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AMMI Biplot Analysis for Stability of Grain Yield in Foxtail Millet [*Setaria italica* (L.) P. Beauv.] Genotypes

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ABSTARCT

Background: Foxtail millet cultivation in India's NEH region holds promise due to its adaptation to diverse environments and high-quality grain. Studying G x E interaction in this region will guide breeding programs to develop foxtail millet varieties adapted to local conditions. The objective of this study was to find out foxtail millet genotypes that produce high yield in diverse environments and to identify ideal mega-environments using additive main effects and multiplicative interaction stability model analysis.

Methods: In this study, 30 genotypes were evaluated at the Research Farm of the SAS, Nagaland University, Medziphema, India. The experiment was conducted during July 2022 to May 2023 involving four different environments. Two environments were rainfed and two were irrigated with weekly intervals. The experiment was conducted in randomized complete block design (RCBD) with three replications in all the environments.

Result: Genotype-environment interactions significantly influenced grain yield across four environments, while replicates were non-significant. Pooled analysis revealed significant genotypic effects and seasonal impacts. AMMI analysis revealed significant effect on environmental and genotypic influences on grain yield and explained 23.47% and 27.94% variability respectively. The AMMI model effectively decomposed the intricate genotype-environment interaction into three principal components (PC1, PC2and PC3), explaining 54.5%, 28.7%and 16.8% of the interaction variance, respectively. AMMI model exposed genotypes *viz* G8, G9, G21and G22 as best performer and stable. AMMI stability value revealed G10 with highest stability (ASV = 0.34) followed by G15, G7, G29, G6and G13 in decreasing order indicating their consistent performance in grain yield across different environments.

Key words: AMMI, ASV, GEI.

INTRODUCTION

Foxtail millet [*Setaria italica* (L.) P. Beauv] is a self-pollinating, C4 cereal crop with a rich history of cultivation dating back to 5000-6000 BC along the Yellow River in China (Li and Wu 1996).The International Year of Millets (2023), a United Nations initiative, aims to raise awareness about the significance of millets as a nutritious and sustainable food source, while promoting their cultivation, consumption and trade.

There are two main groups of methods for analyzing information from multi environmental trails: univariate and multivariate methods. One important multivariate method is the AMMI model, which combines analysis of variance and principal components (PCs) analysis (Gauch, 1992). The first part of AMMI, the additive part, uses analysis of variance, while the second part, the multiplicative part, employs PCs analysis to study Genotype by Environment Interaction (GEI) (Ebdon and Gauch, 2002). The AMMI model is widely used because it provides detailed information about the main effects and GEIand it explains a significant portion of the interaction's variability (Gauch and Zobel, 1997).

In this study, we evaluated 30 foxtail millet genotypes collected from IIMR-Hydrabad to see how they perform in different environments and to find out which ones are the best in yield performance.

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MATERIALS AND METHODS

Experiment location

The investigation was carried out during July 2022 to May 2023 for four different dates of sowing with twenty-five days interval (Table 1). Each sowing date was chosen to create varying environmental conditions, including different temperatures and moisture levels throughout the crop growth stages. Two environments maintained under rained condition and the remaining two environments are maintained under irrigated condition with seven days interval. The experiment was conducted at the Research

Farm of the Department of Genetics and Plant Breeding School of Agricultural Sciences, Nagaland University-Medziphema, India.

Plant materials

Thirty genotypes of Foxtail millet including a check variety collected from Indian Institute of Millets Research (IIMR), Hyderabad were used to assess genetic variability, diversityand stability across different environments. List of 30 genotypes represented in Table 2.

Experimental design and intercultural practice

The experiment used a randomized complete block design (RCBD) with three replications across different environments due to varying site fertility. Each of the three replications had 30 plots (1 m×1 m) spaced 10cm apart, with plants and rows 10 cm and 22.5 cm apart, respectively. The total plot size was 30×5 m, accommodating 90 beds. Recommended agricultural practices were followed throughout.

Data collection

The study embarked on a comprehensive exploration by examining grain yield per plant data across four distinct environmental conditions. This endeavor was driven by the objective of unraveling the intricate patterns of genotype-environment interaction exhibited by the crop within varying agro-climatic settings. This carefully chosen trait was selected based on the comprehensive descriptions and guidelines provided by the Plant Protection Variety and Farmers' Rights Authority (PPV and FR) in 2001, ensuring a robust and standardized framework for analysis.

Statistical analysis

The analysis of variance (ANOVA) was executed using the OPSTAT open-source software to evaluate the pooled dataset. Multiple mean comparison and normality tests were performed using the 'tidyverse,' 'ggthemes,' 'multcompView,' and 'dplyr' packages in R-studio. For the visualization of multivariate stability analysis, GGE biplot and AMMI techniques were employed through the 'Metan' package in R-studio, a tool developed by the R Core Team (Team, 2015).

RESULTS AND DISCUSSION

Analysis of variance

The pooled analysis of variance (ANOVA) was used to examine the interactions between different genotypes and environments. Table 3 presents the results of the pooled ANOVA for all genotypes across various environments, focusing on yield and its components. There were significant variations observed among the different environments (E), genotypes (G) and the interaction between genotypes and environments (G×E). In fact, all the variables studied showed highly significant differences (at 5%) in terms of the environment, genotypeand genotype-environment interaction. These significant differences

Table 1: Environmental description of the experimental site.

Code	Sowing date	Season	Latitude	Longitude	Altitude	Av. Temp		Av. Hum(%)		Rainfall (mm)	Year
						Min	Max	min	Max		
Env-1	01-07-2022	*Kharif*	25°45′ 15.95″N	93° 51′ 44.71 E	310 MSL	31.66	22.30	91.75	69.64	51.92	2022
Env-2	26-07-2022	*Kharif* (Late)	25°45′ 15.95″ N	93° 51′ 44.71 E	311 MSL	32.09	22.84	92.10	69.99	55.19	2022
Env-3	01-01-2023	Summer	25° 45′ 15.95″ N	93° 51′ 44.71 E	312 MSL	29.11	17.40	94.48	61.84	15.58	2023
Env-4	26-07-2023	Summer (Late)	25° 45′ 15.95″ N	93° 51′ 44.71 E	313 MSL	28.28	15.97	95.29	60.11	8.46	2023

Env=Environment, Av. Temp= Average temperature, Av. Hum=Average humidity.

suggest that there is a substantial amount of genetic variation among the evaluated genotypes. Comparable findings are presented in studies conducted by Zhang *et al.*, (2023) on foxtail millet.

Additive main effects and multiplicative interaction (AMMI)

Analysis of variance for the additive model

AMMI analysis of variance (Table 4) for grain yield per plant (g^{-1}) among 30 genotypes in four environments indicated substantial significance of environmental factors (ENV) (P<0.05, F= 111.558, p<0.001), accounting for 23.42% of yield variance. Replicated environments (REP(ENV)) were not significant (P<0.05, F = 1.382, p = 0.2), contributing minimally (0.56%) to variability. Genotypes (GEN) showed high significance (P<0.05, F = 19.025, p<0.001), explaining 27.94% of variability. The genotype-environment interaction (GEN: ENV) was notably significant (P<0.05, F = 4.123, p<0.001), contributing 18.17% to variance. Residuals accounted for 11.75% of variability, suggesting consistent

Table 2: List of genotypes.

Code	Genotype	Code	Genotype	Code	Genotype
G1	ERP 26	G11	ELS 36	G21	FOX 4384
G2	ERP 57	G12	ELS 40	G22	FOX 4386
G3	ERP 82	G13	FOX 4438	G23	FOX 4390
G4	ERP 90	G14	FOX 4440	G24	FOX 4392
G5	ESD 3	G15	FOX 4478	G25	FOX 4394
G6	ESD 46	G16	FOX 4489	G26	FOX 4396
G7	ESD 75	G17	FOX 4330	G27	FOX 4403
G8	ESD 79	G18	FOX 4336	G28	FOX 4428
G9	ELS 20	G19	FOX 4339	G29	FOX 4420
G10	ELS 34	G20	FOX 4341	G30	SURYANANDI

Table 3: Combined analysis of variance for pooled data.

Source of variation	Mean squares				
	Seasons	Rep within season	Genotypes	Year × Season	Pooled
	(DF=3)	(DF=8)	(DF=29)	(DF=87)	(DF=232)
Days to 50% flowering	63,745.27*	2.61	111.69*	34.26*	1
Days to maturity	2,39,669.82*	5.16	129.99*	37.73*	1
Plant height (cm)	38,415.20*	2.52	125.17*	43.80*	1
Panicle length (cm)	262.10*	0.52	36.42*	6.41*	1
Flag leaf length (cm)	60.75*	4.87	45.19*	10.65*	2.8
Flag leaf width (cm)	1.07*	0.27	1.31*	0.31*	0.06
Peduncle length (cm)	989.87*	0.84	23.51*	5.25*	1
No. of basal tillers	93.98*	3.98	5.40*	1.79*	1
Panicle width (cm)	188.85*	2.43	16.90*	3.11*	1
Biological yield (g)	1,236.78*	1.01	34.30*	5.04*	1
Harvest index (%)	2,636.12*	1.71	6.42*	2.97*	1
Fodder yield per plant (g)	423.90*	0.91	36.07*	4.50*	1
Test weight	1.05*	0	0.36*	0.01*	0
Grain yield per plant (g)	490.15*	4.43	60.49*	13.12*	3.2

Table 4: AMMI analysis for grain yield per plant (g^{-1}) of 30 genotypes evaluated in 4 environments.

Source	Df	Sum Sq	Mean Sq	F value	Pr (>F)	Significant levels	Proportion
ENV	3	1473.95	491.32	111.56	0.00	Significant	23.42
REP(ENV)	8	35.23	4.40	1.38	0.20	Non-significant	0.56
GEN	29	1758.61	60.64	19.02	0.00	Significant	27.94
GEN: ENV	87	1143.48	13.14	4.12	0.00	Significant	18.17
Residuals	232	739.51	3.19				11.75
Total	446	6294.28	14.11				
PC1	31	623.12	20.10	6.31	0.00	Significant	54.50
PC2	29	327.82	11.30	3.55	0.00	Significant	28.70
PC3	27	192.55	7.13	2.24	0.00	Significant	16.80

and stable genotype performance across diverse environments.The AMMI model simplifies genotype-environment interaction (GEI) into three components: PC1, PC2and PC3, each with significant F-values (6.31, 3.55 and 2.24, respectively, all at P<0.05). PC1 dominates, explaining 54.5% of variability (SS_{PC1} = 623.1). PC2 contributes 28.7% (SS_{PC2} = 327.8),while PC3 explains 16.8% (SS_{PC3} = 192.5). The cumulative sum of squares for these three axes is 1143.5 units, providing a comprehensive representation of GEI in the model (Table 4).

The AMMI analysis of variance revealed that environmental factors, genotypesand their interaction significantly contribute to the variability in grain yield per plant. The smaller magnitude of the GEI sum of squares compared to genotypes suggests that certain genotypes consistently perform well across different environments, making them valuable for further research or commercialization in agricultural contexts. Similar results are reported by Madhavilatha *et al.* (2022).

AMMI stability biplot[1]

The AMMI model generates valuable visual representations, known as biplots, which facilitate the interpretation of genotype-environment interactions. Genotype IPCA scores serve as indicators of their adaptability across diverse environments. Biplots are valid when the first two IPCAs explain most interaction variation and are often used to interpret AMMI results. However, breeders may need more than two IPCA axes for complex models, especially when stability and high yield across various conditions are sought (Hanamaratti *et al.,* 2009; Verma and Singh, 2024; Balapure *et al*., 2016; Kumar *et al*., 2020; Kesh *et al*., 2021 and Hooda and Hooda, 2019).

Fig 1a, displays IPCA1 scores for both genotypes and environments, plotted against the grain yield per plant in the foxtail millet dataset. Numerical markers in blue denote genotypes, while green lines indicate environments. These environments are typically represented as interconnecting axes originating from their respective averages, signifying the trait averages within those environments. The biplot has a broken vertical line at the centre, representing the experiment's grand mean (14.65 g^{-1}) and a solid horizontal line at IPCA1 axis score = 0. IPCA1 was very important and explained interaction patterns better than other axes. The x-coordinate shows the main effects (means), while the y-coordinate represents the interaction effects (IPCA1). Genotypes and environments positioned to the right of this line exhibit superior yields to the overall mean, while those on the left side demonstrate yields below the overall mean. The intersection of this axis with the vertical axis divides the biplot into four quadrants. The quadrants II and IV have more potential than quadrants I and III.

In the biplot, 16 genotypes (G13, G21, G5, G19, G6, G4, G17, G18, G8, G25, G11, G9, G7, G22, G3 and G1) and one environment (E1), positioned to the right of the grand mean, were identified as high-yielding, whereas their counterparts with lower yields were on the left side of the grand mean. Furthermore, the high-potential environment (E1) was found in quadrant II, indicated by high positive IPCA1 values. Conversely, the least productive

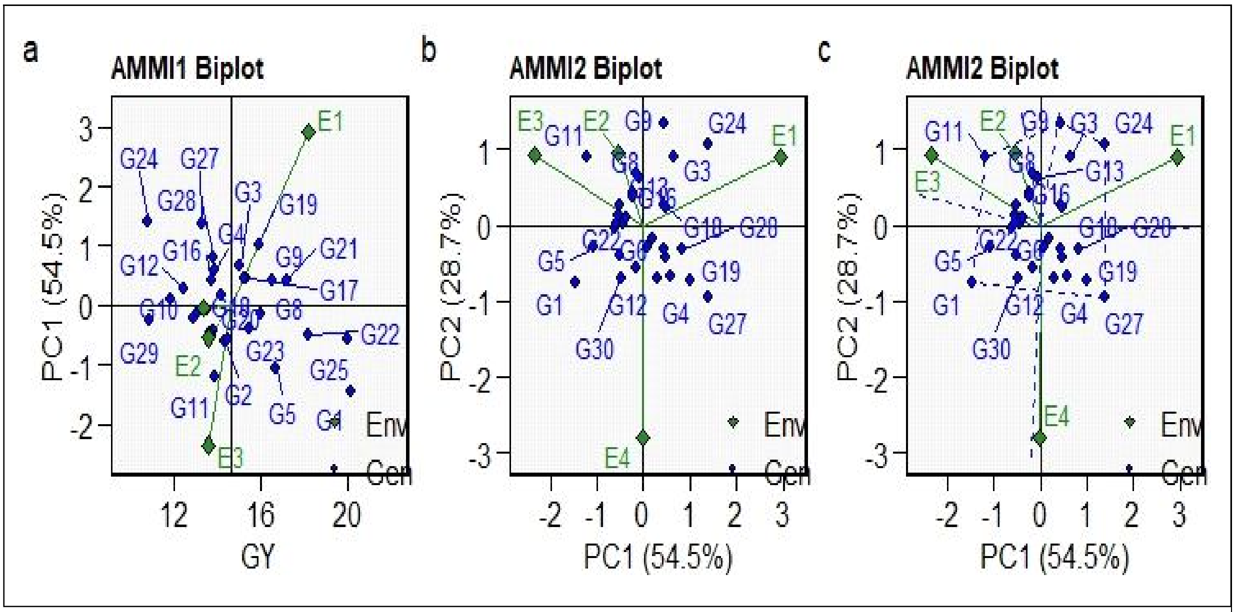

Fig 1: a) AMMI-1 biplot analysis of IPCA 1 score versus grain yield of 30 genotypes under four environmental conditions, b) AMMI-2 biplot analysis of IPCA 1 score versus IPCA 2 score of 30 genotypes under four environmental conditions and c) Polygon view of AMMI-2 biplot based on symmetrical scaling for which-won-where pattern of 30 genotypes under four environmental conditions of grain yield per plant.

environments (E2, E3 and E4) were situated in quadrant III, with negative IPCA1 values. Hanamaratti *et al.* (2009) state that genotypes with low IPCA1 values are considered more stable. Becker and Leon (1988) propose A stable genotype will exhibit minimal variation in its phenotype *(i.e.,* physical characteristics) regardless of the environmental conditions in which it is grown.

In quadrant I, genotypes G24, G27, G28, G4, G16, G15, G10and G12 have positive IPCA scores but below-average yields. Quadrant III includes genotypes G29, G6, G7, G13, G30, G14, G26, G2and G11 with negative IPCA scores and below-average yields. Genotypes G10 (IPCA=0.087), G15 (IPCA=0.183), G12 (IPCA=0.283), G16 (IPCA=0.432), G13(IPCA=-0.0716), G6 (IPCA=-0.153), G7 (IPCA=-0.2316)and G29 (IPCA=-0.239) have IPCA values close to zero, signifying stability with minimal GEI interaction. However, these stable genotypes are non-adaptive and low-yielding, making them unsuitable for cultivation. Quadrant II comprises genotypes G21, G9, G17, G18, G3and G19, characterized by positive IPCA scores and above-average yields. Quadrant IV contains genotypes G8, G23, G22, G25, G5and G1, which have negative IPCA scores but above-average yields. G21 (IPCA=0.425),G9 (IPCA=0.432), G17 (IPCA=0.463), G8 (IPCA=-0.154)and G23 (IPCA=-0.385) thesegenotypes IPCA values are closer to “Zero (0)”, hence these genotypes consider as stable, high yielding, adaptable and exhibits minimum GEI interaction effect and recommended to general cultivation at Nagaland region. Ideal genotypes exhibit high mean yield with stability, making G8, G9, G21and G22 ideal due to their high mean yield and low IPCA scores.Similar results were observed in studies by Khan *et al.*(2021) in Bambara groundnut genotypes.

AMMI-2 stability biplot

The AMMI-2 stability Biplot plotted IPCA1 scores for both genotypes and environments against IPCA2 scores for genotypes and environments. This model uses the first two interaction axes of genotype and environment scores (Vargas and Crossa, 2000). It helps understand genotype-environment interactions and reveals which genotypes perform best in specific conditions. Genotypes near the centre of the Biplot are considered more stable (Purchase,1997).

In the GGE biplot analysis (Fig 1b), PC1 and PC2 capture 54.5% and 28.7% of the total variation, respectively, accounting for 83.2% of the variation. In the AMMI 2 biplot, the environmental scores are joined to the origin by side lines. Sites with short spokes do not exert strong interactive forces. Those with long spokes exert strong interaction. In this AMMI Biplot-2, all environments *viz* E1, E2, E3 and E4 are connected to the origin; among these environments, E2 and E3 exhibit short spokes, indicating limited interaction strength, while E1 and E4 display long arrows, indicating strong interaction forces. Polygonal biplot is used to identify MEs and superior genotypes in different environments. In this biplot, a polygon is drawn from the connection of the genotypes with the maximum distance from the coordinate origin. Genotypes G1, G11, G9, G24 and G27 were located at the farthest distance and formed a polygon (Fig 1c). These five rays divide the biplot into five sectorsand environments are connected to the origin. Thus, two environments, E3 and E2, fell into a similar sectorand the vertex genotype for this sector is G11, suggesting that this genotype achieves ideal performance in those specific environments. Similarly, one environment, E4, fell into a

Table 5: Yield performance and stability of 30 foxtail millet genotypes based on mean grain yield per plant (g-1) and measures of AMMI stability value (ASV).

Genotype	ASV	ASV_R	Genotype	ASV	ASV_R
G1	2.88	30	G16	0.86	10
G2	1.15	19	G17	0.98	14
G3	1.54	22	G18	0.92	13
G4	1.29	21	G19	2.07	26
G5	2.04	25	G20	1.07	18
G6	0.62	5	G21	0.86	11
G7	0.58	3	G22	1.05	16
G8	0.74	7	G23	0.74	8
G9	1.57	23	G24	2.88	29
G10	0.34	1	G25	1.07	17
G11	2.45	27	G26	1	15
G12	0.89	12	G27	2.79	28
G13	0.63	6	G28	1.59	24
G14	0.85	9	G29	0.62	4
G15	0.39	2	G30	1.15	20

ASV = AMMI stability value.

single sectorand the vertex genotype for this sector was G27, while E1 fell into a single sectorand the vertex genotypes for this sector were G24 and G9. Conversely, genotypes in sections without associated environments are less favourable for cultivation across the studied conditions. According to Fig 1b, G2, G25, G14, G15, G20, G23, G18 and G16 were close to the centre and considered to have high grain yield stability. Remain genotypes exhibit medium to instability. The results from this current study support the claims made by Khan *et al*. (2021) using 30 Bambara groundnut varieties in four different environments.

AMMI stability value (ASV)

The AMMI stability value (ASV) for grain yield per plant is a critical metric for assessing the stability of various genotypes within this study. According to the ASV methodology, the genotype with the lowest ASV score represents the highest stability, while a higher score suggests reduced stability (Hassanpanah *et al.* 2016). Consequently, when genotypes are ranked based on ASV scores, G10 emerges as the most environmentally stable genotype, securing the top position with a low ASV of 0.34 and yield is lower than mean yield. Following closely are G15 (ASV = 0.38, Rank = 2), G7 (ASV = 0.57, Rank = 3), G29 (ASV = 0.61, Rank = 4), G6 (ASV = 0.62, Rank = 5) and G13 (ASV = 0.63, Rank = 6). These genotypes demonstrate notable stability in terms of grain yield per plant, signifying their consistent performance across various environmental conditions. Theses genotypes are not suitable for cultivation due to its lower yield performance. ASV values of grain yield per plant are presented in Table 5.

CONCLUSION

This study analyzed data from multiple environments to determine the best conditions for foxtail millet cultivation in Nagaland. We employed various stability analysis methods and compared their results. Our findings point to Environment E1, representing the *kharif* season, as the ideal environment for foxtail millet cultivation in Nagaland. This means that planting during this season is most favourable for good yields. Moreover, we identified specific genotypes that consistently performed well in this region. These genotypes, namely G21 and G22, exhibited stable and reliable performance across different conditions. Therefore, we suggest these genotypes for general cultivation in Nagaland, as they are likely to yield positive results in various agricultural settings. This conclusion is based on a rigorous analysis of multi-environmental data, which provides practical guidance for farmers and cultivators in Nagaland looking to optimize their foxtail millet production.

Conflict of interest

All author’s declaire that they have no conflict of interest.

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