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 analysis
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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 Holeta 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, whereas 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 (9°00’N, 38°38’E), Bekoji (7°15’N, 39°15’E), Debreberhan (9°41’N,39°32’E) and Kofele (7°00’N, 38°45’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

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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 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 (MINTAB, 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-H113/14-49   | MBNVT      | 15    | HB 1963 Released  |            |
| 3     | IBON-H114/15-126  | 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" | 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 = 2.7, 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 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   | 71b  | 130ab| 99c | 44.8a| 27.5bc| 46.6bc| 62.0c | 3805bc| 12.6bc| 78.9bc| 53.2ab|
| 2     | IBON-HI13/14-49           | 76b  | 157ab| 97c | 32e  | 32.6ab| 46.2c | 63.2bc| 3215bc| 12.6bc| 80.3bc| 60.0bc|
| 3     | IBON-HI14/15-126          | 80ab | 156bc| 97c | 28.4c | 32.4bc| 38.6bc| 65.9dc| 3507bc| 12.1bc| 79.2bc| 71.5bc|
| 4     | Bekoji-1 x ND 26333       | 85cd | 135cd| 110b| 38.2cd| 36.4cd| 47.0b | 66.3ab| 2999bc| 12.6bc| 80.1bc| 64.2bc|
| 5     | Bekoji-1 x ND 24263       | 84de | 138d | 114c| 39.6de| 32.6bc| 46.0bc| 65.4bc| 3011bc| 12.3bc| 80.2bc| 60.0bc|
| 6     | IBYT-III 4/2016           | 77c  | 133c | 90c | 44.4bc| 21.3bc| 43.3bc| 64.3bc| 3018bc| 12.2bc| 79.5bc| 59.8bc|
| 7     | Bekoji-1 x Lab No. 128    | 85ab | 155bc| 115c| 46.9cd| 32.6bc| 44.5bc| 65.9bc| 3426bc| 12.8bc| 80.5bc| 61.0bc|
| 8     | IBON-HI 118/2016          | 82bc | 130cd| 103cd| 51.7bc| 33.8bc| 44.5bc| 62.4bc| 3159bc| 11.8bc| 81.1bc| 80.7bc|
| 9     | Bekoji-1 x Traveller      | 81bc | 137d | 109c| 50.8bc| 27.5bc| 46.0bc| 66.0bc| 3254bc| 12.1bc| 81.3bc| 61.6bc|
| 10    | IBON 174/03 x Lab No. 128 | 71a  | 133c | 94c | 61.1bc| 27.5bc| 46.6bc| 63.0bc| 4073bc| 12.6bc| 78.4bc| 56.3bc|
| 11    | Planet                    | 92bc | 146c | 68c | 52.4bc| 21.4bc| 38.8bc| 62.7bc| 1859bc| 10.1bc| 83.4bc| 89.9bc|
| 12    | G 13-64 Belgium           | 78c  | 133bc| 101d| 39.6bc| 33.9bc| 47.2bc| 60.6bc| 2924bc| 12.3bc| 80.0bc| 59.6bc|
| 13    | ICARDA GP-67              | 93bc | 145c | 71c | 44.6bc| 41.2bc| 41.7bc| 64.1bc| 2069bc| 12.0bc| 81.2bc| 72.2bc|
| 14    | IBON-HI 13/14 P# 128      | 98bc | 146c | 62c | 58.4bc| 36.4bc| 32.3bc| 60.9bc| 1706bc| 11.5bc| 82.3bc| 83.6bc|
| 15    | HB 1963                   | 88bd | 144a | 101d| 46.5bc| 27.5bc| 46.3bc| 67.4bc| 2922bc| 11.1bc| 81.9bc| 71.0bc|
| 16    | MN Brite                  | 79bc | 133c | 86c | 59.0bc| 23.7bc| 38.0bc| 65.8bc| 3752bc| 12.6bc| 80.2bc| 57.4bc|
| 17    | Traveller                 | 93bc | 146c | 72c | 50.6bc| 36.3bc| 45.1bc| 62.7bc| 2795bc| 11.7bc| 82.5bc| 74.3bc|
| 18    | IBON 174/03               | 72bc | 130bd| 93c | 48.4d | 28.6bd| 44.3bd| 62.3bc| 3961bc| 13.0bc| 77.9bc| 49.0bc|
| 19    | Miscal-21                 | 77bc | 130bd| 103d | 51.0bc| 31.3bc| 45.1bc| 63.5bc| 3670bc| 13.3bc| 79.8bc| 57.8bc|
| 20    | Acc. #212959A             | 77bc | 126d | 100bd| 47.0d | 50.0c | 28.0c | 51.1bc | 2750bc| 13.3bc| 77.5bc| 62.5bc|
| 21    | Acc. #212959B             | 77bc | 125bc| 97c | 41.4bc| 47.5bc| 29.5c | 53.3c | 2597bc| 12.9bc| 77.9bc| 64.2bc|
| 22    | IBON 15/2018              | 85bc | 137a | 87a | 65.5bc| 20.1d | 49.0c | 62.8bc| 1771bc| 11.8bc| 76.9bc| 66.4bc|
| 23    | EH 1847                   | 77bc | 129c | 105c| 55.7bc| 33.7bc| 42.3bc| 64.6bc| 3597bc| 13.2bc| 80.3bc| 68.0bc|
| 24    | Fatima                    | 96c  | 144a | 66d | 63.3bc| 23.9bc| 38.8bc| 62.0bc| 2191bc| 11.2bc| 83.5bc| 76.5bc|
| 25    | Henrique                  | 89bc | 136c | 77c | 70.2cd| 18.8bc| 37.3bc| 60.1c | 2742bc| 11.9bc| 82.4cd| 74.8cd|

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