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Genotype by Environment Interaction for Protein Content of Malt Barley Genotypes Using the Additive Main Effect and Multiplicative Interaction Effect Model (AMMI) and Genotype Plus Genotype by Environment Interaction (GGE) Biplot

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ABSTRACT

Protein content is a prerequisite for malting quality and it is highly affected by environment. The objective of this study was to quantify the magnitude of genotype by environment interaction and assess the protein content of malt barley genotypes in diversified locations. Eight malt barley genotypes were evaluated in randomized complete block design using three replications at six locations of Tigray, Ethiopia durining 2013/2014 main cropping season. The additive main effect and multiplicative interaction effect model (AMMI) analysis revealed significant differences ($p<0.01$) for genotype, location and genotype by location interaction for protein content. The magnitude of location was twice the magnitude of genotype. Hence, the malt barley genotypes had not a consistent rank across location. The AMMI model clearly indicated the presence of large magnitude of genotype by location interaction which can be partitioned into four significant interaction principal components. The malt barley genotypes were best explained by the AMMI2. Therefore, this model is vital for protein content study in the area. According to the additive main effect and multiplicative interaction effect biplot 1 analysis and ranking biplot f the GGE the genotypes Holkler and Bekoji were stable for desirable protein content for malting. The AMMI1 biplot indicated that locations Korem, Emba-hasti and Astella were unfavorable having protein content less than the grand mean. However, Hashange Mekhan and Hagara-Selam were favorable with protein content greater than the grand mean. In the malting industry protein content is not the only indicator and other quality requirements have to be incorporated for full packaged recommendation of the malt barley genotypes.

Key words: AMMI, GGE, stability, malt, barley

INTRODUCTION

The cultivated barley (*Hordeum vulgare* L.) is originated from its wild progenitor (*Hordeum spontaneum*). It is identical in most respects to present day cultivate barley and this species is still found in abundance in many parts of Asia and North Africa (Harlan *et al*., 1973). In Ethiopia, Barley is one of the most highly cultivated crops with more than 1,700,000 metric t produced on

1,046,000 ha. Barley cultivation is also widely distributed, with more than 4 million smallholder farmers contributing to its production. Barley is among the top five crops in Ethiopia next to Teff, Wheat, Maize and Sorghum (CSA., 2012).

Barley have been breed and selected specifically to produce high yielding and superior malts that provide specific objective to beverages and foods. Low protein level is perhaps the single major consideration in selecting malting barley, which is primarily dependent on genotype by environmental interaction and growing conditions. Relatively low protein is important to malt production because of the negative relationship between the total nitrogen content and the starch content of the kernel (Newman and Newman, 2008).

Malting is the controlled germination of cereals and the primary raw material in the malting industry is barley. In Ethiopia, the demand for malt barley is directly associated with the expansion of the brewery industry. The current malt barley supply is 36,000 metric t but, the demand is 90,000 metric t and even believed to increase in the year 2016 from 110,000-130,000 metric t (USAID., 2012). Effort has been made to tackled the boosting demand and stunted supply of malt barley and different malt barley varieties released, however with the increased demand of barley for malting purpose, the dissemination of improved malt barley genotypes and assessing the protein content in the diversified agro-ecology was not yet done in Tigray Northern Ethiopia.

Various techniques have been developed to analyze genotype by environment interaction and the AMMI and GGE biplot are getting upper hand in the resent years. The Additive Main effects and Multiplicative Interaction (AMMI) model combines analysis of variance for the genotype and environment main effects and principal components analysis of the genotype-environment interaction (multiplicative effect (Zobel *et al*., 1988)).

The GGE biplot analysis is another important model for the evaluation of the genotype performance across testing location and it enables visual evaluation of genotypes, locations and genotype by environment interaction in multi location yield trial (Yan *et al*., 2007). It is also effective tool for genotype evaluation, determining the mean performance and stability and environmental evaluation (the power to discriminate among genotypes in target environment (Yan *et al*., 2000). Hence, to demonstrate the nationally released malt barley genotypes to Tigray region adequate assessment of the genotype by environment and protein content is paramount important and this research is initiated with the objective to evaluate the protein content and quantify the magnitude genotype by environment interaction of the malt barley genotypes.

MATERIALS AND METHODS

Experimental design and methods: The experiment were conducted in the crop season of 2013/2014 in the six barley growing areas of Tigray region, Northern Ethiopia and 8 nationally released malt barley genotypes (Bekoji, Frie-Gebs, Sabini, IBONI174/03, Holker, Bahati and EH-1847) laid out in Randomized Complete Block Design (RCBD) with three replications. Each of the experimental sites and all the treatments were allocated to the experimental plot completely at random. The experimental site contained a total of 6 rows with row spacing of 0.2 m and with a total plot size of 1.2 m by 2.5 m and spacing between plots was 0.5 m while spacing between block was maintained at 1 m. Seed rate was calibrated from the 80 kg ha^{-1} and planting was made by drilling to the six rows. Fertilizer was applied 41 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} at planting and 23 kg N ha^{-1} urea fertilizer was applied in split application during vegetative stage of the crop. For protein analysis the two replications was only considered and from each plot 5 g sample were taken and protein content analysis was done according to the Kjeldahl method.

Statistical analysis: Before combining the data Bartlett's (1974) test was done using the soft ware Minitab 16 for the response v protein content and no series ANOVA assumption violation and the additive main effect and multiplicative interaction effect (AMMI) were done based on the model suggested by Crossa *et al*. (1991) as:

$$y_{ij} = \mu + G_i + E_j + (\sum_{1}^{n} K_n U_{ni} S_{nj}) + Q_{ij} + e_{ij}$$

where, (i = 1, 2…….8: j = 1…….6); Y_{ij} = The performance of the i^{th} genotype in the j^{th} environment, m: The grand mean, G_i: Additive effect of the i^{th} genotype (genotype mean minus the grand mean), K_n: Eigen value of the PCA axis n, E_j: Additive effect of the j^{th} environment (environment mean deviation), U_{ni} and S_{nj}: Scorer of genotype i and environment j for the PCA axis n, Q_{ij}: Residual for the first n multiplicative components and e_{ij}: krror. The GGE biplot was done according to the formula given by Yan *et al*. (2000):

$$y_{ij} - \mu - \beta_J = \lambda 1 \xi i \eta 1_J + \lambda 2 \xi i 2 \eta 2_j + \varepsilon ij$$

Where:
$\lambda 1$ = The singular values of first principal components PC1
$\lambda 2$ = Singular values second principal components PC2
$\xi 1$ = The eigenvectors of genotype I for PC1
$\xi 2$ = The eigenvectors of genotype I for PC2, and
$\eta 1$ = The eigenvectors of environment j
$\eta 2$ = The eigenvectors of environment j for PC1 and PC2, respectively

The analysis for AMMI and GGE was done using the Genestat 16 software.

RESULTS AND DISCUSSION

Analysis of variance for individual environments: The analysis of variance (ANOVA) revealed that there was a significant difference ($p<0.01$) among the malt barley genotypes in the six locations (Table 1). The average protein content across the testing locations range from 10.12-11.12 (Table 1). The location Hagara-selam was with higher protein content and this could be attributed to the low rainfall distribution in the location the result of the study was in agreement

Table 1: Mean of protein content of the eight malt barley genotypes at six locations in the 2013/14 main cropping season

Genotype	Korem	Hashange	Mekhan	Emb-hasti	Astella	Hagra-selam
EH-1847	12.33	12.25	13.07	9.218	9.87	11.03
Bahati	12.01	11.17	10.44	10.828	10.02	9.61
Bekoji	10.68	12.69	10.21	8.844	10.88	11.16
HB-1533	9.68	9.90	11.99	9.894	11.28	11.30
Holker	9.68	10.18	10.34	9.012	10.55	11.55
Sabini	9.61	9.03	11.00	8.314	8.05	10.65
ISBONI	8.90	9.98	10.02	9.275	9.91	12.39
Fire-gebs	8.11	7.61	8.98	9.188	10.78	11.32
Grand mean	10.12	10.35	10.75	9.32	10.16	11.12
LSD	0.722	0.67	0.75	0.58	0.53	0.63
CV	3.00	2.70	3.00	2.60	2.20	2.40

Table 2: AMMI analysis of variance of protein content of eight malt barley genotypes in six locations, in the production year 2013/2014

Source of variation	df	Sum of squares	Mean square	SS of square explained (%)
Total	95	167.65	1.765	
Treatments	47	162.00	3.447**	96.63
Genotypes	7	40.11	5.729**	24.76
Environments	5	36.15	7.23**	22.31
Interactions	35	85.74	2.45**	52.93
Block	6	0.59	0.098	0.36
IPCA1	11	47.32	4.302**	55.19
IPCA2	9	20.33	2.258**	23.71
IPCA3	7	12.42	1.774**	14.49
IPCA4	5	4.85	0.97**	5.66
IPCA5	3	0.83	0.278^{ns}	0.97
Error	42	5.06	0.121	

*,**Significant at $p \leq 0.05$ and 0.01, respectively, ns: Not significant

(Malik, 2012) explained the genotype and environment factors influencing the grain protein content concentration. The genotype EH-1847 were with higher protein content in most of the locations which is undesirable for malting greater than 11.5 grain protein content. The genotype Bekoji was consistent across location and with desirable protein content as well.

Additive main effect and multiplicative interaction analysis (AMMI): The AMMI analysis of variance for the additive main effect revealed significant difference ($p \leq 0.01$) for the testing locations, genotypes and genotype by location interaction (Table 2). The genotype by location interaction explained 52.9% of the total sum of square implying that protein content was highly influenced by the higher magnitude of genotype by location interaction. The magnitude of location was 2 times greater than the genotypes hence the performance of the malt barley genotypes was not consistent across locations. The result was in agreement with Liben *et al*. (2011) who found significant genotype by location interaction in malt barley.

The AMMI analysis of variance for the multiplicative effect was further exploited by decomposing into principal components analysis. The first interaction principal component (IPCA1) captured 55.19% and the second interaction principal component (IPCA2) explained 23.71% and the two interaction principal component analysis cumulatively explained 78.9% of the genotype by location interaction. The postdictive evaluation using the (Gollob, 1968) F-test the four interaction principal components were significant ($p \leq 0.01$). The result was in agreement with Sivapalan *et al*. (2000) recommended an AMMI model with the first four IPCAs predicates well the genotype by location interaction and the result was not consistent with Gauch and Zobel (1988) the two interaction principal component predictive while the reaming contributed to noise and Yan and Kang (2002) stated that most of the interaction occurs in the first few axes. The malt barley genotypes were predicted by the four interaction principal components while the rest of the interaction principal component might be attributed to the noise. Generally the number of the interaction principal component increase with the increasing of the magnitude of the genotype by location interaction and the number of terms to be included in the model without trying predictive assessment of the AMMI model.

AMMI 1 biplot: AMMI analysis provides a graphical representation (biplot) to summarize information on main effects and interaction of both genotypes and environments simultaneously. The closeness between pairs of locations or pairs of genotypes in the biplot is proportional to the response they have to the genotype by location interaction effects (Crossa *et al*., 1991). The

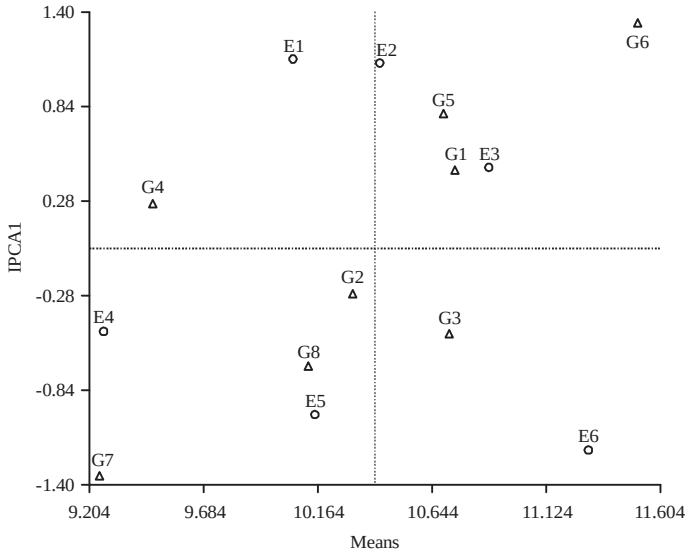

Fig. 1: AMMI biplot for protein content of eight malt barley genotypes in six locations 2013/2014. Genotypes plotted as, Genotypes: G1: Bekoji, G2: Holker, G3: HB-1533, G4: Sabini, G5: Bahati, G6: EH-1847, G7: Fire-Gebs, G8: IBON174/03, B Environments: E1: Korem, E2: Hashange, E3: Mekhan, E4: Emba-hasti, E5: Astella, E6: Hagara-selam

interaction principal component 1 (IPCA1) represented on the y-axis where as the genotypes and environments mean were plotted on the x-axis (Fig. 1). The genotypes G4 (Sabini), G2 (Holker) and G8 (IBNI174/03) were located to the left side of the perpendicular line (grand mean) implying the genotypes was with lower protein content, whereas, the genotypes G3 (HB-1533), G1 (Bekoji), G5 (Bahati) and G6 (EH-1847) were with a protein content greater than the grand mean. The genotype G4 (Sabini), G2 (Holker) and G1 (Bekoji) was moderately stable genotypes for the response variable protein content. The genotype G6 (EH-1847) was the most unstable genotype and with a protein content greater than the standard, which is 11.5% for malting (Fig. 1).

The testing locations E1 (Korem), E4 (Astella) and E5 (Emba-hasti) was unfavorable for protein content located to the left side of the perpendicular line (grand mean). The testing locations E2 (Hashange), E3 (Mekhan) and E6 (Hagara-selam) was favorable testing location for protein content that was located to the right side of the perpendicular line (grand mean). All the testing location was interactive contributing much to the increasing genotype by environment interaction and caused unstable performance of the genotypes for the response variable protein content, except the testing location E4 (Emba-hasti) which attributed to the stable performance of the malt barley genotypes for the response variable protein content (Fig. 1).

Generally the testing locations were diverse both in the main effect and interaction effect and the genotypes were interactive with higher genotype by location interaction and showed unstable performance except Genotype G4 (Sabini) and G2 (Holker). The first interaction principal component (IPCA 1) captured 29% of the variation and with the model fitness of 77% but the malt barley genotypes was more explained by the AMMI 2 biplot model analysis provided a model fitness of 89.36% and the result of the study was in agreement with Bantayehu (2013) that had found best model fitness using the second model AMMI 2.

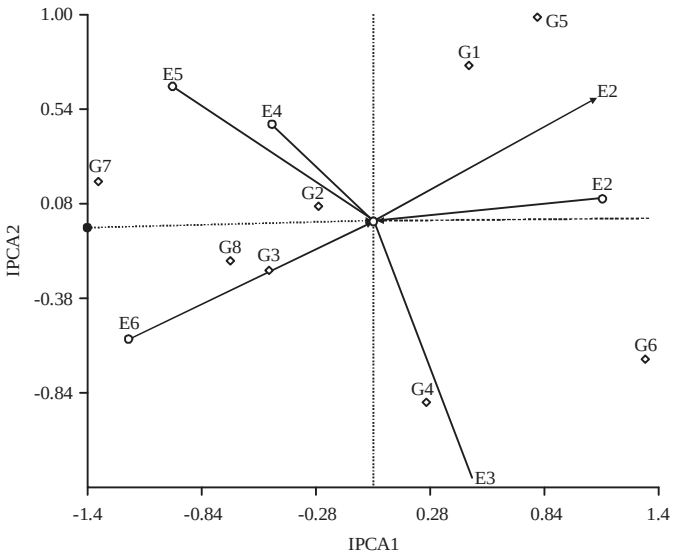

Fig. 2: AMMI 2 biplot for protein content of eight malt barley genotypes tested in six locations 2013/2014. Genotypes plotted as, (A) Genotypes: G1: Bekoji, G2: Holker, G3: HB-1533, G4: Sabini, G5: Bahati, G6: EH-1847, G7: Fire-Gebs, G8: IBON174/03, Environments: E1: Korem, E2: Hashange, E3: Mekhan, E4: Emba-hasti, E5: Astella, E6: Hagara-selam

AMMI 2 biplot: The interaction principal component 1 (IPCA1) was plotted on the x-axis where as the interaction principal component two (IPCA2) plotted on the Y-axis (Fig. 2). The first Interaction Principal Component (IPC1) captured 55.19% and the second interaction principal component explained 23.71% the two interaction principal components cumulatively captured 78.9% of the sum of square of the genotype by environment interaction of malt barley genotypes. When the Interaction Principal Component (IPCA1) was plotted against IPCA2 (Purchase, 1997) pointed out that the closer the genotypes score to the center of the biplot the more stable is the genotype and the reverse is true. The genotype G2 (Holker) was located near to the origin and were stable. The malt barley genotypes G1 (Bekoji), G3 (HB-1533), G4 (Sabini), G5 (Bahati) G6 (EH-1847), G7 (Fire-Gebs) and G8(IBON174/03) were unstable located distant from the origin (Fig. 2).

Genotypes with interaction principal component one (IPCA1) values higher than zero classified as higher protein content while those with PC1 values lower than zero are classified as low protein and low adaptability (Kaya *et al*., 2006). The G1 (Bekoji), G5 (Bahati), G4 (Sabini) and G6 (EH-1847) was genotypes with IPCA1 greater than zero implying that had higher protein content. The genotype G7 (Fire-Gebs), G2 (Holker), G8 (IBONI174/03) and G3 (HB-1533) was with the interaction principal component less than zero implying that the genotypes was with low protein content and adaptability (Fig. 2).

The best malt barley genotype with respect to the testing locations E2 (Hashange) and E1 (Korem) was G5 (Bahati) and G1 (Bekoji). The best genotype with respect the testing location E3 (Mekhan) was the G4 (Sabini) and G6 (EH-1847). The G8 (IBONI174/03) and G3 (HB-1533) were the best malt barley genotypes with respect to the testing location E6 (Hagara-Selam). The genotype G7 (Fire-Gebs) was best with respect to the testing location E5 (Astella). Generally with the consideration of the stability of the genotype and adaptability the tested malt barley genotype G2 (Holker) was better with broad adaption to most of the locations (Fig. 2).

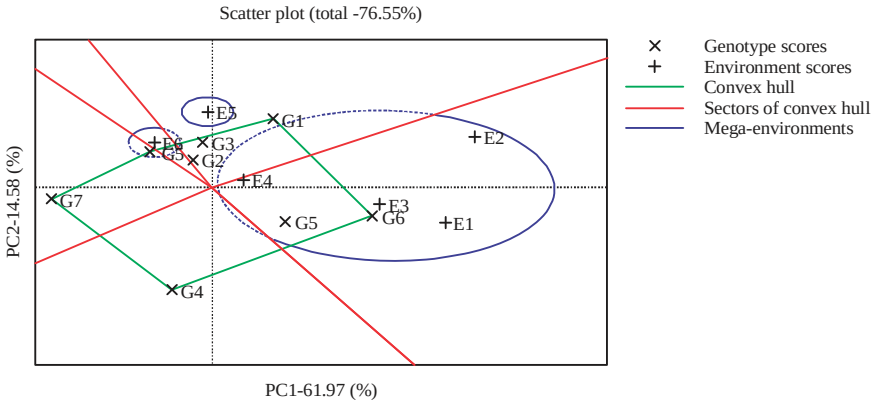

Fig. 3: Which genotype wins where of the GGE biplot for protein content of eight malt barley genotypes in six locations in 2013/2014. Genotypes plotted as A) Genotypes: G1: Bekoji, G2: Holker, G3: HB-1533, G4: Sabini, G5: Bahati, G6: EH-1847, G7: Fire-Gebs, G8: IBON174/03, Environments: E1: Korem, E2: Hashange, E3: Mekhan, E4: Emba-hasti, E5: Astella, E6: Hagara-selam

Which-win-where pattern of genotype by environment interaction of the GGE: Genotypes located on the vertices of the polygon performed either the best or the poorest in one or more environments. The equality lines divide the biplot into sectors and the winning genotype for each sector is the one located on the respective vertex (Yan and Tinker, 2006). The G6 (EH-1847), G4 (Sabini), G7 (Fire-Gebs)) and G1 (Bekoji) were vertex genotypes may perform best or very low since the genotypes located distant from the origin and implying that the genotypes were specifically adapted (Fig. 3). The genotype G2 (Holker), G3 (HB-1533) and G8 (IBON174/03) were genotypes located near to the orgin implying that the genotypes were broadly adapted (Abay and Bjornstad, 2009).

The environments fall into two sections and the genotypes into five sections (Fig. 3). The genotypes G6 (EH-1847) were more adapted to the testing location E3 (Mekhan), E1 (Korem) and E2 (Hashange). The genotypes G1 (Bekoji) G3 (HB-1533) G2 (Holker) were more adapted to the testing location E4 (Emba-hasti). The G7(Fire-Gebs) and G4 (Sabini) were a vertix genotypes with no testing location specifically adapted to it. In the study of the malt barley genotype by environment interaction the testing locations fall into two sectors location E3 (Mekhan), E4 (Emba-hasti), E1 (korem) and E2 (Hashange) categorized in the first sector and the testing location E6 (Hagara-selam) and E5 (Astella) in the second sector (Fig. 3). The polygon view of the GGE provided model fitness of (76.55%) of the genotype by environment interaction data and the AMMI two biplot explained 77% of the genotype by location interaction and both the AMM two biplot and the GGE polygon view biplot can be used in genotype by location analysis of malt barley in the Tigray region northern Ethiopia.

Mean protein content and stability of malt barley genotypes: In breeding programs best genotype is defined as one that is higher yield and stable across wider environments. Higher PC1 scores associated with high mean yield and small absolute PC2 scores associated with high stability (Yan and Tinker, 2006). The Average Tester Coordinate (ATC) with double arrow in Fig. 4 separates genotypes with above average mean and below average means for protein content. The single arrowed line points towards the direction of increasing protein content.

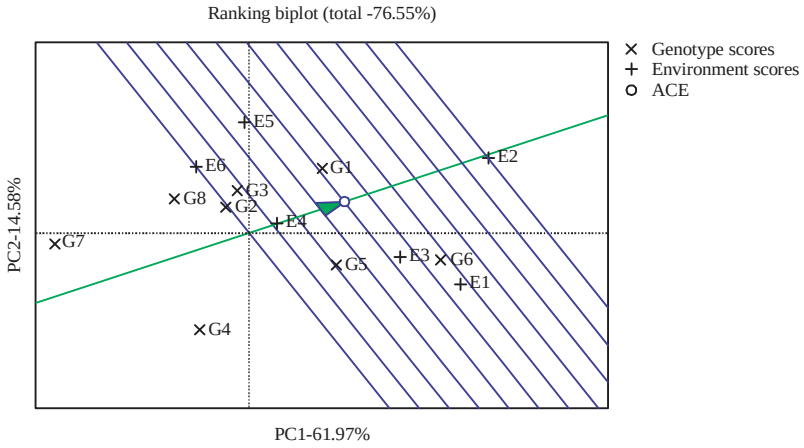

Fig. 4: Mean protein content and stability of the GGE biplot of eight malt barley genotypes in six locations in 2013/2014. Genotypes plotted as A) Genotypes: G1: Bekoji, G2: Holker, G3: HB-1533, G4: Sabini, G5: Bahati, G6: EH-1847, G7: Fire-Gebs, G8: IBON174/03, Environments: E1: Korem, E2: Hashange, E3: Mekhan, E4: Emba-hasti, E5: Astella, E6: Hagara-selam

The genotype G4 (Sabini), G7 (Fire-Gebs) and G8(IBON174/03) were with lower mean protein content below the average tester coordinate (ATC). The genotype G1 (Bekoji), G3 (HB-1533), G2 (Holker) G6 (EH-1847) and G5 (Bahati) were with protein content above the mean. The malt barley genotypes G2 (Holker), G1 (Bekoji), G3 (HB-1533) and G8 (IBON174/03) were stable genotypes nearly placed to abscissa or they were with the shortest vector from the single arrow. The G6 (EH-1847),G5 (Bahati), G4 (Sabini) and G7 (Fire-Gebs) were the most unstable located distant from the single arrowed line and with longer vector (Fig. 4). In multi location trial the consistent performance of genotypes across locations (stability) and acceptable protein content across location is very important hence when the protein content across testing location and stability is simultaneously considered the genotype G1 (Bekoji) was better genotype.

Relationships and discriminating ability of location: The cosine of the angle between the vectors of two environments approximates the correlation between them. The presence of wide obtuse angles indicates strong negative correlation where as an acute angle indicates positive correlation and the association between the environment is 90° they are independent (Yan and Tinker, 2006).

The testing locations E3 (Mekhan), E1 (korem), E4 (Emba-Hasti) and E2 (Hashange had an acute angle that implies the four testing location were positively correlated. The testing location E6 (Hagara-selam) and E5 (Astella) had wide obtuse angle with the testing locations of E1 (korem) and E3 (Mekhan) implying negative correlation between the testing locations. The tentative grouping of the testing location made in to two and this result was in consistent with Dolatabad *et al*. (2010) in hybrid maize (Fig. 5).

Testing location with longest vector from the origin has the highest discriminating power where as the environment with shortest vector discriminate capability is very poor (Yan and Tinker, 2006). The testing locations E2 (Hashahnge) and E1 (Korem) were with longest vector from the

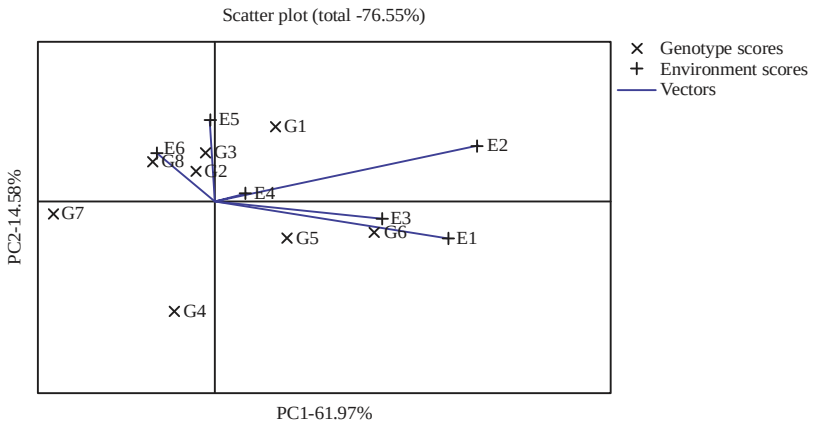

Fig. 5: Relationships and discriminating ability of location using the GGE biplot for protein content of eight malt barley genotypes in 2013/2014. Genotypes plotted as A) Genotypes: G1: Bekoji, G2: Holker, G3: HB-1533, G4: Sabini, G5: Bahati, G6: EH-1847, G7: Fire-Gebs, G8: IBON174/03, Environments: E1: Korem, E2: Hashange, E3: Mekhan, E4: Emba-hasti, E5: Astella, E6: Hagara-selam

origin implying that the testing locations were highly discriminating. The location E3 (Mekhan), E5 (Astella), E1 (Korem) and E6 (Hagar-selam) were moderately discriminating (Fig. 5). The testing location E4 (Emba-Hasti) was less discriminating.

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