factor (Table 8) that accounted for 14.2% of the total variance is related to the traits of number of fertile tillers (0.92) and number of total tillers (0.93), which have a positive and high factor load, and as the factor of performance and performance components is called. The third factor that explained 11.8% of the total variance was related to grain yield (0.93) and biological yield (0.87), which can be defined as the grain yield factor. In a study on barley, (Khajavi, Aharizad *et al.* 2014) identified three factors using factor analysis. They introduced one factor as an effective factor in increasing grain yield that had a positive correlation with the number of grains per spike, grain yield, Thousand-grain weight and harvest index. The fourth factor (Table 8) is loaded by days to heading (0.82) and grain filling period (-0.74), and it accounted for just 9.2% of the total variance. The fifth factor was related to days to stemming (0.92) and days from stemming to heading (-0.88); therefore, the fourth and fifth factors can be defined as phenological factors. The communality value is part of the variance of a variable that is related to common factors. The higher the communality value is, the more accurate it is in estimating the variance of the relevant variable (Jackson 1991). The commonality of most traits (except days to tillering, spike length and leaf proline) was high (Table 8) in non-stress conditions, indicating that the number of selected factors were appropriate. These factors were able to justify the changes in traits well.

Table 9: Results of factor analysis of the studied traits by the varimax method under salinity stress conditions

Traits	**Rotated Factors**								**Communality value**
	FA1	**FA2**	**FA3**	**FA4**	**FA5**	**FA6**	**FA7**	**FA8**	
GY (kg/m^2)	**-0.41**	**-0.015**	**0.85**	**0.12**	**0.06**	**0.028**	**0.16**	**-0.002**	**0.94**
DT (day)	**0.1**	**-0.22**	**-0.64**	**0.25**	**0.06**	**0.2**	**0.21**	**0.23**	**0.67**
DS (day)	**-0.07**	**0.13**	**-0.22**	**0.05**	**-0.31**	**0.8**	**-0.06**	**-0.18**	**0.84**
DH (day)	**0.78**	**018**	**-0.1**	**0.048**	**-0.17**	**0.51**	**0.11**	**-0.05**	**0.96**
DSH (day)	**0.93**	**0.11**	**0.05**	**0.01**	**0.03**	**-0.01**	**0.17**	**0.08**	**0.92**
GFP (day)	**-0.79**	**-0.06**	**0.19**	**0.017**	**0.14**	**-0.06**	**0.07**	**0.23**	**0.75**
DPM (day)	**0.41**	**0.22**	**0.03**	**0.087**	**-0.12**	**0.69**	**0.23**	**0.15**	**0.8**
PH (cm)	**0.38**	**0.31**	**0.47**	**-0.34**	**0.25**	**-0.16**	**0.15**	**0.2**	**0.73**
TGW (g)	**-0.13**	**0.24**	**0.21**	**-0.15**	**-0.03**	**0.26**	**0.6**	**0.22**	**0.63**

BY (kg/m^2)	**0.01**	**0.25**	**0.89**	**0.02**	**0.05**	**-0.04**	**-0.03**	**0.08**	**0.86**
HI (%)	**-0.68**	**-0.3**	**0.35**	**0.21**	**0.057**	**0.076**	**0.35**	**-0.05**	**0.85**
FLL (cm)	**0.02**	**0.88**	**0.21**	**0.06**	**0.13**	**0.03**	**0.05**	**-0.02**	**0.84**
FLW (cm)	**0.2**	**0.86**	**0.098**	**-0.04**	**0.19**	**0.15**	**0.08**	**0.06**	**0.86**
FLA (cm^2)	**0.15**	**0.91**	**0.15**	**-0.02**	**0.21**	**0.12**	**0.08**	**0.04**	**0.94**
NFT	**-0.01**	**0.02**	**0.002**	**0.98**	**-0.04**	**0.03**	**-0.002**	**0.0005**	**0.97**
NTT	**0.02**	**0.016**	**-0.036**	**0.98**	**-0.05**	**0.05**	**-0.007**	**-0.02**	**0.97**
SL(cm)	**-0.52**	**0.15**	**0.028**	**-0.13**	**-0.37**	**-0.08**	**0.18**	**0.006**	**0.49**
GWS (g)	**-0.07**	**0.27**	**0.08**	**-0.076**	**0.89**	**-0.18**	**0.03**	**-0.02**	**0.92**
NGS (g)	**-0.05**	**0.2**	**0.008**	**-0.065**	**0.91**	**-0.12**	**-0.09**	**-0.08**	**0.91**
RWC (%)	**-0.08**	**0.01**	**-0.03**	**-0.01**	**-0.09**	**0.001**	**-0.06**	**0.88**	**0.79**
LChC	**0.06**	**0.06**	**-0.13**	**0.06**	**-0.07**	**0.2**	**0.73**	**-0.21**	**0.65**
LPC (µmol/g)	**0.05**	**-0.45**	**0.05**	**-0.02**	**0.12**	**0.42**	**-0.08**	**0.24**	**0.45**
Eigen Value	**4.42**	**4.16**	**2.45**	**1.91**	**1.47**	**1.14**	**1.1**	**1.06**	**-**
Relative variance (%)	**20.1**	**18.9**	**11.1**	**8.7**	**6.7**	**5.2**	**5**	**4.8**	**-**
Cumulative variance (%)	**20.1**	**39**	**50.1**	**58.8**	**65.5**	**70.7**	**75.7**	**80.5**	**-**

Based on factor analysis under salinity stress conditions (Table 9), the eight factors had eigenvalues greater than one, which explained 80.5% of the data's total changes. The first factor explained 20.1% of the total changes. This factor is mainly defined by days to heading (0.78), days from stemming to heading (0.93) and grain filling period (-0.79) and harvest index (-0.68); Therefore, this factor was named as a phenological factor. The second factor that accounted for 18.9% of the total variance is related to the traits of flag leaf length (0.88), flag leaf width (0.86) and flag leaf area (0.91); Therefore, this factor can be called "flag leaf". The third factor that explained 11.1% of the total variance was related to grain yield (0.85), biological yield (0.89) and day to tillering (-0.64), which can be defined as grain yield factor.

The fourth factor (Table 9) is loaded by number of fertile tillers (0.98) and number of total tillers (0.98), and it accounted for just 8.7 % of the total variance. The fifth factor was related to grain weight per spike (0.89) and number of grains per spike (0.91), and it explained 6.7% of the total variance; therefore, the fourth and fifth factors can be named as "Performance and performance components". Days to stemming (0.8) and days to physiological maturity (0.69) were related to the sixth factor, which explained 5.2% of the total variance, and It was called the "phenological" factor. Thousand-grain weight (0.6) and leaf chlorophyll (0.73) were related to the seventh factor, and relative water content (0.88) was related to the eighth factor, which had the lowest percentage of variance (5 and 4.8%, respectively) and was named as "performance and related traits" factors. The commonality was high (Table 9) in stress conditions, indicating that the number of selected factors were appropriate. These factors were able to justify the changes in traits well.

Conclusion

The correlation analysis results, factor analysis, stepwise regression and path analysis in both non-stress and salinity stress conditions showed that biological yield and harvest index were important traits related to grain yield. Therefore, the main reason for the difference in grain yield of the lines can be attributed to the difference in these traits and can be used in barley breeding programs to select cultivars and high-yielding lines.

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