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Research Article

Identification of foxtail millet (*Seteria italica* **(L.) P. Beauv.) genotypes for multi-season adaptability using GGE biplot analysis in Foothills of Nagaland**

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Abstract

Foxtail millet is a main millets crop of Northeastern region of India. The genotype-by-environment analyses assist in understanding the potential performance of the genotypes over environments. The present study aimed to investigate the 30 foxtail genotypes over the four different environments to identify the genotypes with stable yield in this region. The investigation was carried out during July 2022 to May 2023 for four different dates of sowing with fifty-five days interval. Two environments maintained under rainfed condition and the remaining two environments are maintained under irrigated condition with seven days interval. The experiment was conducted in randomized complete block design with three replications in all environments. Genotype-environment interactions significantly influenced grain yield across four environments, while replicates were non-significant. Pooled analysis revealed significant genotypic effects and seasonal impacts. The "discriminating power vs. representativeness" GGE biplot showed that E4 (showing dates in January 2ndfort night) is the most ideal test environment for foxtail millet elite line selection based on discriminative ability and representativeness. The accessions that performed best in each environment based on "which-won-where" polygon biplot, genotypes G19 (FOX 4392) and G27 (FOX 4420) showed superior and stable performance in E1, however G25 (FOX 4341) and G1 (ELS 20) excelled in E2, E3, and E4.Mean vs stability biplots exposed G1 (ELS 20) is stable and high yielding performance. Ranking genotypes GGE biplot identify the G25 (FOX 4341) is an ideal genotype due to its higher yield and stability compared to the other genotypes.

Keywords: GGE Biplots, Foxtail millet, Tukey's Honest Significant Difference (HSD) Test

INTRODUCTION

Foxtail millet (*Setariaitalica* (L.) P. Beauv.) is a selfpollinating 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). This ancient grain holds significant importance as both a staple food and a valuable source of fodder. Notably, it displays remarkable adaptability to challenging environmental conditions such as drought, extreme temperatures, and high soil salinity (Panneerselvam *et al.,* 2019). 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 (Lata *et al.,* 2013).

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 (Laxmi *et al.,* 2015). The UN named 2023 the International Year of Millets to honor their importance. Millets' nutritional benefits, sustainability, and significance in SDGs are highlighted in this effort. It shares millet cultivation, processing, marketing, and consumer expertise, best practices, and innovations.

Genotype-by-environment interaction (GEI) significantly influences crucial quantitative traits such as yield in

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agriculture (Kiruba *et al.,* 2023). Breeders must carefully consider this interaction when selecting and introducing plant varieties to ensure optimal performance in specific environments (Chandrashekhar *et al*., 2020). However, GEI can also be beneficial, aiding breeders in identifying varieties with either specific adaptation to particular locations or general adaptation across diverse environments. Breeders achieve this by cultivating various plant varieties in different habitats, observing their responses, and selecting the most optimal types that consistently perform well under varying conditions (Fasahat *et al.,* 2016).

Univariate and multivariate approaches are used to analyse multi-environment trial data (Olivoto *et al.,* 2019). The GGE (Genotype and Genotype × 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 × E) patterns (Yan, 2014).

In this investigation, we assessed the performance of 30 foxtail millet genotypes to determine their adaptability to diverse environments and identify the most productive ones under various conditions.

MATERIALS AND METHODS

Experiment location: The study was conducted in four distinct environments and on four distinct sowing dates between July 2022 and May 2023 (**Table 1**), with a gap of 55 days between each sowing. 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 rainfed condition and remaining two environments maintained under irrigated condition with irrigation intervals once in week. The experiment was conducted at the Research Farm of the Department of Genetics and Plant Breeding, School of Agricultural Sciences, Nagaland University, Medziphema, India. The precise coordinates of the research farm are "25 0.450350 N and $95^{\circ}25^{\circ}45^{\circ}$ E" with altitude of 310 m above mean sea level.

Soil sampling and analysis: In all four situations, the top 15 cm of soil was randomly selected from the field. The university lab analyzed this composite sample. The materials were dried in shade and pulverized with a glass mortar and pestle to guarantee nutrient distribution homogeneity and plot representation. After sifting, the sample was tested for chemical characteristics and particle size distribution. These tests measured sand, clay, silt, pH, organic carbon (OC), nitrogen (N), potassium (K), and phosphorus. Results are presented at **Table 2.**

Plant materials: We obtained a collection of one hundred foxtail millet samples, which included reference varieties, from the Indian Institute of Millets Research

Table 1. Environmental description of the experimental site

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

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(IIMR) in Hyderabad. These samples were evaluated during the *Zaid* season in 2022 at same environment. Based on the results of this evaluation, we identified the top 30 (29+1 check) genotypes that showed the highest grain yield specifically in the Medziphema region. These 30 selected samples were used in our study to assess stability across different environments. List of 30 genotypes are presented in **Table 3**.

Experimental design and intercultural practice: The experiment utilized a randomized complete block design (RCBD) with three replications in varying environments due to differing fertility levels. Each replication consisted of 30 plots measuring 1m x 1m, spaced 10cm apart. Plants within a plot had 10cm spacing, with rows separated by 22.5cm. Replications were 30cm apart, forming a 30m x 5m plot with 30cm spaces before the first and after the third replication. In total, 90 beds were used across all environments, and recommended agricultural practices were adhered to throughout the experiment.

Data collection: The research conducted a thorough investigation, analysing the amount of grain yield per plant (g) each plant produced in four different environments. The goal was to understand how the crop interacts with its surroundings in various agricultural settings.

Statistical analysis:The statistical analysis involved various techniques and software tools to examine the combined dataset. To evaluate the data, the OPSTAT open-source software was used for analysis of variance (ANOVA). Additionally, R-studio was utilized along with specific packages like 'tidyverse,' 'ggthemes,' 'multcompView,' and 'dplyr' for multiple mean comparison, normality tests, and visualization through GGE biplot, using the 'Metan' package developed by the R Core Team (Team, R. 2015).

Table 3. List of selected genotypes based on the mean yield

RESULTS AND DISCUSSION

Analysis of variance : The pooled analysis of variance (ANOVA) was used to examine the interactions between different genotypes, environments for grain yield per plant. **Table 4 and 5** presents the results of the individual ANOVAs of each environment and pooled ANOVA for all genotypes across various environments, focusing on grain yield per plant. The ANOVA model demonstrates its capacity by explaining 89% of the data variability from environment-1, with highly significant genotypic effects. The replicates effect is, however, not significantly different. Similarly, the model effectively accounts for 87% of the variability in data from environment-2, with highly significant genotypic effects. The replicates effect is, however, not significantly different. Furthermore, the model adeptly captures 88% of the variability in environment-3 data, underscoring the significant impact of genotypic effects. Replicates effects continue their non-significant pattern. Interestingly, the model explains 89% of the variability in environment-4 data, with significant genotypic effects and varying replicates effects, suggesting potential experimental variations. Pooling data across environments, the model captures 58% of the variability, indicating genotypic significance and illustrating how seasons impact outcomes across the four environments. The box plot (**Fig. 1**) represents the variation of four seasons. Similar Results were reported by Gupta *et al.* (2016) found substantial variability in treatment and block effects across centres, with high

variability explained in Bathinda, Hisar, and IARI centres. Navgaon, Ludhiana, and Teri centres exhibited varying significance patterns.

Mean Performance : The Tukey's Honest Significant Difference (HSD) Test was performed on four environments and with a significance level (alpha) set at 0.05. A comparative analysis of the means within Environment-4 reveals a high degree of similarity, suggesting that the observed differences between group means may not attain statistical significance levels detectable by the Tukey's HSD Test.

Grain yield per plant is a critical metric in assessing crop productivity, and it is heavily influenced by environmental factors. In E1, the top-performing genotypes were G9 $(22.2 g⁻¹)$, G19 (21.77 g⁻¹), and G25 (21.57 g⁻¹), with scores indicating their remarkable yield potential in this particular environment. Conversely, G29 (13.4 g⁻¹), G30 (14.9 g⁻¹), and G10 (15.27 g⁻¹) showed the lowest grain yields in E1, underscoring their sensitivity to this environment. Moving on to E2, G1 (20.7 g⁻¹), G25 (20.07 g⁻¹), and G9 (17.37 g^{-1}) emerged as the top performers, highlighting their adaptability to the conditions in this environment. On the other hand, G24 (9.467 g⁻¹), G28 (9.533 g⁻¹), and G27 (9.667 g^{-1}) struggled with lower yields in E2. In E3, G1 (20.73 g⁻¹), G5 (19.5 g⁻¹), and G22 (19.6 g⁻¹) were the standout genotypes, demonstrating their strength and

Table 5. Combined Analysis of variance for pooled data of 4 environments on grain yield per plant

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E₁ $E2$ F3 E4 **S= GRAMS PER PLANT**

Fig 1. Box plot representation of 30 foxtail millet genotypes performance for grain yield across the four environments Fig 1. Box plot representation of 30 foxtail millet genotypes performance for grain yield across the four environments

adaptability with their respective scores. Conversely, G24 (7.733 g^{-1}) , G27 (8.6 g^{-1}) , and G12 (9.567 g^{-1}) were among the lowest performers in this environment. Finally, in E4, G1 (21 g⁻¹), G22 (17.87 g⁻¹), and G25 (18.6 g⁻¹) continued to exhibit strong yields. In contrast, G24 (6.4 g⁻¹), G29 (8.333 g^{-1}) , and G11 (10.03 g^{-1}) recorded the least favourable results in E4. The outcomes of the Tukey's Honest Significant Difference (HSD) Test across these four environments are presented in **Table 6**.

GGE biplot graphical analysi: Various trials are carried out in all regions for major crops every year. Plant breeders and agronomists conduct these trials to find better genotypes and recommend superior cultivars to growers. Crop traits can be grouped into three categories: target traits (economically valuable, like crop yield), explanatory traits (related to target traits), and marker traits (easily measured and less influenced by the environment). In multiyear variety data analysis, the key method is GGE biplot analysis. The challenge needs to be more balanced and complete data due to changing genotypes over the years. Two strategies are used: 1) Analyzing yearly and summarizing results; 2) Evaluating consistency of patterns in grouping test locations and genotypes across years (Yan, 2014).

The GGE biplot results showed that the primary and second principal components accounted for 65.06% and 17.51% of the grain yield (GY) variability, totaling 82.57%. This indicates strong support for the biplot's credibility in elucidating genotype and genotype by environment interaction (GEI) variations, as the first two principal components capture a significant portion of the variance. When these two components fall short of explaining most of the data variability, it suggests the complicated nature

of GEI (Yan, 2014), but it does not condense the biplot invalid (Yan and Wu, 2008). As Yan and Tinker (2006) noted, when a biplot can account for at least 60% of the data's variance, it becomes a valuable tool for identifying meaningful patterns in genotype-environment interactions (MEs).

The AEA (The average environment axis) is a line with a single arrow in the biplot. It starts from the biplot origin and goes towards the average environment in **Fig. 2**. This arrow indicates higher genotypic values for the genotypes it points to (Yan, 2014). The AEC (average environment coordination) is a coordinate system with the AEA as the horizontal axis. It has a double-arrowed line that goes through the biplot origin and is perpendicular to the AEA. The arrows on the AEC (average environment coordination) point outward from the origin and indicate higher instability for the genotypes, regardless of the direction (Yan, 2014). The AEC ordinate distinguishes between genotypes with below-average and aboveaverage means. All locations are on the same side of the AEC in this biplot, indicating that the G/GE in this dataset is sizable and that the AEA is meaningful for genotype evaluation. If the locations are placed on both sides of the AEC ordinate, then the G/GE in the dataset would be too small for the AEC to be reliably used for genotype evaluation. Additionally, the average yield of genotypes can be estimated by projecting their markers onto the AEC abscissa (Kaya *et al.,* 2006). In this study, genotype G3 (FOX 4394), G18 (FOX 4489), G17 (FOX 4478), G23 (ESD 46), G19 (FOX 4392), G8 (FOX 4403), G9 (FOX 4428), G21 (FOX 4330), G5 (ERP 82), G22 (ESD 75), G25 (FOX 4341), and G1 (ELS 20) showed above average mean yields and remained genotypes (from G20-G24) exhibit beloved average mean yield. The

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 Table 6. Environmental Wise Treatment Means and Multiple Comparison Procedure Results under Tukey's HSD test

| Genotype | E1 | E ₂ | E ₃ | E ₄ | Mean |
|----------------|--------------|----------------|----------------|----------------|-------|
| G ₁ | 18.13 defgh | 20.70 a | 20.73 a | 21.00a | 20.14 |
| G ₂ | 15.93 hijk | 13.73 defghi | 14.46 bcde | 13.13 fghijkl | 14.32 |
| G ₃ | 21.40 ab | 14.00 defghi | 13.53 cdef | 11.13 jklmn | 15.02 |
| G4 | 17.96 defghi | 13.23 efghij | 9.83 ghij | 14.40 defgh | 13.86 |
| G ₅ | 17.66 efghij | 13.36 efghij | 19.50 a | 16.16 bcde | 16.68 |
| G ₆ | 15.53 ijkl | 11.633 hijkl | 11.70 defghi | 13.23 fghijkl | 13.02 |
| G7 | 16.03 hijk | 12.366 ghijk | 12.66 cdefgh | 10.53 klmn | 13.01 |
| G8 | 19.60 bcdef | 15.833 cde | 15.80 bc | 12.76 fghijklm | 16.00 |
| G ₉ | 22.20a | 17.36 bc | 15.10 bcd | 11.43 ijklm | 16.53 |
| G10 | 15.26 jkl | 10.56 jkl | 10.16 fghij | 11.33 jklm | 11.84 |
| G11 | 15.40 jkl | 12.20 ghijkl | 17.86 ab | 10.03 mn | 13.88 |
| G12 | 15.90 hijk | 11.26 ijkl | 9.56 hij | 13.10 fghijkl | 12.46 |
| G13 | 17.40 Fiji | 12.90 fghij | 13.26 cdefg | 10.43 lmn | 13.50 |
| G14 | 15.80 hijkl | 13.63 defghi | 13.36 cdef | 12.46fghijklm | 13.82 |
| G15 | 17.90 defghi | 13.26 efghij | 12.20 defgh | 13.30 efghijkl | 14.17 |
| G16 | 18.53 cdefg | 13.06 efghij | 11.53 efghi | 11.63 hijklm | 13.69 |
| G17 | 20.13 abcd | 12.60 fghij | 13.40 cdef | 15.13 cdef | 15.32 |
| G18 | 19.93 Abcde | 15.30 cdef | 12.53 cdefgh | 13.20 fghijkl | 15.24 |
| G19 | 21.76 ab | 13.30 efghij | 11.86 defghi | 16.56 bcd | 15.88 |
| G20 | 16.13 ghijk | 14.56 cdefg | 14.26 cde | 12.80 fghijklm | 14.44 |
| G21 | 21.40 ab | 16.26 cd | 14.33 cde | 16.66 bcd | 17.17 |
| G22 | 20.66 abc | 14.40 defgh | 19.60 a | 17.86 bc | 18.14 |
| G23 | 17.46 fghij | 15.86 cde | 14.53 bcde | 13.80 defghij | 15.42 |
| G24 | 19.53 bcdef | 9.461 | 7.73j | 6.400 о | 10.78 |
| G25 | 21.56 ab | 20.03 ab | 19.70 a | 18.600 ab | 19.98 |
| G26 | 16.33 ghijk | 12.23 ghijkl | 14.73 bcde | 11.66 ghijklm | 13.74 |
| G27 | 20.23 abcd | 9.661 | 8.60 ij | 14.53 defg | 13.26 |
| G28 | 20.26 abcd | 9.531 | 12.13 defgh | 13.33 efghijk | 13.82 |
| G29 | 13.401 | 12.03 ghijkl | 9.53 hij | 8.33 no | 10.82 |
| G30 | 14.90 kl | 12.60 fghij | 12.66 cdefgh | 14.26 defghi | 13.61 |

length of the average environment vector, in relation to the biplot size, indicates how much the genotypes main effect matters compared to genotype-environment interaction (GEI). A longer vector signifies a greater importance of the genotype's main effect, making selection based on mean performance more meaningful (Yan,2014). In this study, the average environment vector's length was enough to choose genotypes based on their average yield performance. Genotypes G21 (FOX 4330), G5 (ERP 82), G22 (ESD 75), G25 (FOX 4341), and G1 (ELS 20), which had above-average yields, were selected, while the others were discarded. A longer projection on the AEC ordinate, in any direction, indicates that a genotype has a stronger genotype-environment interaction (GEI). This means it is less consistent and more variable across different environments or the opposite (Yan, 2014). For instance, genotypes G21 (FOX 4330), G22 (ESD 75),

G25 (FOX 4341), G3 (FOX 4394), G18 (FOX 4489), G17 (FOX 4478), G8 (FOX 4403), G23 (ESD 46) and G9 (FOX 4428) were more stable as well as high yielding. Conversely, G5 (ERP 82), G19 (FOX 4392) and G1 (ELS 20) were more variable but high yielding.

An ideal genotype should ideally have the highest mean performance and absolute stability, meaning it performs exceptionally well in all environments. This ideal genotype is represented by a long arrow pointing to it in **Fig. 3**. While such an ideal genotype may not exist in reality, it serves as a reference for evaluating other genotypes. The closer a genotype is to this ideal, the more desirable it is. To visualize this, concentric circles were drawn around the ideal genotype as the centre to show the distance between each genotype and the ideal one. In this evaluation, the genotypes' PC1 and PC2 units are in the original yield

Fig 2. Average environment coordination (AEC) views of the GGE-biplot based on environment-focused Fig 2. Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling for the means performance and stability of genotypes. scaling for the means performance and stability of genotypes.

units. Therefore, the units of the AEC abscissa (mean yield) and ordinate (stability) are also in the original yield units. The original yield units also measure the distance between genotypes and the ideal genotype. This ranking method assumes that stability and mean yield are equally important, as proposed by (Yan, 2002). **Fig. 6** shows that G25(FOX 4341) positioned at the centre of the concentric circles, is an ideal genotype due to its higher yield and stability compared to the other genotypes. G21 (FOX 4330), G1 (ELS 20), and G22 (ESD 75), found on the next circle, can also be considered desirable genotypes. The remaining genotypes are less stable.

"Which Own Where" biplots visually represent megaenvironments and facilitate the identification of superior genotypes made-to-order to specific environments. These biplotsplot genotypic means against the IPCA-1, where each genotype is represented as a line with the IPCA serving as the slope. Such biplots are referred to as "which own where" biplots (Yan, 2014). Polygonalbiplot 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 the biplot are perpendicular to the sides of the polygon or their extensions. In the GGE biplot (**Fig. 4**), genotypes

G25 (FOX 4341), G1 (ELS 20), G27 (FOX 4420), G19 (FOX 4392), G24 (ERP 57), G29 (ELS 34), and G11 (FOX 4336) were located at the farthest distance and formed a polygon. These six vertexes and no equality lines divide the biplot into six sectors, and the environments fall into 2 of them. Three environments—E4, E2, and E3—fell into a similar section, and the genotypes at the corners of this section were G25 (FOX 4341) and G1 (ELS 20). This suggests that these genotypes performed exceptionally well in those specific environments.

On the other hand, one environment, E1, fell into its single section, and the genotypes at the corner of this section were G27 and G19. This indicates that these genotypes were the highest-yielding ones for this particular environment. Conversely, genotypes located in sections without associated environments are not as suitable for cultivation across the studied conditions. Among these, Genotypes G24, G29, and G11 were positioned in such sections, suggesting they may not perform well in the tested conditions.

A test location that cannot effectively distinguish between cultivars does not give us any useful information. Another critical aspect of a test location is how well it represents the environment we are interested in (Yan, 2014).

Which-won-where view of the GGE biplot

Fig 4. Polygon views of the GGE-biplot based on symmetrical scaling for the which-won-were pattern for genotypes and environmentsFig 4. Polygon views of the GGE-biplot based on symmetrical scaling for the which-won-were pattern for

ideal genotype showing G+G×E interaction effect of 30 foxtail millet genotypes under four environments for
orain vield per plant **ideal genotype showing G+G×E interaction effect of 30 foxtail millet genotypes under four environments Fig 5. The GGE biplot 'Discriminativeness vs. Representativeness' pattern for genotype comparison with grain yield per plant.**

Suppose a test location does not accurately represent the target environment. In that case, it could be more helpful and can lead to misleading results because it only provides partial information about the tested cultivars (Yan, 2014). An "ideal test location" is like a theoretical spot that's defined to have the most extended vector among all locations, and it is perfectly representative, meaning it does not contribute to genotype-environment interactions (GE) and sits right on the AEA. The closer an accurate location is to this ideal one, the better it is as a core test location (Yan, 2014). The concepts of discriminativeness and representativeness in GGE biplots are crucial for identifying ideal environments that can effectively distinguish between genotypes. Using average environmental coordinates (AEC) and test environments helps us visualize Environments I, II, III, and IV more effectively and represent them in **Fig.5**. Environment II is characterized by short vectors, suggesting it has average discriminative power, representing the average performance of genotypes. The length of the environment vector roughly corresponds to the standard deviation within each environment, indicating how distinct that environment is. Environments with longer vector lengths have higher standard deviations, indicating a more vital ability to distinguish between genotypes. E3 stands out with its long vector, signifying significant discriminative power and highgenotype performance. Notably, E4 has a narrower angle with the AEA, making it more representative compared to other environments. This study, E4 is identified as the most representative environment, while E3 stands out for its strong discriminative capacity.

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., G1 and G25 exhibited stable and reliable performance 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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