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Genotype by Environment Interaction and Stability Analysis for Grain Yield in White Seeded Tef [*Eragrostis tef* (zucc.)Trotter] Genotypes in Western Oromia, Ethiopia

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Abstract

Tef [*Eragrostis tef (Zucc.) Trotter* L.] is a most important cereal crop in Ethiopia in terms of production, consumption and cash. The study was carried out to investigate grain yield stability and genotype by environment interaction for 18 genotypes conducted in the potential high land areas of Western Oromia, Ethiopia for two consecutive years (2020 to 2021) using Randomized Complete Block Experimental Design with three replications. The study of variance for grain yield using the AMMI model indicated highly significant variation for genotypes, environment, and genotype-environment interactions. Environment accounted for 18.7% of the variance in grain yield, 17.9% for genotypes, and 61.5% for genotypes. The first IPCA component accounted for 47.9% of the interaction effect and revealed the two models were fit. Genotypes G15, G10, G4, G1, and G3 had the lowest AMMI stability value (ASV), indicating stability; genotypes G16, G14, G9, G7, G2, and G5 had the highest ASV value, indicating instability. From over all analysis genotype G1 and G3, showed a high mean grain yield, lowest GSI, ASV and stable compared to other genotypes in the study. As a result, G1 and G3 were identified as the best genotypes for future breeding programs and potential release in Western Oromia, Ethiopia's highlands.

Keywords

AMMI, ASV, Genotypes, Stability, Tef

1. Introduction

Tef (*Eragrostis Tef Zucc* L). Trotter is Ethiopia's most significant cereal crop in terms of production, consumption, and cash crop value. In Ethiopia, tef is cultivated on around three million hectares yearly, involving over 7.1 million families, with a total grain yield of about 5.7 million tons. [1]. Tef accounts for almost 30% of the total cultivated area and one-fifth of the gross grain yield of all cereals grown in the country [2] Tef adapts to extreme environmental conditions and persist in diverse socio ec nomic conditions. Tef's agro-

nomic merits include broad and versatile agro-ecological adaptation; tolerance to both drought and water-logging conditions; fitness for various cropping systems and crop rotation schemes; usefulness as a reliable and low-risk catch crop at times of failures of other long-season crops such as maize and sorghum due to drought or pests; and little vulnerability to pest and disease epidemics in its major growing regions [3, 4]. In terms of dietary qualities, tef grain is gluten-free and contains all eight essential amino acids, as well as high contents

of fiber, minerals, and vitamins [5]. In addition, in terms of forage, it has high feed quality, crude protein content, fast growth rate, and its suitability for multiple harvests [6].

The interaction of genotype and environment affects crop performance and adaptation to various environments [7]. One of the most serious concerns in plant breeding research is properly analyzing genotype $\times$ environment (G x E) interactions using data from multi-environment trials [8]. Different models were developed to investigate the interaction of GEI. Some of the most commonly used stability models for estimating the magnitude of G x E interactions include additive main effects and multiplicative interaction (AMMI) and genotype by environment interaction (GEI) [9, 10] to identify high-yielding and better adapted genotypes [10]. The AMMI model is a hybrid model that incorporates both additive and multiplicative components of a two-way data structure, allowing a breeder to make precise predictions about genotypic potential and environmental influences on it [11]. It has been heavily used because it incorporates both the classical additive main effects for GEI and the multiplicative components into an integrated least square analysis, making it more effective in the selection of stable genotypes [12]. AMMI uses ordinary ANOVA to analyze the main effects (additive part) and principal component analysis (PCA) to analyze the non-additive residual leftover by the ANOVA [7, 13]. The effectiveness of the AMMI procedure has been demonstrated by various authors using multi-location data in tef [8-14]. G x E interaction study, or assessing genotypes for broad and particular adaptation to a microenvironment, is crucial for

yield stability of tef cultivars [15]. As there are very limited studies on G x E in tef crop, the importance of conducting more studies across major tef growing environments has been suggested [4, 16]. The GGE biplot is based on principal component analysis and graphically displays the two-way (genotype x environment) data matrix [17]. It allows for mega-environment analysis ('which - won - where' pattern), genotype evaluation (mean vs. stability), and test environment evaluation (discriminating power vs. representativeness) [14, 15, 17, 21]. Understanding G x E interaction helps breeders determine the best breeding strategy, choose locations and input systems, and develop crop varieties for different agro-ecologies. Therefore, this study was designed to assess the magnitude of GEI, adaptability, and stability of various white seeded genotypes for grain yield in the study area.

2. Materials and Methods

2.1. Description of Experimental Sites

The experiment was conducted at three potential tef growing of Horo, Chalia and Jimma Arjo Districts. These districts are situated in Horo Guduru, West Shewa and East Wellega zones of Western Oromia, Ethiopia. As a result, Shambu is in Horo district, Gedo is in Chaliya district and Arjo is in Jimma horo district (Table 1 & Figure 1).

Table 1. Descriptions of study area and physio-chemical properties of soils.

Districts	Soil Parameters	Result	Soil Status	Remark	Climate Data	
					Temperature (℃)	Rainfall (mm)
Horo (Shambu)	pH (H$_2$O)	5.38-5.63	Strong Acid to Moderate		10.78-22.32	1566
	%OC	3.08-4.46	high			
	%OM	3.93-6.09	Moderate to high			
	%TN	0.20-0.37	Moderate to high			
	avaP	3.73-4.68	Low	Bray II Method		
Chaliya (Gedo)	pH(H$_2$O)	4.49-5.18	Very strong acid to strong Acid		11-28	900-1400
	%OC	2.2-3.88	Moderate to High			
	%OM	3.80-6.69	Moderate to high			
	%TN	0.19-0.32	Moderate to high			
	avaP	3.75-5.92	Low	Bray II Method		
Jimma Arjo (Arjo)	pH(H$_2$O)	4.45-5.98	Very strong acid to Moderate acid		16.8-36.5	1200-2200
	%OC	1.17-2.11	Low to Moderate			
	%OM	2.02-3.63	Low to Moderate			

Districts	Soil Parameters	Result	Soil Status	Remark	Climate Data	
					Temperature (℃)	Rainfall (mm)
	%TN	0.1-0.18	Low to Moderate			
	avaP	2.74-3.93	Low	Bray II Method		

Source: Bako Agricultural Research Center Laboratory Result, 2022.
Key: pH (H₂O) =Power of Hydrogen, OC=Organic Carbon, OM=Organic Matter, TN=Total Nitrogen, avaP=available Phosphorus
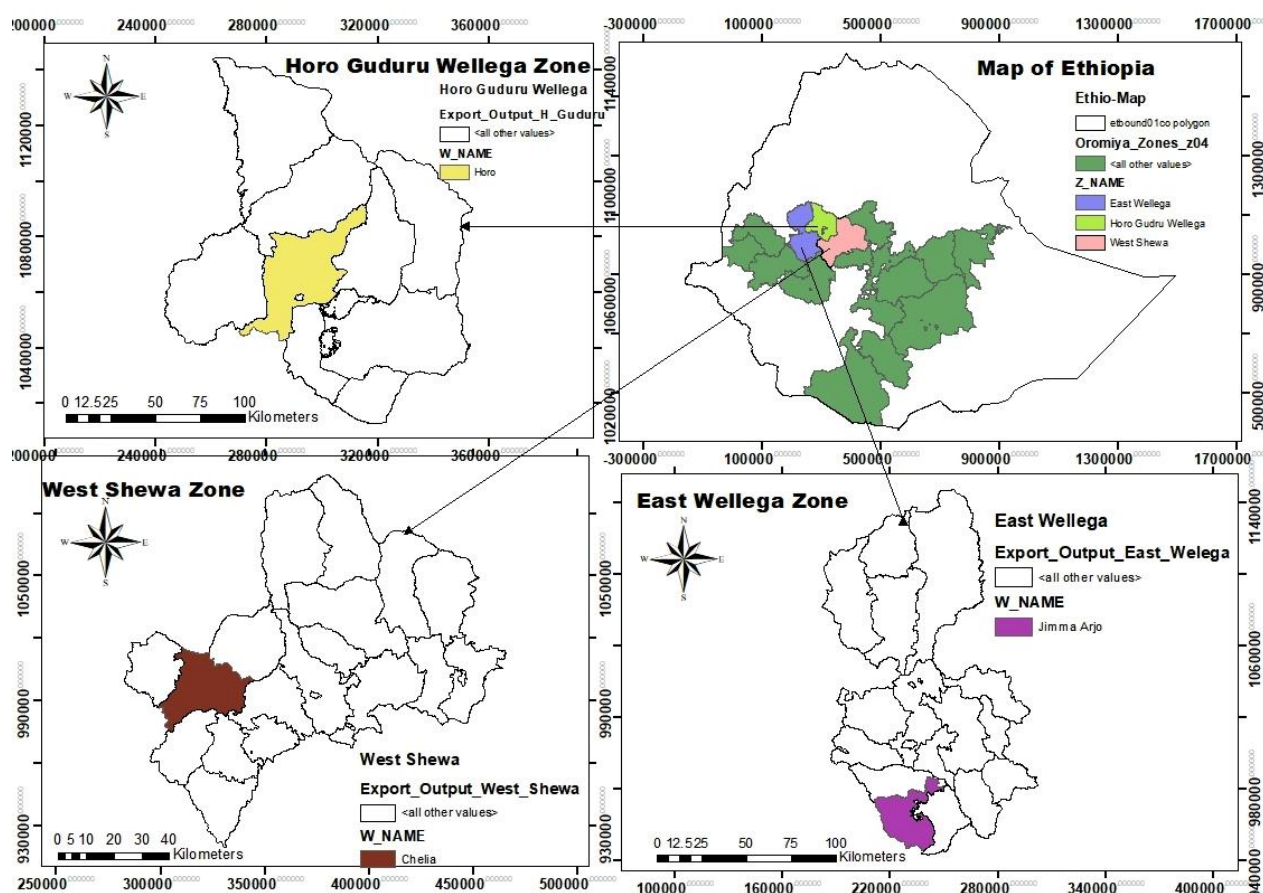

Figure 1. Geographical location of map of selected districts of western Oromia.

2.2. Experimental Materials and Design

Sixteen promising genotypes were used for this study including two tef varieties released from Bako agriculture research center. The experiment was conducted using Randomized Complete Block design with three replications on a plot size (experimental unit) of 2m x 2m (4m²) each with 0.2m of row spacing. The distance between block was 1.5m and between plots was 1.0m. Fertilizer rate of 100/50 kg DAP/UREA at planting and 10 kg/ha of seed rate will used. Other agronomic practices were applied uniformly as required.

Table 2. Detailed descriptions of the planting materials.

No.	Entry code	Genotypes code
1	G1	BK-01-1817
2	G2	BK-01-0217
3	G3	BK-01-0917
4	G4	BK-01-1017
5	G5	BK-01-0317

No.	Entry code	Genotypes code
6	G6	BK-01-0617
7	G7	BK-01-7617
8	G8	BK-01-7717
9	G9	BK-01-3817
10	G10	BK-01-1617
11	G11	BK-01-4717
12	G12	BK-01-7217
13	G13	BK-01-2717
14	G14	BK-01-2917
15	G15	BK-01-3017
16	G16	BK-01-2417
17	Check	Dursi
18	Check	Local

G= genotype, BK-01-1817= (BK=Bako. 01=first collection for Bako, 1817= accession no.

2.3. Data Collection

Grain yield (g) of each plot was measured, sun-dried and the measured grain yield value (g) has converted to kilogram per hectare for data analysis.

2.4. Data Analysis

The analysis was performed by using R computer software. The R package 'metan' and "ggplot2" were specifically designed to analyze multi-environmental trials (METs) [19] This package provides a workflow-based procedure with sequential functions for evaluating commonly used parametric and nonparametric stability statistics [20]. The 'metan' package offers comprehensive tools for managing, analyzing, and visualizing MET data. It has been successfully used to quantify yield stability in a variety of crops, [21-23]

3. Results and Discussion

3.1. Mean Grain Yield (kgha^{-1}) of White Tef Genotypes Tested over Location and Years

The highest grain yield obtained from G1 (BK-01-18179) 2.55 tone ha^{-1} whereas least mean grain yield was obtained from G7 (Bk-01-7617) 1.39 tone ha^{-1}. The mean grain yield across locations ranged from the highest 2.62 tone ha^{-1} for Shambu in 2021 cropping season, and the lowest 1.07tone ha^{-1} for Gedo in 2020 cropping season (Table 3). The grand mean for grain yield across locations and years was 1.88 tone ha^{-1}. (table 3)

Table 3. Mean grain yield (tone ha^{-1}) of white tef genotypes tested over location and years.

Environments							
Row Labels	Arjo 2020	Arjo 2021	Gedo 2020	Gedo 2021	Shambu 2020	Shambu 2021	Grand Total
G1	2.56	2.57	2.48	2.50	2.58	2.62	2.55
G10	2.07	1.26	1.40	1.99	2.14	1.88	1.79
G11	2.18	1.20	1.39	2.11	1.89	2.02	1.80
G12	1.71	1.20	1.35	1.47	1.46	1.92	1.52
G13	1.60	1.17	1.23	1.74	1.61	1.77	1.52
G14	2.04	1.90	1.90	2.34	1.94	2.37	2.08
G15	2.38	2.14	2.32	2.11	2.19	2.20	2.22
G16	2.33	1.84	2.15	2.64	2.24	2.35	2.26
G17	1.99	2.06	2.03	2.04	2.03	2.02	2.03
G18	1.78	1.72	1.63	1.91	1.82	1.81	1.78
G2	2.06	1.19	1.07	1.54	1.57	2.02	1.58
G3	2.47	2.54	2.49	2.60	2.56	2.52	2.53
G4	1.99	1.24	1.31	1.91	1.67	1.69	1.64
G5	2.00	1.23	1.55	1.75	1.29	2.25	1.68

Environments							
Row Labels	Arjo 2020	Arjo 2021	Gedo 2020	Gedo 2021	Shambu 2020	Shambu 2021	Grand Total
G6	2.11	1.28	1.18	1.85	1.47	1.82	1.62
G7	1.63	1.23	1.06	1.24	1.38	1.77	1.39
G8	2.31	1.79	1.27	2.28	1.85	2.02	1.92
G9	1.93	1.44	2.16	2.00	2.02	2.14	1.95
Grand Total	2.06	1.61	1.67	2.00	1.87	2.07	1.88

3.2. AMMI Analysis

To identify high-yielding and stable genotypes across the environments, the AMMI1 biplot was generated. AMMI analysis (Table 1) showed that the effects of genotype, environment and genotype by environment interaction (GEI) were highly significant ($p < 0.01$). In this study, it was observed that there were significant differences in the environment, genotype, and their interactions. Significant variance at 1% level which explained 18.7% of the total variation whereas the GEI accounted for 17.9%, and the genotypes captured 61.5% of the total sum of squares (Table 4). Similar significant variation for the genotypes by environment interaction, and the environments were reported by [25, 10] in tef, [26, 27] in sorghum, [28] in maize and [29] in wheat highly

Significant difference was obtained for the first three PCA. According to [30] AMMI with two, three, or four IPCA axes is the most effective predictive model. In the current study, the AMMI analysis revealed that the first three interaction PCs accounted for a total of 85.4% of the interaction sum of squares. According to [31] the interaction sum of squares is best explained by the first two principal component axes. In the current study, the IPCA4's contribution of 9.70% to the interaction sum of squares is considered noise. PC1's mean squares significantly impacted grain yield GEI ($P < 0.001$) (Table 4). The significant interaction result indicates that genotypes react differently in different contexts. The high variation in genotype traits for tef genotype grain yield demonstrated in the present study is consistent with previous reports on genotype variability by other authors [10, 23, 26, 27, 32].

Table 4. ANOVA for grain yield of 18 white seeded tef genotypes for the AMMI Model.

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)	Proportion (%)	Accumulated
ENV	5	10.90	2.18	22.87	0.00	18.68	
REP(ENV)	12	1.14	0.10	2.19	0.01	2.4	
GEN	17	35.91	2.11	48.53	0.00	61.5	
GEN: ENV	85	10.46	0.12	2.83	0.00	17.9	
PC1	21	5.01	0.24	5.48	0.00	47.9	47.9
PC2	19	2.16	0.11	2.62	0.00	20.7	68.6
PC3	17	1.75	0.10	2.37	0.00	16.8	85.3
PC4	15	1.02	0.07	1.56	0.09	9.7	95
PC5	13	0.52	0.04	0.92	0.53	5	100
Residuals	204	8.88	0.04				
Total	408	77.76	0.19				

DF = Degree of freedom, Sum sq = sum of square, Mean sq = Mean of square, IPCA = Interaction principal component axis

The AMMI1 biplot plots the means of the genotypes and

environments against their PCA1 (Figure 2A). The AMMI1

biplot revealed that those environments located at Arjo 2021 and Gedo 2020 produced the lowest yields across the genotypes. Similarly, G7 and G2 showed the minimized grain yield average across environments but stable while G1, G3 and G13 are high yielding across all locations presented in the current research but unstable. G8 is ideal genotype (Figure 2A).

G8, G17, G2, G5, and G11 have higher G×E (far from the origin), making them more sensitive to environmental changes and thus better suited to their surroundings. These genotypes are thought to be more stable and respond better to environmental changes than other genotypes and recommended for specific area adaptation [33, 34]
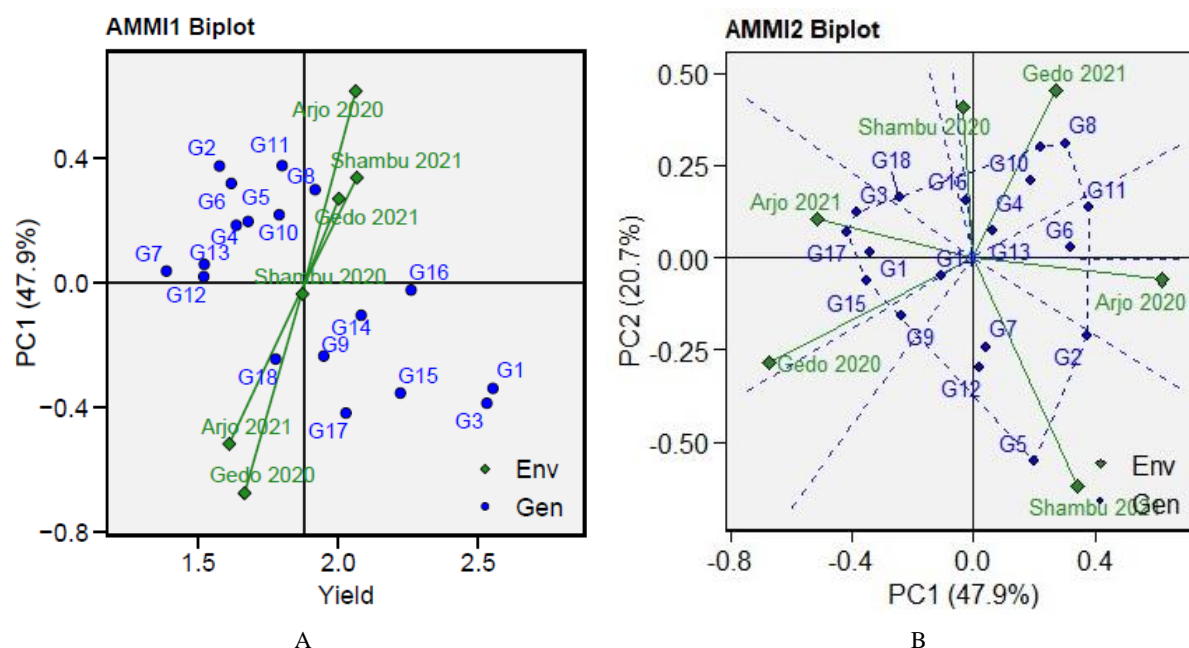

Figure 2. (A) The "AMMI1" biplot displays the main effect (Grain Yield) and IPC1 effect values explaining the relationship among tested white tef genotypes and environments. (B) The "AMMI2" biplot displays the main axes of G+GEI effect (IPCA1 and IPCA2) values for the tested genotypes and environments.

In contrast, the widely adapted genotypes G14, G13, G1, G4, and G6 had fewer interactions because they were closer to the origin and thus less susceptible to environmental changes and those genotypes were recommended for wider adaptation (Figure 2b). However, the other genotypes interacted with insignificantly similar outcomes, as reported by [35].

Based on AMMI stability value (ASV) and = weighted average of absolute scores (WAAS) genotypes G13 (0.03), G16 (0.16,) G14 (0.24) and G12 were with low ASV and WAAS were the most stable (Table 5). This analysis also confirmed that G17, G2 and G11 were the most unstable genotypes in the present study with ASV value 0.97, 0.90 and

0.88, respectively (Table 5). However, stable genotypes would not predictably provide the best yield performance and therefore identifying genotypes with high grain yield together with consistent stability across growing environment is important. Therefore, genotype selection index (GSI) which combine both mean yield and stability in a single index have been introduced to further detect high yielding genotypes with stable yield performance, through diverse growing environments [8, 12, 33, 36]. In the present study genotype selection Index (GSI) showed that the most stable and high yielding genotypes were G1 and G3 whereas, G17, G2, G11 and G6 were the least stable (Table 5).

Table 5. Mean grain yield, Stability Parameters, ASV and GSI for 18 white seed color tef genotypes tested across years.

GEN	Mean R	Mean R	ASI	ASI_R	GSI	ASV	ASV R	WAAS	WAAS R
G1	2.55	1	0.16	13	14	0.78	13	0.22	10
G10	1.79	10	0.12	9	19	0.59	9	0.23	11
G11	1.80	9	0.18	15	24	0.88	15	0.28	16

GEN	Mean R	Mean R	ASI	ASI_R	GSI	ASV	ASV R	WAAS	WAAS R
G12	1.52	17	0.06	5	22	0.30	5	0.08	3
G13	1.52	16	0.03	1	17	0.16	1	0.07	2
G14	2.08	5	0.05	3	8	0.24	3	0.07	1
G15	2.22	4	0.17	14	18	0.82	14	0.22	9
G16	2.26	3	0.03	2	5	0.16	2	0.10	4
G17	2.03	6	0.20	18	24	0.97	18	0.27	15
G18	1.78	11	0.12	8	19	0.59	8	0.19	7
G2	1.58	15	0.19	16	31	0.90	16	0.29	17
G3	2.53	2	0.19	17	19	0.79	14	0.26	13
G4	1.64	13	0.10	6	19	0.48	6	0.16	6
G5	1.68	12	0.15	10	22	0.72	10	0.25	12
G6	1.62	14	0.15	11	25	0.74	11	0.21	8
G7	1.39	18	0.05	4	22	0.26	4	0.13	5
G8	1.92	8	0.16	12	20	0.76	12	0.31	18
G9	1.95	7	0.12	7	14	0.56	7	0.27	14

ASV=AMMI Stability value, GSI=Genotype selection Index, WAAS= weighted average of absolute scores

3.3. GGbiplot Analysis

Which won where View of the GGE bi-plot

The GGE biplot polygon view based on the which-won-where structure of a mega-environment test approach is the simplest and most efficient method for detecting the genotype and its environmental interactions. The first two principal components (PC1 and PC2) obtained by singular value decomposition of environment-centered data of grain yield explained 82.93% of the total effect on grain yield variation, with PC1 and PC2 accounting for 82.93% and 6.73% of variability, respectively (Figure 3) using environment-centered data. In the which-won-where view of the GGE biplot, a polygon is drawn on genotypes that are furthest from the biplot origin circumscribing all other genotypes and used for interpreting GEI and detecting superior genotypes across different environments [37, 38]. The genotypes at the corner of each section of those environments had the highest yield for the corresponding environments. The genotypes joined by the polygon are the farthest from the origin and called vertex genotypes. Those vertex genotypes located in the same sector or close to a specific environment are considered the best genotypes for this environment. [39, 40, 41]. Accordingly, G1 best perform at Shambu 2021 followed by Arjo 2020. The equality lines, perpendicular lines drawn to each side of the polygon, divide the biplot into sectors [39, 40]. The pattern in Figure 3 suggests that the target environment could be divided into two different mega-environments. The first mega-environment is

composed of environments Shambu 2021, Arjo 2020, Shambu 2020 and Gedo 2021 with genotype (G3, and G16, as a winner genotypes (Figure 3). The second Mega-environment is composed of Arjo 2021 and Gedo 2020 represented by genotype G3, G15, and G9 were vertex genotypes and a winner. Genotypes found closer to the origin of the axes have wider adaptation and less responsive for environment variation [37, 39, 41]. Accordingly, G14, G5 and G7 were found to be genotypes of wider adaptation [41, 42].
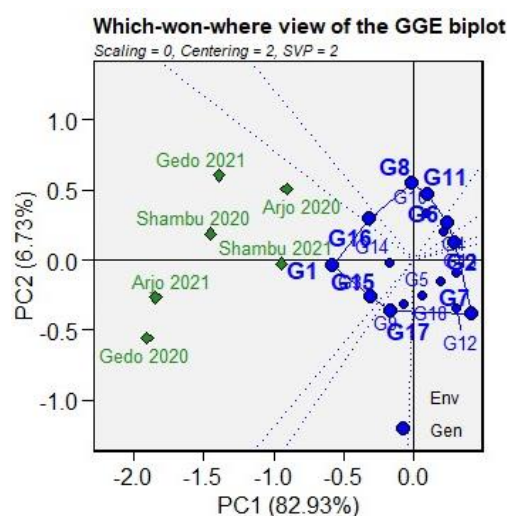

Figure 3. *The which-won-where view of the GGE scatter bi-plot showing white seeded tef genotypes with best performance in each environment and mega environments (MGEs) for grain yield.*

3.4. GGE Biplot—Means Versus Stability Model and Ranking of White tef Genotypes' Performance

The genotypes' mean performance versus stability biplot is a useful visual tool for distinguishing the tested genotypes (Figure 4A, B). This biplot depicts the two PCs (1 and 2) that, by additive percentage, explain the G + GE effects.

The single-arrowed line in the biplot (Figure 4A) represents the average environmental average (AEA), indicating higher mean performance across the tested genotypes. In terms of the AEA, the average environment corresponding to the average values of the two PCs is indicated by the arrowhead in Figure 4A and circled in Figure 4B. The genotypes in the circle are considered the best genotype. The perpendicular line to the AEA is known as the average ordinate environment (AOE), and its intersection represents both average mean performance and high stability. Other perpendicular lines linking the genotypes to the AEA explain the genotype's stability. According to comparison biplot for genotypes based on concentric circle, G1, G3, G9 and G14 were found near the concentric circle. This indicated genotypes are with high grain yield and stable (Figure 4B). Similar result was reported by [21, 37, 39]. The closeness of genotypes to the AEA explains their stability across environments. By using the ranking of the biplot, the ideal genotype is (G3), being in the center of the circle being high yield and the best adaptability among the other genotypes. To rank genotypes from worst to best, follow the AEA direction [21, 37, 42]. Accordingly, G7<G12<G13<G2…<G14<G17<G15<G16<G3<G1 (Figure 4A).
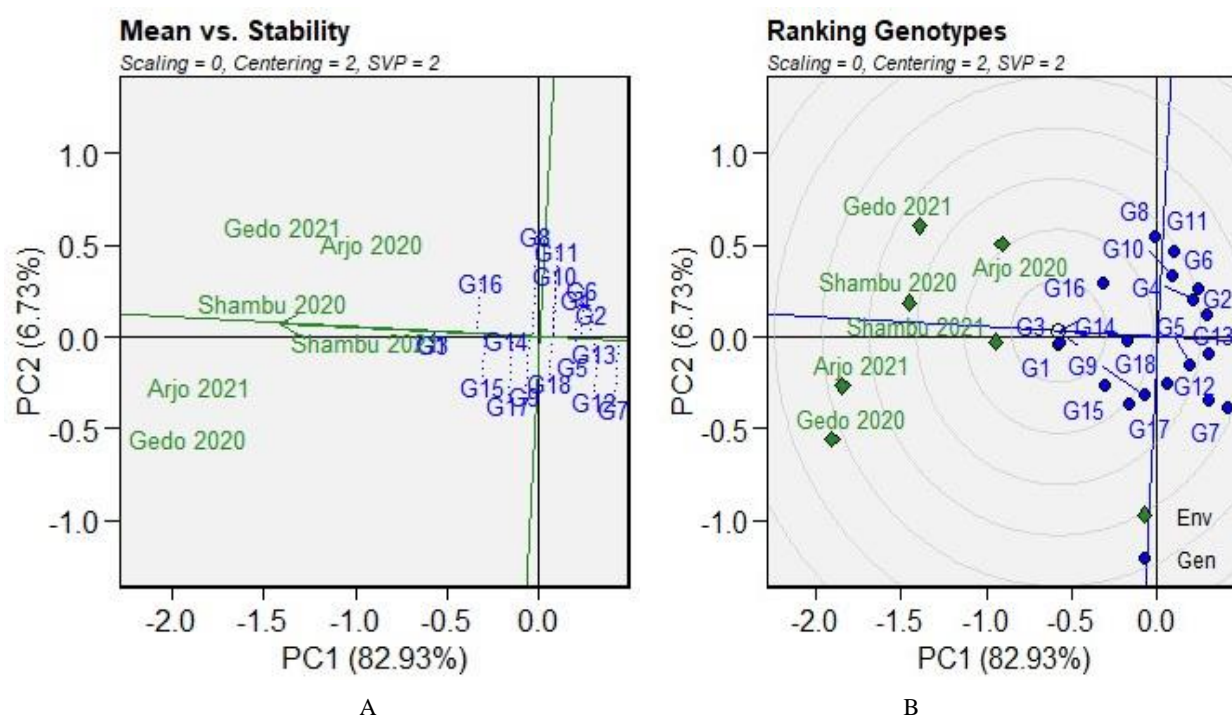

Figure 4. (A) The "mean versus stability" model describing the interaction effect of the tested tef genotypes evaluated across six environments. (B) The "ranking genotypes" model of biplot to assess the ideal genotype. The tested genotypes are 18 (G1:G34 in blue color) grown in four locations in the two consecutive years.

Discriminative vs. representativeness and ranking environments relative to an ideal environment

In a variety performance trial, evaluating test environments is critical for determining the most desirable genotypes for a mega environment. Figure 5 depicts the "discriminating ability vs. representativeness" view of the GGE biplot. The distance between the environmental markers and the biplot origin is a measure of its discriminative ability [43-45]. Test environments with longer vectors are more discriminative of genotypes, whereas test environments with short vectors provide little information about genotype differences [44, 45].

As a result, of the six environments evaluated, Gedo 2020, followed by Arjo 2021, were the most discriminating of the genotypes, while Arjo 2020 was the least discriminating of all test environments. The ideal test environment discriminates between genotypes and is representative of the target environments [40, 45, 46]. [46] define representativeness of a testing environment as the angle between the environment vector and the abscissa of the average environment axis. The smaller the angle, the more representative the test environment [47]. Thus, Shambu 2021 and Shambu 2020 were identified as more representative environments.
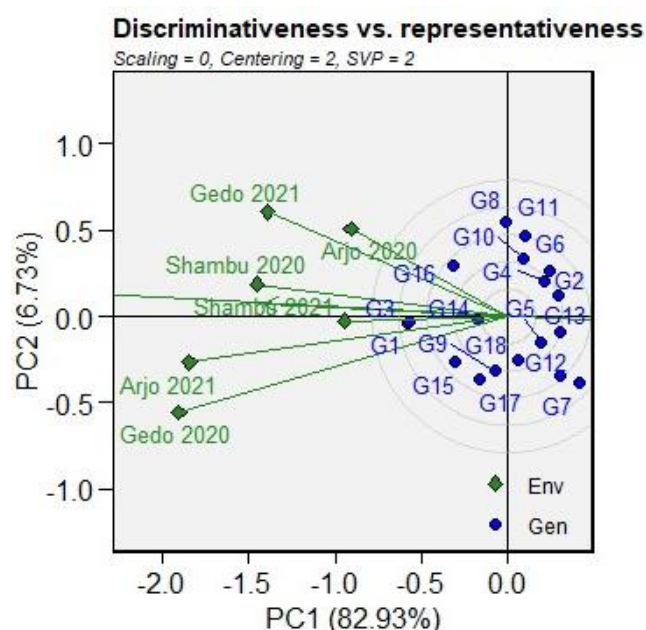
Figure 5. The "discriminating power vs. representativeness" view of the GGE biplot based on 18 tef genotypes tested at five test environments.

4. Conclusion

The current study found that the Genotype had the greatest impact on tef grain yield, followed by the Environment and the interaction of Genotype by environment. The potential and diversity of of the genotypes identified for future breeding programs. The presence of a G ×E interaction for grain yield indicates how the environment affects the trait's expression. The GGE biplot model was effective for analyzing and visualizing the G x E pattern, as well as identifying the most high-yielding and stable genotype, discriminating ability, and representativeness of the test environments. The G ×E interaction resulted in a change in genotype performance rankings across environments. Genotypes G1 (BK-01-1817) and G3 (BK-01-0917) had high mean grain yield and most stable among all tested genotypes across environments. In addition, AMMI analysis of variance effect revealed that highly significant difference for genotypes, environment interaction. Shambu 2021 environment was identified as high yielding and best desirable testing environment for tef. As a result, the AMMI, GGE biplot, and which-won-where results revealed that genotypes G1 and G3 are widely adaptable genotypes that can be recommended for release as varieties.

Acknowledgments

Abbreviations

AEA	Average Environmental Average
AMMI	Additive Main Effects and Multiplicative Interaction
AOE	Average Ordinate Environment
ASV	AMMI Stability Value
G x E	Genotype by Environment
GEI	Genotype by Environment Interaction
GSI	Genotype Selection Index
MET	Multi-Environmental Trials
WAAS	Weighted Average of Absolute Scores

Author Contributions

Girma Chemeda: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing

Natol Bakala: Data curation, Formal Analysis, Investigation, Software, Validation, Visualization, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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