Original Research Article

INVESTIGATION OF THE RELATIONSHIP BETWEEN GRAIN YIELD WITH STUDIED TRAITS UNDER NORMAL AND SALT STRESS CONDITIONS IN BARLEY CULTIVARS (*HORDEUM VULGAR* L.) USING MULTIVARIATE ANALYSIS

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ABSTRACT: In order to evaluate the relationship between different traits on grain yield in barley, 148 modern European two-row spring barley cultivars were planted in two alpha lattice designs with five incomplete blocks of thirty plots in two replications at both normal and salt stress (EC=12 dsm^{-1}) conditions at Agriculture and Natural Resources Research Center, Yazd, Iran in 2014-15. Data for 11 characteristics were compiled then subjected to multivariate analysis to study variability within the hybrids. Significant variations were observed among the hybrids for the measured characteristics. The result of principal component analysis, stepwise regression and path analysis indicated that biological yield, harvest index, thousand grain weight and grain filling period were as the most important effective components of grain yield in both normal and salt stress conditions. As a consequence, these traits can be used as the most selection criteria for improving grain yield in barley breeding program at early generations.

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1.INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide and is an important source for feed, malt, and human food. Its importance is derived from the ability to grow and produce in marginal environments, such as drought, low temperature and salinity (Hayes et al., 2003). The genetic diversification and the adaptability to a broad range of ecological conditions have highly strengthened the salinity tolerance in barley. These factors might have raised a rich gene pool with a large variation in adaptation to salinity. Salinity stress is a major constraint to agricultural food production because it decreases crop yield and restricts the use of agricultural land. It was estimated that salinity affected nearly 950 million ha (6%) in the world (Babu et al., 2007). Approximately 20% of agricultural land in the world and nearly half of all irrigated land suffer from salinity (Flowers and Yeo, 1995; Munns, 2005). The area affected by salinity in South, South East, North and Central Asia covered 25% of the total soil. Some of the most severe problems in soil salinity occur in arid and semiarid regions of the world. Besides these regions, salinity also affects agriculture in coastal regions and areas affected by low-quality irrigation water. Salt tolerance of barley plants is affected by soil conditions. In dry saline soils, the primary limiting factors for plant growth and development are high pH and sodicity (Barrett-Lennard, 2003; Colmer et al., 2005).   Genetic diversity between genotypes is usually estimated by measurements of physiological and morphological differences of quantitative and economically important traits. The disadvantages of this conventional approach are the cost of time and labour during the measurements, and the influences of environmental factors. Often, these disadvantages are exacerbated in salt-tolerance breeding. For example any change in the environment such as temperature, light or humidity can dramatically change the transpiration driving forces and, subsequently, ion uptake (Jaiwal 1997), Such changes may alter salt tolerance among genotypes. It is important to note that morphological characters are often limited in their numbers and may not adequately represent actual genetic relationships among genotypes. Improvement in grain yield and related traits and grain quality for different end uses is essential for the development of genotypes. Multivariate data analysis facilitates a graphic display of the underlying latent factors and an interface between individual samples and variables (Nielsen and Munck, 2003). Principal component analysis (PCA) has been widely used in plant sciences for a reduction of variables and grouping of genotypes. Principal component analysis is defined as "a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables". PCA can be used to drive a two dimensional scatter plot of individuals, such that the geometrical distance among individuals in the plot reflect the genetic distances among them with minimal distortion. Aggregates of individuals in such a plot will reveal sets of genetically similar individuals (Warburton and Crossa, 2000). Path analysis enables us to distinguish the direct impact of each yield component from indirect effects caused by mutual relationships among them (Emam & Borjan, 2000). For the same purpose, plant modification experts utilize path analysis

method as a technique for determining significance of attributes affecting the yield (Jafari et al., 2003). The objective of this study was the investigation of genetic diversity, determining effective traits on yield in barley cultivars under normal and salt stress conditions using multivariate methods which provide valuable information for plant breeders who are interested in researching the agronomic traits of barley and breeding new high yielding barley cultivars in future.

2. MATERIALS AND METHODS

A total of 148 modern European two-row spring barley cultivars were used, representing commercial germplasm used all over north-west Europe in the past 15 years (see Kraakman et al. 2004 for more details). Barley cultivars were provided by wageningen university and Research Center. This experiment was conducted at Agriculture and Natural Resources Research Station of Yazd (31o 55' N, 54o 16' E, 1213 m alt),Iran in 2014-15.   The cultivars were planted in two alpha lattice designs with five incomplete blocks of thirty plots in two replications at both normal and salt stress (EC=12 dsm^{-1}) conditions, where the plot size was 0.45 * 2 meters dimensions consisting of three 2-meter lines, 15 cm apart from each other. Plant samples were measured for the following traits: Days to tillering (X1) Days to steming (X2) Days to heading (X3) Days from steming to heading (X4) Grain filling period (X5) Days to maturity (X6) Plant height (X7) Thousand grain weight (X8) Grain yield (X9) Biological yield (X10) Harvest index (X11). The data were tested for the normal distribution of frequency using Kolmogorov-smirnov test in SPSS software (SPSS, 2004). Then, for analysis of variance, a general linear model (GLM) procedure, principal component analysis, Stepwise regression and path analysis were used SAS (SAS Institute, 2003),Path analysis and Minitab14 software's.

3. RESULTS AND DISCUSSION

Analysis of variance of data showed significant differences among genotypes in all measured traits (P≤0.01) (Table 1, 2.) which demonstrates high diversity among landraces under study in both normal and salt stress conditions.

Table 1. Analysis of variance of investigated traits in normal conditions.

Source	Df	Mean Squares										
		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Rep	1	28.83**	554.88**	6.16$^{n.s}$	444.08**	28.21$^{n.s}$	60.75*	1056.56**	9.36$^{n.s}$	2.65**	3.55**	0.107**
Block(rep)	8	12.04**	539.06**	12.44*	590.96**	19.18*	15.35$^{n.s}$	107.03*	44.77**	0.21**	0.93*	0.0085**
Genotype	149	6.86**	48.18**	27.38**	39.61**	19.16**	20.63**	135.79**	19.35**	0.098**	0.67**	0.003*

Error	141	3.72	20.29	6.17	19.8	8.86	9.43	44.53	4.93	0.064	0.37	0.002
R² (%)	-	68.6	80.8	72.78	79.87	70.48	71.01	77.9	82.37	67.68	68.23	67.74
CV (%)	-	6.08	6.64	2.9	9.3	7.7	1.87	11.6	9.3	21.83	17.4	12.9
StD	-	1.84	5.11	3.7	4.55	3.04	3.1	8.14	3.16	0.22	0.56	0.04
Mean	-	30.4	76.97	125.98	49.01	39.75	165.72	70.46	33.95	0.997	3.2	0.31

CV: Coefficient of Variation, StD:Standard Deviation, * and ** respectively significant at 0.05 and 0.01 probability levels

Table 2 . Analysis of variance of investigated traits in salt stress conditions.

| Source	Df	Mean Squares										
		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
Rep	1	82.16**	1474.08**	52.92**	968.4**	0.03[n.s]	50.43*	156.96*	187.23**	5.43**	92.44**	0.0064*
Block(rep)	8	5.78**	23.85[n.s]	28**	56.1**	22.68[n.s]	22.08**	155.43**	7.98[n.s]	0.24**	3.34**	0.0024[n.s]
Genotype	149	6.16**	31.81**	70.23**	56.28**	28.11**	32.58**	75.38**	23.35**	0.082**	0.61**	0.0043**
Error	141	2.13	11.96	6.29	18.85	12.16	7.15	31.32	10.21	0.05	0.32	0.0014
R² (%)	-	77.72	79.16	92.37	78.67	71.83	83.44	74.1	72.15	73.37	82.18	76.96
CV (%)	-	4.56	4.22	4.7	15.4	12.45	2.57	10.07	12.4	29.9	21.93	18.4
StD	-	1.73	3.89	5.97	5.26	3.8	4.03	5.85	3.4	0.21	0.61	0.046
Mean	-	37.94	92.2	126.35	34.14	30.46	156.8	58.07	27.44	0.72	2.8	0.252

1. Principal components analysis

According to principal components analysis in normal condition (Table 3 and Figure 1), five principal components (PC) had Eigen values >1 and accounted for 84.41% of the total variance in the data. The proportions of the total variance attributable to the first three PC were 23.42, 21.22 and 16.9%. The importance of traits to the different PC can be seen from the corresponding eigen vectors which are presented in Table 4. The results showed that grain filling period, grain yield and days to heading had the highest loadings in PC1 indicating their significant importance for this component. On the other hand, other traits were less important to PC1. Days to steming, days to maturity and grain yield are the main traits of PC2. For PC3 traits such as days from steming to heading and plant height were the most important, whereas days to maturity, days from steming to heading and harvest index mainly contributed to PC4. The fifth PC was derived mainly from the variance of days to tillering and thousand grain weight traits.

Table 3. Principale components analysis of investigated traits in normal conditions.

Component	Eigen Value	Proportion Variance (%)	Cumulative Variance (%)
1	2.58	23.42	23.42
2	2.33	21.22	44.64
3	1.86	16.9	61.54
4	1.39	12.66	74.2
5	1.12	10.22	84.41

Table 4. Eigen vectors (loadings) of the first five principal components in normal conditions.

Traits	PCA1	PCA2	PCA3	PCA4	PCA5
X1	-0.078	0.080	0.286	-0.204	0.7
X2	-0.319	0.4	-0.342	-0.249	-0.0099
X3	-0.426	0.37	0.224	0.234	0.048
X4	0.0107	-0.146	0.568	0.47	0.05
X5	0.445	0.093	-0.103	-0.369	0.167
X6	-0.074	0.536	0.168	-0.823	0.221
X7	0.158	0.062	0.471	-0.432	-0.13
X8	0.312	-0.228	-0.0518	0.0246	0.45
X9	0.43	0.4	-0.018	0.256	-0.101
X10	0.375	0.326	0.239	-0.018	-0.352
X11	0.242	0.237	-0.329	0.474	0.289
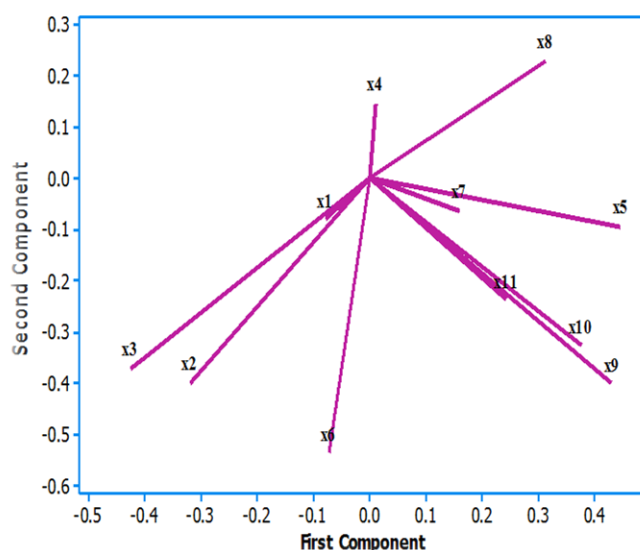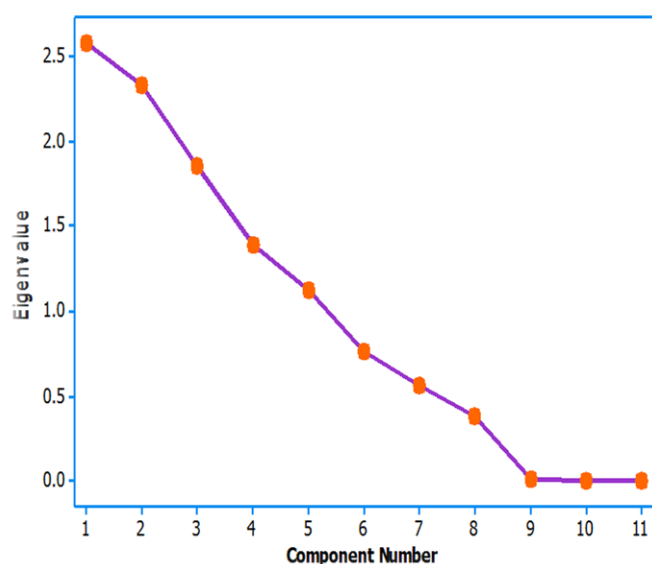
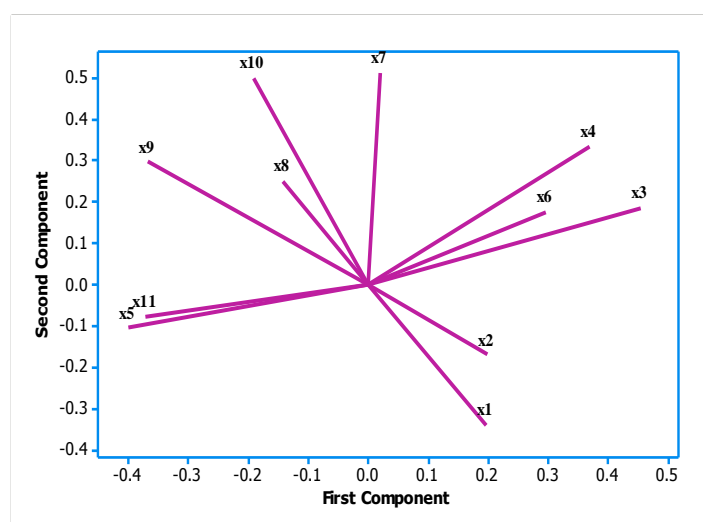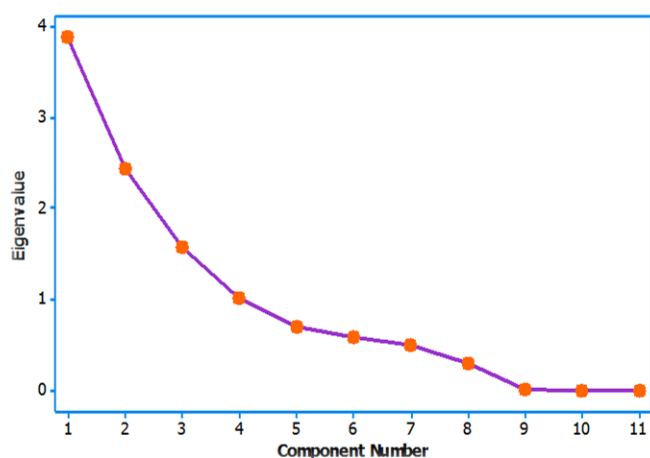**Figure 1. Principle components analysis of investigated traits in normal conditions.**

According to principal components analysis in salt stress condition (Table 5 and Figure 2), four principal components (PC) had Eigen values >1 and accounted for 80.9% of the total variance in the data. The proportions of the total variance attributable to the first three PC were 35.27, 22.14 and 14.32%. The importance of traits to the different PC can be seen from the corresponding Eigen vectors which are presented in Table 6. The results showed that days to heading had the highest loadings in PC1. Plant height and biological yield are the main traits of PC2. For PC3 traits such as days to steming and days to maturity were the most important indicating their significant importance for this component, whereas thousand grain weight mainly contributed to PC4.

Table 5. Principle components analysis of investigated traits in salt stress conditions.

Component	Eigen Value	Proportion Variance (%)	Cumulative Variance (%)
1	3.88	35.27	35.27
2	2.44	22.14	57.41
3	1.58	14.32	71.73
4	1.01	9.17	80.9

**Table 6. Eigen vectors (loadings) of the first five principal components
in salt stress conditions**

Traits	PCA1	PCA2	PCA3	PCA4
X1	0.196	-0.338	0.059	0.386
X2	0.198	-0.168	0.613	-0.14
X3	0.455	0.185	0.249	-0.015
X4	0.37	0.335	-0.17	0.086
X5	-0.4	-0.1	0.14	0.22
X6	0.297	0.176	0.501	0.183
X7	0.02	0.513	-0.16	0.189
X8	-0.143	0.251	0.039	0.751
X9	-0.367	0.297	0.306	-0.161
X10	-0.193	0.5	0.14	-0.309
X11	-0.372	-0.077	0.346	0.15

Figure 2. Principle components analysis of investigated traits in salt stress conditions.

2. Regression analysis

To determine the contribution of traits effects in determining yield, the stepwise multiple linear regression method was used. Results of stepwise analysis are shown in Table 7. In normal conditions, biological yield (R^2= 62.2%), harvest index (R^2= 35.3%), thousand grain weight (R^2= 0.07%) and grain filling period (R^2= 0.09%) entered to the model as significant traits that highly contributed to grain yield variations. These traits explained 97.69% of total variations of grain yield under non-stress conditions. The model of stepwise regression was as below:

$$Y=0.008+0.03091X_1+3.243X_2-0.0019X_3+0.0024X_4$$

In this model Y, X_1, X_2, X_3 and X_4 denote for grain yield, biological yield, harvest index, thousand grain weight and grain filling period respectively. The significant coefficient in the successful regression equation indicating these attributes are to be effective in increasing yield. The above equation showed that biological yield, harvest index, and grain filling period had positive impact and thousand grain weight negative effect on increasing grain yield. Dadashi et al (2010) using stepwise regression and at the 5% level three traits such as grain per spike and grain weight introduced as an effective traits on the yield.

Table 7. Stepwise regression models for grain yield (Y) prediction in barely genotypes under normal conditions.

Step	Variable Entered	Parameter Estimate	Partial R-Sequare	Model R-Sequare	F Value
1	Biological yield (X_1)	0.3091	0.6221	0.6221	243.59[**]
2	Harvest index (X_2)	3.243	0.3532	0.9753	2099.05[**]
3	Thousand grain weight (X_3)	-0.0019	0.0007	0.9760	4.35[*]
4	grain filling period (X_4)	0.0024	0.0009	0.9769	5.82[*]

Table 8. Stepwise regression models for grain yield (Y) prediction in barely genotypes under salt stress conditions.

Step	Variable Entered	Parameter Estimate	Partial R-Sequare	Model R-Sequare	F Value
1	Biological yield (X_1)	0.274	0.6188	0.6188	240.3[**]
2	Harvest index (X_2)	2.766	0.3521	0.9709	1778.82[*][*]
3	Days from steming to heading (X_3)	-0.0018	0.0014	0.9723	7.15[**]
4	grain filling period (X_4)	-0.0022	0.0007	0.9729	3.67[*]
5	Days to tillering (X_5)	-0.0037	0.0006	0.9736	3.47[n.s]

The model of stepwise regression for grain yield and other traits under salt stress indicated that biological yield, harvest index, days steming to heading, grain filling period and days to tillering had higher contributions to grain yield variations (Table 8). The model (R^2= 97.36%) for grain yield (Y) and entered traits including biological yield (X_1), harvest index (X_2), days steming to heading (X_3), grain filling period (X_4) and days to tillering (X_5) was as follow:

$$Y= -0.052+0.274 X_1+ 2.77 X_2-0.0018X_3-0.0022X_4-0.0037X_5$$

This model shows that all entered traits except days to tillering had significant effects on grain yield. The above equation showed that biological yield and harvest index had positive impact t on increasing grain yield. Afzali Far et al (2011) according to the stepwise regression analysis traits such as total grain yield, biomass and plant height introduced as an effective traits on the yield.

3. Path analysis

In path analysis, grain yield was supposed as the function variable and the rest traits were introduced as independent variables. As the path analysis results suggest, biolgical yield showed the greatest direct effect on grain yield in both normal stress and salt stress conditions; its total effect was 0.79 (Table 9,10). After biolgical yield, harvest index showed the greatest direct effect on grain yield improvement. Their total effects were 0.65 and 0.7 in both normal and salt stress conditions, respectively. Harvest index exhibited the largest indirect effect (through improvement of thousand

grain weight) on grain yield in both environments. Furthermore, biolgical yield, in additions to its direct effect, indirectly influenced grain yield enhancement (through increasing thousand grain weight). Seyed-Aghamiri et al (2010) that based on path analysis, biological yield 0.992 had the highest positive direct effect on grain yield is similar with the results of this study about Harvest index and spike weight, but it was unlike each other about biological function.

Table 9. Path coefficients of direct and indirect effects of the traits with grain yield under normal conditions.

Traits	Direct effect	Indirect effect via			Total effect correlation
		Biolgical yield	Harvest index	Thousand grain weight	
Biolgical yield	**0.73**	-	0.05	0.012	0.79[**]
Harvest index	**0.6**	0.04	-	**0.11**	0.65[**]
Thousand grain weight	0.032	0.00053	0.006	-	0.1[n.s]
Residual Error	0.15				

Table 10. Path coefficients of direct and indirect effects of the traits with grain yield under salt stress conditions.

Traits	Direct effect	Indirect effect via			Total effect correlation
		Biolgical yield	Harvest index	Thousand grain weight	
Biolgical yield	**0.69**	-	0.1	**0.128**	0.79[**]
Harvest index	**0.59**	0.09	-	**0.13**	0.7[*]
Thousand grain weight	0.01	0.0012	0.0015	-	0.25[n.s]
Residual Error	0.16				

4. CONCLUSION:

The result of principal component analysis, stepwise regression and path analysis indicated that biological yield, harvest index, thousand grain weight and grain filling period were as the most important effective components of grain yield in both normal and salt stress conditions. As a consequence, breeding methods for selection of higher these traits along with better agronomical practices are necessary for increasing grain yield. Among the significant positive coefficient correlation of biological yield and harvest index in the regression equation and high direct effect in path analysis indicating these attributes are to be effective in increasing yield, therefore selection on the basis of these traits in this matter would likely to be most useful for increasing grain yield in practical barely breeding program because of their direct positive contribution to grain yield improvement.

CONFLICT OF INTEREST

The authors declared that they have no competing interests.

REFERENCES

1. Afzali Far A, Zahravi M, Bihamta M. Evaluation of tolerant genotypes to drought stress the barley spantanyum iran in Karaj region. Journal of Agronomy and Plant Breeding. 2011; 7(1): 25-44.

2. Babu S, Sheeba A, Yogameenakshi P, Anbumalarmathi J, Rangasamy P. Effect of salt stress in the selection of salt tolerance hybrids in rice (Oryza Sativa L.) under in vitro and in vivo condition. Asian Journal of Plant Science. 2007; 6(1): 137-142.

3. Barrett-Lennard E. The interaction between water logging and salinity in higher plants: causes, consequences and implications. Plant Soil. 2003; 253: 35-54.

4. Colmer T, Munns R, Flowers T. Improving salt tolerance of wheat and barley: future prospects. Aust J Exp Agr. 2005; 45: 1425-1443.

5. Dadashi M, Norinia AA, Morteza-Asghar M, Azizi-Chakhrchaman Sh. Evaluate the correlation a number of physiological and morphological characteristics of naked barley varieties with grain yield. Journal of Eco-physiology of crop plants and weeds.2010; 15: 29-40.

6. Emam Y, Borjan AR. Yield and yield components of 2 winter wheat cultivars in response to rate and time of foliar application. J Agri Sci Tech. 2000; 2: 263-270.

7. Flowers TJ, Yeo AR. Breeding for salinity resistance in crop plants: where next? Australian Journal of Plant Physiology. 1995; 22: 875-884.

8. Hayes PM, Castro A, Marquez-Cedillo L, Corey A, Henson C, Jones BL, Kling J, Mather D, Matus I, Rossi C, Sato K. Genetic diversity for quantitatively inherited agronomic and malting quality traits. In von Bothmer R, Knüpffer H, van Hintum T, Sato K eds. Diversity in barley (Hordeum vulgare L.). Amsterdam, the Netherlands: Elsevier Science Publishers, 2003.

9. Jafari AA, Connolly V, Walsh EJ. Genetic analysis of yield and quality in full sib families of perennial ryegrass (Lolium perenne L.) under two cutting managements. Irish Jour Agric Food Res. 2003; 42: 275-292.

10. Jaiwal P. Strategies for improving salt tolerance in higher plants. In: Flowers T, Yeo A, (ed) Breeding for salt resistance in plants, Scientific Publisher, Enfield, New Hampshire, 1997.

11. Kraakman ATW, Niks RE, van den Berg PMMM, Stam P, Van Eeuwijk FA. Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics. 2004; 168: 435–446.

12. Munns R. Genes and salt tolerance: bringing them together. New Phytologist. 2005; 167: 645-663.

13. Nielsen JP, Munck L. Evaluation of malting barley quality using exploratory data analysis. I. Extraction of information from micromalting data of spring and winter barley. J Cereal Sci. 2003; 38: 173-180.

14. SAS Institute. Release 9.1. SAS Institute, Inc, Cary NC USA, 2003.

15. Seyed-Agamiri SMM, Mostafavi Kh, Amd Mohammad A. Reviews relationships between yield and its components, under normal and drought conditions on cultivated barley genotypes using path analysis. Fifth International Conference on New Ideas in agriculture. Islamic Azad

University Khorasgan Branch. Isfahan, 2010.

16. SPSS Inc. SPSS User's Guide. Ver. 14. SPSS Inc. Chicago IL. USA, 2004.

17. Warburton M, Crossa J. Data analysis in the CIMMYT Applied Biotechnology Center for Fingerprinting and Genetic Diversity Studies. CIMMYT, Mexico, 2000.