Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.85, 2021

Multivariate Analysis of Malt Barley Genotypes for Different Malt Quality and Agronomic Traits in Ethiopia

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Abstract
Barley is one of the widely grown cereal crop in the highlands of Ethiopia. Twenty five malt barley genotypes were evaluated using a 5 × 5 simple lattice design at Holetta, Bekoji, Debreberhan and Kofele locations to group tested malt barley genotypes, to characterize traits that contribute to total variability and to determine genetic variability among malt barley genotypes. The tested genotypes showed significant variation for all eleven agronomic and malt quality traits considered in this experiment. The candidate genotype (IBON-HI 118/2016) showed relatively better malt quality and agronomic performance. The first three principal components (PCs) contributes 85% total variability. Days to heading, maturity and malt quality traits (protein, extract and friability), plant height and grain yield contribute chiefly for 50% percent variability explained by PC 1. Based on cluster analysis the tested genotypes grouped into three clusters (C) consisted of 15 (C-I), 8 (C-II) and 2 (C-III) genotypes. C-I contain genotypes which had relatively better grain yield. Whereas, C-II consists of barley genotypes with better malt qualities. Thus, crossing among genotypes from these two clusters could give better genetic recombination for important malt quality and agronomic traits.
Keywords: Cluster analysis, genetic variability, principal component
DOI: 10.7176/ALST/85-03
Publication date: January 31st 2021

Introduction

Barley is the fifth most important cereals of Ethiopia. It accounts for about 6.42 % of the total area and 5.63 % of the gross grain production of cereal crops, more than 3.7 million small holder farmers engaged in barley production (CSA, 2019). Barley has deep roots in the conception habits of Ethiopians. It used to prepare traditional foods, such *as injera, besso, chiko, genfo, kolo, kinche* and *kitta*; local beverages, *tella, borde, areki* and *atmit*. Currently, in Ethiopia, there is a high demand of malt barley with the introduction of new breweries and malt factories (Bekele *et al*., 2005; Kifle, 2016). This make barley an important cash crop for farmers found in high lands of the country. Malt barley genotypes have specific qualities (Low protein content (9.5-11), high extract (>80%), high friability (>80%)) required to use for malting purpose. Therefore, beside the major agronomic traits, the malt barley breeding activities focus on these quality parameters. In Ethiopia, the national research system released many malt barley varieties, where some of them are widely cultivated across the country. The Holetta Agriculture research center is one of the major breeding center for malt barley improvement. It conducts selection activities from lines derived from its own crossing activities and introduced materials from international sources. The program handles many genotypes and deal with main traits. A multivariate analysis used to evaluate such data set which contains more than two variables at once (Kumar *et al*., 2013). Cluster and principal component analysis are popular multivariate analysis techniques used to group and characterize genotypes. The former used to partition a set of data into clusters, where objects within the cluster are similar to one another, while dissimilar to objects in other clusters (Han *et al*., 2012). Principal component analysis used to reduce the dimensionality of large data sets into a smaller one that still contains most of the information in the large set (Jaadi, 2019). This study was, therefore, aimed to group malt barley genotypes into similar groups, to characterize traits that contribute to total variation and to determine genetic variability among malt barley genotypes.

Materials and methods

In this experiment, twenty five malt barley genotypes were grown in simple lattice design. The genotypes used in the present study were extracted from malt barley yield trials (national variety, preliminary variety and observation nursery trials), parental performance trial and malt barley released varieties (Table 1). Except EH 1847, Fatima and Henrike all the genotypes included in the trial serve as a parent in the 2019 off-season crossing activity. This experiment was carried out at Holetta (9º00'N, 38º38'E), Bekoji (7º15'N, 39º15'E), Debreberhan (9º41'N,39 º32'E) and Kofele (7 º00'N, 38º45'E) sites during 2019 main cropping seasons. The genotypes were sown in ten-row plots each having 2.5m length and 2m width. In the experiment, eleven agronomic and malt quality traits were measured. These include days to 50 % heading, days to 50 % maturity, plant height (cm), scald severity (%), net blotch severity (%), thousand kernel weight (gm), hectoliter weight ($Kghl^{-1}$), grain yield ($Kgha^{-1}$), protein content (%), extract (%), and friability (%). Scald and net blotch disease severity recorded by visually estimating the percentage of leaf area diseased and rated using the Saari and Prescott (1975) scale. The malt quality traits were analyzed following Near infrared spectroscopy (NIRs) technique using Bruker Tango instrument at Holetta quality

laboratory. All crop management practices were followed as per the recommendation for each location.

The analysis of variance was analyzed using lmer4 package of R-software (Douglas *et al*., 2015), where location and genotype considered as random and fixed effects, respectively. The mean separation was done using emmeans package of R-software (Russell, 2019). The multivariate analyses were made based on the mean values for the eleven traits and 25 barley genotypes over the four locations. For cluster and principal component analysis, the mean data were first pre-standardized to mean zero and variance unity to avoid bias due to differences in measurement scales. Multivariate statistical analysis was done using MINTAB statistical computer package, version 19 (MINITAB, 2020) and the points where local peaks of the pseudo F statistic join with small values of the pseudo t^2 statistic followed by a larger pseudo t^2 for the next cluster fusion were observed to decide the number of clusters (SAS Institute, 2002).

Table 1. The list of genotypes used in the study

Entry	Genotype	Source	Entry	Genotype	Source
1	IBON 174/03 x Traveller	MBNVT	14	IBON-HI 13/14 P# 128	NPPT
2	IBON-HI13/14-49	MBNVT	15	HB 1963	Released
3	IBON-HI14/15-126	MBNVT	16	MN Brite	Released
4	Bekoji-1 x ND 26333	MBPVT	17	Traveller	Released
5	Bekoji-1 x ND 24263	MBPVT	18	IBON 174/03	Released
6	IBYT-HI 4/2016	MBPVT	19	Miscal-21	Released
7	Bekoji-1 x Lab No. 128	MBPVT	20	Acc. #212959A	Accession line
8	IBON-HI 118/2016	MBPVT	21	Acc. #212959B	Accession line
9	Bekoji-1 x Traveler	MBPVT	22	IBON 15/2018	MBON
10	IBON 174/03 x Lab No. 128	MBPVT	23	EH 1847	Released
11	Planet	Released	24	Fatima	Released
12	G 13-64 Belgium	NPPT	25	Henrike	Released
13	ICARDA GP-67	NPPT			

MBNVT=Malt barley national variety trial, MBPVT=Malt barley national trial, NPPT=National parental performance trial, MBON=Malt barley observation nursery

Results and discussion

The analysis of variance combined over locations showed significant differences among the genotype for all traits (Table 2). This variation offers ample chances to select better genotypes for direct release or donor parent in crossing program. Similarly, mean squares due to location and genotype by location interaction were also highly significant ($p < 0.01$) for all traits (Table 2). This is in line with the findings reported from evaluations of various barley genotypes for different traits (Rodriguez *et al*., 2008; Zerihun, 2011; Abtew *et al*., 2015; Thomas *et al*., 2019,2020; Thomas 2020).

Table 2. Mean squares from the combined analysis variance of 12 traits for 25 malt barley genotypes over four locations

	Df	DHE	DMA	PLH	SC	NB	TKW	HLW	GYLD	PR	EX	FR
gen	24	493.3**	344.7**	1925.4**	940.9**	497.9**	251.84**	79.4**	3741306**	4.431**	24.4**	799.39**
loc	3	669.8**	4201.2**	3176.4**	11288.6**	15818**	1313.81**	1043.4**	53269595**	43.91**	257.1**	2100.31**
gen:loc	72	32.5**	34.6**	49.3**	325.3**	235.8**	22.26**	7.6**	923985**	0.65**	1.21**	101.67**
loc:rep	4	7.3	37*	207.5**	449.7*	99.3	5.52	3.88	911145*	0.96**	1.59*	58.46
loc:rep:row	32	9.3*	11.7	40.1*	247.7	47.5	10.1	3.7	516255*	0.70**	1.02*	32.36
residuals	64	5.0	12.6	21.6	156.4	58.4	9.46	3.02	273424	0.24	0.60	29.73
CV		2.71	2.62	5	25.1	24.4	7.3	2.8	17.2	4	0.96	8.27
Mean		82.45	135.5	92.7	49.8	31.3	42.2	63.1	3042.0	12.26	80.4	65.95

*,** significant at P=0.01 and 0.05 level respectively, Df= degree of freedom, DHE=days to heading, DMA=days to maturity, PLH= plant height, SC= scald severity, NB= net blotch, TKW= thousand kernel weight, HLW= hectoliter weight, GYLD= grain yield, PR= protein, EX=Extract, FR= Friability

Significant mean differences were observed among the barley varieties for grain yield. Entry #10 and #18 demonstrated top ranking grain yield performance, though not significantly different from the other 15 released and elite materials included in the trial (Table 3). Concerning malt quality traits ICARDA GP-67, HB 1963,

Traveller, Planet, IBON-HI 118/2016 and Fatima showed good performance (Table 3). In addition, there was a large variability in mean plant height, which ranged from 62-115 cm. Mostly, the imported malt barley genotypes had relatively less resistance to scaled and the two accession lines (Entry #20 and #21) showed less resistance to net blotch. On the other hand, IBON-HI 13/14 P# 128, Fatima, Traveller, Planet and ICARDA GP-67 had the maximum heading date, but the released variety IBON 174/03 and its crosses had early heading date. Regarding malt quality traits Planet, Fatima, Traveller and HB 1963 showed good performance (Table 3). So, these genotypes can serve as parents in the malt barley crossing program. Moreover, the candidate genotypes IBON-HI 118/2016, which had good malt quality and agronomic performance can be recommended for variety verification trial.

Table 3. Overall means for eleven traits of 25 malt barley genotypes tested during 2019 main season at Holetta, Bekoji, Debreberhan and Kofele locations

Entry	Genotype	DHE	DMA	PLH	SC	NB	TKW	HLW	GYLD	PR	EX	FR
1	IBON 174/03 x Traveller	71^{jk}	130^{def}	99^{c-f}	44.8^{d-h}	27.5^{cde}	46.6^{abc}	62.6^{f-j}	3806^{abc}	12.6 $^{a-e}$	78.9^{k-n}	53.2^{kl}
2	IBON-HI13/14-49	76^{h-k}	131^{c-f}	97^{d-g}	32.9^{gh}	36.2^{a-d}	46.2^{a-d}	63.3^{b-j}	3215^{a-f}	12.5 $^{b-f}$	80.8^{fgh}	60.5^{ijk}
3	IBON-HI14/15-126	80^{e-h}	136^{bcd}	97^{d-g}	28.4^{h}	32.4^{b-e}	38.6^{fg}	65.9^{a-d}	3507^{a-f}	12.1 $^{d-h}$	79.2^{j-m}	71.5 $^{c-g}$
4	Bekoji-1 x ND 26333	85^{cde}	137^{b}	110^{ab}	38.2^{fgh}	36.4^{a-d}	47.0^{ab}	66.3^{ab}	2999^{b-h}	12.6 $^{a-e}$	80.4^{f-i}	64.2 $^{e-j}$
5	Bekoji-1 x ND 24263	84^{def}	138^{b}	114^{a}	39.6^{fgh}	32.6^{b-e}	46.0^{a-d}	65.4^{a-g}	3091 $^{b-g}$	13.2^{ab}	80.2^{g-j}	60.0^{ijk}
6	IBYT-HI 4/2016	77^{gh}	133^{b-e}	90^{ghi}	44.4^{d-h}	21.3^{de}	43.3^{b-e}	64.3^{a-h}	3018^{b-h}	12.2 $^{d-h}$	79.5^{i-l}	59.8^{ijk}
7	Bekoji-1 x Lab No. 128	85^{cde}	135^{bcd}	115^{a}	46.9^{c-g}	32.6^{b-e}	44.5^{bcd}	65.9^{a-e}	3426^{a-f}	12.8 $^{a-d}$	80.5^{f-i}	61.0^{h-k}
8	IBON-HI 118/2016	82^{efg}	130^{def}	103^{bcd}	51.7^{b-f}	33.8^{b-e}	44.5^{bcd}	62.4^{g-j}	3159^{a-g}	11.8 $^{f-j}$	81.1^{efg}	80.7^{abc}
9	Bekoji-1 x Traveler	81^{e-h}	137^{b}	109^{ab}	50.8^{b-f}	27.5^{cde}	46.0^{a-d}	66.0^{abc}	3254^{a-f}	12.1 $^{d-h}$	81.3^{def}	61.6 $^{g-k}$
10	IBON 174/03 x Lab No. 128	71^{k}	133^{b-e}	94^{e-h}	61.1^{a-d}	27.5^{cde}	46.6^{abc}	63.0^{e-j}	4073^{a}	12.6 $^{a-e}$	78.4^{mno}	56.1^{jkl}
11	Planet	93^{ab}	146^{a}	68^{kl}	65.0^{ab}	21.4^{de}	38.8^{fg}	62.7^{e-j}	1859^{ijk}	10.1 ^{k}	83.4^{ab}	89.9^{a}
12	G 13-64 Belgium	78^{gh}	133^{b-e}	101^{cd}	39.6^{fgh}	33.9^{b-e}	47.2^{ab}	66.0^{abc}	2924^{c-h}	12.3 $^{c-g}$	80.0^{hij}	59.6^{ijk}
13	ICARDA GP-67	93^{ab}	145^{a}	71^{jk}	44.6^{d-h}	41.2^{abc}	41.7^{d-g}	64.1^{a-i}	2069^{h-k}	12.0 $^{e-i}$	82.1^{cde}	72.2^{c-f}
14	IBON-HI 13/14 P# 128	98^{a}	146^{a}	62^{l}	58.4^{a-e}	36.4 $^{a-d}$	33.2 ^{hi}	60.9^{ij}	1706^{k}	11.5^{hij}	82.3^{bcd}	83.6^{ab}
15	HB 1963	88^{bcd}	144^{a}	101^{cd}	46.5^{c-g}	27.5^{cde}	46.3^{abc}	67.4 ^{a}	2922^{c-h}	11.1 ^{j}	81.9^{cde}	71.0^{c-h}
16	MN Brite	79^{fgh}	133^{b-e}	86^{i}	59.0^{a-d}	23.7 ^{de}	38.0^{fg}	65.8 $^{a-f}$	3752^{a-d}	12.6 $^{a-e}$	80.2^{g-j}	57.4^{i-l}
17	Traveller	93^{ab}	146^{a}	72^{jk}	50.6^{b-f}	36.3^{a-d}	45.1^{a-d}	62.7 $^{d-j}$	2795^{d-i}	11.7 $^{g-j}$	82.5^{abc}	74.3^{b-e}
18	IBON 174/03	72^{ijk}	130^{def}	93^{fgh}	48.4^{b-g}	28.6^{cde}	44.3^{bcd}	62.3 $^{g-j}$	3961^{ab}	13.0^{ab}	77.9^{no}	49.0^{l}
19	Miscal-21	77^{g-j}	130^{def}	103^{bcd}	51.6^{b-f}	31.3^{cde}	45.1^{a-d}	63.5^{b-i}	3670^{a-e}	13.3 ^{a}	79.8^{h-k}	57.8^{i-l}
20	Acc. #212959A	77^{g-j}	126^{f}	100^{cde}	47.0^{c-g}	50.0^{a}	28.0^{l}	51.1 ^{k}	2750^{e-i}	13.3 ^{a}	77.5^{o}	62.5^{f-k}
21	Acc. #212959B	77^{gh}	125^{f}	97^{d-g}	41.4^{e-h}	47.5^{ab}	29.5^{ij}	53.3 ^{k}	2597^{f-k}	12.9^{abc}	77.9^{no}	64.2^{e-j}
22	IBON 15/2018	85^{cde}	137^{b}	87^{hi}	65.5^{ab}	20.1^{e}	49.0^{a}	62.8 $^{c-j}$	1771^{jk}	11.8 $^{f-j}$	78.6^{lmn}	66.4^{d-i}
23	EH 1847	77^{ghi}	129^{ef}	105^{bc}	55.7^{a-f}	33.7^{b-e}	42.3^{c-f}	64.6 $^{a-h}$	3597^{a-e}	13.2 ^{a}	80.3^{f-i}	60.8^{ijk}
24	Fatima	96^{a}	144^{a}	66^{kl}	63.3^{abc}	23.9^{de}	38.8^{efg}	62.0^{hij}	2191^{g-k}	11.2^{ij}	83.5^{a}	76.5^{bcd}
25	Henrike	89^{bc}	136^{bc}	77^{j}	70.2^{a}	18.8^{e}	37.3^{gh}	60.1 ^{j}	2742^{e-j}	11.9^{e-i}	82.4^{bcd}	74.8^{bcd}
	CV	2.7	2.6	5.0	25.1	24.4	7.3	2.8	17.2	4.0	0.96	8.27
	Mean	82.5	135.5	92.7	49.8	31.3	42.2	63.1	3042	12.26	80.4	65.95

DHE=days to heading, DMA=days to maturity, PLH= plant height, SC= scald severity, NB= net blotch, TKW= thousand kernel weight, HLW= hectoliter weight, GYLD= grain yield, PR= protein, EX=Extract, FR= Friability

The cluster analysis based on average linkage method and Euclidean distance measure group 25 tested genotypes to three major clusters (Figure 1). The largest cluster (C-1) contains 15 genotypes which include all genotypes selected from preliminary and national variety yield trials. In addition, this cluster contains nationally released malt barley varieties, IBON 174/03, Miscal-21, EH 1847 and MN Brite. The second cluster comprised of the registered malt barley genotypes Planet, Fatima, Henrike and Traveller (Table 4). Recently released popular malt barley genotype (HB 1963) and other three genotypes selected from ICARDA materials also included in C-2. The third cluster (C-3) contains two accession lines selected from barley acid tolerance screening trial (Table 4). Similarly, Amabile *et al*., 2014, group thirteen malt barley genotypes in two similarity group. However, Enyew *et al*. (2019), Gupta *et al*. (2009) and Mekonnon *et al*. (2014), group 48, 207 and 102 barley landrace accessions in six and five distinct clusters, respectively. Generally, the cluster analysis group the genotypes of similar origin in the same cluster and these genotypes assembled in one cluster had similar agronomic performance and malt quality. In addition, the lower number of clusters reported in the present study were could be due to selections

applied on previous generations of these tested genotypes using common traits.

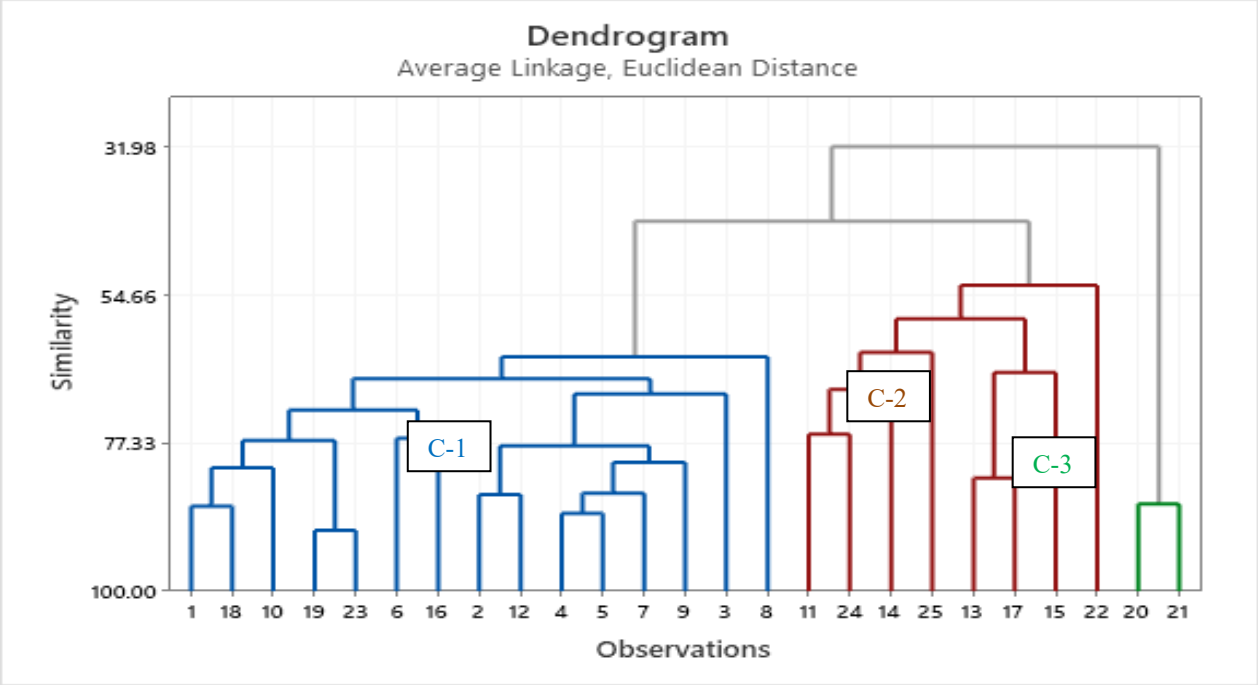

Figure 1. Dendrogram of seventy genotypes based on average linkage and Euclidean distance of 11 traits evaluated at four locations

Table 4. List of barley genotypes grouped in three clusters (average linkage euclidean distance cluster analyses) using 11 traits evaluated at four locations

Clusters	No. of genotypes	Name of tested malt barley genotypes
I	15	IBON 174/03 x Traveller, IBON 174/03, IBON 174/03 x Lab No. 128, Miscal-21, EH 1847, IBYT-HI 4/2016, MN Brite, IBON-HI13/14-49, G 13-64 Belgium, Bekoji-1 x ND 26333, Bekoji-1 x ND 24263, Bekoji-1 x Lab No. 128, Bekoji-1 x Traveler, IBON-HI14/15-126 and IBON-HI 118/2016
II	8	Planet, Fatima, IBON-HI 13/14 P# 128, Henrike, ICARDA GP-67, Traveller, HB 1963 and IBON 15/2018
III	2	Acc. #212959A and Acc. #212959B

Based on cluster mean analysis, cluster II consisted of barley genotypes with late spike emergence and maturity (Table 5). This cluster consists of genotypes having good malt qualities (protein, extract, friability), lower grain yield and relatively susceptible to scald. Whereas, cluster I showed the highest mean grain yield and plant height values. Cluster III, which contained of two accession lines, characterized by low malt quality and high net blotch values (Table 5). Generally, cluster mean values confirmed that these genotypes which had higher malt qualities showed a low mean grain yield and vice versa. Consequently, crosses among genotypes found in these different clusters could give genotypes which have a good agronomic performance and malt qualities in the subsequent segregating generations.

Table 5. Mean values of three cluster for the 11 quantitative traits

Entry	**DHE**	**DMA**	**PLH**	**SC**	**NB**	**TKW**	**HLW**	**GYLD**	**PR**	**EX**	**FR**
Cluster I	78	133	101	46.2	30.6	44.4	64.5	3430	12.6	79.9	60.9
Cluster II	92	143	76	58.0	28.2	41.3	62.8	2257	11.4	82.1	76.1
Cluster III	77	125	98	44.2	48.7	28.7	52.2	2674	13.1	77.7	63.4

DHE=days to heading, DMA=days to maturity, PLH= plant height, SC= scald severity, NB= net blotch, TKW= thousand kernel weight, HLW= hectoliter weight, GYLD= grain yield, PR= protein, EX=Extract, FR= Friability

In principal component analysis (PCA), 85% of the total variation were explained by the first three principal components (PCs) and these PCs have Eigenvalue greater than one (Table 6). PC1 accounted for 50 % of the variation among the genotypes under investigation. Days to heading, maturity and malt quality traits (protein, extract and friability), plant height and grain yield contribute more for the percent variability explained by PC 1. Besides, PC2 contributed about 23 % of the total variation originated mainly from variation in hectoliter weight,

thousand kernel weight and net blotch severity. The third PC explained 12% of the variations observed among 25 malt barley genotypes. Scald and net blotch severity contribute largely to these variability (Table 5). Likewise, in Enyew *et al*. (2019) study the first PC alone explained about 50% of the total variance mainly due heading date, plant height and grain yield, biomass yield. However, Abebe *et al*., (2010) and Enyew *et al*. (2019) reported more contribution of thousand kernel weight for percent variation explained by PC1.

Table 6. Eigenvectors and eigenvalues of the first three principal components for 11 traits of 25 malt barley genotypes evaluated at four locations

Variable	PC1	PC2	PC3
DHE	**0.394**	-0.043	-0.210
DMA	**0.371**	0.162	-0.217
PLH	**-0.336**	0.159	-0.235
SC	0.220	-0.003	0.683
NB	-0.135	-0.421	-0.528
TKW	-0.066	0.559	-0.096
HLW	0.036	0.584	-0.214
GYLD	**-0.335**	0.233	0.122
PRO	**-0.380**	-0.096	-0.015
EXT	**0.358**	0.180	-0.144
FRI	**0.371**	-0.142	-0.132
Eigen value	5.48	2.51	1.30
Proportion	0.50	0.23	0.12
Cumulative	0.50	0.73	0.85

Conclusion

The tested genotypes showed significant variation for all traits, which helps to select genotypes for direct release or donor parents in crossing program. The significant genotype by environment interactions of all traits indicated that the performance of genotypes was not consistent across test locations. Besides, the principal components analysis showed that malt quality traits (protein, extract, friability), phenological traits, plant height and grain yield chiefly contribute for variation recorded by PC 1. The cluster analysis grouped twenty five barley genotypes in three clusters. Genotypes found in same cluster have similar performance, cluster I contain relatively high yielding genotypes, whereas high malt quality yielding genotypes found in cluster II. The crosses between these genotypes found in different clusters could result better segregates which had good malt and agronomic performance.

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