

## GGE biplot analysis on the performance of wheat genotype for hectolitre weight and mega environments identification in north western Ethiopia

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### Abstract

Grain quality traits like hectolitre weight are quantitatively inherited and as a result their phenotypic performance is highly influenced by genotype, environment, and the interaction between the two. The present study was conducted in north western Ethiopia across six test locations during the 2008 and 2009 cropping seasons to study the nature of GEI on hectolitre weight of bread wheat and to classify environments based on the performance of genotypes. Randomised complete block design with three replicates was used on each site. A total of 12 advanced bread wheat lines along with standard and local checks were tested and data were analysed on hectolitre weight. Both the main effects of genotypes and environments, and their interaction significantly ( $P \leq 0.01$ ) contributed for the observed phenotype. The environment explained 46.6 % of the total variation while GEI and genotype explained 15.6% and 10%, respectively. The first two principal components of a GGE biplot explained 67% of the variation. ETBW5344 was the most stable and near ideal genotype for regional release while ETBW5345 was the most variable genotype. The six locations were divided in to two mega environments ETBW5345 and ETBW4992 being the two best genotypes in each of the mega environments. Among the test sites Adet was the most representative and most discriminating environment while Sirinka was less desirable as a testing site for hectolitre weight. Trials across several years need to be done to firmly conclude the presence of the two mega environments.

**Keywords:** hectolitre weight, gene by environment interaction, bread wheat, biplots analysis.

**Abbreviations:** AEC\_Average environment coordination, G\_Genotype, GEI\_genotype by environment interaction, GGE\_Gene plus Genotype by environment, MET\_multi environment trial, PCA\_principal component analysis.

### Introduction

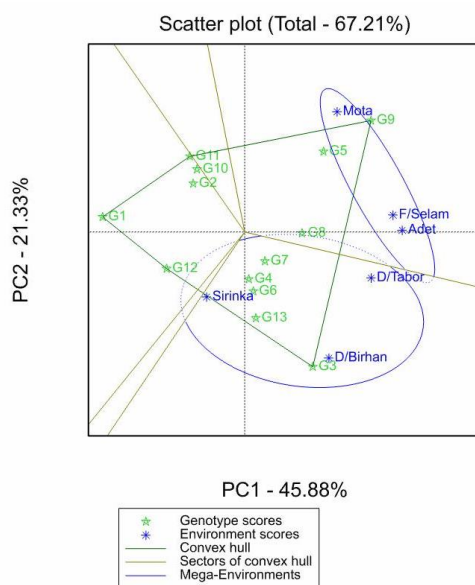
Bread wheat is one of the major cereals grown in the world. According to the USDA (2013) report, a total of 221.8 million hectares of land was covered by wheat with a total grain production of 697.01 million metric tonnes in the 2011/2012 cropping season. In the same season Ethiopia produced a total of 3.3 million metric tonnes of wheat grain on 1.5 million hectares of land with an average productivity of two tonnes per hectare. Bread wheat accounts for about 60% of the national wheat production in Ethiopia. Though wheat is grown in such a vast area in the country the average productivity is low by the standards of world average. The low level of productivity can be ascribed to poor growing conditions and/or lack of well adapted varieties. The diverse agro ecology (Tesemma et al., 1998) of the region may also have imposed significant genotype by environment interaction (GEI) that hampers crop improvement (Yan and Kang, 2003). Knowledge on the nature and magnitude of GEI is helpful to determine whether there is a need to develop a widely adapted cultivar for all environments of interest, or specifically adapted cultivars for specific target environments (Bridges, 1989; Yan et al., 2007; Yan and Kang, 2003). Breeders generally want to minimize GEI and generate varieties that are adapted to wider areas as it is easier and cost effective both in terms of variety evaluation

and seed multiplication (Matus-Cadiz et al., 2003). However, the need to develop a widely adapted variety is dependent up on the kind of interaction prevailing (Yan and Kang, 2003). GEI can be either quantitative in which case there is no change in genotypes ranking; or qualitative when change in ranking happens. Qualitative (crossover) interaction is the type of interaction that poses a challenge to breeders (Romagosa and Fox, 1993). If crossover (qualitative) interaction happens consistently, then the breeding environments may be classified in to mega environments and specifically adapted varieties can be developed for each sub environment separately (Busey, 1983; Yan et al., 2007). The selection of widely adapted genotypes that perform well across many locations is more economical despite the fact that it is at the cost of some yield gains from specifically adapted genotypes (Yan and Kang, 2003). Wheat breeding in Ethiopian has long been established mainly focusing on developing high yielding and widely adapted varieties which has overlooked grain quality improvement (Dessaegn et al., 2011; Tarekegne and Labuschagne, 2005). Measuring productivity based primarily on grain yield is disguising as high grain yield doesn't have strong correlation with several quality traits (Govindaraj et al., 2009; Kibite and Evans, 1984; Rankin, 2009).

**Table 1.** Global location and average weather data of the 6 test sites.

Location	Latitude	Longitude	Altitude (masl)	Rainfall (mm)	Temperature (C <sup>0</sup> )		Soil type
					Max.	Min.	
Adet	11 <sup>0</sup> 17'N	37 <sup>0</sup> 43'E	2240	1315	25.7	9	Nitosol
Mota	11 <sup>0</sup> 5'N	37 <sup>0</sup> 52'E	2487	1012	n/a	n/a	Nitosol
F/ Selam	10 <sup>0</sup> 42'N	37 <sup>0</sup> 16'E	1917	950	28	12	Nitosol
D/ Tabor	11 <sup>0</sup> 51'N	38 <sup>0</sup> 1'E	2706	1580	n/a	n/a	Nitosol
Sirinka	11 <sup>0</sup> 45'N	39 <sup>0</sup> 36'E	1850	1023	25	13	Eutric vertisol
D/Birhan	9 <sup>0</sup> 36'N	39 <sup>0</sup> 29'E	2828	893	24	6	Vertisol

n/a= not available.



**Fig 1.** Polygon views of GGE biplot based on environmental scaling for the ‘which-won-where’ pattern of genotypes and environments.

Hectolitre weight is one among the various grain physical quality traits that can be used to predict flour protein content which is associated with flour baking quality (Schuler et al., 1995). It measures the density of grain and is calculated as kilograms per hectolitre (kg/hl) which is recognized as a standard quality parameter across all milling industries (GTA, 2013; Schuler et al., 1995). Depending on the purpose of the grain, different markets have different standards set for hectolitre weight. Generally the milling grades have a minimum hectolitre weight of 76 kg/hl everywhere except in Australia (GTA, 2013). Low hectolitre weight indicates that the grain did not form properly or has not maintained its intended form which may be caused by frost damage during protein deposition, lack of moisture during grain filling among others. Quality traits are quantitatively inherited and as a result their performance is influenced by genotype, environment and the interaction between genotype and environment (Ding et al., 2011; Eagles et al., 1995; Ramya et al., 2010; Uhlen et al., 1998). Multi environment evaluation of genotypes across diverse locations/environments before release of a new variety is a common practice. Previous studies done on different crops and genotypes tested across different locations and years witnessed the presence of significant GEI thereby influencing the selection and recommendation of cultivars in north western Ethiopia (Ayalew and Wondale, 2010; Bantayehu, 2009; Muhe and Assefa, 2011). However, studies explaining the nature of GEI on grain quality traits are so far limited in the region. Consequently there lacks documented information regarding the effect of genotype by environment interaction on grain quality of bread wheat in north western Ethiopia. The objectives of this research were 1) to study the nature and

extent of genotype by environment interaction on hectolitre weight, and 2) to analyse the similarities and differences between the testing locations.

## Result

### Genotypic performance

Highly significant ( $p \leq 0.01$ ) differences were detected for both main effects (genotypes and environments) and their interaction (Table 2). The overall mean hectolitre weight of the tested genotypes (77.2 kg/hl) met the world minimum standard for industrial processing (GTA, 2013). Means for genotype effect were separated using Tukey’s honestly significant difference test at 95% confidence interval. The standard check (Gasay) was out yielded by five genotypes and G9 was the top yielding based on the overall mean of all data sets (Table 3). Large proportion of the variation was explained by the environmental effect (46.6%) followed by the GEI effect (15.6%). Genotypes accounted for 10% of the overall variation. There still remains large proportion of variation left unexplained by the model pooled into the error term (26.7%).

### Mega environments and best genotypes

Based on the GGE analysis the first two principal components explained about 67% of the total interaction variation. The six test locations segregated in to two distinct sectors of the ‘which-won-where’ biplot each sector having different genotypes performing best (Fig. 1). G3 was the best genotype at D/Birehan, Sirinka and D/Tabor areas whereas

**Table 2.** ANOVA of the combined data over 10 environments (6 sites and 2 years, 2008 and 2009).

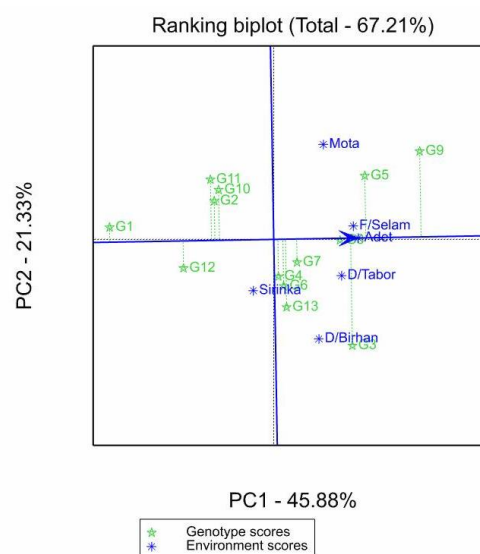
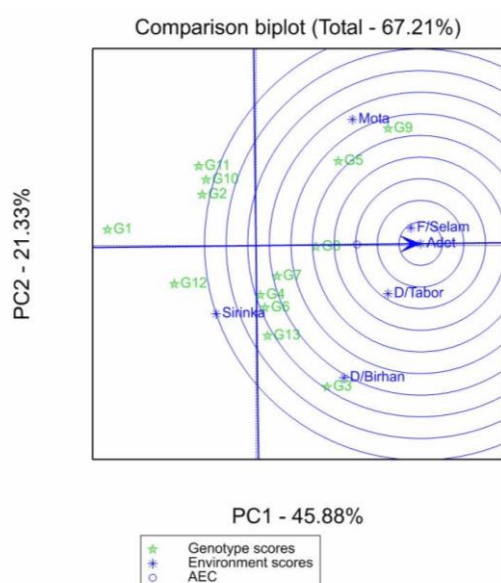
Source	DF	Adj MS hLW	Proportion TSS
Environment	9	223.3**	46.6%
Genotype	12	37.9**	10.5%
Environment X Genotype	108	6.3**	15.6%
Error	258	4.5	26.7%
SE		2.07	

\*Adj MS hLW= adjusted mean square for hectolitre weight, TSS= total sum square

**Table 3.** Grouping of the 13 genotypes using Tukey's honestly significant difference test at 95.0% level confidence.

Genotype code	Rank	Genotype	Mean hLW
ETBW 5341	1	G9	79.32 <sup>a</sup>
ETBW 5330	2	G3	78.18 <sup>ab</sup>
ETBW 5345	3	G5	77.84 <sup>abc</sup>
ETBW 5339	4	G8	77.83 <sup>abcd</sup>
ETBW 5346	5	G6	77.65 <sup>abcd</sup>
HAR3730 (Gasay)-St.Check	6	G13	77.58 <sup>abcd</sup>
ETBW 5348	7	G7	77.57 <sup>abcd</sup>
ETBW5344	8	G4	77.11 <sup>bcd</sup>
ETBW4992	9	G10	76.38 <sup>cde</sup>
ETBW 5304	10	G2	76.3 <sup>cde</sup>
ETBW 5013	11	G11	76.29 <sup>cde</sup>
ETBW 5231	12	G12	76.05 <sup>de</sup>
ETBW 5303	13	G1	75.01 <sup>e</sup>

\*hLW= hectolitre weight



**Fig 2.** GGE biplot based on environment focused scaling for comparison of the environments with the ideal test environment.

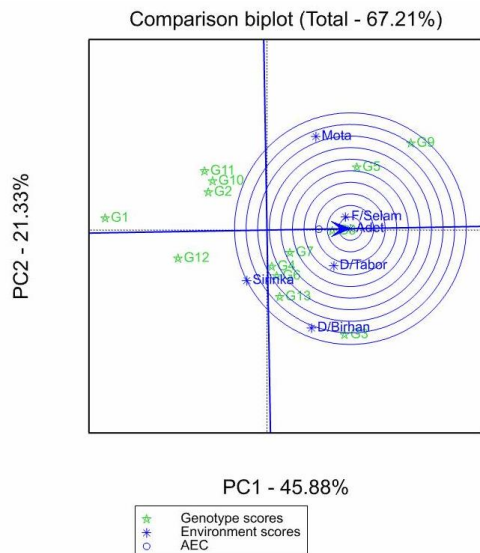
G9 was the best at Adet, F/Selam, and Mota environments. Adet F/Selam and D/Tabor were among the best locations for hectolitre weight evaluation. Adet was found near the ideal test environment of the average environment coordination (AEC) biplot (Fig. 2) followed by F/Selam while D/Tabor was a near average test site. The mean scores of Sirinka and Debre Birehan were below the average environment coordinate point. Sirinka had negative PC1 score and it had an obtuse angle between the test sites except with D/Birhan. Genotypes G3, G5, G8 and G9 were the only genotypes having mean scores greater than the average environment coordinate point based on genotype focussed GGE biplot

**Fig 3.** Average environment coordination (AEC) views of the GGE biplot based on genotype focused scaling for the means performance ranking and stability of genotypes.

(Fig.3). G8 was the most stable genotype as it was subtended by relatively low PC2 score. G1, G2, and G10-12 were among the undesirable genotypes (negative PC1) while G3 was the most variable genotype having the highest PC2 projection (Fig. 4).

### Discussion

The primary focus of Ethiopian wheat breeding projects were improving grain yield (Dessalegn et al., 2011; Tarekegne and Labuschagne, 2005).



**Fig 4.** GGE biplot based on genotype focused scaling for comparison of genotypes with the ideal genotype.

As a result breeding for grain quality didn't receive considerable attention in the past years. However the grain quality dictates the end use of the whole produce. As most other quality traits are, hectolitre weight is highly affected by the environmental factors in addition to the genetic composition of the genotype (Ding et al., 2011). Highly significant differences were detected for all sources of variation calling for the need to consider these factors carefully in MET analysis for hectolitre weight evaluation. The proportion of variation explained by the environment is larger (46.6%) than the proportion explained by the rest of the factors (Table 2). There still was a sizable genotypic variation (10%) that can be exploited for hectolitre weight improvement. A comparable estimate of variation was reported on grain yield of a malting barley trial (Bantayehu, 2009), G and GEI explaining nearly 9%, and 14% of the total variation, respectively. High environmental variation indicates that the heritable portion of the observed variation is relatively low and improvement for hectolitre weight may not be proportional to the observed phenotypic variation. The high control of GEI over the phenotypic variation further complicates selection for hectolitre weight genetic improvement as the phenotype will no longer be good predictor of genotype in the presence of high GEI (Tesemma et al., 1998; Yan and Kang, 2003). Large and diverse germplasm shall be screened to widen the genetic base upon which selection is practiced. In this particular study both qualitative and quantitative types of interaction were observed. In the case of quantitative interaction the best genotype performs best even if there may be a universal change in the mean performance of all of the genotypes tested across environments which does not affect the genotype to be selected. Qualitative (crossover) interaction however is the kind of interaction that makes MET data interpretation and selection complex (Yan et al., 2007). G3 was the best genotype at D/Birehan, Sirinka and D/Tabor while G9 was the best at the rest of the test environments (Adet, Mota and F/Selam) (Fig. 1). The two groups of environments had different genotypes performing best which imposes managerial challenge. Whenever there is a consistent differential ranking of genotypes, the test environments can be divided into to groups of locations that share the same best genotype namely mega environments (Busey, 1983; Yan et al., 2007; Yan and Kang, 2003).

Among the test environments, Adet, Finote Selam, Mota and Debire Birehan were having a good discriminating power. Mota and Debire Birehan sites are important for culling less suitable genotypes (Discriminating) while Adet and Finote Selam (discriminating and representative) sites are useful to generate superior varieties (Fig. 2). According to Yan et al. (2007), environments having long vectors and large angles with the AEC abscissa are good in culling unstable genotypes while sites with long vector and smaller angles with the average environment axis are suitable for selecting superior genotypes. An ideal test environment is one that has both discriminating ability among genotypes and representative of all other environments for widely adapted variety improvement (Yan, 2001; Yan et al., 2007). Based on this research Adet was a near ideal site having high discriminating power and very low angle from the average environment axis (Fig 2). The details of this study also indicated that the Finote Selam site can be left out in case of budget or seed constraints without drastically affecting the quality of data to be generated from the rest of the locations in the presence of Adet site. The overall desirability of a genotype is a combination of high yield and stability in performance. An ideal genotype is one that has the highest yield and an absolute stability (Yan and Kang, 2003). However to get an ideal genotype is not easy. Genotypes closer to the ideal genotype are the most desired genotypes (Yan et al., 2007; Yan and Kang, 2003). Concentric circles rippling around the average environmental coordinate (AEC) of a genotype focussed GGE biplots (Fig. 4) encompass genotypes that are relatively similar in their overall desirability (Kaya et al., 2006; Yan and Kang, 2003). Based on this criterion G8, G5 and G7 were the only genotypes under the desirable genotypes for wider adaptation. Despite their highest yield G3 and G9 in the two mega environments, they were grouped with the low yielding and less desirable genotypes in terms of overall suitability for wide adaptation. The GGE biplots clearly filtered out the best genotypes unlike the combined ANOVA using mixed model and the most conservative mean separation (Table 3). GGE analysis was reported to be a handy tool for MET data (Kaya et al., 2006; Yan, 2001; Yan and Kang, 2003; Yan and Tinker, 2006). Based on the present study genotype ETBW 5339 (G8) was the most stable and widely adapted genotype for regional release for hectolitre weight. Decision to divide breeding locations into mega environments does not solely depend on the biological and statistical analyses of GEI. Having a separate breeding program for each of the mega environments demands more logistics and research staff. In addition to the challenge to generate varieties for different mega environments, the seed multiplication and distribution is another crucial thing to be considered before implementing specific adaptation breeding. Specific adaptation breeding will also be highly dependent on public research institutions as its scope is limited to be a viable seed market for private seed companies. Therefore, the pros and cons of breeding for specific adaptation need to be considered before embarking on it. Trials across several years and locations will enable to firmly conclude the presence of the two mega environments.

## Materials and Methods

### Plant materials

A multi environment trial was conducted using 12 advanced bread wheat genotypes (Table 3) along with local checks for each location and one standard check (Gasay) in the 2008 and 2009 cropping seasons across six testing sites. Plots size of 3

m<sup>2</sup> (1.2 m \* 2.5 m), each plot consisting of six rows 20 cm apart, were arranged in randomised complete block design replicated three times. The four middle rows were harvested and data on hectolitre weight was measured as kilograms per hectolitre based on standard procedure (AACC, 1983).

#### Test locations and years

Multi environment trial was conducted during the 2008 and 2009 main cropping seasons at Adet, Mota, Finote Selam, Debre Tabor, Debre Birhan, and Sirinka agricultural research stations. Average weather data and geographical coordinates of the test sites are presented in table 1. Fertilizer rate, seed rate, and crop cultivation were applied based on agronomic recommendations for each site.

#### Statistical analysis

##### Analysis of variance

The experiment at Sirinka and Debre Birhan stations was conducted only for one season (2009). Combined analysis over years and locations was done separately for Adet, Mota, Finote Selam and Debre Tabor; and the genotype by year interaction was found to be non-significant. Then data were combined for the ten environments in total (the two seasons for the four of the environments were considered as separate environments) using Minitab 16 software (Minitab Inc., 2013) after testing for homogeneity of variance. The local checks for each of the environments were excluded from the combined analysis since each environment had its own local check. The following mixed model was used:  $y_{ijk} = \mu + G_i + E_j + GE_k + \varepsilon_{ijk}$ , where  $y_{ijk}$  is the observed mean,  $\mu$  is the grand mean,  $G_i$  is the genotype effect,  $E_j$  is the environment effect,  $GE_k$  is interaction between G and E and  $\varepsilon_{ijk}$  is the error effect. Environment and, as a result genotype by environment interaction were considered random. Mean comparison for the fixed effects was carried out using Tukey's test.

##### Biplot analysis

GGE biplot analysis was conducted on the mean best linear unbiased estimate (BLUE) values of 12 bread wheat genotypes in the respective locations using GenStat 15 (VSN International, 2012). The two years (2008 and 2009) data for the first four locations (Adet, Mota, Finote Selam, Debre Tabor) was averaged as the year by genotype interaction was not significant for these locations.

#### Conclusion

In conclusion, variation among the tested genotypes was highly significant. Two genotypes significantly outperformed the current standard check variety in the region which shows the possibility of releasing a new variety following the national variety verification process. This present study has also identified the presence of two mega environments for hectolitre weight improvement in north western Ethiopia. Adet was the most suitable taste location for wheat breeding in the region. However, research across many years is required to firmly conclude the identified mega environments and devise a breeding strategy for the two mega environments separately.

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