RESEARCH PAPER

Selection of barley advanced lines at rainfed conditions using regression and cluster analyses

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Abstract

Due to the low heritability, direct selection for yield has usually low efficiency at rainfed conditions. Therefore, plant breeders may improve yield indirectly through selection of yield contributing traits. The purpose of this study was to suggest a method for indirect selection of promising lines through yield contributing traits. This method was tested on a set of 18 advanced lines of winter barley cultivated at Gachsaran Agricultural Rainfed Research Station, Iran, for two years from 2009-10 to 2010-2011. The experimental design was a randomized complete block with four replications. Grain yield along with eight yield-related traits were assayed. In this method, initially, yield contributing traits were detected using a multiple linear regression analysis. Then, the obtained predictors were weighted by the corresponding regression coefficients. Selection of lines was carried out based on cluster analysis. Estimation of correlation coefficients revealed that, grain yield (GY) was positively correlated with plant height, weight of hectoliter and the number of grains per spike (GS). The highest and lowest broad-sense heritability belonged to GS and GY, respectively. Cluster analysis determined that, genotypes 7-B-Gachsaran, 8-BNYT-Gachsaran and 12-B-Gonbad had the best level of yield predictors in both years and therefore, were the most stable genotypes.

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Introduction

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop behind wheat, rice, and maize (Taketa *et al.*, 2008).

In barley genetic improvement, hybridization of parents look for desirable agronomic traits, and afterward the best recombinants are selected among offsprings. One of the main purposes of genetic improvement in plants is developing genotypes with high yield potential and with the ability to maintain grain yield in different environments (Inostroza *et al.*, 2007).

Cereal genetic improvement has been mainly based on empirical selection of grain yield per se. However, this methodology is not optimum since grain yield is a character with low heritability (Richards *et al.*, 2002). It is argued that an indirect selection methodology, based on comprehension of crops at physiological level, may help improving grain yield. Such methodologies may complement conventional breeding programs (empirical) and thus accelerate improvement of grain yield (Araus *et al.*, 2002; Reynolds *et al.*, 2005).

To improve yield indirectly, the knowledge of the relationships among yield and yield-contributing traits is important.

Correlation coefficient analyses help researchers to distinguish significant relationship between traits. Some researchers have studied correlation coefficients between morphological traits in barley (Chand *et al.*, 2008; Eshghi and Akhundova, 2009; Kole, 2006; Peighambari *et al.*, 2005). Also, stepwise regression can reduce effect of non-important traits in regression model, in this way traits accounted for considerable variations of dependent variable are determined (Agrama, 1996). Beal (2005) suggested a likelihood-based regression analysis where two criterions namely Akaike information (AIC) and adjusted R-square determine the best regression model.

After identifying yield predictors, selection of genotypes with maximum yield would be a key step. In this way, cluster analysis is a suitable solution to group and select desirable genotypes.

Indirect selection methods are typically based on index selection. However, these methods usually require some complicated calculations to estimate the phenotypic and genetic variance-covariance matrices. Meanwhile, plant breeders should have profound knowledge about the proper use of the corresponding formulas.

In this paper we would like to suggest a method, "which can be done easily using statistical software" for indirect selection based on phenotypic values using regression and cluster analyses.

Materials and methods

Plant materials and studied traits

Plant materials of this research comprised eighteen genotypes including sixteen advanced lines along with two local cultivars of winter barley (Table 1). The field trial was arranged in a randomized complete block design with four replications conducted at the experimental field of Gachsaran, Research Center, Iran (Latitude:30°,10′ N, Longitude:50°,50′ E, Altitude:699.5 m), during two years from 2009-10 to 2010-11. The seeds were sown in plots with size of 6m² with space of 15cm. The studied traits were, grain yield per plot (GY), plant height (PH), thousand kernel weight (KW), days to heading (DH), days to maturity (DM), weight of hectoliter (WH), number of grains per spike (GS), spike length (SL) and peduncle length (PL). Traits were measured on 10 plants randomly selected from each plot.

Statistical analyses

First, the normality of the data distribution was assayed. The homogeneity of the error variances was evaluated by using Bartlett test. Then, combine analysis of data was done using Proc GLM of SAS statistical software according to the following statistical model:

$Y_{ijk} = \mu + t_i + (r|a)_{jk} + a_k + (ta)_{ik} + e_{ijk}$

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where: Y_{ijk} is the observation of the trait i in the replication j in the year k; μ is a constant common to all observations; t_i is the effect of the trait i; $(r|a)_{jk}$ is the effect of the replication j in the year k; a_k is the effect of the year k; $(ta)_{ik}$ is the effect of the interaction between the trait i and the year k; e_{ijk} is the error associated to the observation Y_{ijk}.

Correlation coefficients, have been estimated using SPSS software release 15. The broad sense heritability (h_{bi}^2) was estimated based on the below equation:

$$h_{bi}^2 = \frac{\sigma_{gi}^2}{\sigma_{pi}^2}$$

Where: σ_{gi}^2 and σ_{pi}^2 are the genotypic and phenotypic variances of trait i. The phenotypic and genotypic variances were calculated using multivariate restricted maximum likelihood estimation through SAS code written by Holland(2006).

For each year, yield contributors were determined using likelihood-based multiple linear regression (MLR) analysis (based on mean traits) via SAS code suggested by Beal (2005). In this technique, the best regression model is determined based on Akaike information (AIC) and adjusted R-square criterions.

It was assumed that, grain yield was related to the other measured traits using the multiple linear regression model defined as:

$GY = \alpha + \beta_1 x_1 + \cdots + \beta_p x_p + \varepsilon$

Where GY is grain yield, $x_1, \ldots, x_p$ are the explanatory variables $\alpha, \beta_1, \ldots, \beta_p$ are the model parameters, and ε is the residual error term.

Then, the obtained yield contributors in each year were weighted by the corresponding regression coefficients. In fact, by assigning weight to contributors, their importance in cluster analysis got proportional to their importance in determining GY.

To select genotypes with desirable level of yield contributors, cluster analysis was applied by using WARD method. Lance and Williams (1967) have recommended to use one of the squared distance measures when choosing Average, Centroid, Median, or Ward as the linkage method. Therefore, squared Euclidian distance was used as the interval measure. The optimal stopping point for merging clusters was estimated according to the sudden jump in the distance coefficient printed in the agglomerative schedule. The validity of clusters was assayed using discriminant analysis.

Results and discussion

Analysis of variance and heritability of traits

Combined analysis of variance showed that the main effects of genotype were significant for all the traits of study (p< 0.01) indicating a high diversity among studied experimental material (Table 2). The effect of year did not have significant effect on GS. The coefficient of variation was varied from 1.71 for DM to 17.15 for PL. Grain yield had the lowest value of the broad sense heritability among the traits of study showing the intensive influence of environmental factors on this trait (13.45%). This low heritability was the main motivation for proposing the present method. However, some researchers such as Peighambari *et al.* (2005), Eshghi and Akhundova (2009), Kole (2006) and Chand *et al.* (2008) have reported relatively higher heritability for GY (more than 50%). It seems that environmental conditions or the type of the plant materials used in the experiment would be the source of such differences. Like Chand *et al.* (2008) report, a high heritability was observed for GS indicating a high transmitting ability to the next generation (Table 2). Also, this trait would be useful for developing selection indices in order to improve GY with regard to significant positive correlation between GS and GY.

Mean yield comparisons and correlation coefficients between traits

The average yields are shown in Table 1. In 2009-10, genotypes 7-B-Gonbad, 11-BNYT-Gachsaran and 13-B-Moghan had the highest means with 2.71, 2.70 and 2.64 kg plot⁻¹, respectively. Also, in 2010-11, 7-B-Gonbad, 2-B-Moghan and 10-B-Moghan had the best level of grain yield (3.43 kg plot-1). The differences observed between the mean values of traits recorded

in each year have been probably due to differences in rainfall patterns. This conclusion was in agreement with findings of other studies. For example, Al-Yassin *et al.* (2005) pointed out that, about one half of the variation in grain yield between locations and years was related to differences in total annual rainfall.

Also, a review by Ceccarelli *et al.* (2004) revealed that, the percent variance of grain yield explained by total rainfall is varied from 6% to 63% in barley. Also, Saeed and Francis (1984) found that cropping season rainfall and temperature had a significant effect on yield and contributed to the GE interaction.

Table 1. Profile of eighteen winter barley advanced lines studied during 2009-10 and 2010-11.

No	Line/variety	Parentage/pedigree	Mean of grain yield per plot (kg/6m²)	
			2009-10	2010-11
1	10-B-Moghan	Alger/Ceres//Sls/3/ER/Apm/4/Wi2197/Mazurkal ICB92-0944-OAP-OAP	2.46 abc	2.87 abc
2	13-B-Moghan	Moroc09-75/Wi2291/Wi2269	2.64 ab	2.40 bcd
3	2-B-Moghan	Rhn-03//Lignee 527/As 45 ICB93-0815-OAP-5AP-OAP-OAP	2.43 abc	3.16 ab
4	6-B-Moghan	Wi2291/Tipper ICB93-1156-OAP-22AP-OAP-OAP	2.04 cde	2.68 abcd
5	12-B-Moghan	Hyb 85-6//As46/Aths*2 ICB91-0736-OAP-OAP-OAP	2.36 abcd	2.68 abcd
6	16-B-Moghan	Arizona5968/Aths//Avt/Attiki	1.69 e	2.10 cd
7	14-B-Moghan	BKF/Maguelone1604/3/Apro//SV	2.48 abc	2.22 bcd
8	7-B-Gachsaran	Alanda/5/Aths/4/Pro/Toli//Cer*2/Toli/3/5106/6/Avt/. -8G -3 G	2.28 abcd	2.62 abcd
9	9-B-Gachsaran	Bda/Cr. 115/Pro/Bc/3/Api/Cm67/4/ Giza121/... -9G -2 G	2.54 abc	1.86 d
10	11-BNYT-Gachsaran	Emir/Nacta//As907/3/Avt_(9-9)ACSAD-1290-6AP-OTR-OAP-6AP-OAP-OAP	2.70 a	2.42 bcd
11	8-BNYT-Gachsaran	Lth/3/Nopal//Prol/11012-2/4/Kabaa-03ICB94-0498-OAP-3AP-OAP-OAP	2.39 abc	2.43 bcd
12	6-BNYT-Gachsaran	Himalaya-12/Plaisant ICBH95-0630-OAP-OAP-16AP	2.57 abc	2.57 abcd
13	5-BNYT-Gachsaran	MoB1337/Wi2291//Bonita//Weeah/3/Atahualpa ICB98-0563	2.12 bcde	2.77 abcd
14	7-B-Gonbad	Weeah11/wi2291/Bgs/3/ER/Apm//Ac253 ICB94-0707-OAP-OAP	2.71 a	3.43 a
15	11-B-Gonbad	26216/4/Arar/3/Mari/Aths*2//M-ATT-73-337-1 ICB94-0517-37AP-OAP	2.17 abcde	2.47 bcd
16	12-Bgonbad	MK1272//Manker/Arig8/3/Alanda ICB93-0448-OAP-6AP-OAP	2.43 abc	2.81 abcd
17	LB	Local variety	1.86 de	2.16 cd
18	Izeh	Local variety	2.61 ab	2.73 abcd

Letters that do not share letters are significantly different (α = 0.05). The values correspond to averages of four replications.

Table 3 shows the Pearson's correlation coefficients between studied traits. In this study, Grain yield showed positive correlation with PH (0.247**). This result was in agreement with findings of Kole (2006) while, Prasad *et al.* (1979) reported negatively. Also positive significant correlations were found between GY and traits WH (0.194*) and GS (0.249**). Peighambari *et al.* (2005) and Moreno *et al.* (2003) reported similar association between GY and GS. Also, GS was negatively correlated with KW (-0.309**) showing a compensatory relationship between them. Unlike the findings of Moreno *et al.*

(2003) and Ahmad (2005), in this research, the correlation coefficient between GY and KW was not significant (Table 3). Cycle traits (DH and DM) showed positive correlation with PL and WH.

Multiple linear regression and Cluster analysis
Table 4 shows the MLR analysis results. Many researches have used regression analysis to study the relationship between grain yield and its contributors (Whittingham *et al.*, 2006) . In 2009-10, the detected yield contributors were KW, DH, DM, and GS and the corresponding regression equation was: GY=296.4 +

3.4×KW - 8.4×DH + 5.5×DM + 1.5×GS. Also PH, DH, DM, GS and PL were regressed with GY in 2010-11 and the corresponding regression equation was GY= -580.9 + 3.4×PH - 9.5×DH + 12.8×DM + 2.2×GS − 13.1×PL. In this study, GS, DH and DM had more important role in predicting GY as they appeared in both of the two regression models. Moreno *et al.* (2003) reported association between GY and ears per m2, kernels per ear and weight of thousand kernels. Like the report of Moreno *et al.* (2003), KW regressed with GY in the model estimated for 2009-10.

Table 2. Combined analysis of variance of 10 measured traits studied over 18 advanced lines of barley from 2009-10 to 2010-11.

S.O.V	d.f	Mean squares								
		PH	KW	DH	DM	WH	GS	GY	SL	PL
G	17	269.74**	114.75**	95.83**	101.78**	46.39**	1273.59**	16458.14**	9.82**	26.11**
Y	1	7.20$^{n.s}$	1686.47**	498.78**	1150.34**	35478.86**	11.67$^{n.s}$	47596.69**	8.12**	674.09**
R(Y)	6	85.28*	1.83$^{n.s}$	15.03*	34.51**	35.17**	9.41$^{n.s}$	3522.02$^{n.s}$	1.003$^{n.s}$	6.71**
G × Y	17	59.73n$^{n.s}$	10.6**	6.13$^{n.s}$	13.68**	13.13**	25.53**	8525.26$^{n.s}$	1.26*	2.17$^{n.s}$
Error	102	35.29	3.30	5.10	3.33	5.46	4.96	5667.67	0.71	1.81
C.V %		7.02	4.40	2.06	1.71	4.91	6.80	16.27	12.39	17.15
σ²G		29.63	13.02	11.21	11.01	4.16	156.01	991.61	1.07	2.99
σ²P		71.03	18.14	16.57	16.93	11.54	166.11	7373.68	1.92	4.89
h² %		41.71	71.76	67.66	65.06	36.03	93.92	13.45	55.87	61.17

PH, plant height; KW, thousand kernel weight; DH, days to heading; DM, days to maturity; WH, weight of hectoliter; GS, grains per spike; SL, spike length; PL, peduncle length; GY, grain yield per 6 m².

S.O.V, Source of variations; Y, effect of year; R(Y), replication within year; G, genotype; G×Y, Genotype by year interaction; C.V%, coefficient of variation; σ²G , Genotypic variance; σ²P , Phenotypic variance; *h²%*, broad sense heritability; R.S, Response to selection.

∗∗ and ∗ mean significant at the 1% and 5% levels of probability, respectively and n.s means non-significant

Table 3. Correlation coefficients between traits studied over 18 advanced lines of barley from 2009-10 to 2010-11.

	PH	KW	DH	DM	WH	GS	SL	PL	GY
PH	1								
KW	-0.033	1							
DH	-0.124	0.114	1						
DM	-0.012	0.206*	0.814**	1					
WH	-0.044	0.627**	0.408**	0.553**	1				
GS	0.091	-0.309**	-0.120	-0.122	-0.057	1			
SL	0.119	0.144	-0.006	0.162	0.210*	-0.430**	1		
PL	0.349**	0.515**	0.240**	0.350**	0.658**	0.085	0.095	1	
GY	0.247**	0.088	-0.101	0.106	0.194*	0.249**	-0.012	0.137	1

PH, plant height; KW, thousand kernel weight; DH, days to heading; DM, days to maturity; WH, weight of hectoliter; GS, grains per spike; SL, spike length; PL, peduncle length; GY, grain yield per plot (6 m²).

∗∗ and ∗ mean significant at the 1% and 5% levels of probability (2-tailed) , respectively.

Results of cluster analysis are shown in Figure 1. Also, the characteristics of clusters have been summarized in Table 5. Results showed that, four clusters were constructed in each year.

For 2009-10, GS 'as the most important trait' was varied from 22.13 for cluster 2 to 48.5 for cluster 3. Thousand kernel weight was also varied from 35.12 for cluster 1 to 43.95 for cluster 4. However, cluster 4 was not considerable because it had very low value for GS (20.75). This result was predictable with regard to the negative correlation coefficient between GS and KW. In fact, the simultaneous rise of these two important characters was impossible. Therefore cluster 3 was still the best cluster. Because of the negative regression coefficient between GY and DH (-

8.4), the smaller amounts of DH was preferred and thus cluster 3 with 84.42 days was the best cluster. In total, for 2009-10, cluster 3 comprising three genotypes viz. 7-B-Gachsaran, 8-BNYT-Gachsaran, 12-Bgonbad had a desirable, high level of the traits taken to the analysis, viz. KW, DH, DM and GS (Fig. 1 and Table 5).

Table 4. Multiple linear regression analysis for association between grain yield and the eight related traits measured over 18 barley promising lines from 2009-10 to 2010-11.

Year	RMSE	Intercept	PH	KW	DH	DM	WH	GS	SL	PL	Adj Rsq	AIC
2009-10	60.7	296.4	.	3.4	-8.4	5.5	.	1.5	.	.	0.2	596.0
2010-11	86.1	-580.9	3.4	.	-9.5	12.8	.	2.2	.	-13.1	0.3	647.3

Dependent variable: GY

RMSE, Root Mean Squared Error; Adj Rsq, Adjusted R-square; AIC, Akaike information criterion.

PH, plant height; KW, thousand kernel weight; DH, days to heading; DM, days to maturity; WH, weight of hectoliter; GS, grains per spike; SL, spike length; PL, peduncle length.

The clustering obtained for 2010-11 was not very different from that for 2009-10. Four clusters were detected. Likewise, cluster 4 consist of three genotypes viz. 7-B-Gachsaran, 8-BNYT-Gachsaran, 12-Bgonbad had a desirable value and was the best cluster for 2010-11 because it had a high level of the traits taken to the analysis, viz. PH (101.18 cm), DH (87.75), DM (122.92), GS (50.33) and PL (11 cm) (Fig. 1 and Table 5).

Table 5. Results of cluster analysis based on yield contributing traits over 18 promising lines of barley from 2009-10 to 2010-11.

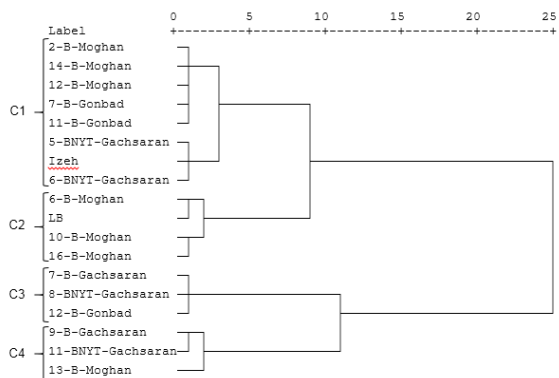|	2009-10						2010-11					
Cluster	Mean ± standard error					Cluster	Mean ± standard error					
	KW (g)	DH	DM	GS	N		PH (cm)	DH	DM	GS	PL(cm)	N
1	35.12±0.8	91.09±0.76	123.03±0.65	46.56±0.54	8	1	95.44±1.7	94.84±0.67	127.44±0.5	45.88±1.58	9.86±0.4	8
2	36.23±1.74	92.75±0.62	124.75±0.18	22.13±0.48	4	2	101.46±4	95.75±0.87	129.56±0.21	24.13±0.38	9.94±1.15	4
3	37.73±1.94	84.42±0.36	112.58±0.55	48.5±0.5	3	3	87.42±2.41	91.25±1.98	123.75±1.18	21.5±0.3	10.09±1.41	3
4	43.95±1.93	86.25±1.59	118.33±1.46	20.75±0.52	3	4	101.18±1.19	87.75±0.66	122.92±0.3	50.33±1.56	11±0.92	3
Total	38.2575	88.6275	119.6725	34.485	18	Total	96.375	92.3975	125.9175	35.46	10.2225	18

PH, plant height; KW, thousand kernel weight; DH, days to heading; DM, days to maturity; GS, grain per spike; PL, peduncle length.

Cluster analysis has been carried out based on the Ward method and squared Euclidian distance measure.

Before cluster analysis, traits taken to analysis were weighted by corresponding regression coefficients.

Conclusion

The statistical approach proposed in this study should be useful for identifying traits influencing grain yield and identifying the most promising genotypes. Since the proposed method is applied separately for each year therefore, two clustering structure were obtained. Selection of the most promising genotype across two years was done based on the results of cluster analysis applied separately for each year.

A
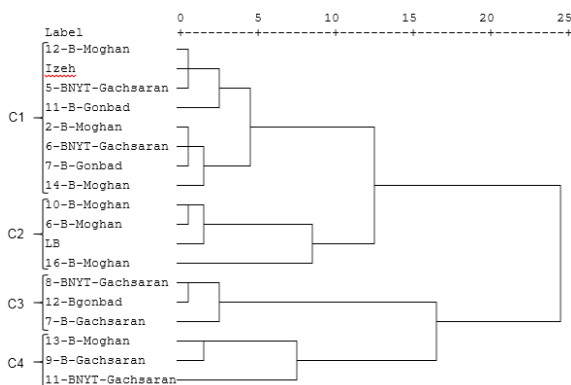
B

Fig. 1. Cluster analysis of 18 promising lines of winter barley in 2009-10 (A) and 2010-11 (B) using Ward's method and squared Euclidean distance coefficient, based on contributors weighted by corresponding regression parameters.

These were genotypes grouped in the best clusters viz. 7-B-Gachsaran, 8-BNYT-Gachsaran, 12-Bgonbad and have been considered as the best genotypes. It seems that, selection of the most promising genotypes would be more accurate when more number of environments is included in the experiment.

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