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## Characterization of Foxtail Millet Genotypes for Selecting Candidate Parental Lines and Important Traits to Initiate the Foxtail Millet Breeding in the Foothills of North- East Region of India

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**Abstract:** Foxtail millet is an authenticated crop in the ethnic tribes of Nagaland and plays a major role in their traditions. The development of hybrids in foxtail millet is a big task due to the minute flower morphology and self-pollinated crop. Hence collection, characterization, and identification of stable yielders in pure line mixtures in this region prioritize foxtail millet breeding. The objective of this current study was to investigate 30 foxtail genotypes across four distinct environments, aiming to identify stable performance genotypes in the region. The investigation was carried out from July 2022 to May 2023, incorporating four different sowing patterns with a 25-day interval. Two environments were maintained under rainfed conditions, while the remaining two were under irrigated conditions. A randomized complete block design with three replications was used in all environments. AMMI analysis revealed significant ( $P < 0.05$ ) genetic variation observed among the all traits for genotypes, environments, and genotype X environment interaction. Four genotypes (G8, G9, G21, and G22) exhibited stable performance under various testing conditions for grain yield. The AMMI-2 biplot highlighted that E1 and E4 exhibit strong environmental interaction for all traits, while E3 and E2 exhibit weak environmental interaction. Multi-trait stability index module revealed genotypes G17, G18, G21, and G14 were superior performance under multi trait combination.

**Key Words:** AMMI, BLUP, Foxtail millet, MTSI

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### Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is self-pollinated and C4 millet crop, which was extensively grown in the world among the millet. This ancient grain holds significant importance as both a staple food and a valuable source of fodder (Li et al., 2021). Notably, it displays remarkable adaptability to challenging environmental conditions such as drought, extreme temperatures and high soil salinity (Zhanget al., 2023). Foxtail Millet stands as one of the oldest cultivated

millet varieties globally, with a presence in approximately 23 countries across Asia, Africa and the Americas (Madhaviathaet al., 2022). In 2023, India produced almost 50% of the world's 6 million tons of foxtail millet, a vital crop. It covered 0.87 lakh ha in India and produced 0.66 lakh tons at 762 kg/ha in 2015-16 (Hariprasana K, 2023). The UN announced the 2023 as the International Year of Millets to honor their importance. Millets' nutritional benefits, sustainability and significance in sustainability development goals are highlighted in this effort. It shares millet cultivation, processing, marketing and consumer expertise, best practices and innovations.

General and specific adaptability of a genotype is important aspect for commercial cultivation of a crop in wide range environments. Stability performance of genotypes in terms of yield under varied agro ecological zones have been assessed through univariate and multivariate statistical modules (Madhaviathaet al., 2022).

One important multivariate method is the Additive Main effects and Multiplicative Interaction (AMMI), which combines analysis of variance and principal components (PCs) analysis (Taleghaninet al., 2023). The first part of AMMI, the additive part, uses analysis of variance, while the second part, the multiplicative part, employs principal components (PCs) analysis to study Genotype by Environment Interaction (GEI) (Taleghaninet al., 2023). The AMMI model is widely used because it provides detailed information about the main effects and genotype X environment interaction (GEI), and it explains a significant portion of the interaction's variability (Nagarajaet al., 2023). The GGE (Genotype and Genotype  $\times$  Environment interaction) biplot is a graphical tool used in the field of plant breeding and agricultural research to analyse and visualize complex genotype by environment interaction ( $G \times E$ ) patterns (Ataeiet al., 2019). The aim of current study was to evaluate  $G \times E$  interaction under different sowing dates at foothills of Nagaland using 30 foxtail millet genotypes to evaluate stability and their performance, in relation to grain yield and agronomic traits and to identify mega environment and stable genotypes in this region.

## **Materials and methods**

### **Experiment location:**

The entire experiment was conducted between July 2022 and May 2023 with four different sowing patterns (Table 1.). Each date of sowing was believed to create varied environmental conditions during the crop's growth. Among the four environments, two are maintained under rained conditions, while the remaining two are maintained under irrigated conditions at irrigation intervals of once a week. The entire experiment was conducted at the Research Farm of the Department of Genetics and Plant Breeding, School of Agricultural Sciences, Nagaland University, Medziphema, India. India. The coordinates of the research

farm are “250450350 N and 950250450 E,” with an altitude of 310 m above mean sea level. Soil nutrient analysis was conducted in every environment, and it is presented in Table 2.

**Plant materials:**

We've collected 100 foxtail millet genotypes, including national and zonal check varieties, from the Indian Institute of Millets Research (IIMR), Hyderabad. We evaluated these genotypes during the Zaid season in 2022 in the same environment and selected the best 29 genotypes and one national check variety based on the mean performance of grain yield. We used these 30 genotypes to conduct further stability studies in this region. List of genotypes presented in Table 3.

**Experimental design and intercultural practice:**

The experiment was carried out through a randomized complete block design (RCBD) with three replications in all environments. Each replication consisted of 30 plots, with each plot measuring 1 x 1 meter. Replication to replication had a 75-cm spacing. Plant to plant and row to row had 10 x 22.5 cm spacing in the plot. A total of 90 plots were used across four environments. Recommended agricultural practices were adhered to throughout the experiment.

**Data collection:**

A total nine quantitative characters of foxtail millet were taken for experimentation. These characteristics were chosen based on descriptions and guidelines provided by PPV&FR in 2001 (DUS). For each characteristic, data were gathered from five randomly sampled plants from each genotype and in each replication. The quantitative data encompassed various traits, including days to 50% flowering (DF), plant height (PH) (cm), panicle length (PL) (cm), flag leaf length (FL) (cm), flag leaf width (FW) (cm), peduncle length (PDL) (cm), total tiller numbers per plant (NT), fodder yield per plant (FY) (g), and grain yield per plant (GY) (g). Days to 50% flowering and days to maturity data were collected on plot basis.

**Statistical analysis**

R-studio was utilized for visualization of GGE biplots and AMMI techniques using the 'Metan' package developed by the R Core Team (Team, R. 2015). The multi-trait stability index carried out by the 'Metan' package (Olivoto et al., 2019; Olivoto & Nardino, 2021).

## Results and Discussion

### Additive main effects and multiplicative interaction (AMMI)

#### AMMI analysis of variance

The AMMI analysis of variance was conducted to investigate the impact of environmental factors (ENV), replicated environments (REP (ENV)), genotypes (GEN), and the interaction between genotype and environment (GEN: ENV) on yield and yield attributes across multiple experimental conditions. AMMI analysis revealed significant ( $P < 0.05$ ) genetic variation observed among the all traits for genotypes, environments, and genotype X environment interaction. All traits of AMMI ANOVA are represented at Table 4.

In the AMMI model, we simplify the interaction between genotypes and environments (GEI) into three main components: PC1, PC2, and PC3, each accompanied by a significance level at  $P < 0.05$ . Regarding days to 50% flowering, genotypic variance contributed 34.5% to the overall variance, while environment and G×E interaction contributed 4.8% and 30.9%, respectively. In terms of plant height, genotypic variance constituted 41.9% of the overall variance, with environment and G×E interaction contributing 8.28% and 22.1%, respectively. Panicle length displayed genotype, G×E interaction, and environment effects explaining 44.9%, 21.8%, and 0.3% of the variance, respectively. The breakdown for flag leaf length and width showed genotype accounting for 32.4%, 33.7%, environment for 4.5%, 2.8% and G×E interaction for 22.9%, 22.4% of the total variance. Peduncle length and number of tillers per plant were characterized by genotype contributing 38.3%, 19.8%, environment 2.4%, 7.0% and G×E interaction 21.3%, 18.7% to the variation. In grain and fodder yield, G×E interaction explained 18.1%, 18.3, while genotype and environment contributed 27.9%, 50.1% and 23.4%, 1.47% to the total variance, respectively. Comparable findings are presented by Khan et al., (2021) in 30 Bambara groundnut genotypes and Taleghaninet al., (2023) 18 sugar beet genotypes.

#### AMMI biplot analysis

##### AMMI-1 stability Biplot

The AMMI model generates valuable visual representations, known as biplots, which facilitate the interpretation of genotype-environment interactions (Gabriel, 1978). Genotype IPCA scores serve as indicators of their adaptability across diverse environments (Purchase, 1997). 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 (Hanamarattiet al., 2009) and (Zhanget al., 2023).

Based on the AMMI stability biplot-1, the genotypes namely G14, G23, G16, and G9 were identified stable performances for days to 50% flowering (Fig 1a). G23 and G28 were stable performances genotypes for plant height (Fig 1b). G18 and

G30 were reported as stable performance genotypes for panicle length (Fig 1c). G18 is determined as stable performance genotype for flag leaf length (Fig 1d, e). G24 and G1 were identified as stable performance genotypes for peduncle length (Fig 1f). G18, G16 and G25 were exhibited as stable performance genotypes for number of tillers per plant (Fig 1g). G13, G17 and G18 were determined as stable performance genotypes for fodder yield per plant (Fig 1h). G8, G9, G21, and G22 were stable performance genotypes for grain yield per plant (Fig 1i). These genotypes had nearly zero scores on the first PCA1 axis, suggesting that they were minimally affected by the environment and all these genotypes perform above the average mean values. Similar findings reported by Enyewet al., (2021) stability analysis for grain yield and other agronomic traits in sorghum.

### **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 (Boratkar, 2023). It helps in understanding the genotype-environment interactions and reveals which genotypes perform best in specific conditions (Dela et al., 2023). Genotypes near the center of the Biplot are considered more stable (Babiker et al., 2024).

The AMMI stability biplot-2 illustrated the environment scores for IPCA1 and IPCA2 across all traits. In this AMMI2 biplot, environments with low IPCA1 and IPCA2 scores, positioned near the origin, contribute significantly to genotype stability while having a minimal impact on genotype-environment (GE) interaction. (Enyewet al., 2021). In this study, AMMI2 biplots indicated that all environments were positioned far from the biplot origin for all traits under current study. Similar study reported by Enyewet al., (2021) in sorghum.

The figures (Fig 2a-h) show how different genotypes performed across various environments for specific plant characteristics. For days to flowering (Fig 2a), the first two principal component (PC) axes accounted for 83.7% of the total variation, with genotypes G30, G26, G11, G25, G23, G14, and G20 being the most stable across environments. Similarly, in terms of plant height (Fig 2b), these axes explained 81.1% of the variation, highlighting G21, G28, G6, G2, G16, G20, and G30 as the most stable genotypes. Panicle length (Fig 2c) variation was explained by 77.7% of the sum of squares on the first two PC axes, with G15, G13, and G30 noted as the most stable genotypes. Flag leaf length (Fig 2d) showed 82.2% of the variation accounted for by these axes, identifying G9, G1, G10, and G5 as the most reliable across environments. Flag leaf width (Fig 2e) exhibited 83.6% of the variation explained by the first two PC axes, highlighting G3, G28, G19, G14, and G15 as stable genotypes. Peduncle length (Fig 2f) showed 77.8% of the variation explained by these axes, emphasizing G5, G17, G14, G13, and G25 as stable genotypes. Number of tillers per plant (Fig 2g) saw 89.1% of the variation

explained by the first two PC axes, highlighting G25, G28, G17, and G4 as the most stable genotypes. Fodder yield (Fig 2h) exhibited 86.9% of the variation explained by these axes, showing G25, G14, G2, and G15 as the most stable genotypes across various environments. Lastly, grain yield per plant (Fig 2i) exhibited 83.2% of the variation explained by the first two PC axes, highlighting G25, G28, G17, and G4 as the most stable genotypes. A 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 that have the maximum distance from the coordinate origin. The rays' lines in biplot that is perpendicular to the sides of the polygon or their extensions. A polygonal biplot view of all traits presented in Figure 3a-i.

### **Multi-trait stability index (MTSI)**

The Multi-Trait Stability Index (MTSI) was applied to data from nine yield traits (Figure 4), ranking genotypes from highest to lowest MTSI. Genotypes with the highest MTSI values are positioned at the center of the circle, while those with the lowest values are on the outermost circle. Some genotypes were marked with red dots, indicating they were selected based on their MTSI values, with a selection intensity of 15%. The black dots represent genotypes that were not selected.

Notably, G2 achieved the top rank, followed by G25, G18, and G1, indicating these are the most desirable and stable genotypes. Genotypes like G21 and G8 are clustered near the center, suggesting they may possess interesting attributes worth further investigation. The selected genotypes demonstrated higher average values across all traits, aligning with our selection goals. Overall, selecting these genotypes resulted in a favorable selection differential across all traits. Previous studies by Sharifi et al., (2021) and Koundinya et al., (2021) have emphasized the MTSI's effectiveness in assisting plant breeders in selecting superior genotypes across multiple traits using data from various environments.

### **Conclusion**

The present study concludes that the Environment E1, representing the kharif season, as the ideal environment for foxtail millet cultivation in Nagaland. This indicates that planting during this season is highly favourable for good yields. Genotypes viz., G25 and G18 exhibited stable and reliable performance of grain yield across different conditions. The present study suggests that these genotypes are highly recommended 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.

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**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 (KE)	25 <sup>0</sup> 45' 15.95'' N	93 <sup>0</sup> 51' 44.71 E	310 MSL	31.66	22.30	91.75	69.64	51.92	2022
Env-2	26-07-2022	Kharif(Late)(KL)	25 <sup>0</sup> 45' 15.95'' N	93 <sup>0</sup> 51' 44.71 E	311 MSL	32.09	22.84	92.10	69.99	55.19	2022
Env-3	01-01-2023	Summer (SE)	25 <sup>0</sup> 45' 15.95'' N	93 <sup>0</sup> 51' 44.71 E	312 MSL	29.11	17.40	94.48	61.84	15.58	2023
Env-4	26-01-2023	Summer(Late) (SL)	25 <sup>0</sup> 45' 15.95'' N	93 <sup>0</sup> 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

**Table 2. Characterization of soil properties of the experimental region**

Determination	Field-1	Field-2	Field-3	Field-4
Physical analysis	Value			
Sand (%)	42.8	43.4	42.9	45.1
Silt (%)	24.9	26.7	35.1	34.5
Clay (%)	32.2	29.8	21.9	14.2
Textural classes (USDA)	Clay loam	Clay loam	Loam	Sandy Loam
Chemical analysis	Value			
pH	4.68	5.49	6.48	5.74
Organic matter (%)	0.89	0.98	0.94	1.03

Available nitrogen (Kg ha <sup>-1</sup> )	193.56	197.94	195.75	207.20
Available phosphorus (Kg ha <sup>-1</sup> )	17.08	17.56	16.05	16.85
Available potassium (Kg ha <sup>-1</sup> )	124.54	128.36	121.87	120.89

<b>Table 3. List of selected genotypes based on the mean yield</b>			
<b>ACC. No</b>	<b>IC. No</b>	<b>Source</b>	<b>Code</b>
ELS 20	IC 0621991	Andhra Pradesh	G1
FOX 4438	IC 0077702	West Bengal	G2
FOX 4394	IC0610541	Andhra Pradesh	G3
FOX 4339	IC 0597715	Andhra Pradesh	G4
ERP 82	IC 0622113	Tamil Nadu	G5
FOX 4384	IC 0610531	Andhra Pradesh	G6
FOX 4396	IC 0610543	Andhra Pradesh	G7
FOX 4403	IC 0610550	Andhra Pradesh	G8
FOX 4428	IC 0850064	Unknown	G9
ESD 79	IC 0618660	Maharashtra	G10
FOX 4336	IC 0597710	Andhra Pradesh	G11
FOX 4386	IC 0610533	Andhra Pradesh	G12
ERP 26	IC0622071	Tamil Nadu	G13
ESD 3	IC 0618597	Maharashtra	G14
ELS 40	IC 0622003	Andhra Pradesh	G15
ERP 90	IC 0622117	Tamil Nadu	G16
FOX 4478	IC 0078006	Uttar Pradesh	G17
FOX 4489	IC 0078200	Tamil Nadu	G18
FOX 4392	IC 0610539	Andhra Pradesh	G19
FOX 4390	IC 0610537	Andhra Pradesh	G20
FOX 4330	IC	Arunachal	G21

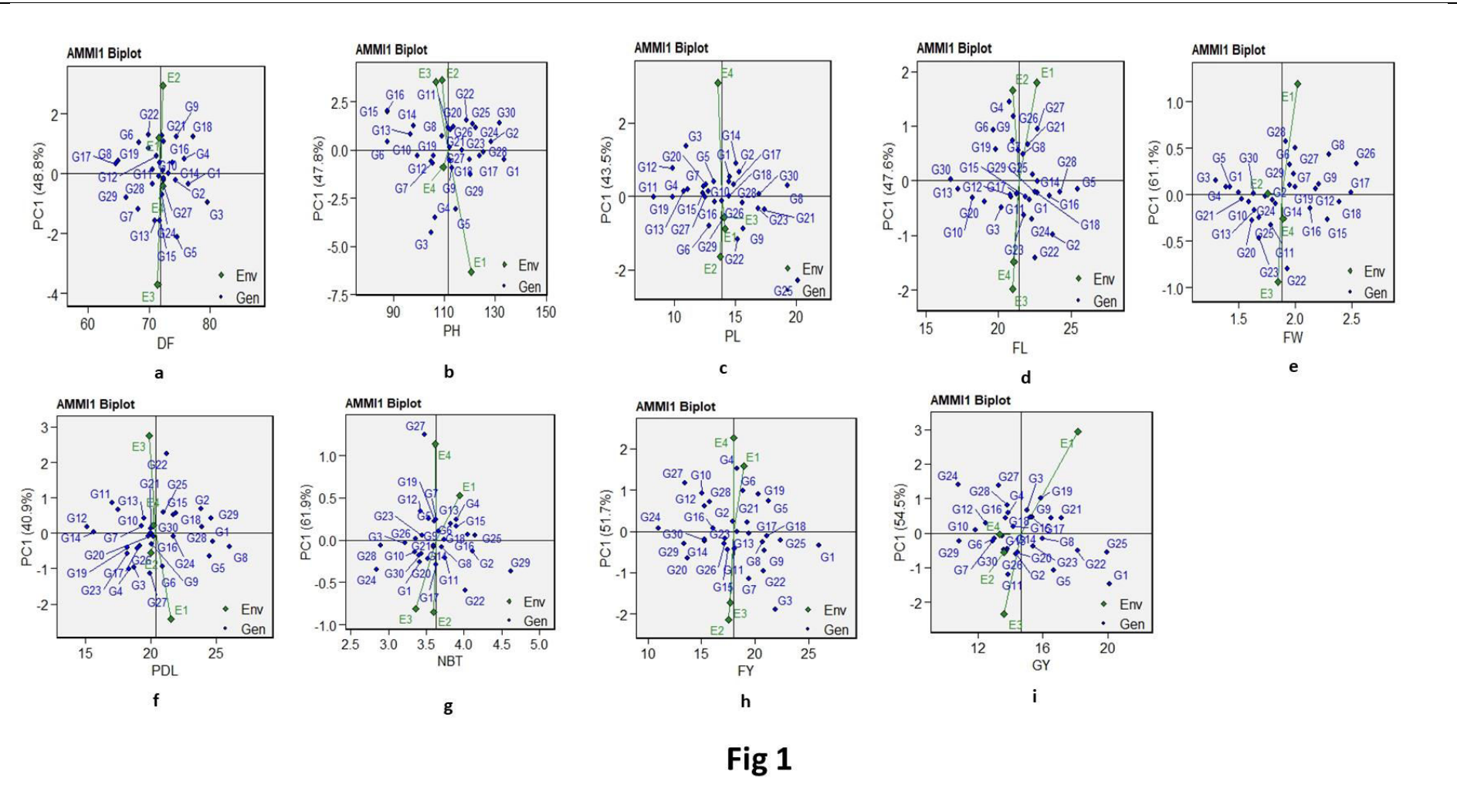
	0596783	Pradesh	
ESD 75	IC 0618657	Maharashtra	G22
ESD 46	IC 0618634	Maharashtra	G23
ERP 57	IC 0622094	Tamil Nadu	G24
FOX 4341	IC 0597722	Andhra Pradesh	G25
FOX 4440	IC 0077761	Gujarat	G26
FOX 4420	IC 0613573	Andhra Pradesh	G27
ELS 36	IC 0621999	Andhra Pradesh	G28
ELS 34	IC 0621998	Andhra Pradesh	G29
Surya Nandi	Check	Andhra Pradesh	G30

**Table 4. AMMI ANOVA for grain yield and related traits of 30foxtail Millet genotypes across four environments**

		DF			PH			PL			FL		
Source	Df	Sum Sq	Mean Sq	% variation	Sum Sq	Mean Sq	% variation	Sum Sq	Mean Sq	% variation	Sum Sq	Mean Sq	% variation
ENV	3	47.04	15.68	41.80	10506.20	3502.07	8.29	19.06	6.35	0.34	182.05	60.68	4.51
REP(ENV)	8	20.50	2.56	18.21	189.11	23.64	0.15	12.94	1.62	0.23	38.99	4.87	0.97
GEN	29	3885.79	133.99	34.50	53137.75	1832.34	41.93	2549.12	87.90	44.95	1310.29	45.18	32.49
GEN:ENV	87	3482.56	40.03	30.90	28058.94	322.52	22.14	1237.93	14.23	21.83	926.58	10.65	22.97
PC1	31	1698.99	54.81	48.80	13400.61	432.28	47.80	537.90	17.35	43.50	441.04	14.23	47.60
PC2	29	1217.15	41.97	34.90	9330.44	321.74	33.30	423.18	14.59	34.20	320.97	11.07	34.60
PC3	27	566.42	20.98	16.30	5327.89	197.33	19.00	276.85	10.25	22.40	164.57	6.10	17.80
Residuals	232	332.19	1.43	2.95	6793.43	29.28	5.36	614.66	2.65	10.84	648.70	2.80	16.08
Total	446	11250.64	25.23		126744.36	284.18		5671.64	12.72		4033.18	9.04	
		FW			PDL			NBT			FY		
Source	Df	Sum Sq	Mean Sq	% variation	Sum Sq	Mean Sq	% variation	Sum Sq	Mean Sq	% variation	Sum Sq	Mean Sq	% variation
ENV	3	3.20	1.07	2.84	161.96	53.99	2.47	15.82	5.27	7.00	106.54	35.51	1.47
REP(ENV)	8	2.13	0.27	1.89	33.58	4.20	0.51	11.66	1.46	5.16	22.14	2.77	0.31
GEN	29	38.04	1.31	33.77	2509.29	86.53	38.32	44.94	1.55	19.89	3633.24	125.28	50.16
GEN:ENV	87	27.36	0.31	24.28	1396.26	16.05	21.33	42.40	0.49	18.76	1329.80	15.29	18.36
PC1	31	16.71	0.54	61.10	571.46	18.43	40.90	26.26	0.85	61.90	687.95	22.19	51.70
PC2	29	6.15	0.21	22.50	514.58	17.74	36.90	11.55	0.40	27.20	468.00	16.14	35.20
PC3	27	4.49	0.17	16.40	310.23	11.49	22.20	4.58	0.17	10.80	173.85	6.44	13.10
Residuals	232	14.59	0.06	12.95	1050.18	4.53	16.04	68.75	0.30	30.43	822.24	3.54	11.35
Total	446	112.67	0.25		6547.52	14.68		225.97	0.51		7243.77	16.24	
		GY											
Source	Df	Sum Sq	Mean	%									

			<b>Sq</b>	<b>variation</b>									
<b>ENV</b>	3	1473.95	491.32	23.42									
<b>REP(ENV)</b>	8	35.23	4.40	0.56									
<b>GEN</b>	29	1758.61	60.64	27.94									
<b>GEN:ENV</b>	87	1143.48	13.14	18.17									
<b>PC1</b>	31	623.12	20.10	54.50									
<b>PC2</b>	29	327.82	11.30	28.70									
<b>PC3</b>	27	192.55	7.13	16.80									
<b>Residuals</b>	232	739.51	3.19	11.75									
<b>Total</b>	446	6294.28	14.11										

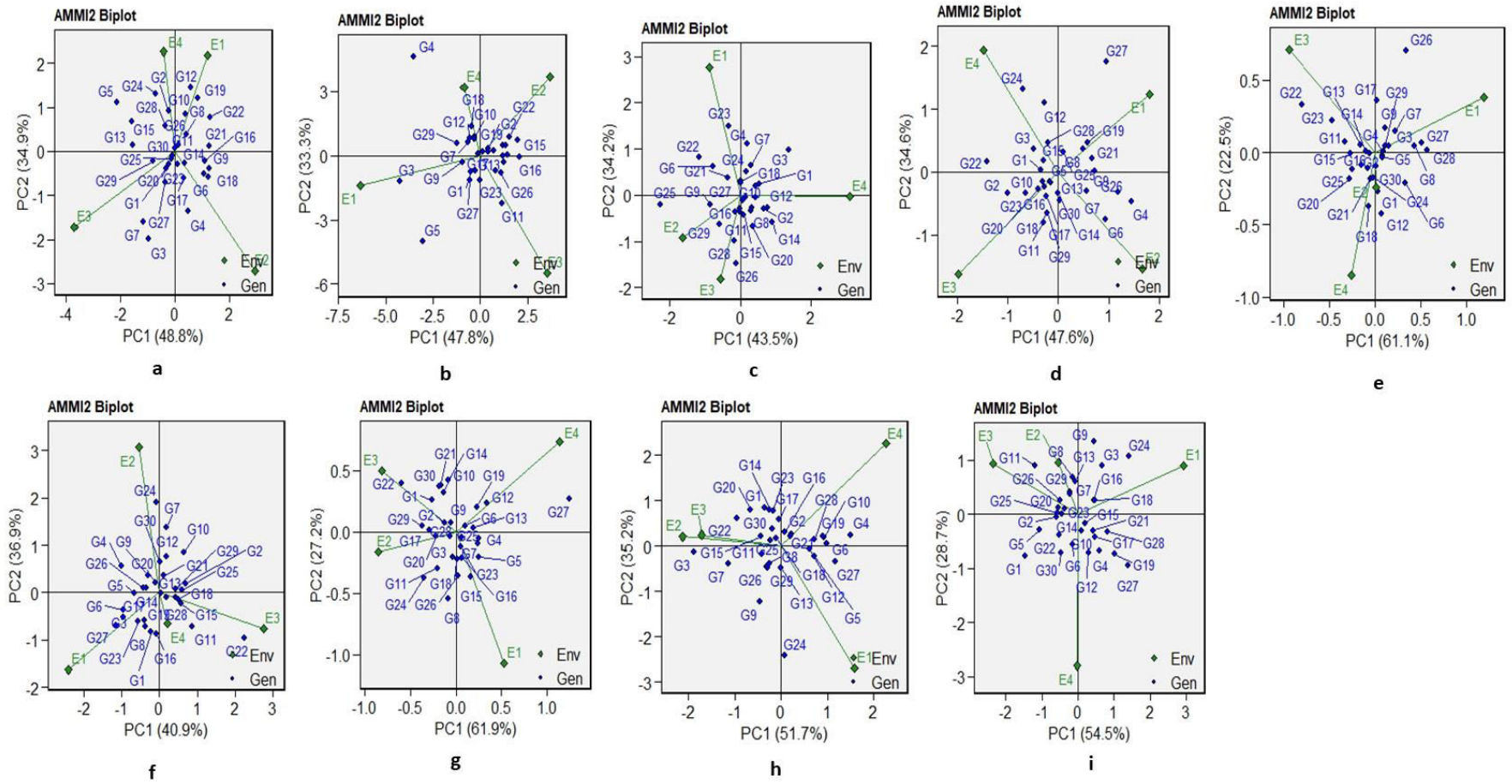
**Fig 1. AMMI 1 biplot for grain yield and related traits of 30 foxtail Millet genotypes across four environments**



**Fig 1**

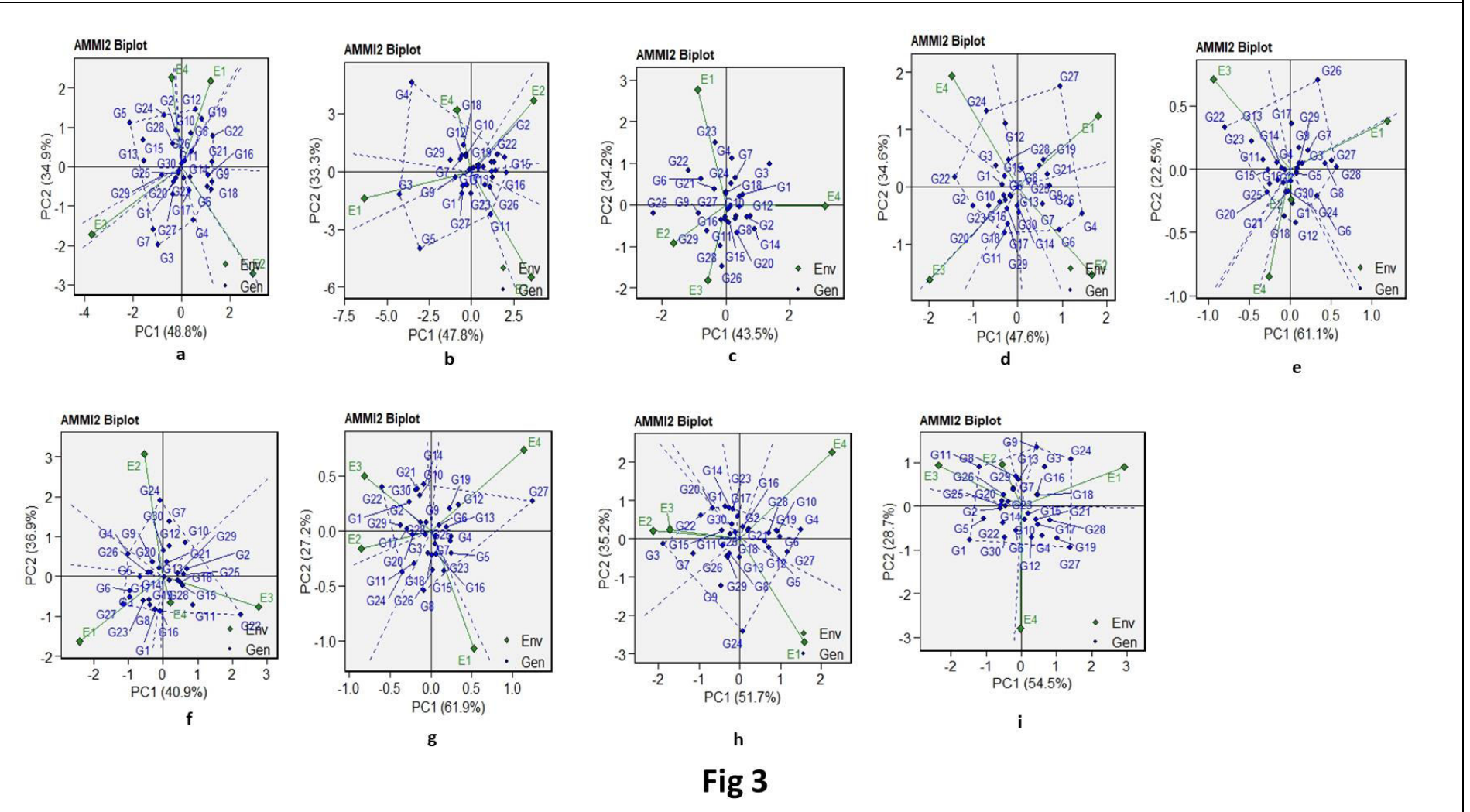


**Fig 2. AMMI 1 biplot for grain yield and related traits of 30 foxtail Millet genotypes across four environments**



**Fig 2**

**Fig. 3. Which-won-where pattern for grain yield and related traits of 30 foxtail Millet genotypes across four environments**



**Fig 3**

Fig 4. Ranking of 30 foxtail millet genotypes in ascending order based on MTSI index.

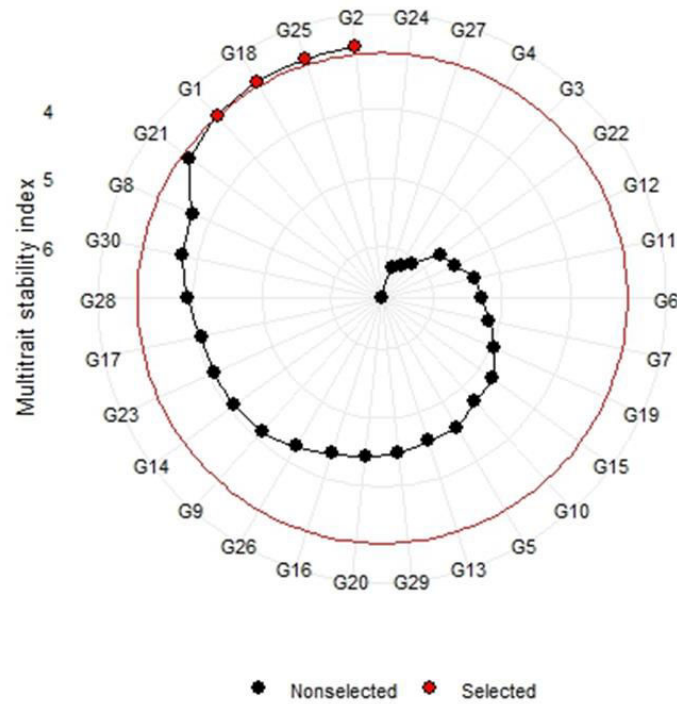


Fig 4