

Vol.7, Issue.1, pp.72-78, February (2020)

E-ISSN: 2347-7520

Multilocation Evaluation for yield and Yield Related Traits in Three-Way Cross Maize Hybrids in Kenya

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Available online at: www.isroset.org

Received: 05/Feb/2020, Accepted: 20/Feb/2020, Online: 28/Feb/2020

Abstract— Maize (*Zea mays L.*) is the third most important crop after wheat and rice worldwide. It is the main staple food in Kenya. The objective of this study was to evaluate genotype by environment interactions and yield stability of twenty three-way cross hybrids at four locations in Kenya evaluated in two seasons. The experiment was conducted in an alpha lattice design (Incomplete Randomized Block Design) with three replications. There was significant variation for grain yield among the genotypes, locations and their interaction. Stability analysis was evaluated using the joint regression, additive main effects and multiplicative interactions (AMMI) and GGE biplot methods. The environmental and genotypic means ranged from 2.72 to 7.67 and 2.39 to 5.56 respectively. The regression coefficient (β i) and deviation from regression (s²di) values of these genotypes ranged from 0.55 to 1.64 and 0.02 to 0.59 respectively. There were also significant differences for genotypes, environments and genotype by environment interaction for the AMMI analysis of variance. The total proportion of variation contributed by genotypes, environments and genotype by environment interaction was 8.82%, 76.03% and 9.17% respectively. When considering the Pi, β i, S² di and the AMMI biplot analysis, the most stable genotype in the high yielding category in this study considering all stability parameters was WE-CMT-TWC-1001 (G1) followed by WE-CMT-TWC-1003 (G3) and WE-CMT-TWC-1020 (G20). The best genotype with both high mean yield and high stability was WE-CMT-TWC-1003 (G3). The genotypes identified could be utilized as reference for genotype evaluation and tested further for selection.

Keywords— Multilocation trial, grain yield, three way cross maize hybrids

I. INTRODUCTION

Maize (*Zea mays L.*) is the main staple food for many nations and third most important crop after wheat and rice worldwide [1,2]. It is the main staple food in Kenya estimated to contribute to about 68% of daily per capita cereal consumption, 35% of total dietary energy consumption and 32% of total protein consumption thereby indicating that Kenya's national food security is strongly linked to production of adequate quantities of maize to meet an increasing domestic demand [3].

In plant breeding, the process of identifying genotypes with high yield potential and yield stability across environments is a fundamental activity [4]. Identification of stable genotypes by plant breeders is usually difficult due to the presence of genotype by environment interaction (GEI) [5]. GEI causes the relative ranking of genotype performance to change across environments and thereby affecting breeding progress [6]. Due to GEI effect, genotypes with wide adaptation are rarely identified [7].

Various stability analyses using the GEI have been developed in order to identify genotypes with good yield performance and yield stability across different environments [8]. These stability analysis methods include, additive main effects and multiplicative interaction model (AMMI), principal component analysis (PCA), linear regression analysis, analysis of variance (ANOVA) and GGE biplot analysis [9]. The ANOVA describes the main effects with no information on individual genotypes and locations, which are elements of the interaction and the AMMI uses the principal component analysis to explain genotype performance by incorporating the use of ANOVA and PCA [10]. The AMMI explains the main effects of genotypes, environments and their interaction by combining the additive components in a single model for the main effects of genotype and environment and multiplicative components of their interaction [11].

The linear regression analysis proposed by Eberhart and Russell, [12] classifies variation in genotype performance into predictable (regression) and unpredictable (deviation from regression) evaluating yield and stability respectively [13]. According to this model, a relatively lower value of regression coefficient (β i) around 1 (β i-1) will mean a genotype is less responsive to environment and therefore more adaptive [4]. Deviation from regression (s²di) if significantly different from zero will mean the genotype is less stable across environments and if not significantly different, the genotype is stable [12]. If the phenotypic index (pi) is negative, the genotype has a low grain yield and if phenotypic index is positive, the genotype has a high grain yield [14]. The environmental index (Ij) reflects the

suitability of an environment to hybrid maize production. Negative environmental index reflects a poor environment while positive environmental index reflects a favorable environment for the hybrid maize production [15].

The GGE (genotype plus genotype-by-environment) biplot analysis combines the genotype and genotype by environment effects in genotype evaluation [6]. It uses graphic axes to identify candidate genotypes in the mega environments (groups of environments sharing the same test genotypes) [16]. The GGE biplot also incorporates ANOVA and PCA by classifying genotypes and genotype by environment interaction sum of squares together by use of the PCA method [17].

Because of increasingly importance of maize production in Kenya there is need to improve its production and thereby improve food security. This will be achieved by growing high yielding and stable maize varieties. Therefore, this study aims to evaluate the yield performance and yield stability of three way cross maize hybrids across four environments in Kenya using AMMI, GGE biplot and joint regression methods.

II. METHODOLOGY

Germplasm:

The experimental materials used in the study were 20 three way cross hybrids and four commercial local check varieties (Table 1). The 20 three way crosses were obtained from the Kenya Agricultural and Livestock Research Organization (KALRO) breeding program.

Experimental sites:

The experiments were conducted at KALRO Kakamega, KALRO Katumani, KALRO Kiboko and KALRO Kitui in Kenya. The agro-climatic descriptions of the four experimental sites are presented on table (Table 2).

Experimental design:

The 20 three way cross hybrids together with four local checks were planted in an Alpha Lattice Design (Incomplete Randomized Block Design) with three replications in all the locations for two seasons. Each hybrid was sown in two row plot of 5.0 m. Two seeds were planted in each hill and thinning was later done to one plant per hill. Plant spacing was 0.75 m between rows and 0.25 m between hills.

A recommended application of fertilizer for nitrogen (60 kg N ha⁻¹) and phosphate (60 kg P_2O_5) was applied for each location to ensure healthy and vigorous plants. The experimental sites were also kept free of weeds by hand weeding throughout the growth cycle of the plants. Supplemental irrigation was done when necessary.

Data collection and analysis:

Data on yield and yield related characters was collected according to the standard protocols provided by CIMMYT [19].

Analysis of variance for every location was done for grain yield and yield related characters using the SAS computer program [20]. Bartlett's test was used to evaluate the homogeneity of error variances before the combined analysis of variance across environments.

The stability analysis for genotype by environment interaction was estimated using the AMMI model [21,22]. In this model, the contribution of every genotype and every environment to the genotype by environment interaction is estimated using the GGE biplot whereby genotype mean yields and environmental means are plotted against the first interaction principal component axes scores (IPCA1). AMMI analysis computational program is supplied by Durate and Zimmermann [22].

The regression model stability parameters, regression coefficient (β i) and deviation from regression (S²di) were calculated according to the method proposed by Eberhart and Russell [12]. The t-test was used to test the significant differences among the β i values and unity while the F-test was used to test significance of the S²di values.

III. RESULTS

ANOVA for grain yield (t ha⁻¹) and yield related traits

The analysis of variance for grain yield within locations showed significant differences for genotypes (Table 3). The analysis of variance also showed significant differences for grain yield (GY), number of plants harvested (NP), number of ears harvested (NE), grain moisture content percentage (MOIST) and ear aspect (EA) across genotypes, locations and their interaction (Table 4).

Eberhart and Russell joint regression model

The environmental and genotypic means ranged from 2.72 to 7.67 and 2.39 to 5.56 respectively. Twelve genotypes had a higher grain yield (positive phenotypic index) and also twelve genotypes had a lower grain yield (negative phenotypic index). Kiboko (-0.78), Katumani (-1.08) and Kakamega (-1.54) were poor environments for hybrid maize production while Kitui (3.41) was the best for hybrid maize production. The regression coefficient (β i) and deviation from regression (s²di) values of these genotypes ranged from 0.55 to 1.64 and 0.02 to 0.59 thus showing that these genotypes responded differently to different environment (Table 4).

According to the joint regression model, the most stable genotypes as indicated by the lowest (s^2 di) values were WE-CMT-TWC-1017 with a genotype mean of 4.35 which was ranked eleventh with a phenotypic index (pi) of 0.09, regression coefficient (β i) of 1.03 and deviation from regression (s^2 di) of 0.02 then followed by WE-CMT-TWC-1008 with genotype mean of 4.16 (ranked fourteenth) with pi value of -0.10, β i value of 0.81 and s^2 di value of 0.05 and WE-CMT-TWC-1003 with genotype mean of 5.27 (ranked second) with pi value of 1.01, β i value of 0.80 and s^2 di value of 0.06. The most unstable genotype as indicated by the highest s^2 di value was WE-CMT-TWC-1007 with a

mean of 3.93 (ranked eighteenth) with pi value of -0.34, β i value of 1.64 and s²di value of 0.59. The genotype was classified as the most unstable because its s²di value was significantly different from zero and bi value was significantly different from 1 as compared to the rest (Table 5).

Additive main effects and multiplicative interaction (AMMI) analysis

The combined analysis of variance (ANOVA) according to the AMMI 2 model indicated that there were highly differences significant (p≤0.01) for genotypes, environments and the interaction of genotype by environment (Table 4). The IPCA were ordered according to decreasing importance [8]. All genotypes showed highly significant differences for the first IPCA scores (Table 6). The total variation explained (%) was 8.82% for genotypes, 76.03% for environments and 9.17% for genotype by environment interaction and the two IPCA axes explained 88.4% of the genotype by environment interaction. The first IPCA captured 66.02% of the total interaction sums of squares in 36% of the interaction degrees of freedom and the second IPCA captured 22.38% of the interaction sum of squares in 33% of the interaction degrees of freedom (Table 6).

The analysis of the GGE biplot

The GGE biplot gives a visual expression of the relationship between the first principle component analysis (IPCA) and the means of the genotypes and environments.

The IPCA scores of genotypes indicate the stability or adaptation of the genotypes to the environments. The greater the IPCA score, whether negative or positive (as it is a relative value) the more specifically adapted is a genotype to certain environments. The more the IPCA scores are close to zero, the more adapted or stable the genotype is across all the environments sampled. The environment scores from AMMI analysis relating to interaction also have a meaningful interpretation in that, environments with large IPCA scores are more discriminating of genotypes while environments with IPCA scores near zero show little interaction across genotypes and low discrimination among genotypes [8].

From the biplot analysis, environments are categorized into four parts i.e Quadrants I (top left) and IV (Bottom left) as lower yielding environments and Quadrants II (top right) and III (bottom right) as the high yielding environments. Therefore, the high yielding environment as indicated by the biplot analysis is Kitui and lower yielding environments are Kakamega, Katumani and Kiboko. Kitui showed more discrimination for genotypes as compared to Kakamega, Katumani and Kiboko (Figure 1).

The genotypes categorized under favorable environments considering the IPCA 1 scores with above average means were G1 (WE-CMT-TWC-1001), G3 (WE-CMT-TWC-1003), G18 (WE-CMT-TWC-1018), G20 (WE-CMT-TWC-1020) and G14 (WE-CMT-TWC-1014). Among them, G18 (WE-CMT-TWC-1018) was considered more

stable with IPCA values close to zero. The genotypes categorized under low yielding environments are categorized into the upper and lower left quadrants of the biplot. G21 (PH3253 (Local check)) was categorized as the most unstable genotype according to the AMMI model. Genotypes that are close to environment indicates their better adaptation to that environment, therefore G7 (WE-CMT-TWC-1007) was the best adapted to Kiboko and Katumani while G24 (WH505 (Local check)) was best adapted to Kakamega.

Since IPCA2 also played an important role (22.38%) of explaining the genotype by environment interaction, IPCA1 scores were plotted against IPCA2 scores to further explain the adaptation (figure 2). G1 (WE-CMT-TWC-1001), G21 (PH3253 (Local check)) G24 (WH505 (Local check)), G10 (WE-CMT-TWC-1010) and G7 (WE-CMT-TWC-1007) were categorized as unstable genotypes. G9 (WE-CMT-TWC-1009), G12 (WE-CMT-TWC-1012), G15 (WE-CMT-TWC-1015), G18 (WE-CMT-TWC-1018), and G19 (WE-CMT-TWC-1019) were categorized as moderately stable genotypes. G17 (WE-CMT-TWC-1017) was categorized as the most stable according to the model.

When considering the regression model, AMMI analysis and the GGE biplot, the most stable and ideal genotypes in the high yielding category in this study were WE-CMT-TWC-1001 (G1), WE-CMT-TWC-1003 (G3) and WE-CMT-TWC-1020 (G20). The best genotype with both high mean yield and high stability was WE-CMT-TWC-1003 (G3). The genotypes can therefore be recommended as reference for genotype evaluation and tested further for selection.

IV. DISCUSSION

Genotype performance

Significant mean squares for grain yield from the analysis of variance for the four locations indicated that the mean yield of genotypes differed from location to location due to environmental diversity. Similarly, there were also significant differences among the genotypes thus indicating that the genotypes differed in their yield potential across locations. The presence of significant genotype by location interaction indicated the differential in performance of genotypes across environments, therefore, genotypes performed well in one environment and performed poorly in another environment. Similar results have been reported whereby a change in environment cause genotype by environment interaction on maize [23,16].

The highly significant differences ($p \le 0.01$) in genotypes, locations and genotype by location interaction for grain yield indicated the need to develop varieties that are adapted to particular environmental conditions and varieties that are exceptional in their stability across environments [24].

The significant mean squares for locations and genotypes for days to tasseling, days to silking, plant height, ear height, number of ears, moisture, grain texture and husk cover indicate that the genetic expressions of these traits were affected by environmental conditions at the four locations [25].

Stability analysis

The genotype by environment interaction for grain yield was significant hence showing that the stability parameters (β i and s²di) estimated by linear response to change in environment were not the same for all genotypes across environments. These findings are in agreement with different authors in their study on yield stability of maize genotypes [26,27]. They reported that genotypes, environments and genotype by environment interactions had significant effect on yield of maize genotypes.

The AMMI analysis of variance for all the genotypes indicated that there were large sum of squares and highly significant mean squares for environment hence indicating that the environments were diverse with large differences among the environmental means causing most of the variation in the grain yield. These results are in agreement with the findings of [28,17] who declared significant all the genotypes, environment and genotype by environment effects in the ANOVA of AMMI. Previous research also reported that environment contributed the largest portion of the total variance whereby 80% and above of total sum of square variance is contributed by environment while 10% is contributed by genotype and environment interaction [5].

Genotype performance and stability across environments

A good genotype must have both high mean yield performance and also be stable for selection for broad adaptation [29]. Therefore GGE biplot, regression coefficient (β i) and deviation from regression (s²di) were used to determine the mean performance and stability of genotypes for grain yield because of the significant interaction for grain yield alone.

According to the joint regression model, a stable variety has β i values close or equal to unity (1) and s²di values close or equal to zero (0) [30]. This method has been widely used but it has some difficulties that can be realized from the analysis of the results (Table 4) whereby the genotype PH3253 (Local check) was the most stable when considering s²di values but unstable when considering the β i values. This makes it difficult on using the method alone to recommend high yielding and stable varieties for future production. Similar results were reported by different authors [26,31]. The biplot showed the pattern of variability of genotypes, environments and their interaction, however, different scaling methods for biplot puts different weight on means and stability thereby causing the choice of scaling method to affect the ranking of the genotypes in relation to mean performance and stability [32,33].

Contrary to this, genotype PH3253 (Local check) was high yielding but unstable. This fact can cause a serious challenge to plant breeders in variety selection because the highest yielding genotypes may not be preferred by farmers due to their instability across environments. This finding is also in agreement with [34] who reported that high interaction caused difficulties in selection of high yielding genotypes due to their inconsistency to perform across different environments.

V. CONCLUSION AND FUTURE SCOPE

From the study conducted, yield performance of maize was highly affected by environmental change. There is also need to test the maize hybrids for more environments to promote breeding efficiency for genotype stability across environments. The most preferred genotype was WE-CMT-TWC-1003 (G3), followed by WE-CMT-TWC-10020 (G20) and WE-CMT-TWC-1001 (G1). These hybrids need to be tested further and thereafter be commercially released in order to increase maize production in Kenya in order to increase food security. **Figures and Tables**

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Genotype No.	Genotype Code	Source
1	WE-CMT-TWC-1001	KALRO
2	WE-CMT-TWC-1002	KALRO
3	WE-CMT-TWC-1003	KALRO
4	WE-CMT-TWC-1004	KALRO
5	WE-CMT-TWC-1005	KALRO
6	WE-CMT-TWC-1006	KALRO
7	WE-CMT-TWC-1007	KALRO
8	WE-CMT-TWC-1008	KALRO
9	WE-CMT-TWC-1009	KALRO
10	WE-CMT-TWC-1010	KALRO
11	WE-CMT-TWC-1011	KALRO
12	WE-CMT-TWC-1012	KALRO
13	WE-CMT-TWC-1013	KALRO
14	WE-CMT-TWC-1014	KALRO
15	WE-CMT-TWC-1015	KALRO
16	WE-CMT-TWC-1016	KALRO
17	WE-CMT-TWC-1017	KALRO
18	WE-CMT-TWC-1018	KALRO
19	WE-CMT-TWC-1019	KALRO
20	WE-CMT-TWC-1020	KALRO
21	PH3253 (Local check)	PIONNER
22	DK8031 (Local check)	MONSANTO
23	WE1101 (Local check)	AATF/KALRO
24	WH505 (Local check)	WSCO
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KALRO: Kenya Agricultural and Livestock Research Organization AATF: African Agriculture Technology Foundation WSCO: Western Seed Company

Table 2. Geographical and Climatic data for four sites (locations) used in the study

Site	Geographic Location			Mean annual	Temperature (°C)		Agro-ecology and soil type	Source
	Longitude	Latitude	Altitude	Rainfall	Min	Max		

				(mm)				
Katumani	37°32' E	1°35' S	1580	30 582 13.9 24.7 Semi arid with Loamy sand so		Semi arid with Loamy sand soil	[18].	
Kiboko	37°75' E	2°15' S	993	548	548 17.0 30.6 Semi arid with ferrasols to ferric luvis		Semi arid with ferrasols to ferric luvisol	[6].
						soils		
Kakamega	34°45' E	0°16' N	1585	1995	13.0	28.6 Sub humid with basaltic loam so		[6].
Kitui	38°1'E	1° 22' S	1100	775	14.0	34.0 Arid to semi arid with red sandy soil		[1].

Table 3. Mean square from ANOVA and percentage of variance components for grain yield (tha⁻¹) evaluated for the genotypes in each location (averaged over two seasons)

		K	lakameg	a	Katumani		Kiboko			Kitui			
Source	df	MS	SS	%SS	MS	SS	%SS	MS	SS	%SS	MS	SS	%SS
Bloc/Rep	3	1.44**	4.32	10.67	0.09ns	0.27	0.93	1.59*	4.76	13.28	4.00*	12.00	9.00
Genotype	23	1.32**	30.35	74.94	1.03**	23.63	81.04	1.00*	23.10	64.41	4.11**	94.63	70.95
Error	21	0.28	5.83	14.40	0.26	5.26	18.04	0.38	8.00	22.31	1.27	26.75	20.06
Total	47		40.51			29.15			35.86			133.38	
CV%		19.38			15.72			17.74			14.71		

df = degrees of freedom, MS = mean squares, SS = sum of squares, %SS = percentage sum of squares

Table 4. Mean square from ANOVA for yield (tha⁻¹) and yield related traits evaluated for the genotypes across four locations (averaged over two seasons)

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Source	df	GY	NP	NE	MOIST	EA
Replication	1	3.30	70.08	27.00	2.90	0.00
Genotype	23	3.82**	205.17**	268.41**	17.99**	0.93**
Location	3	252.65**	1456.06**	1788.85**	268.82**	19.37**
Gen × Loc	69	1.32**	21.19*	30.52*	7.28**	0.49*
Error	95	0.59	12.75	20.31	4.24	0.32
S.E (Mean)		0.77	3.57	4.51	2.06	0.56
L.S.D (0.05)		1.53	7.09	8.95	4.09	1.12
CV%		18.10	9.80	12.10	10.30	20.40
Mean		4.26	36.30	37.35	20.01	2.76

df = degrees of freedom, GY = grain yield, NP = number of plants, NE = Number of ears, MOIST = moisture content, EA = ear aspect *and** = Significant at 5% ($p \le 0.05$) and 1% ($p \le 0.01$) respectively

Table 5. Joint regression stability analysis for grain yield (tha⁻¹) across four locations (averaged over two seasons)

Genotype	Pedigree	Locations			Across		P index				
		Kakamega	Katumani	Kiboko	Kitui	Envrmnts	Rank	(Pi)	βi	s²di	Rank
1	WE-CMT-TWC-1001	3.88	4.61	4.11	9.67	5.56	1	1.30	0.83	0.08	5
2	WE-CMT-TWC-1002	2.11	2.32	3.21	6.67	3.57	22	-0.69	1.08	0.09	7
3	WE-CMT-TWC-1003	3.52	4.14	3.90	9.54	5.27	2	1.01	0.80	0.06	3
4	WE-CMT-TWC-1004	2.79	3.73	4.12	7.52	4.54	9	0.28	1.10	0.11	10
5	WE-CMT-TWC-1005	2.26	4.11	3.69	6.94	4.25	13	-0.01	1.11	0.26	22
6	WE-CMT-TWC-1006	3.44	3.30	2.56	9.93	4.81	5	0.55	0.65	0.11	11
7	WE-CMT-TWC-1007	2.74	3.12	4.37	5.49	3.93	18	-0.34	1.64	0.59	24
8	WE-CMT-TWC-1008	2.59	2.55	3.14	8.35	4.16	14	-0.10	0.81	0.05	2
9	WE-CMT-TWC-1009	1.96	3.35	3.92	6.98	4.05	16	-0.21	1.05	0.20	20
10	WE-CMT-TWC-1010	3.19	3.56	4.56	7.12	4.61	8	0.35	1.27	0.19	19
11	WE-CMT-TWC-1011	2.85	3.50	3.20	6.62	4.04	17	-0.22	1.31	0.12	12
12	WE-CMT-TWC-1012	2.71	3.35	3.99	7.53	4.39	10	0.13	1.06	0.08	6
13	WE-CMT-TWC-1013	2.81	3.33	2.90	6.12	3.79	19	-0.47	1.44	0.17	17
14	WE-CMT-TWC-1014	4.04	3.26	2.97	9.86	5.03	4	0.77	0.68	0.14	14
15	WE-CMT-TWC-1015	1.59	3.43	3.41	7.91	4.08	15	-0.18	0.83	0.12	13
16	WE-CMT-TWC-1016	2.58	3.64	4.38	6.72	4.33	12	0.07	1.25	0.27	23
17	WE-CMT-TWC-1017	2.93	3.22	3.58	7.66	4.35	11	0.09	1.03	0.02	1
18	WE-CMT-TWC-1018	2.71	3.18	4.49	8.64	4.75	6	0.49	0.84	0.10	8
19	WE-CMT-TWC-1019	2.27	2.18	3.59	6.90	3.73	21	-0.53	1.02	0.16	16
20	WE-CMT-TWC-1020	4.30	3.88	3.46	8.79	5.11	3	0.85	0.89	0.18	18
21	PH3253 (Local check)	2.83	2.21	2.72	10.78	4.63	7	0.37	0.55	0.07	4
22	DK8031 (Local check)	3.32	2.30	2.69	6.72	3.76	20	-0.51	1.08	0.25	21
23	WE1101 (Local check)	1.58	2.38	2.81	6.10	3.22	23	-1.04	1.15	0.10	9
24	WH505 (Local check)	0.31	1.85	1.78	5.63	2.39	24	-1.87	0.99	0.14	15
	Mean	2.72	3.18	3.48	7.67	4.26					
	Env. Index (Ij)	-1.54	-1.08	-0.78	3.41						
	LSD (0.05)					1.53					

 s^2 di, β i and Pi = deviation from regression, regression coefficient and phenotypic index respectively

Table 6. Analysis of variance (ANOVA) based on the AMMI model for grain yield (t ha⁻¹) for the genotypes across four environments (averaged over two seasons)

Source	df	SS	MS	Total variation	G×E explained	Cumulative
Total	191	996.80	5.22	explained (70)	(70)	(70)
Environments (E)	3	757.90	252.65**	76.03		
Blocks within (E)	4	6.10	1.54*			
Genotypes (G)	23	87.90	3.82**	8.82		
Gen × Env (G×E)	69	91.40	1.32**	9.17		
IPCA 1	25	70.40	2.81**		66.02	
IPCA 2	23	13.40	0.58		22.38	88.4
Residual	92	53.40	0.58			

df = degrees of freedom, MS = mean squares, SS = sum of squares, *and** = Significant at 5% ($p \le 0.05$) and 1% ($p \le 0.01$) respectively



(PCA) axis 1 mean yield (tha⁻¹) for the genotypes grown in four environments. The vertical line represents the grand mean of the experiment while the horizontal line is PCA axis 1=0.



Figure 2. Biplot of interaction principal components analysis (PCA) axis 1 versus axis 2 for grain yield (tha⁻¹) for the genotypes grown in four environments

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ACKNOWLEDGMENT

The author acknowledge the Centre Director, KALRO -AMRI, (Kenya Agricultural and Livestock Research Organization - Agricultural Mechanization Research Institute) for hosting research, providing the germplasm and research facilities. The author thank KALRO-AMRI Kiboko and Katumani staff for assisting in managing field experiments and data collection.

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