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Integrating Multivariate and Univariate Statistical Models to Examine Protein, Moisture, and Gluten Stability in Ethiopian Tetraploid Wheat Varieties under Irrigation Condition

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ABSTRACT

Protein, moisture and gluten content of 12 popular Ethiopian tetraploid wheat varieties were investigated for genotype-environment interaction and stability performance under irrigation conditions. The experiment was carried out at nine different sites using a randomized complete block design with three replications. Qualitative data such as gluten, protein and moisture contents were collected. Multivariate (ANOVA, AMMI and GGE biplot) and univariate (b_i, S^2d, σ_i, W_i^2, YS_i and CV_i) analysis models were applied to identify stable varieties for qualitative traits. The AMMI analysis of variance revealed that the main effects (genotype and Environment) had a highly significant ($p < 0.001$) influence on the protein, moisture and gluten contents. The G × E interaction had a significant ($p < 0.05$) impact on moisture and protein contents, and a highly significant ($p < 0.001$) influence on gluten. Environmental variance was the most significant source of variation, accounting for 71.5% (gluten), 71.1% (moisture) and 33.2% (protein) of the total variation; the larger environmental variance may be attributed due to the variations in soil types and climatic conditions among environments. Univariate statistical analysis models showed that Utuba was stable across the testing environments for protein, gluten, and moisture contents. The GGE biplot clustered the nine test locations into 4, 4 and 3 mega environments for gluten, moisture, and protein, respectively. Both statistical analyses (univariate and multivariate) proved that the protein, gluten, and moisture contents of the Utuba variety were stable across the test environments. Therefore, Utuba is recommended for further demonstration and popularization in test locations with similar agro-ecologies.

KEYWORDS: quality traits; multivariate; stability; univariate

ABBREVIATIONS

AMMI, additive main effect and multiplicative interaction; ANOVA, analysis of variance; bi, regression coefficient; CVi, coefficient of variance; S^2d, deviation from regression; GGE, genotype main effect (G) plus genotype by environment interaction (GE); GEI, genotype-environment interaction; IPCA, instrumented principal components analysis; MS, mean of square; YSi, Kang's stability statistic; σi^2, Shukla's stability variance; SSA, Sub-Saharan Africa; RCBD, randomized complete block design; W^2i, Wricke's ecovalence

INTRODUCTION

Durum wheat (*Triticum turgidum* ssp., $2n = 4x = 28$, AABB) is the most ancient cultivated form of tetraploid wheat species in Africa and therefore Ethiopia is the major durum wheat producer in sub-Saharan Africa (SSA), having with 0.6 million ha of area coverage. In Ethiopia, durum wheat nearly accounts for 15%–20% from the total of wheat production and it's covered more than 30% of the whole area wheat acreage [1,2]. Durum wheat majorly grown in the highlands of Ethiopia, which lies between 6° and 16°N and 35° and 42°E, at altitudes ranging from 1500 to 3000 meters above sea level [3]. Durum wheat has multiple uses such as raw material of several foods like pasta, macaroni, biscuit, cake, and for traditional foods, others and has great contribution in the feeding of Africa population. Durum wheat is an economically important crop because of its unique features related to grain end use products [4]. Several authors [5–7] reported the uniqueness of the Ethiopian tetraploid wheat. Ethiopian tetraploid wheat has distinct characteristics, such as adaptability in wide environments, responsiveness to low inputs (nitrogen) and tolerance to biotic and abiotic stresses. Durum wheat has various traits of interest such as resistance to yellow rust [8], environmental stability and high quality of its products [9].

Durum wheat quality is highly dependent of the genotypes, fluctuations in biotic and a biotic environmental factors and agricultural production technology packages [7,10]. Among the environmental factors, high temperatures and humidity during grain filling [5,11], distribution of precipitation [12] and nitrogen fertilization [13], Soil fertility, fertilization and water availability are the main factors affecting the quality stability [14] Environmental conditions are known to have a significant influence on end-use quality characteristics, but the relative magnitude of environment, genetic and genotype × environment (G × E) effects on quality is unclear [15].

The G × E interaction effects on durum wheat pasta quality have been studied by several groups of researchers [14,16,17], who found that environment and year, significantly affect protein content, sedimentation volume, gluten index and yellow pigment content [18]. Moreover, test weight, kernel size and virtuousness are also important, as they are

strongly related to semolina yield and brightness appearance of semolina [19]. Interactions between G and E are significant because they provide information about the effect of various environments on variety adaptability and are used to evaluate the performance stability of breeding materials. Assessment of diverse genotypes across locations and over time is now not only necessary for selecting and recommending high-yielding cultivars, but also for identifying acceptable sites that represent the optimal environment.

Several statistical methods have been proposed for analysis stability with the aim of explaining the information contained in the GE interaction data matrix. The most employed parametric univariate approaches are Wricke's ecovalence (W_i^2), Shukla's stability variance (σ_i^2), deviation from regression (S^2d), and linear regression slope (b_i) and coefficient variation (CV_i). Contrarily, a nonparametric technique such as Kang's stability statistic (YS_i). According to [20], genotypes with $b_i = 1$ and $s^2d_i = 0$ is considered as a stable genotype. Genotype with low Shukla's stability variance [21] and Wricke's ecovalence [22] are regarded as stable. According to [23], genotype identified as stable if CV_i was less than average while a YS_i value greater than the mean performance is considered a stable genotype [24]. The multivariate stability measures, AMMI and GGE, are another most commonly methods used to estimate stability of genotypes in multi-location trials. Two types of biplot models that are extensively used are AMMI (the additive main effects and multiplicative interaction) biplots and GGE (genotype + genotype × environment) biplots. Introduced by [25], the additive main effects (G and E) and multiplicative interaction (GE) model, or AMMI model, combines ANOVA and PCA in a single model. GGE biplots display both G (genotype) and G × E (genotype by environment), which are the major sources of variation.

MATERIAL AND METHODS

Experimental Design and Methods

The study was conducted in 2021 off-season at nine wheat-growing locations (medium to high altitude ecology) of the different part of Oromia under irrigation conditions. These locations represent the main multi-location variety testing sites for the Oromia region wheat improvement program for mid to highland agro-ecologies i.e., (Shambu, Hareto, Arjo, Sinana, Dodola, Mechera, Delo Mena, Dero Lebu and Fiche) (Figure 1). Twelve durum wheat varieties (Table 1) were used in experiment arranged in randomized complete block design (RCBD) with three replicates. Plots 3 m long and 10 rows, with spacing of 0.3 m between rows and 0.5 m between plots were used. Distance between blocks was 1.5 m. The fertilizers (Urea = 100 kg ha^{-1}, NPS = 100 P_2O_5 kg ha^{-1}) were applied based on previous practice in the irrigable areas. Urea Fertilizer application was on split basis; half at planting and half at 25–30 days after planting and NPS applied all at planting. NPS is a compound fertilizer

containing nitrogen, phosphorous and sulfur with the ratio of 19% N, 38% P_2O_5 and 7% S and Urea (46N-0-0). All experimental plots irrigated uniformly using furrow irrigation methods in 10 days interval until the wheat crop reached physiological maturity. Other management practices performed as per previous recommendations. The grain from each genotype and replication was collected on a plot basis. The grain quality traits were investigated at the Sinana Grain Quality Laboratory. The grain quality traits studied were protein, gluten and moisture contents were determined using MINIFRA SmarT grain analyser [26] for each plot. The Mininfra SmarT analyzer transmits light with a wavelength range of 800–1064 nm.
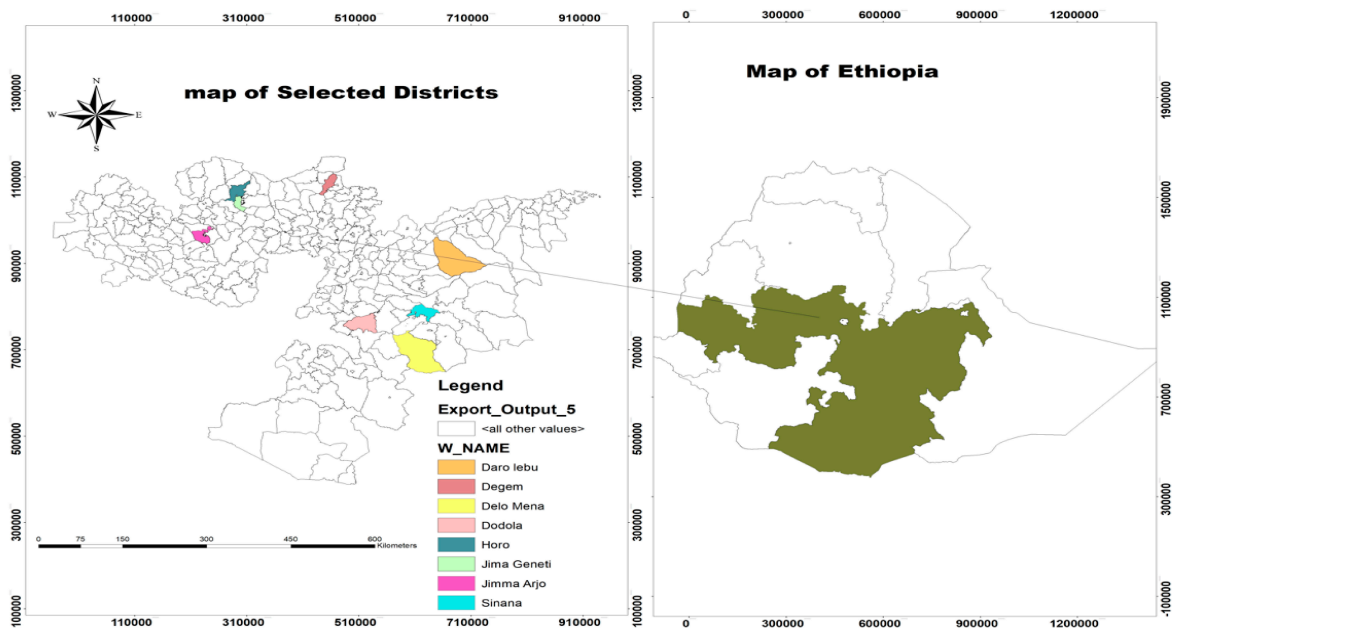
Figure 1. Map of study areas of Shambu/Horo, Hareto/Jimma Geneti, Arjo/Jima Arjo, Sinana, Dodola, Fiche, Delo Mena, Daro Lebu and Dodola in Oromia, Ethiopia.

Table 1. List of durum wheat varieties used in the study.

Variety	Year of released	Center of released
Bulala	2017	Sinana agricultural research center
Mukiye	2012	Debre zeit agricultural research center
Dambi	2009	Debre zeit agricultural research center
Yerer	2002	Debre zeit agricultural research center
Werer	2009	Debre zeit agricultural research center
Mangudo	2012	Sinana agricultural research center
Don matteo	2018	CGS Italian
Ude	2002	Debre zeit agricultural research center
Toltu	2010	Sinana agricultural research center
Utuba	2009	Debre zeit agricultural research center
Dire	2012	Sinana agricultural research center
Alemtena	2015	Debre zeit agricultural research center

Statistical Analysis

Primary statistical tests, such as Bartlett's homogeneity test was used to examine whether the residuals' variance was homogeneous and the Anderson-Darling normality test for determining whether the data are normally distributed were evaluated. The qualitative parameters, including protein, gluten and moisture contents, of twelve durum wheat varieties in nine environments were collected and subjected to analysis of variance (ANOVA) using R software to determine the presence of variation among genotypes, locations, years, genotype by location, genotype by years, and genotype by location by years (genotype by environmental interaction). G (genotypes) is a fixed effect, while E (environment, which may be a combination of locations and years), genotype by environment interaction and block/replication were considered as random factors [27]. However, the major constraint of ANOVA is the inability to distinguish genotype differences in non-additive terms such as GEI [28]. If there is a significant interaction between the genotype and the environment, then an additional statistical analysis was carried out to determine the stability level of the evaluated genotypes across the entire nine environments.

Two types of stability statistical model were utilized to examine the stability of quality traits in durum wheat varieties: univariate and multivariate. The univariate stability parameter measure includes corrected means by least squares (Y), regression coefficient (bi) and deviation from regression (S^2d), Coefficient of variance (CVi) and Wricke (Wi^2) Eco valence, yield stability statistic (YSi) and Shukla's stability variance (σi^2). Multivariate stability such as GGE and AMMI biplot were estimated using R and PBSTAT software. GGE biplots and AMMI are graphical images to exemplify G × E interaction and genotype ranking based on mean and stability. Graph generated is based on multi environment evaluation (which-won-where pattern) and tested environment correlation and discriminative versus representative. The GGE and AMMI biplots were constructed using the first and second principal components (PC1 and PC2) that were derived by subjecting environment-centered means of quality traits to singular value decomposition.

RESULT AND DISCUSSION

AMMI Analysis of Variance for Genotype, Environment and Genotype by Environment Interactions

The result of AMMI analysis of variance for protein, gluten and moisture content in durum wheat for 12 genotypes and 9 environments are presented in Table 2. The AMMI analysis of variance revealed that quality traits such as protein, moisture and gluten contents were significantly ($p < 0.001$) influenced by main effects (genotypes and environments) (Table 2). These results were in line with the previous findings [29–31] that environmental conditions, along with genotype, are

of great significance in durum wheat quality traits. A significant level of environmental variance was found in this study, which might be attributed to climatic factors such temperature fluctuations, precipitation, soil fertility, nitrogen concentration fluctuations and humidity during grain filling. Different authors [10,32] also reported that quality traits of durum wheat is strongly affected by genotypes, as well as changes in biotic and abiotic environmental factors. The main elements influencing the stability of crop variety attributes are, according to [11–13], climatic conditions such high or low temperatures, humidity during grain filling, precipitation distribution, nitrogen concentration, soil fertility and water availability. Protein, gluten and moisture contents of durum wheat were significantly ($p \leq 0.05$) affected by genotype × environment interactions. Similarly, [33] also reported that the genotype and environmental influence were highly significant ($p < 0.001$) for protein and gluten, whereas genotype × environment interactions were affect significantly ($p \leq 0.05$) protein and gluten contents in durum wheat. Environmental variance was the most significant source of variation, accounting for 71.5% (gluten), 71.1% (moisture) and 33.2% (protein) of the total variation (Table 2). A large sum of squares for environments indicated the environments are diverse, with large differences among environmental means causing most of the variation in durum wheat quality traits such protein, moisture and gluten contents. Several researchers have also reported the high influence of environment and genotype × environment interaction in determining durum wheat quality [28,30,32,34].

The presence of G × E interaction was clearly demonstrated by the AMMI model (Table 2) in which eight of the principal component axes were explained. IPCA-I significantly ($p < 0.001$) affected the G × E interaction for all studied traits, accounting for 34.4% of protein, 47.1% of gluten and 48% of moisture in the total interaction. The interaction was explained by IPCA-II in terms of protein (31.8%), moisture (18.8%) and gluten (21.6%) of the total interaction. Many researchers witnessed that the best accurate AMMI model prediction can be made using the first two IPCA [35]. The remaining interaction principal component axes captured mostly non-predictive random variation and did not fit to predict validated observations [25,36]. Based on this, the first two interaction principal components explained for 66.2% (protein), 66.8% (moisture) and 68.7% (gluten) of the total variation (Table 2). The two principal components (IPCA-IPCA-II and I) together captured above 50% interaction principal components. Several authors also reported for various crops that significant and greater percentage of G × E interaction (>50) was explained by the first two IPCA score [37,38].

Table 2. Analysis of variance for protein, moisture and gluten contents using Additive Mean Effect and Multiple Interactions (AMMI) model.

Source of variation	Degree of freedom	Protein			Moisture			Gluten		
		MS	G × E explained (%)	Variance explained (%)	MS	G × E explained (%)	Variance explained (%)	MS	G × E explained (%)	Variance explained (%)
Environment	8	10.92**	-	33.17866	84.57***	-	71.10504	858.9***	-	71.5
Replication	9	0.39ns	-	1.365188	1.9**	-	1.870632	19.38**	-	1.8
Genotype	11	6.96***	-	29.35921	3.03***	-	3.503757	26.98 ***	-	3.1
G × E	88	0.38*	-	12.83123	0.92*	-	8.523987	9.67**	-	8.9
PC1	18	0.61***	34.4	-	2.12***	48	-	22.26***	47.1	-
PC2	16	0.23ns	31.8	-	0.95ns	18.8	-	11.47*	21.6	-
PC3	14	0.14ns	12.9	-	0.80ns	13.9	-	7.96ns	13.1	-
PC4	12	0.12ns	9.8	-	0.78ns	11.6	-	6.89ns	9.7	-
PC5	10	0.04ns	4.6	-	0.29ns	3.6	-	4.10ns	4.8	-
PC6	8	0.04ns	3	-	0.28ns	2.8	-	2.79ns	2.6	-
PC7	6	0.05ns	1.8	-	0.18ns	1.3	-	1.53ns	1.1	-
PC8	4	0.01ns	1.2	-	0.11ns	0	-	1.23ns	0	-
Residuals	99	0.2746	-	-	0.621767	-	-	5.70	-	-

Note: *** = $p < 0.001$, ** = $p < 0.01$, * = $p \leq 0.05$, ns = non-significant.

Table 3. Means (corrected by least squares) (Y), regression coefficient (bi), deviation from regression (S^2d), Wricke's ecovalence (W^2i), Shukla's stability variance (σi^2), Kang's stability statistic (YSi) and Coefficient of variance (CVi) for protein, moisture and gluten contents among 12 durum wheat tested in eight locations.

Genotype	Proteins							Moisture							Gluten						
	Y	CVi	bi	s^2di	σi^2	Wi^2	YSi	Mean	CVi	bi	s^2di	σi^2	Wi^2	YSi	Mean	CVi	bi	s^2di	Wi^2	σi^2	YSi
Alemtena	11.11	8.16	1.12*	−0.08ns	0.08	0.35	4	14.17	14.65	1.07ns	−0.01ns	0.58	2.23	3	29.88	22.61	1.09*	0.59ns	26.45	6.97	1
Bulala	11.01	6.95	0.89ns	0.02ns	0.21	0.77	0	14.59	12.73	1.2ns	−0.06ns	0.56	2.18	10	31.11	19.96	1.02ns	−0.12ns	19.11	4.76	10
Danbi	11.07	5.66	0.73**	−0.05ns	0.22	0.81	2	14.08	11.79	0.86**	−0.11ns	0.5	1.97	2	30.11	17.83	0.88**	−1.64ns	12.59	2.81	3
Dire	11.09	7.15	0.94ns	−0.02ns	0.18	0.68	3	14.23	15.99	1.17**	0.08ns	0.98	3.56	5	30.31	24.91	1.23**	0.47ns	38.31	10.53	2
Don Matteo	11.13	8.72	1.20**	−0.07ns	0.14	0.54	5	14.23	10.84	0.73**	0.26ns	1.73	6.07	−4	30.1	17.24	0.78**	3.17*	56.24	15.91	−6
Mangudo	11.23	6.31	0.88*	−0.09ns	0.06	0.27	9.5	14.02	13.22	0.88**	0.48*	1.68	5.91	−7	30.56	20.11	1.03ns	9.01***	83.24	24	−1
Mukiye	11.24	8.62	1.21**	−0.08ns	0.11	0.46	11	14.24	15.59	1.15**	−0.03ns	0.7	2.63	6	30.33	22.63	1.11*	0.73ns	28.41	7.55	5
Toltu	11.29	9.36	1.04ns	0.39***	1.04	3.56	4	13.74	16.85	1.19**	0.13ns	1.13	4.07	−3	29.11	25	1.18**	1.05ns	36.22	9.9	−3
Ude	11.04	5.38	0.74**	−0.11ns	0.09	0.39	1	14.78	11.87	0.90*	−0.03ns	0.6	2.29	12	32.34	15.38	0.80**	−0.62ns	27.29	7.22	13
Utuba	11.23	6.77	0.95ns	−0.10ns	0.01	0.1	9.5	14.31	12.6	0.95ns	−0.21ns	0.14	0.79	7	30.39	18.81	0.94ns	−2.01ns	6.73	1.05	6
Werer	11.13	9.05	1.20**	0.02ns	0.32	1.14	6	14.71	14.28	1.01ns	0.61**	1.83	6.42	3	31.94	21.79	1.05ns	7.12**	70.65	20.23	3
Yerer	11.53	8.39	1.10ns	0.10ns	0.44	1.54	12	15.27	12.52	0.98ns	0.04ns	0.64	2.45	15	33.51	16.79	0.91*	−0.29ns	20.39	5.15	15

Note: *** = $p < 0.001$, ** = $p < 0.01$, * = $p \leq 0.05$, ns = non-significant.

Univariate Stability

Six univariate stability parameters, namely, regression coefficient (bi) and deviation from regression (S^2d), Coefficient of variance (CVi), Wricke (Wi^2) Eco valence, yield stability statistic (YSi) and Shukla's stability variance (σi^2) were used to determine the stability of the quality traits in durum wheat genotypes.

Higher linear component value than non-linear value suggested the probability of prediction for yield performance over the environments [39]. Hence, linear regression coefficient (bi) and non-linear deviation from regression (S^2d) of G × E interactions were considered for stability analysis [20,40]. According to [20], a genotype is considered stable if the residual mean squares from the regressions model on the environment index are small. Genotype is said to be stable across a wide range of environment if it has a regression coefficient not different from unity (bi = 1) and the regression deviation is not different from zero ($S^2d = 0$). The high value of regression (bi > 1) indicates that the variety is more responsive for the input rich environment, while the low value of regression (bi < 1) is an indication that the variety may be adopted in a low environment [41]. In this investigation, the regression coefficient value ranged from 0.73 (Dambi) to 1.21 (Mukiye) for protein, 0.73 (Don matto) to 1.19 (Toltu) for moisture and 0.78 (Don matto) to 1.23 (Dire) for gluten (Table 3). According to the coefficients of linear regression slope [40,42] modified regression coefficient and α parameter of [43] regression model, variety "Utuba" could be considered the most stable variety across the test locations in protein, moisture and gluten contents. According to [41,44], Mukiye variety is relatively more responsive for the input-rich environment that could be greater adaptation to specific high-yielding environments, and the Danbi variety could be adopted in a low environment could be performing well in low productivity environments for protein, moisture and gluten contents. According to deviation from linear regression method [20] variety Bulala is the most stable with the value of −0.02 (protein), 0.06 (moisture) and 0.12 (gluten) (Table 3).

According to [21,22], a genotype with low W^2i and σi^2 is considered stable. Shukla's stability variance (σi^2) is strictly a measure of stability, rather than performance while Wricke's ecovalence (W^2i) defines the contribution of each genotype to the G × E interaction sum of squares. According to these two parameters, genotypes with the least stability variance σi^2 and Wricke's ecovalence W^2i is ranked highest and considered the most stable. As a result, variety Utuba is considered stable for the quality traits studied. According to [5] the range of variables indicates the level of interaction in response to genotypes across environments. Genotypes with the lowest interaction variance are less responsive to the environment, while larger variances indicate environmental influences.

According to [23], genotype identified as stable if the Coefficient of variability (CVi) value was less than average. In this regard varieties with their coefficient variation (CVi) below the average in protein (7.54), moisture (12.58) and gluten (20.26) contents are Bulala, Danbi, Ude and Utuba (Table 3). [45] developed a yield-stability statistic (YSi) as selection criteria once the G × E interaction is significant and demonstrates the significance of emphasizing stability performance for yield selection. Hence, genotypes with a YSi value greater than the mean protein (5.58), moisture (4.08) and gluten (4.0) are considered stable. Based on this result, Utuba and Yerer varieties were the most stable for protein, moisture and gluten contents (Table 3).

Polygon View of GGE Biplot (Which-Won-Where Pattern)

The GGE biplot simultaneously study of the genotype main effect (G) and the genotype by environment interaction (GE) effect. The G and GE are the two major sources of variation for genotype evaluation in multi-environment trials (MET) [46]. The generated biplot is specifically used for mega-environment to show the which-won-where pattern based on genotype mean performance and stability across the tested environments [47]. The 'which-won where' pattern is an effective tool for visualization of the patterns between genotypes and environments interaction [46]. It also shows the presence or absence of crossover G × E interaction, which explains the possibility of the existence of different mega-environments [48].

In GGE biplot, a polygon was drawn by joining the vertex genotypes, which were placed far from the origin, with straight lines and hence, all the other genotypes were enclosed within the polygon [49]. The genotypes, which placed far from the biplot origin (vertex genotypes), are the poorest or best performing in some or in all tested environments [36]. The winning cultivar is located at the vertex where two sides of the polygon join, whose perpendicular lines form the sector's borderline. As shown in Figure 2a,b,c, the vertex genotypes for Protein were Bulala, Yerer, Toltu and Danbi where as Toltu, Mangudo, Ude, Yerer, Werer and Dire were the vertex genotypes for both gluten and moisture traits. These genotypes perform better or worse in some or all environments because they are the furthest from the biplot origin [36]; they are thought to be especially suited genotypes because they are more sensitive to changes in the environment. On the other hand, the variety, which was located near the origin, was less responsive than the corner (vertex) varieties. Hence, the Utuba and Mangudo were located to be near the biplot origin for protein, moisture and gluten, and they were less responsive to environmental variation than the vertex varieties (Figure 2a,b,c). [35] also reported that the genotype at the vertex of each sector had the highest yield in the environment that falls within that particular sector. According to [47], genotypes inside a polygon closer to the origin are less affected by environmental changes. According to [47]

Utuba and Bulala varieties could be less responsive in all evaluated environments because they are located close to the polygon's origin for gluten, moisture and protein traits (Figure 2a,b,c). Varieties fall in the vertex where no environment falls in the sector show that such genotype gave a poor stable across the environments [47]. According [47], the Toltu variety was the least stable across environments since its location in the vertex, where no environment falls within the sector.

In "which-won-where" GGE biplot, lines from the origin divide the biplot into different sectors and create different mega environments [50]. The nine testing environments were clustered into three, four, and four mega-environments of the tested durum wheat genotype for protein, moisture and gluten levels, respectively (Figure 2a,b,c).

The 3 mega-environments formed for protein content (Figure 2a): The first mega-environment included one environment (Shambu) and three varieties (Alemtena, Dire and Toltu), Toltu is the vertex variety of the group. The second mega-environment contained a single environment (Harato) and variety (Mangudo). The third mega-environment encompasses many environments (Sinana, Dodola, Daro lebu, Dalo mana, Arjo and fiche) with the winning varieties of Bulala, Ude, Done matteo, Mangudo and Utuba. Bulala was located in the vertex.

Four mega-environments constituted for moisture content (Figure 2b): Group I consisted of a single environment (Harato) and three varieties (Utuba, Danbi and Mangudo), with mangudo as the vertex genotype. Group II included two environments (Sinana and Dodola) and a single variety (Ude). Group III had three environments: Fiche, Arjo and Shambu Delo mena, as well as two genotypes: Bulala and Yerer, with yerer as the vertex genotype. There was only one environment (Daro-Labu) and one variety (Werer) in Group-IV.

The four mega environments formed for gluten content (Figure 2c): Group I had a single environment (Harato) and four varieties (mangudo, Done matteo, Bulala, Utuba and Danbi), Mangudo variety was the vertex genotype for this group. Group II included three environments (Dodola, Arjo and Sinana) and a single variety (Ude). Group III included two settings (Fiche and Shambu) and one variety (yerer). Group IV included two environments (Delo Mena and Dero Lebu) and one variety (Werer).

The identifying mega-environments could be useful in managing the genotype-by environment interactions and then generalizing the results to similar agroclimatic locations [51]. According to this finding, the target environment is separated into four different mega-environments for moisture and gluten, and three distinct mega-environments for protein.

(**a**)

(**b**)

(**c**)
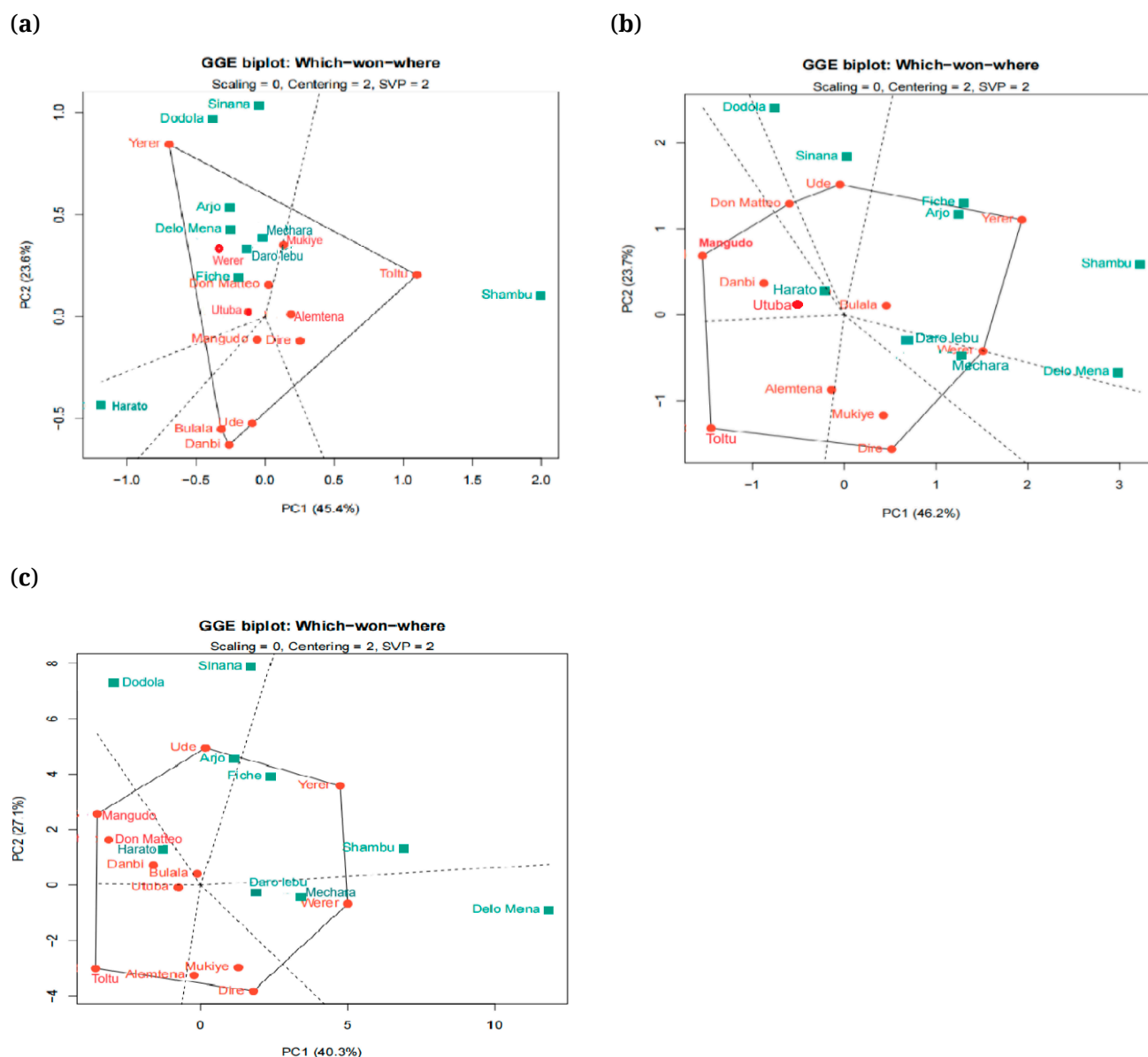
Figure 2. The which-won-where view of the GGE biplot to show which durum wheat variety stable in which environment for (**a**) Protein, (**b**) Moisture and (**c**) Gluten contents.

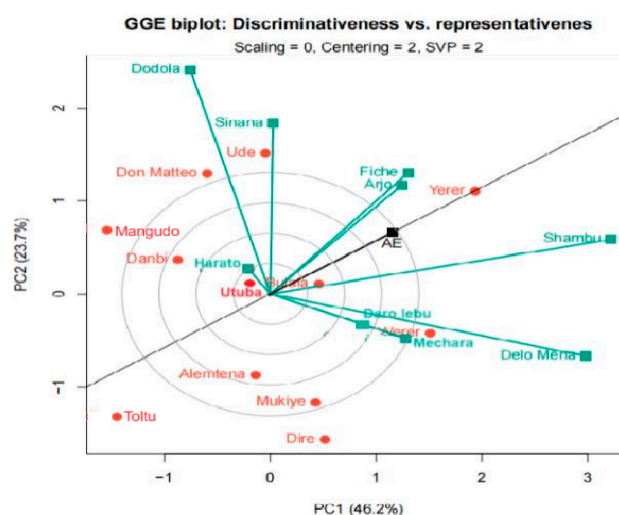**Relationship among Environments and Discriminative vs Representativeness**

The angle between the vectors of two environments has a meaningful relation with the correlation coefficient between them and are used to group the test environments [52]. Environment IPCA1 and IPCA2 scores had both positive and negative scores which give rise to the crossover non-crossover GEI, leading to disproportionate genotypes yield differences across environments [35]. The angle between the vectors of two environments is related to its correlation coefficient [50]. Acute angles indicate a positive correlation, obtuse angles a negative correlation and right angles no correlation [36]. The relationships among the nine test environments in the present study are presented in

Figure 3a,b,c. According to [36], the strong similarity was observed among Sinana, Delo Mena Dodola, Arjo and Fiche environment, and between Harato and Daro Labu for protein contents. Whereas Harato, Shambu and Dodola negative association among each other's. Between Sinana and Dodola, and Arjo and Fiche locations the existence of strong positive correlation between them for moisture contents. Shambu and Dalo Mena, and Arjo and Harato had strong correlation between those environments for gluten contents. In this study, there is existence of strong relationship among/between in some environments for protein, moisture and gluten, indicating that the obtained information was very similar therefore testing environment could be reduced to minimize cost without significantly affecting the validity of information. [50,53,54] also reported that the presence of close association among test environments suggests that the same information about genotypes could be obtained from few test locations, and hence by dropping one or two environments from each group can reduce cost of multiplications replicated trials. On the other hand, Arjo and Harato had an angle > 90 and negatively correlated with Dodola, sinana, Fiche and Dalo Mena for gluten contents (Figure 3c). Furthermore, Dodola had obtuse angle (>90°) with Delo Mena, Shambu and Daro Labu that it has negative correlation with these environments for moisture contents (Figure 3b). Thus, if environments were negatively correlated, genotypes performing best in one environment would perform less in the other environment and vice versa.
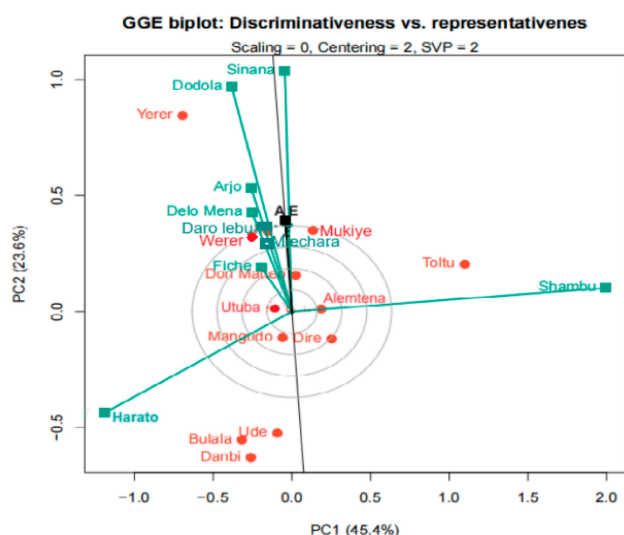
(a) (b)

Figure 3. GGE biplot view showing the relationship among the testing environments and discriminative and representativeness for (**a**) Protein, (**b**) Moisture and (**c**) Gluten contents.
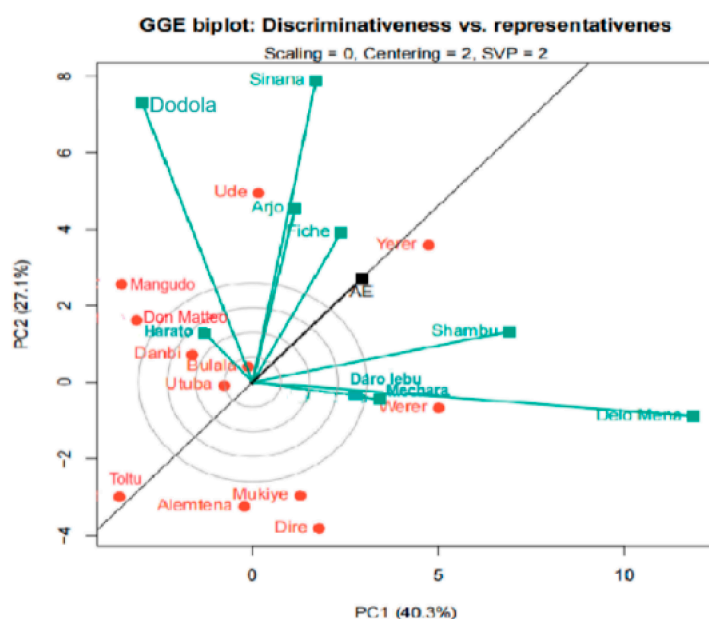
(c)

Figure 3. *Cont.*

CONCLUSION

Protein, gluten and moisture in durum wheat are among the most critical elements that affect the end-use quality of wheat-based food products. This study investigated 12 common Ethiopian tetraploid wheat cultivars to identify a variety with consistent protein, gluten and moisture contents across several environments for commercial cultivation in Ethiopia. Multivariate (ANOVA, AMMI and GGE biplot) and univariate (bi, S^2d, σi^2, Wi^2, YSi and CVi) models were used to identify stable varieties for qualitative traits in durum wheat. The AMMI analysis of variance revealed that the environment, genotype, and G × L interaction had a significant effect on protein, moisture and gluten contents in durum wheat varieties. Environmental factors were the leading cause of variation, accounting for 71.5% (gluten), 71.1% (moisture) and 33.2% (protein). Univariate statistical analysis models showed that Utuba was stable across the testing environments for protein, gluten and moisture traits. The GGE biplot clustered the nine test locations into 3, 4 and 4 mega environments for protein, gluten and moisture, respectively, with the utuba variety winning in the majority of the environments. Based on the univariate and multivariate models' analysis, Utaba variety is the most stable and widely adaptable across the test locations, and it is unaffected by environmental fluctuations. To sustainably strengthen row materials for the pasta industry and produce a high-quality product, it is essential to scale out the Utuba variety across large areas. It could also be utilized in breeding programmes to improve quality attributes in durum wheat, as well as stable breeding material for commercial production.

DATA AVAILABILITY

The corresponding author will make data related to the study available upon request.

AUTHOR CONTRIBUTIONS

The contributions of all authors to the preparation of this manuscript are listed below: Geleta Gerema was in charge of data management and analysis, as well as full paper writing and experiment supervision; Girma Mengistu was in charge of project planning, data analysis, and manuscript editing; and Tilahun Bayissa and Urgaya Balcha were in charge of data management, review manuscript and experimental supervision.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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