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Delineation of genotype × environment interaction and identifying superior red sorghum [*Sorghum bicolor* L. Moench] genotypes via multi-trait-based stability selection methods

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Abstract

Red sorghum, a versatile crop with high nutritional and biochemical value, was evaluated for agronomic and grain quality traits using 27 genotypes across diverse environmental zones of Tamil Nadu. The data collected were subjected to Additive Main Effects and Multiplicative Interaction (AMMI) and Genotype-by-Environment Interaction (GGE) biplot models to recommend a specific genotype for the particular locality. The analysis of variation of the pooled data revealed a significant interaction effect between genotype and environment for the majority of the examined traits. Correlation analysis revealed a significant positive association of panicle weight and a negative association of micronutrient content with grain yield. The G × E was smaller than the genetic variation of grain yield as it portrayed the maximum contribution of genotypic effects (50.2%). GGE biplot showed E2 as a highly discriminating environment for grain yield and also identified environment-specific genotypes viz., G11 for E1, E3 and G6 for E2 environments. The GGE biplots recommended G9 and G14 as high-yielding stable genotypes, outperforming the check variety PAIYUR 2 (G15) by 15.83% and 36.16% respectively, across all environments, particularly in E1 and E3. The genotypes G14, G18, G21 and G26 shown wide adaptation and excelled as biofortified genotypes with significant Fe and Zn contents. Multi-trait stability evaluation approaches such as multi trait stability index (MTSI), multitrait genotype–ideotype distance index (MGIDI), multi-trait index based on factor analysis and genotype-ideotype distance (FAI-BLUP), multitrait mean performance and stability index (MTMPS) and Smith-Hazel index assuming 15% selection intensity were adopted. G14 consistently ranked as the top and steady performer in all evaluation methods. The identified genotype demonstrated exceptional reliability, high yield potential, and early maturity, making them suitable candidates for variety and hybrid development, as well as ideotype breeding programs aimed at ensuring food and nutritional security.

Keywords Red sorghum, Stability, G × E interaction, Multi-trait selection index

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) ranks as the 5th most important and widely grown cereal crop cultivated across the world, particularly in arid and semiarid tropical regions [1]. It belongs to the Poaceae (Gramineae) family and is predominantly a self-pollinated diploid species ($2n = 2x = 20$) [2]. Global sorghum production reached an estimated 59.5 million metric tonnes (MMTs) in 2024, an 11.21% increase from the previous year. Among the various cultivated varieties of sorghum, red sorghum is particularly noteworthy for its abundance of bioactive compounds and gluten-free nature, making it an attractive option for a range of applications [3]. Its essential nutritional qualities, including protein (4.4–21.1%), carbohydrates (32.1–72.5 g), dietary fiber (9.7–14.3 g), and minerals (P, Ca, Fe, Zn), make it a staple food for millions residing in arid and semiarid regions [4]. These attributes have attracted substantial interest from scholars and created market demand for red sorghum worldwide, particularly in the fields of food, feed, and pharmaceuticals [5]. The adaptability of sorghum allows it to thrive in diverse conditions and exhibit superior drought tolerance to that of other cereals [6]. One of the challenging quantitative traits with a high degree of environmental interaction is grain yield. Therefore, it is vital to base selection decisions on an evaluation of yield stability rather than mean performance under various environmental circumstances [7]. Given India's diverse and demanding environment, genotype-by-environment interaction (GEI) and the absence of an effective multitrait-based selection approach pose significant challenges in breeding climate-resilient red sorghum suitable for adaptation to non-conventional regions. Memon, et al. [8] highlighted numerous stability approaches for rating genotypes and determining appropriate environments. Researchers often use the additive main effects and multiplicative interaction (AMMI) model and GGE biplots to analyze multilocation trial data [9]. However, the AMMI model excludes genotypic effects. To address this, Yan, et al. [10] developed the exploratory biplot Genotype × Genotype × Environment (GGE), which includes both principal genotype and GEI effects. This makes the GGE biplot a valuable tool for identifying ideal genotypes and testing conditions, offering genotype performance analysis across environments [11].

In the final stage of red sorghum genotype development, breeders prioritize selecting supreior genotype by considering grain yield alongside other key agronomic traits can improve breeding efficiency. But, breeders have faced difficulties incorporating multiple trait datasets without encountering multicollinearity in the screening process. As a result, multitrait-based stability selection methods are designed to help breeders maintain sustainable gains in principal traits, such as grain yield, while also maintaining genetic progress in secondary characteristics.

These indices include the multitrait stability index (MTSI), the multitrait genotype-ideotype distance index (MGIDI), the multitrait mean performance and stability (MTMPS), the multitrait index based on factor analysis, the genotype-ideotype distance (FAI-BLUP index) and the Smith–Hazel index [12–16]. Additionally, multitrait selection models have been extensively employed in multilocation trials to investigate the performance of red sorghum genotypes [17].

Selection indexes like the Smith-Hazel (SH) index are widely employed to stream line trait selection but often face limitations in plant breeding, particularly in early and more advanced stages, as noted by studies like Bhering, et al. [18], Jahufer and Casler [19], Dalló, et al. [20], Olivoto, et al. [12] and Woyann, et al. [21]. To address these challenges, Olivoto and Nardino [15] developed the Multi-Trait Genotype-Ideotype Distance Index (MGIDI), a factor analysis-based index designed for simultaneous selection and treatment recommendation across multiple traits. Its effectiveness was validated through Monte Carlo simulations, demonstrating its ability to achieve desired trait gains under varying scenarios, including different genotype numbers, trait sets, and correlation structures.

Therefore, a comparative study of the stability and five multitrait models was also conducted to determine the most supreior genotypes. Given this background, our study addresses the following array of objectives: (1) evaluate and suggest genotypes with specified and distinct features during summer offseason; (2) select the steady and better performing genotypes across multiple locations using AMMI and GGE biplot; and (3). From a nutritionist's/breeder's view, attempts were made to identify suprerior high yielding and nutritious genetic stocks through multi trait selection indexes.

Materials and methods

Plant genetic materials and environments

A total of 27 red sorghum genotypes, including cultures and landraces (Table S1), collected from different regions of Tamil Nadu were employed for assessment in the summer of 2024 across three locations in the southern agroclimatic zone of Tamil Nadu, India, namely, E1: Madurai—at the foothills of Yanamalai, E2: Paramakudi—in a semidry southern ecosystem zone on the southern bank of the Vaigai River and E3: Kovilpatti—part of a network of 25 Agrometeorological Cooperating Centers established by the ICAR. These diverse locations provide various environmental conditions (Table S2) for evaluating red sorghum genotype performance. A randomized block design (RBD) with three replications was used in each environment. Each genotype was grown in three 3 m rows, with 45 cm between rows at all locations (E1, E2, and E3). Agronomic practices and plant protection measures were carried out during the crop growth season according to the recommendations provided in the TNAU Crop Production Guide for Agriculture (2020). Weed management involved hand weeding at 15 and 45 days after sowing. To ensure accuracy and minimize border effects, border rows were excluded from the data collection. The experiment followed necessary agronomic procedures and plant protection strategies. Specific details, including plot size, sowing date, and spacing, are provided in Table S2.

Evaluation of morphological traits

Eleven morphological traits were assessed at the flowering, maturity, and postharvest stages. Phenotyping for days to 50 percent flowering (DTF) was conducted on a plot basis. The average values for key yield-related adaptive traits, including plant height at fifty percent flowering (PH, in cm), number of leaves per plant (NL), leaf width (LFW, in cm), leaf length (LLH, in cm), stem girth (SG, in cm), panicle weight (PW, in g), panicle length (PL, in cm), panicle width (PWI, in cm), thousand-grain weight (TGW, in g), and single-plant yield per plant (YIELD, in g), were recorded for five randomly selected plants from each genotype in each location from 2024 (Table S3) [17].

Evaluation of nutritional traits

The iron (Fe) and zinc (Zn) contents in the grains were also analyzed via an atomic absorption spectrophotometer at 248.3 and 213.8 nm, respectively. Seeds of each genotype were selected at random and processed with a ball grinder. The analysis followed standard protocols and was measured in mg/kg, as described by Madhusudhana, et al. [22]. The analysis employed the mean data, which was the average value of each genotype from all three environments (E1, E2, and E3).

Statistical analysis

The data on biometrical and micronutrient traits collected across the three environments were analyzed via combined analysis of variance. In line with the linear mixed model methodology described by Khandelwal, et al. [11], genotypes and GEI were treated as random effects, allowing for the estimation of variance components attributable to GEI. The stability and superiority of red sorghum genotypes was assessed via a variety of stability and selection models, including AMMI, GGE biplot, MTSI, MTMPS, MGIDI, the FAI-BLUP index, and the Smith−Hazel index. BLUPs were derived for random effects, including genotypes and GEI, to evaluate stability and genetic superiority. The selection of AMMI, GGE biplot, and Multi trait selection methodologies was guided by the objective of identifying genotypes that not only demonstrate high yield but also maintain stability across diverse environments. The AMMI model was employed to decompose GEI into principal components, providing insight into the adaptability and stability of genotypes but might oversimplify interactions at higher-order components. This complements the GGE biplot, which visualizes the interaction patterns and facilitates the identification of ideal genotypes for micronutrient content and yield. Along with this the Multi trait selection methods further integrates multi-trait data, ensuring the selection of genotypes that meet both agronomic and nutritional criteria. This multi-pronged approach ensures robust and objective genotype evaluation. These statistical analyses were performed via RStudio, with R version 4.0 used as the underlying computational engine. Specifically, we employed the "metan" R package to conduct a suite of analyses [23]. This approach allowed for a comprehensive examination of the data, leveraging both traditional and modern statistical techniques to assess genotype stability and performance across environments.

AMMI and GGE analysis

Various methods have been developed to analyze data from multi-environment trials, which are generally classified into two main categories: univariate and multivariate methods. Among the multivariate approaches, the AMMI model holds significant importance. Bartlett's test was used to assess the homogeneity of experimental error variances [24]. Once the homogeneity of error variances was confirmed, variance analysis for each trait was conducted using the AMMI model. Using AMMI analysis, the data (YIELD) were analyzed as described by Bradu and Gabriel [25]. A primary envision employing this approach offered information about the additive and nonadditive components, which were revealed via analysis of variance (ANOVA) and principal component analysis (PCA). The significance of location, genotype, and their interaction was assessed at different probability levels via the F test. The AMMI biplots were constructed by charting the primary effects against the first PC axis and between the first two PC axes. Furthermore, adopting the approaches of Farshadfar [26] and Atta, et al. [27], AMMI stability values (ASVs) and the yield stability index (YSI) were used to rank the genotypes on the basis of stability and mean performance. The AMMI model is often used together with the GGE biplot graphical model to identify mega-environments as well as winning genotypes in each mega-environments. Equation used to perform stability analysis by the AMMI method [28]

$$Y_{ge} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge}$$

where Y_{ge} is the yield of genotype g in environment e; μ is the grand mean; α_g is the genotype deviation from the grand mean; β_e is the environment deviation; λ_n is the singular value for IPCn and correspondingly λ^2_n is its eigenvalue; γ_{gn} is the eigenvector value for genotype g and component n; δ_{en} is the eigenvector value for environment e and component n, with both eigenvectors scaled as unit vectors; and ρ_{ge} is the residual.

Yan and Kang [29] and Zobel, et al. [30] suggested that the analysis and interpretation of intricate data structures across several environments be carried out effectively via the site regression model genotype–genotype–environment interaction (GGEI) biplot. The GGE analysis also indicated that the first two main components account for the majority of the total variation. They are optimal for generating GGE biplots via environment-centered data and the symmetrical singular value partitioning (SVP) approach. They are best suited for creating GGE biplots with environment-centered data and the symmetrical singular value partitioning (SVP) approach [29]. A unique feature of the GGE biplot is its ability to identify which genotype performs best in a specific environment or subgroup based on the visual representation [31]. An experimental mean versus stability biplot was created via the row metric preservation approach for genotype assessment. Graphic analysis of GGE biplot was done based on single value decomposition as follows

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j1} + \varepsilon_{ij}$$

where, Y_{ij} is the mean of ith genotype in jth environment, μ is the mean of all genotypes, β_j is the main effect of jth environment, λ_1 and λ_2 are the special quantities for the first and second components, respectively, ξ_{i1} and ξ_{i2} are the special vectors of genotypes, and η_{j1} and η_{j2} are the environmental vectors of first and second components, respectively, and ε_{ij} is the remaining quantity for the ith genotype in jth environment.

Furthermore, a Y x WAAS biplot with four quadrants was used to group the genotypes into distinct quadrants, and the mean performance on the X-axis and stability according to the WAAS values on the Y-axis were used.

Multi-Trait Stability Selection Methods

To assess genotype stability, singular value decomposition (SVD) was applied to theBLUP matrix for genotype–environment interaction (GEI) effects via a linear mixed model (LMM). Stability was measured by the weighted average of absolute scores (WAAS), and the WAASBY index was used to select genotypes for both average performance and stability [12]. The genotype with the lowest MTSI score represents the ideal type, showing superior steady performance across environments. The following genotypes were selected with a 15% selection intensity:

$$MTSI_i = \sum_{j=1}^{f} \left[(F_{ij} - F_j)^2\right]^{0.5}$$

where MTSIi is the multitrait stability index for the ith genotype, F_{ij} is the jth score of the ith genotype, and F_j is the jth score of the ideotype. The MTSI scores were plotted to differentiate between genotypes that were selected and those that were not.

The multitrait mean performance and stability index (MTMPS) is derived from the MTSI with only the difference as calculated via Wricke's ecovalence (Wi) as opposed to the WAASB indices [12, 14, 15].

The MGIDI was calculated following the methodology of Olivoto and Nardino [15] via the following form.

ula:

$$MGIDI_i = \sqrt{\left(\sum_{j=1}^{f} (Y_{ij} - Y_j)^2\right)}$$

Here, MGIDIi represents the multitrait genotype–ideotype distance index for the ith genotype, and Y_{ij} is the jth factor score of the ith genotype (i = 1, 2,., t; j = 1, 2,., f), with t and f being the number of genotypes and factors, respectively, and is the Y_j score of the ideotype.

The strengths and weaknesses of the genotypes were evaluated by calculating the percentage of the MGIDI for the ith genotype attributed to the jth factor (ωij) as follows.

$$\omega_{ij} = \frac{\sqrt{D_{ij}^2}}{\sum_{j=1}^{i} \sqrt{D_{ij}^2}}$$

where D_{ij} is the distance between the ith genotype and the ideal genotype for the jth factor. A trait with a low contribution indicates that the genotypes within such a trait are close to the ideal genotype.

After the ideotype is defined, each genotype's spatial probability is calculated by estimaiting its location from the ideotype. This assessment helps in assessing the genotypes. The formula for calculating the Multi-Trait Index Based on Factor Analysis and Genotype-Ideotype Distance (FAI-BLUP Index) is as follows:

$$P_{ij} = \frac{\frac{1}{d_{ij}}}{\sum_{i=1;j=1}^{i=n;j=m} \frac{1}{d_{ij}}}$$

Here, P_{ij} represents the probability that the ith genotype (i = 1, 2, ..., n) is similar to the jth genotype (j = 1, 2,

…, m), whereas d_{ij} denotes the genotype–ideotype distance from the ith genotype to the jth ideotype, which is calculated via the standardized average Euclidean distance [13].

The equation for calculating the Smith–Hazel index (SH) in the standard approach for evaluating multitrait stability with 15% selection intensity is as follows [16]:

$$I_{ij} = \sum_{k} b_k \overline{Y}_{ik}$$

Ii represents the index value for the ith progeny, where b_k is the coefficient of weight for trait k, and Y_{ik} denotes the progeny i phenotypic mean for trait k. The values of b_k are determined via the formula b = P − 1Gxa, where P − 1 is the matrix inverse of average phenotypic covariances between traits, G is the matrix of genotypic variances and covariances for progeny means across traits, and a is the vector of economic weights assigned to the traits.

Results and discussion

Mean performance and pooled ANOVA

A joint ANOVA revealed significant differences concerning each characteristic for the genotypes being examined. The genotype mean squares showed that the genotypes displayed considerable variation across the locations. Notably, genotype had the most significant effect on all the traits, followed closely by location (Table 1). For example, the highest variance among genotypes was observed in DTF, which accounted for 85.3% of the total variance. E3 had the shortest DTF (88.72), whereas E2 had the longest DTF (92.71) across all environments (Fig. 1 and Table S4). These findings suggest that genetic factors have a major influence on DTF. As specified in Table 1, the Genotype × Environment Interaction (GEI) accounted for 6.4% of the variance in DTF, indicating that genotype performance varies across environments. The genotypic variance for the YIELD was 50.2%, indicating that genetic differences are a key factor in YIELD variability and highlighting the substantial potential for genetic improvement. The GEI had a notable influence on the YIELD, with a 28.8% contribution, suggesting that genotype performance varies significantly with different environments. This underscores the importance of considering environmental adaptability when selecting genotypes. For the other traits, the environmental variance was substantial, with PH accounting for 48.8% of the variance, followed by PW (43.3%), PWI (29.0%), and NL (27.9%). The environment contributed 21.0% of the variance in YIELD, indicating its significant but secondary role compared with the genotype and G × E interactions. This suggests that while environmental factors do influence the YIELD, they are not the primary determinants of its variability. In this study, most traits presented considerable GEIs, demonstrating that the genotype stability of key traits such as PW, YIELD, Fe and Zn should

Table 1 Pooled analysis of variance of 27 red sorghum genotypes evaluated in three environments during summer 2024

Source of variation	Environment (E) (df: 2)		Genotype (G) (df: 26)		GEI (df: 52)		Residual (df: 156)		
Trait	Mean Squares	% (G + E + GEI)	MS	% (G + E + GEI)	MS	% (G + E + GEI)	MS	CV (%)	Mean Over Environments
DTF	220.2**	8.3	2258.9**	85.3	170.5**	6.4	35.2	6.5	90.9
LLH	146.6**	16.0	409.1**	44.6	361.1**	39.4	42.4	8.4	76.0
LFW	1.0**	4.8	9.4**	45.2	10.3**	49.9	0.7	10.4	8.3
NL	6.8**	27.9	7.6**	31.2	9.9**	40.8	1.6	11.9	10.7
PL	40.3**	15.2	168.1**	63.6	55.7**	21.1	7.2	10.8	24.4
SG	0.5**	15.2	2.3**	66.7	0.6**	18.2	0.2	12.1	3.5
PWI	11.2**	29.0	22.4**	58.1	5.0**	12.9	0.9	11.9	7.7
PH	6362.4**	48.8	2620.3**	20.1	4056.5**	31.1	894.8	11.6	253.3
PW	1139.9**	43.3	818.6**	31.1	676.2**	25.7	75.9	10.7	80.3
TGW	102.0**	25.8	197.5**	50.0	95.3**	24.1	12.4	11.4	31.4
YIELD	458.5**	21.0	1095.3**	50.2	627.7**	28.8	54.4	10.1	72.2
Fe	154.0**	19.7	555.9**	71.1	72.1**	9.2	11.9	9.1	37.2
Zn	36.5**	26.8	85.3**	62.7	14.3**	10.5	3.2	9.4	18.7

df degree of freedom, *MS* Mean square

** Significant at the 0.01 probability level, *GEI* Genotype–environment interaction, *CV%* Coefficient of variation, *DTF* Days to 50% flowering, *LLH* Leaf length, *LFW* Leaf width, *NL* Number of leaves/plant, *PL* Panicle length, *SG* Stem girth, *PWI* Panicle width, *PH* Plant height, *PW* Panicle width, *TSW* Thousand-seed weight, *YIELD* single-plant yield, *Fe* red sorghum grain iron content, *Zn* red sorghum grain zinc content

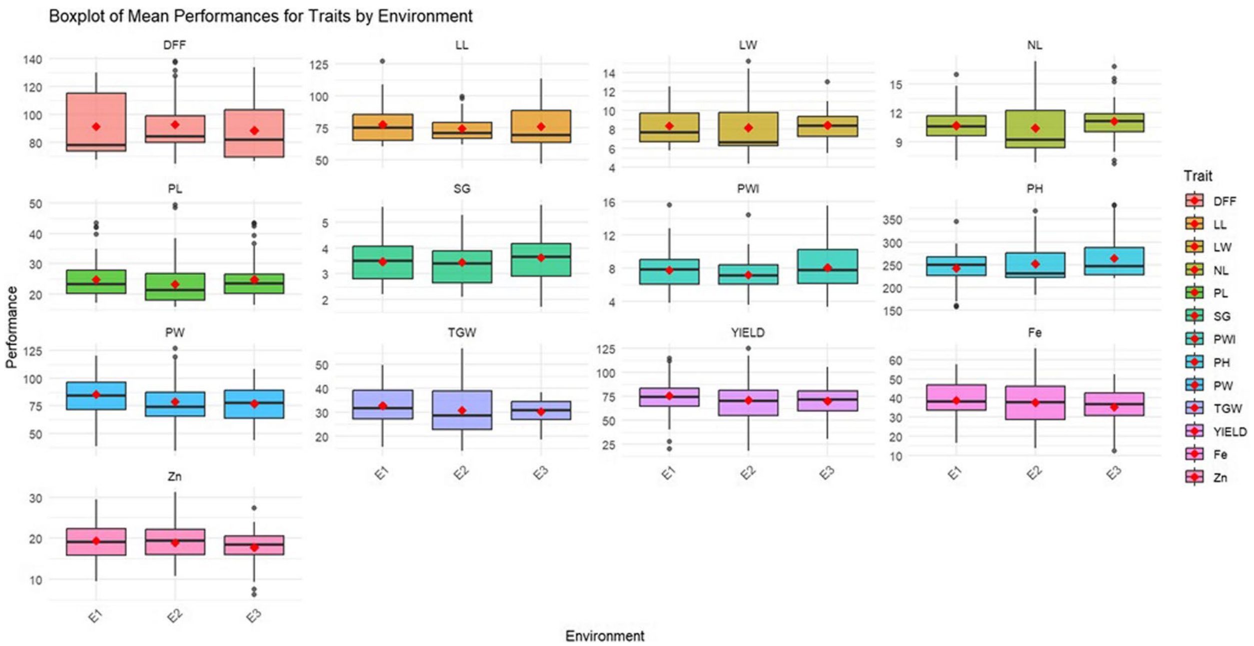

Fig. 1 Box plots depicting a visual summary of the mean performances of the studied traits across all environments during the summer of 2024. DTF: days to 50% flowering, LLH: leaf length, LFW: leaf width, NL: number of leaves/plant, PL: panicle length, SG: stem girth, PWI: panicle width, PH: plant height, PW: panicle weight, TSW: thousand-seed weight, YIELD: single-plant yield, Fe: grain iron content, Zn: grain zinc content, E1: Madurai, E2: Paramakudi, E3: Kovilpatti

be further investigated. For example, LFW had the highest GEI variance at 49.9%, followed by NL (40.8%), LLH (39.4%), and PH (31.1%) (Table 1). Worede, et al. [32] noted substantial influences of genotypic and environmental impacts on traits such as DTF, PH, PW, TGW, and YIELD. Behera, et al. [17] reported very large GEI impacts for most traits except SG. Enyew, et al. [6] also reported notable outcomes for all the biometrical traits studied. On the basis of these findings, Worede, et al. [32] and Enyew, [6] advised further stability investigations to implement the AMMI model. The mean single plant yield was 72.2 g across environments, ranging from 70.03 g (E3) to 75.51 g (E1) (Tables 1 and S4). The panicle weight ranged from 76.70 g (E3) to 85.49 g (E1), with an average of 80.3 g per plant (Tables 1 and S4). The iron content ranged from 35.4 g (E1) to 38.73 g (E3), with 37.22 g per plant on average (Tables 1 and S4). Similarly, Table 1 shows the means for other traits across environments: 90.9 (DTF), 76.0 (LLH), 8.3 (LFW), 10.7 (NL), 24.4 (PL), 3.5 (SG), 7.7 (PWI), 253.3 (PH), 37.2 (Fe) and 18.7 (Zn). The boxplot (Fig. 1) illustrates the average trait values across different environments. Enyew, et al. [6] reported an average grain yield of 78.14 g among 324 genotypes, and similar trends were observed for other agronomical traits. Behera, et al. [17] reported comparable results for NL and PL across locations, whereas Worede, et al. [32] reported similar results for TGW across locations.

Genotype-by-environment interaction analysis

The AMMI ANOVA results revealed that the genotype, environment and GXE interaction (GEI) effects influence the most significant variations in the additive component of the entire sum of squares for all traits (Table 1). These findings indicate that environmental changes also play a critical role in shaping the expression of most traits. GEI was also significant for all traits at the 1% probability level. It caused variation in genotypes ranking in different environments for all studied traits. Such a result has been reported in almost all similar studies [17, 33] Research by Enyew, et al. [6] highlighted substantial genotypic variance in PH, PL, PW, and YIELD. Their study also revealed notable location–genotype interactions, with significant differences at the 0.001 significance level observed for these traits. In a similar vein, Behera, et al. [17] demonstrated considerable genotypic variance in PH, LLH, DTF, and PL. These findings also pointed to significant location–genotype interactions for these characteristics. Comparable outcomes were reported by Worede, et al. [32] in their evaluation of sorghum genotype stability and adaptability. Their study, which employed the AMMI model, examined yield across 13 genotypes in 3 locations over a 2-year period. The GEI impact was further broken down by the AMMI model into two interaction components that are usually defined as multiplicative effects (Table S5). These components were identified through principal component analysis [34] and are illustrated in Table S5 and Figure S1. Yan, et al. [10] posit that strong GEI effects can hinder genetic improvements in quantitative traits such as YIELD and PW. However, in the present study, the GEI percentage was low to moderate, suggesting the potential for improvement in most traits across various environmental conditions.

AMMI Biplot

Considering the significant effect of GEI for all studied traits, multiplicative effects analysis was performed to identify stable genotypes based on AMMI's model. AMMI model with two significant IPCs is the best predicted model [30, 35]. This study included an array of biplots that broadcast different performing genotypes across different traits, focusing on PW, YIELD, Fe and Zn. The AMMI I exploratory graph or biplot was designed with the X-axis representing the trait's mean across environments, highlighting the principal effects [34], whereas the Y-axis displayed the first interactive principal component axis (IPCA 1) score, addressing the multiplicative effects (Fig. 2). The standard description for this AMMI biplot is as follows: a genotype or environment with a PC score near zero indicates minimal interaction. Conversely, if both the environment and the genotype share the same sign for their PC scores, this signifies that they interact positively with one another [36–38].

A significant genotype–environment interaction (GEI) was detected for the traits PW, YIELD, Fe and Zn, explaining 25.7%, 28.8%, 9.2%, and 10.5% of the variance, respectively (Table 1). Two interacting principal components (PCs) were further separated from this interaction. The first two multiplicative components, IPCA1 and IPCA2, sufficiently explained 100% of the GEI (Table S5 and Figure S1). Of these, the first component accounted for the largest share of the variance, ranging from 53.7% to 88.5%, indicating that IPCA 1 accounted for the most of the overall variance (Table S5). In terms of the YIELD, environments E2 and E3 were located to the immediate left of the mean yield line, indicating that they were less productive. In contrast, E1, positioned dextrally, revealed a highly productive environment, as noted byPawar, et al. [39]. Furthermore, E3 had the shortest vector and was positioned closest to the origin; thus, it was determined to be the least variable environment. The other environments, with longer vectors, presented higher levels of interaction (Fig. 2B).

For PW, E1 was more productive than the other environments were, as it was positioned to the right of the mean line. However, it exhibited relatively less frequent interactions with genotypes, as it was positioned closer to the center with the second smallest vector (Table S7 and Fig. 2). In contrast, a different trend was noted for micronutrient content. With respect to Fe and Zn, E2 and E3 were recognized as the most interactive and productive environments (Table S7 and Fig. 2). Interestingly, these same environments were revealed to be less productive for the YIELD and PW. Among them, E3 demonstrated the least interaction, with the minimal length vector and a position near the origin (Table S7 and Fig. 2). This result suggests an inverse relationship between productivity and micronutrient accumulation. Specifically, genotypes tend to accumulate higher concentrations of micronutrients when they are grown in environments that are less productive for YIELD and PW (Fig. 2). This finding highlights the intricate interactions between environmental conditions, yield components, and nutrient accumulation in the studied genotypes.

The current findings are corroborated by Assefa, et al. [33], who reported that sorghum varieties have an IPCA 1 axis variance of 75.03%. In this study, genotypes G9 and G14 were located on the dextral side closest to the overall mean line, with IPCA 1 values of nearly zero and high mean grain yields (Fig. 2B). These genotypes demonstrated minimal environmental interactions, as evidenced by their alignment along the IPCA 1 line (Fig. 2B and Table 2). G14 also showed the minimal interaction for PW (Table S7 and Fig. 2A). For micronutrient content, G18 had the lowest IPCA 1 value and was the most productive genotype for iron content, whereas G17 had the least interaction for zinc content (Table S7, Fig. 2C, D). These results highlight the stability and productivity of specific genotypes across different traits, providing valuable insights for breeding programs targeting yield and nutrient content improvement in diverse environments.

In this further research, IPCA 2 was utilized to further analyze the GEI given its significant role in the study (Table S5). To further explore IPCA2, an AMMI II exploratory graph was designed (Fig. 3). In this plot, the polygon in the form of dotted lines results from joining the genotypes located at the vertices, and the vertical projection from the genotype to the environment vector indicates the extent of interaction with a particular environment. For grain yield, the AMMI 2 biplot revealed that E2 was highly interactive for G6, which contributed largely to the GEI (Fig. 3B). Among the three environments, E2 was revealed to be more interactive for genotypes, whereas E3 had short vectors, indicating the least interaction and nearly stable performance (Fig. 3B). E1 was highly interactive for genotype G10. Notably, among all the genotypes, G11 produced exceptionally high grain yields (Table 2). With respect to panicle weight, G6 and G25 were highly responsive at E2. G10 and G19 strongly interact with variations in E1 (Fig. 3A). For the Fe content, E1 and E3 strongly interacted with G3 and G16, respectively. Environment E2 was identified to be more

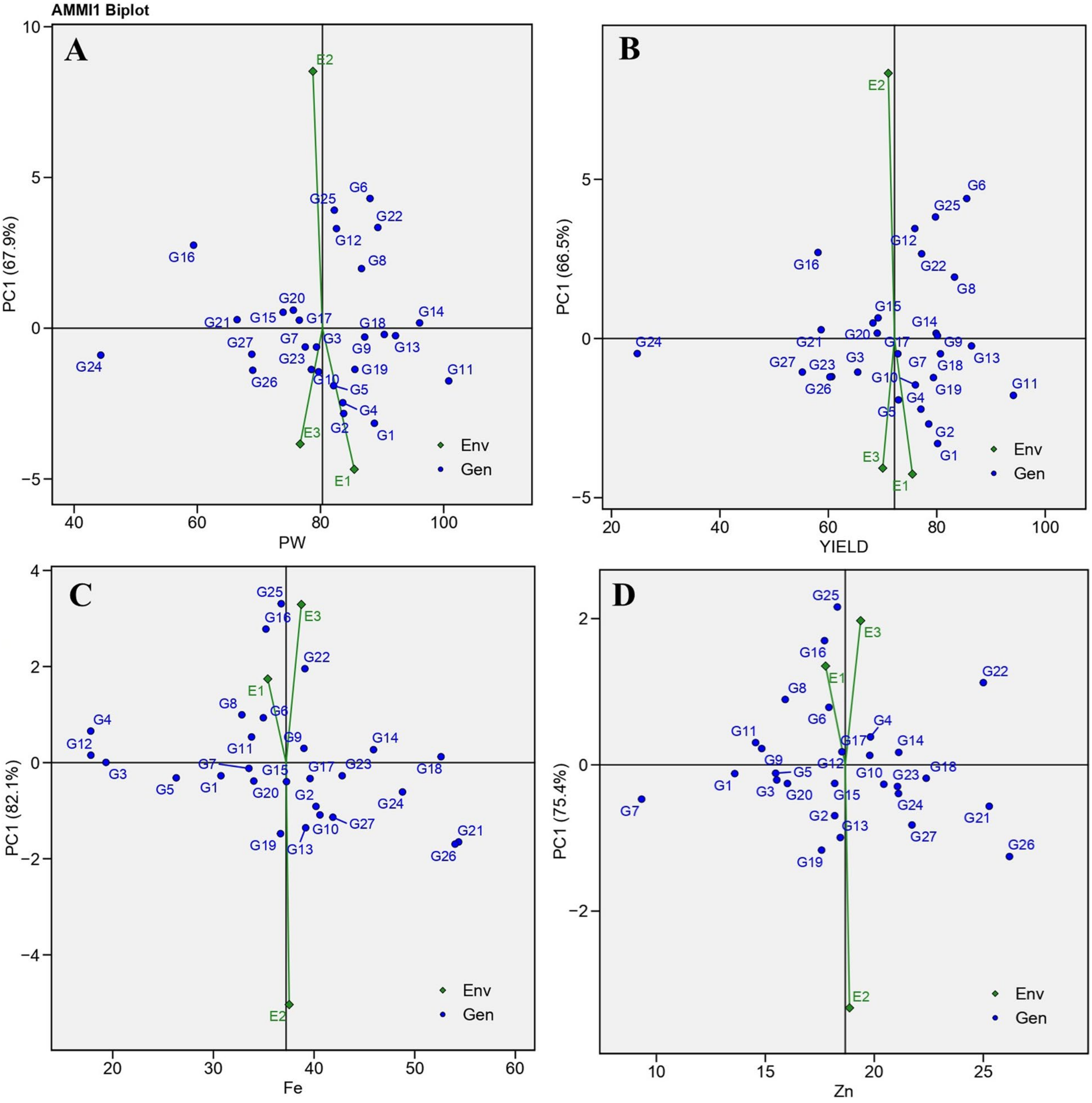

Fig. 2 AMMI I biplot of 27 red sorghum genotypes (blue dots) tested in three environments (green dots) from summer–2024: **A** PW: panicle weight, **B** YIELD: single-plant yield, **C** Fe: iron content, **D** Zn: zinc content. PC: principal component

interactive than the other factors were, whereas E3 had the minimal interaction with the genotypes (Fig. 3C). The Zn content exhibited a strong interaction with genotype G3 in environment E1, which had a prolonged vector, indicating highly variable locations for the Zn content. E2 was interactive for genotypes G26 and G27, which possessed short vectors, suggesting consistent Zn uptake from particular locations (Fig. 3D). These findings provide insights into the valuable complex interactions between environments and genotypes for various traits, which can inform breeding strategies and environmental management practices.

Worede, et al. [32] outlined a biplot analysis for AMMI II, where environments CH13 and JR13 presented greater interactions, indicating greater genotype discriminating ability. KB13 and KB14 exhibited moderate interactions, whereas JR14 and CH14 contributed the least to the genotype−environment interaction (GxE). These

Table 2 PC values, means across the environment, and WAAS scores of 27 genotypes evaluated across three different locations from summer–2024

GENOTYPES	Mean over environment	PC1	PC2	WAAS
G1	80.155	−3.299	−1.381	2.657
G2	78.515	−2.685	2.225	2.531
G3	65.422	−1.056	−2.649	1.59
G4	77.078	−2.219	0.580	1.67
G5	72.933	−1.928	−1.529	1.794
G6	85.527	4.396	−0.331	3.034
G7	72.818	−0.480	−0.253	0.404
G8	83.277	1.929	0.157	1.336
G9	80.123	0.088	−0.554	0.244
G10	76.060	−1.458	4.270	2.400
G11	94.133	−1.786	−0.690	1.419
G12	75.958	3.454	0.552	2.482
G13	86.407	−0.234	1.416	0.63
G14	79.880	0.164	−1.023	0.452
G15	69.175	0.648	−1.103	0.800
G16	58.092	2.702	−0.030	1.807
G17	69.028	0.168	−0.865	0.401
G18	80.677	−0.480	−0.928	0.63
G19	79.385	−1.228	3.167	1.877
G20	68.233	0.488	−0.498	0.491
G21	58.665	0.275	1.620	0.725
G22	77.212	2.660	1.755	2.357
G23	60.623	−1.199	−1.618	1.339
G24	24.757	−0.473	−1.039	0.663
G25	79.762	3.818	−0.473	2.697
G26	60.290	−1.208	2.042	1.487
G27	55.203	−1.055	−2.823	1.647
E1	75.512	−4.260	6.036	4.855
E2	71.054	8.332	0.091	5.571
E3	70.032	−4.072	−6.127	4.761

PC Principal component, *WAAS* Weighted average of absolute score, *E1* Madurai, *E2* Paramakudi, *E3* Kovilpatti

findings suggest that JR14 and CH14 are more representative but less discriminating environments. In a study by Enyew, [6], AMMI 2 analysis of panicle weight (PAWT) among 324 sorghum genotypes revealed that G148 and G73 presented high PAWT and stability. Conversely, the mean grain yield of genotypes G206, G213, and G306 was high, but their placement distant from the biplot origin suggested poor stability. Madhusudhana, et al. [40] conducted AMMI II analysis of the Fe and Zn contents in sorghum. Their findings revealed that Deesa and Hyderabad environments had lower GEIs for Fe, whereas Coimbatore and Solapur exhibited strong interactions. For Zn, Hyderabad and Palem presented lower GEIs, whereas Solapur presented strong interactions. These studies collectively demonstrate the utility of AMMI-II biplots in identifying stable genotypes and characterizing environments further by using discriminating potentiality and representativeness for various traits in sorghum breeding programs.

Correlation relationships among multiple traits across environments

Correlation analysis by Karl Pearson was conducted, and a network plot was designed on the basis of mean data across environments, following the method of Singamsetti, et al. [41] (Fig. 4 and Table S8). The network plot (Fig. 4) revealed a significant positive correlation between GY and PW. Additionally, the YIELD was positively correlated with PW and PH, but the Zn and Fe contents were negatively correlated. A strong positive correlation ($r = 0.69$) was also observed between the Zn and Fe contents in sorghum (Table S8). The DTF was negatively associated with the YIELD but was significantly positively correlated with the Zn and Fe contents (Table S8). The network plot further indicated that traits such as PL, LFW, NL, PH, LLH, YIELD, PW, TGW, PWI, and SG clustered closely together, forming one group. In contrast, DTF, Fe, and Zn formed separate clusters. Cluster 1 primarily included yield-related traits and plant morphological characteristics, whereas Cluster 2 included phenological traits (DTF) and micronutrients (Fe and Zn). The trait closeness within clusters was obtained via multidimensional clustering, as suggested by Gower [42]. A similar analysis was carried out by Enyew, et al. [6], who reported a strong correlation ($r = 0.91$) between the YIELD and PW. Madhusudhana, et al. [40] also reported a strong and positive correlation between micronutrients (Fe and Zn). In contrast, Phuke, et al. [43] reported a significant negative correlation between micronutrients and grain yield across six evaluated environments. Similarly, Ng'Uni, et al. [44] reported a negative correlation between micronutrients and yield.

GGE biplot analysis

The GGE biplot graphical analysis method was employed to examine the variation among genotypes, environments, and their interactions (GEI) [31]. The first two principal components (PCs) accounted for a substantial proportion of the variance across all studied traits, affirming the relatively high validity of the biplot in capturing the variations in genotypes and genotype-environment interactions (GEI). However, if the combined variance explained by the first two PCs is insufficient, it suggests a more complex nature of the GEI [45]. Nonetheless, this does not imply that the biplot lacks validity[46].

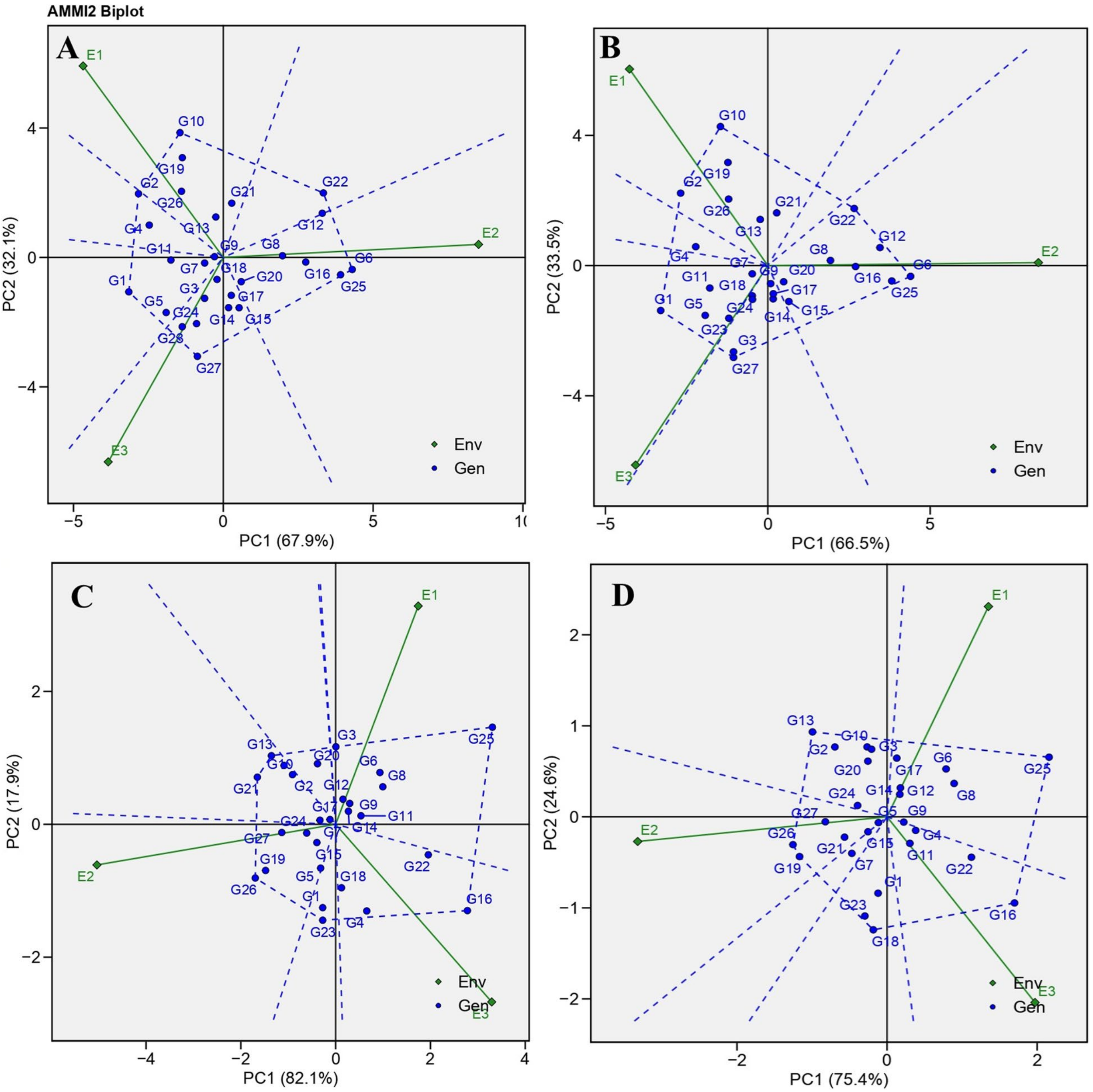

Fig. 3 AMMI II biplot developed using the PC I and PC II values of 27 red sorghum genotypes (blue dots) evaluated in three environments (green dots) from summer–2024: **A** PW: panicle weight, **B** YIELD: single-plant yield, **C** Fe: iron content, **D** Zn: zinc content

Which-Won-Where and What?

The polygon view of GGE, which shows where and what biplot unfolds the ideal genotype for an area of interest. The polygon in this biplot is developed by connecting the vertex genotypes (Fig. 5) [11]. It is further divided by the dotted rays, which extend from the plot's origin and run vertically to the boundaries of the polygon, into numerous unique quadrants. Gauch and Zobel [47] and Zobel, et al. [30] proposed that this distinction facilitates the endorsement of a genotype for an environment in particular. According to Memon, et al. [8], the genotype positioned at the extremity performs efficiently in that particular environment. This GGE biplot depicted two megaenvironments for the YIELD. E1 and E3 share a single megaenvironment, suggesting that the top-performing genotype, G11, is similar in these environments (Table S6). Similarly, E2 represents a single megaenvironment in which G6 is identified as a winning genotype,

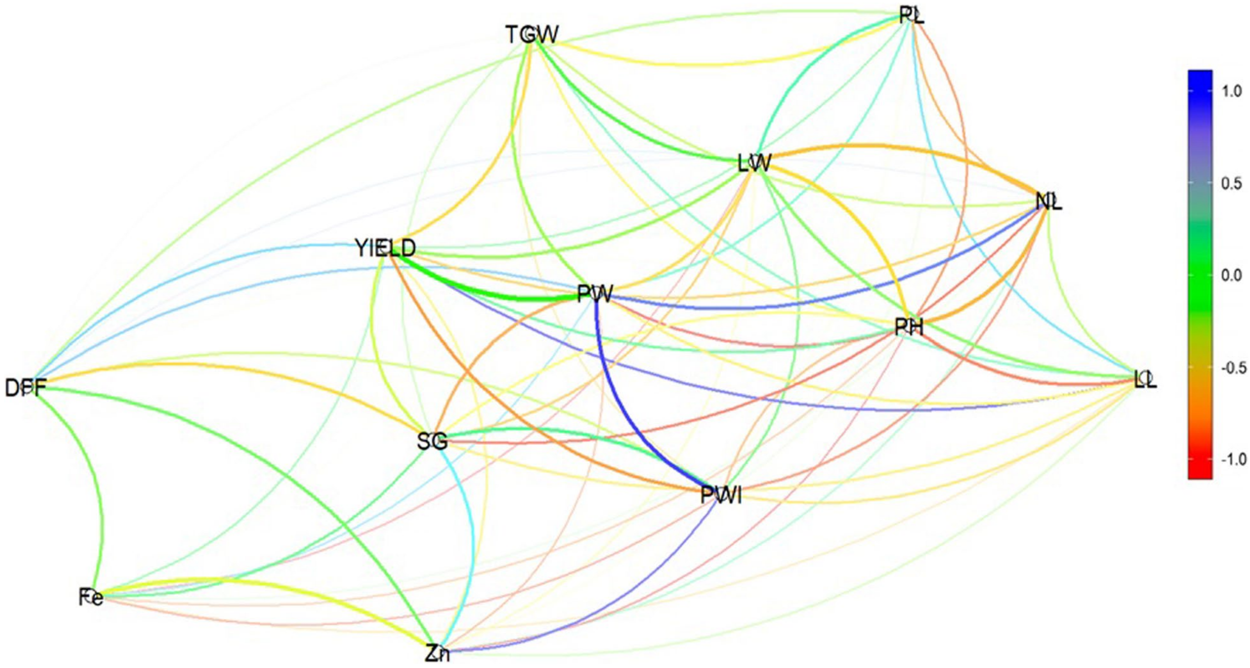

Fig. 4 Pearson's correlation network plot. DTF: days to 50% flowering, LLH: leaf length, LFW: leaf width, NL: number of leaves per plant, PL: panicle length, SG: stem girth, PWI: panicle width, PH: plant height, PW: panicle width, TSW: thousand-seed weight, YIELD: single-plant yield, Fe: grain iron content, Zn: grain zinc content

which also has a negative PC2, suggesting better performance under stressed or low-yield conditions (Fig. 5B and Table S6). Genotypes G17, G14 and G15 are closer to the origin, suggesting stable performance of the YIELD across environments (Table S6). G16 and G24 were not part of any megaenvironment, suggesting a low-yield genotype in nearly all of the environments (Table S6).

Like the YIELD, the PW depicted two megaenvironments (Fig. 5A). E1 and E3 share a common mega environment with G11 as a competitive genotype for panicle weight. Similarly, the unsettled environment E2 created a singleton mega environment with G22 and G6 as the winning genotypes (Table S6). However, genotypes G16 and G24 presented poorer performance in all environments, as they did not take part in any mega-environments (Table S6). In Fig. 5C, the plot reveals two distinct megaenvironments: one represented by E2 and the other by E1 and E3. The genotypes G3, G4, G19, G26, G21, G18, and G25 form the vertices of a polygon. G18 stands out as the top performer in environments similar to E1 and E3, whereas G26 and G21 are likely the best in conditions resembling E2 (Table S6). The fact that E1 and E3 share the same sector suggests that they favor similar top-performing genotypes, whereas E2 occupies a separate sector, indicating that it supports different genotypes. G22 appears to perform best in environments such as E1 and E3, whereas G26 excels under conditions akin to E2 (Table S6). G25, positioned higher on the positive PC2 axis, may indicate better performance under specific environmental conditions (Fig. 5D). Genotypes G17, G15, and G10, located closer to the origin, show greater stability across environments, whereas those at vertices G25, G22, and G26 demonstrate greater responsiveness to environmental variation (Table S6).

This result aligns with the conclusions ofTeressa, et al. [48], who evaluated sorghum hybrids for crop grain yield performance in moisture-stressed regions. They identified a single megaenvironment consisting of Kobo and Sheraro, where vertex sorghum genotypes 23 and 9 excelled. Additionally, they observed Mieso as a distinct mega-environment, with genotype 33 emerging as the top-performing hybrid.

Mean vs Stability analysis

An exploratory two-dimensional plot utilizing the first two PCs was made to compare yield against stability as per Memon, et al. [8] and Patel, et al. [49]. This plot serves the purpose of genotype evaluation because it was generated by applying the row metric preservation technique (Fig. 6). From the standpoint of average environment coordination (AEC), genotype stability was highlighted, and the arrow pointing on the AEC line represented the trajectory of the rising mean. The most stable genotypes are revealed by a tiny dotted line sketched

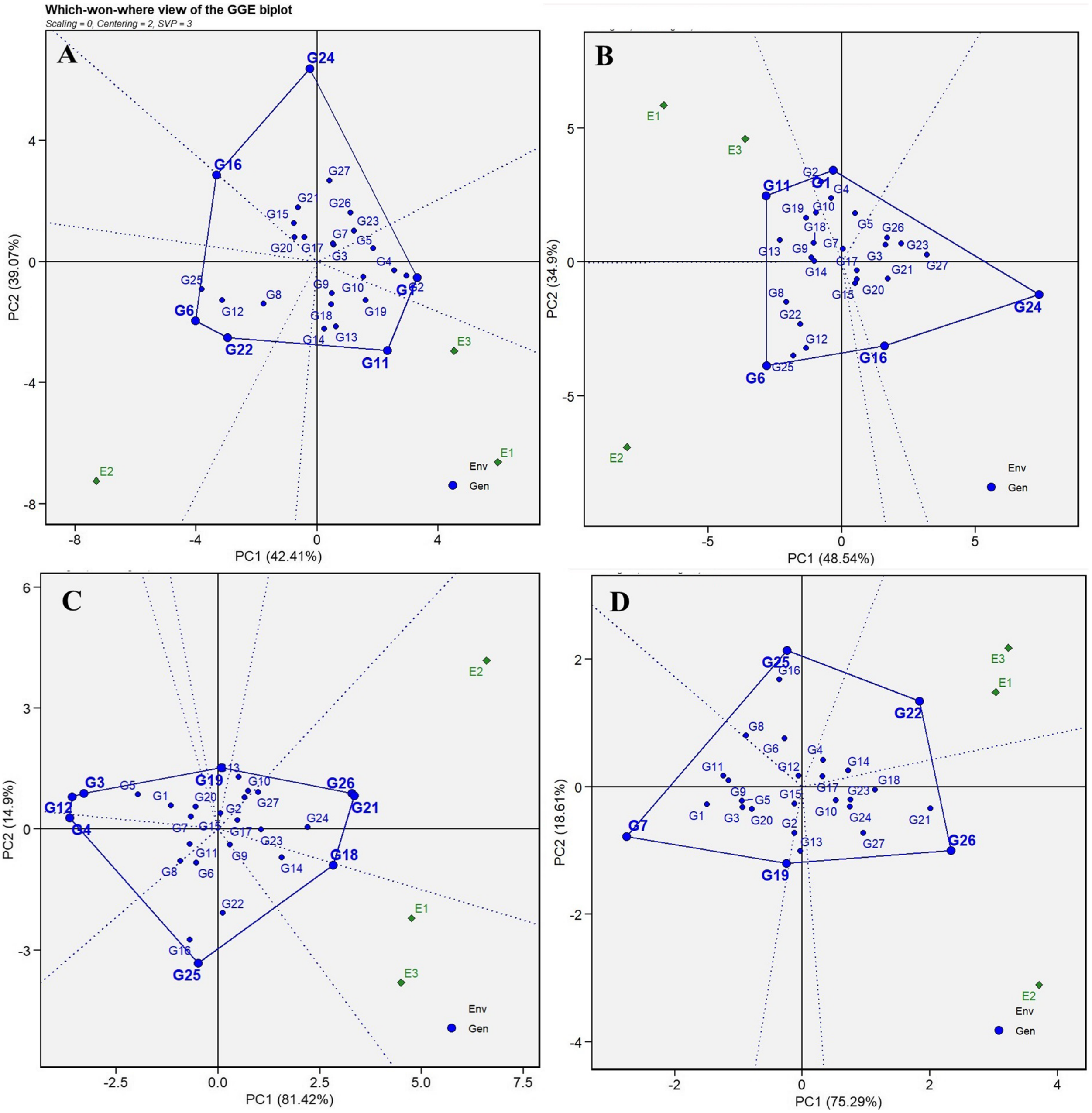

Fig. 5 View of 27 red sorghum genotypes (blue dots) tested in three environments (green dots) during the summer of 2024. **A** Panicle weight, **B** single-plant yield, **C** iron content, **D** zinc content

vertically to the AEC line. As shown in Fig. 6B, genotypes G9 and G14 were stable and presented better yield production, while G11 was a high-yielding genotype across every environment, but it interacted with changing environmental conditions. Similarly, G14, G13 and G9 were better-performing genotypes with notable stability for PW (Fig. 6A). G11 also performed differently in E1/E3 than in E2. G14, G17, and G18 were positioned in the advancing direction along the grain iron and zinc AEC line, with the smallest number of dotted rays, indicating excellent stability with significant micronutrient content and suggesting wider adaptation (Fig. 6C, D). Genotypes G26 and G21 presented the most elevated Fe and Zn levels and were suggested for specific adaptations. Teressa, et al. [48] used this mean vs. stability biplot to interpret the most stable high-yield genotype, G55, followed by G28 and 34, which can be considered genotypes with superior mean yield enactment and stability

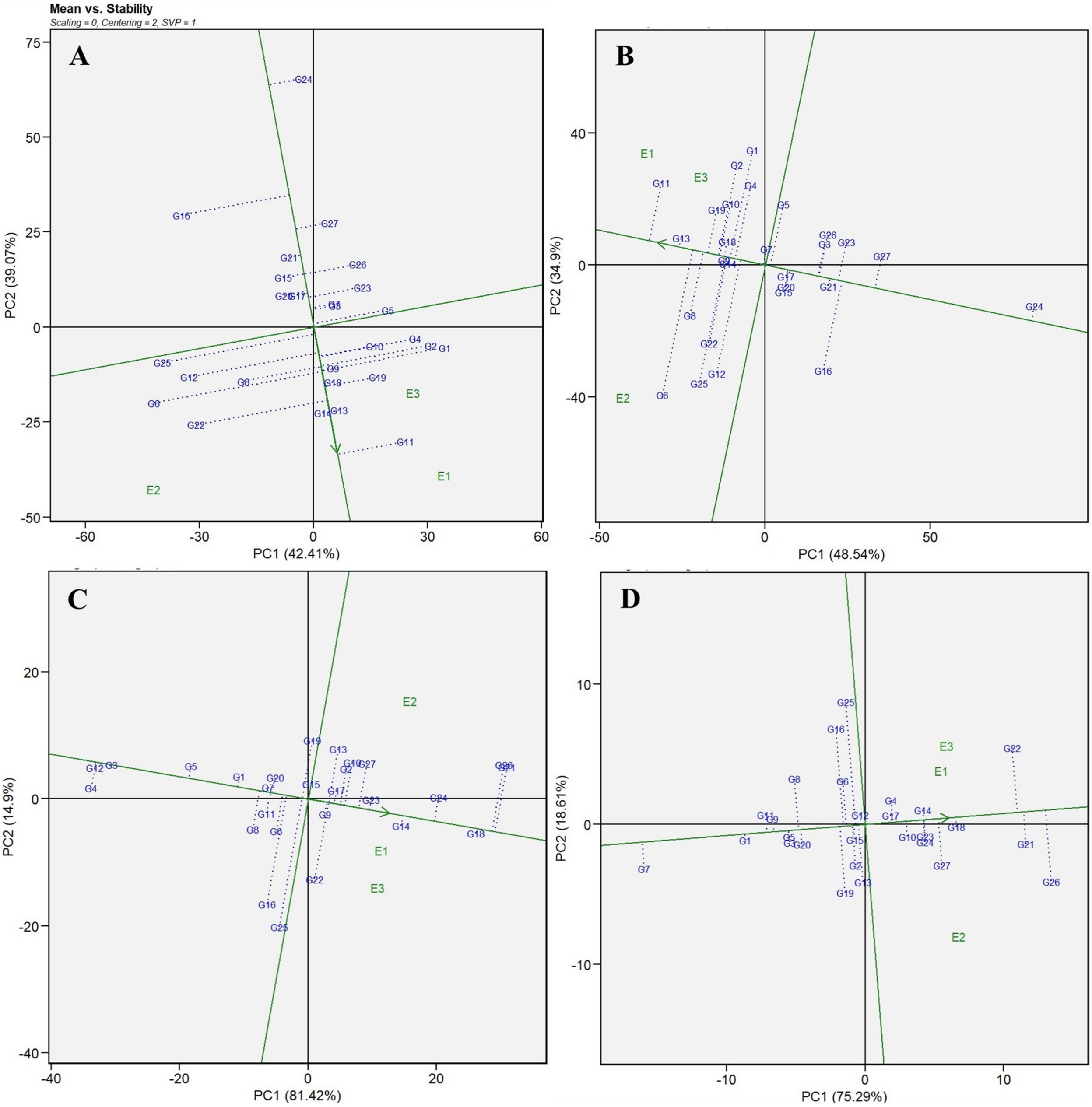

Fig. 6 Mean vs. stability GGE biplot of 27 red sorghum genotypes (blue dots) evaluated in three environments (green dots) during the summer of 2024. **A** Panicle weight, **B** single-plant yield, **C** iron content, **D** zinc content

performance. Similarly, a comprehensive analysis using a GGE biplot was executed by Alwala, et al. [50] to determine stable and high-performing maize genotypes.

Discriminativeness vs. Representativeness (DVR) view of stability

This DVR biplot partitioned into four quadrants was developed via environment-focused centering and the symmetrical method of singular-value partitioning. The cosine angle formed between two environmental vectors illustrates the relationship between them. According to Memon, et al. [8], an obtuse angle implies a negative association, whereas an acute angle signifies a positive relationship. Additionally, there is no relationship between environments when the angle is exactly 90°. Patel, et al. [49] further explained the discriminating capacity of the environment, reflected by the extent of the environment vector on the exploratory biplot, which corresponds to the standard deviation of that environment. To assess the representativeness of an environment, the average environment axis (AEA) is used. As the angle formed by the AEA and environmental vector inclines, representativeness decreases, and vice versa. For grain yield, environments E1 and E3 form an acute angle, indicating a positive correlation between them. Conversely, E2 forms obtuse angles with both E1 and E3, suggesting a negative association with these environments (Fig. 7B). E2 has a lengthy vector, indicating that it has the greatest discriminating power for yield. E3, having the minimal angle with the AEA, is the most representative environment, whereas E1 and E2 have larger angles, indicating that they are less representative of the average environment (Fig. 7B). For PW, a similar pattern is observed, with E1 and E3 showing a positive correlation. E2 forms obtuse angles with both, suggesting a negative correlation. E3 has the smallest angle with the AEA, making it more representative of the environment, whereas E1 and E2, with larger angles, are less representative. E1 has the longest vector, making it the most discriminating environment. E1 and E2 are highly discriminating but

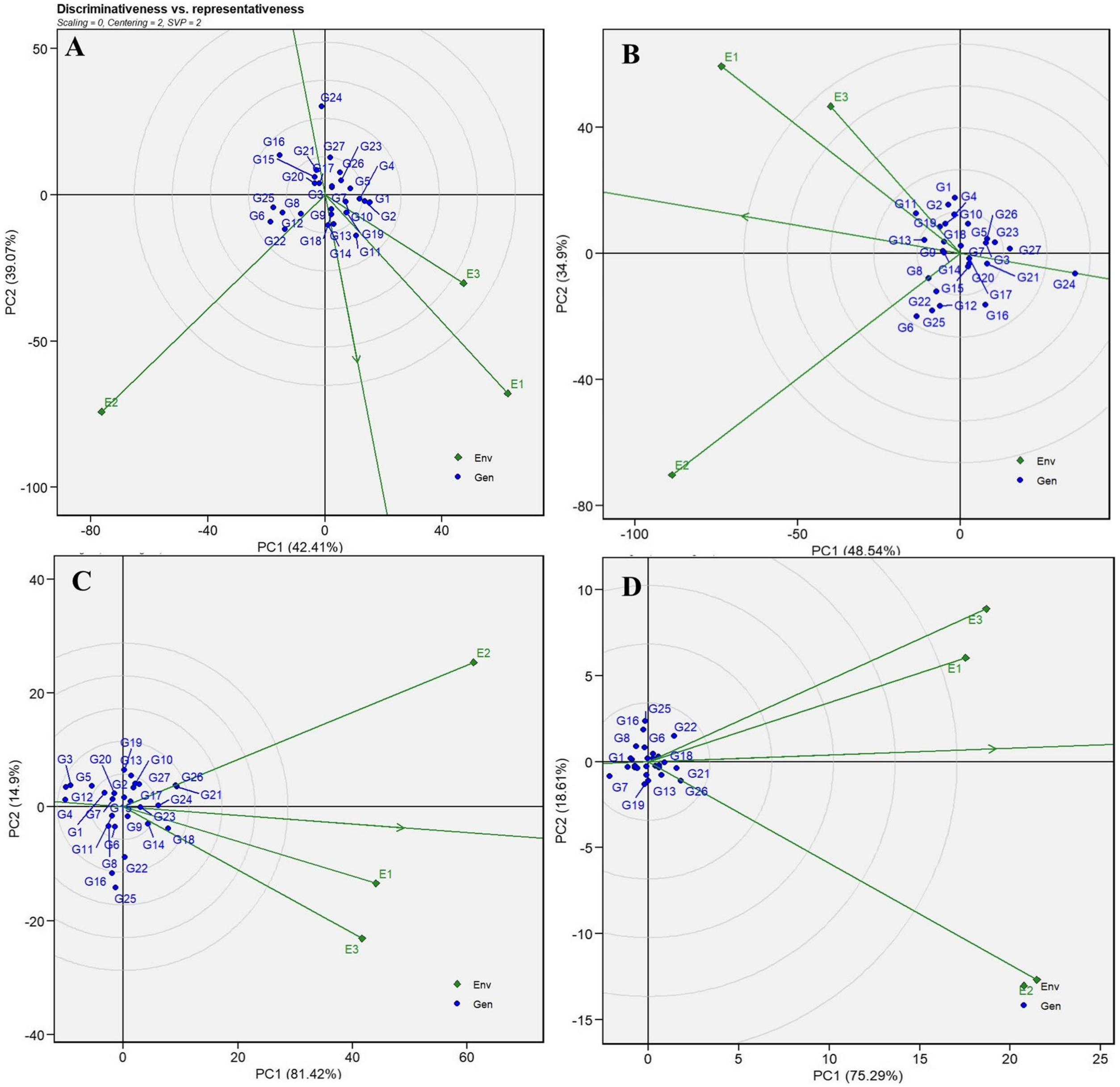

Fig. 7 GGE biplot of the discriminativeness and representativeness of 27 red sorghum genotypes (blue dots) evaluated in three environments (green dots) during the summer of 2024. **A** Panicle weight, **B** single-plant yield, **C** iron content, **D** zinc content

less representative of average conditions (Fig. 7A). In the case of iron (Fe), E1 is identified as a more representative environment because of its minuscule angle with the AEA. E2 has the extended vector, indicating the highest discriminating power. E1 combines both good discriminating ability and representative ability (Fig. 7C). For zinc (Zn), E2 has the highest discriminating power, whereas E1 is more representative. E3 is similar to E1 in terms of representativeness but has a slightly better discriminating ability (Fig. 7D). The biplot also provides insight into the ideal genotypes, represented by circles drawn around the origin. For grain yield, G17 and G18 were identified as ideal genotypes, whereas G3 and G7 were ideal for panicle weight. With respect to Fe and Zn, G2 and G15 are located within the innermost circle, suggesting that they are ideal genotypes for micronutrient content.

Y × WAAS biplot

The mean performance × WAAS biplot developed by combining productivity and stability (WAASB) classifies genotypes into four portions (Fig. 8, Table 2 and S7), enabling the simultaneous selection of genotypes with superior trait values and stability across the included variables, as described by Patel, et al. [49] and Yadav, et al. [51]. In the biplot (Fig. 8B), environments E2 and E3 are located in the Y × WAAS biplot's first quadrant, indicating more unstable genotypes with low productivity but strong discriminating power for grain yield. Moreover, G6, G19, G22, G2, and four other genotypes, along with environment E1, are in the second quadrant of the biplot, showing high yield production but poor stability, suggesting the need for focused efforts in this environment to increase grain yield, as noted by Patel, et al. [49]. The third quadrant contains ten genotypes characterized by greater stability (as indicated by lower WAAS values) but relatively lower productivity. Alternatively, the remaining nine genotypes, including G14, G9, and G11, in the fourth quadrant, exhibited both high stability and outstanding yield performance (Table 2 and Fig. 8B). Similarly, for panicle weight, two genotypes, including E2 and E3, are in quadrant I, eight genotypes (including E1) are in quadrant II, ten genotypes are in quadrant III, and six genotypes are in quadrant IV, with the left-over environments falling into quadrant II (Table S7 and Fig. 8A). For the iron (Fe) content, all 27 red sorghum genotypes were also divided across the four quadrants (Fig. 8C). G16 and E3 are placed in quadrant I, with six genotypes in quadrant II, whereas nine and ten genotypes are allocated to quadrants III and IV, respectively (Table S7 and Fig. 8C). A similar pattern was observed for the zinc (Zn) content, with six genotypes each in quadrant I, two genotypes in quadrant II, ten genotypes in quadrant III, and the remaining nine genotypes in quadrant IV (Table S7 and Fig. 8D).

Multitrait Stability Index (MTSI)

Memon, et al. [8] reported that plant breeders commonly use the classical stability parameters proposed in the model of Eberhart and Russell. However, relying solely

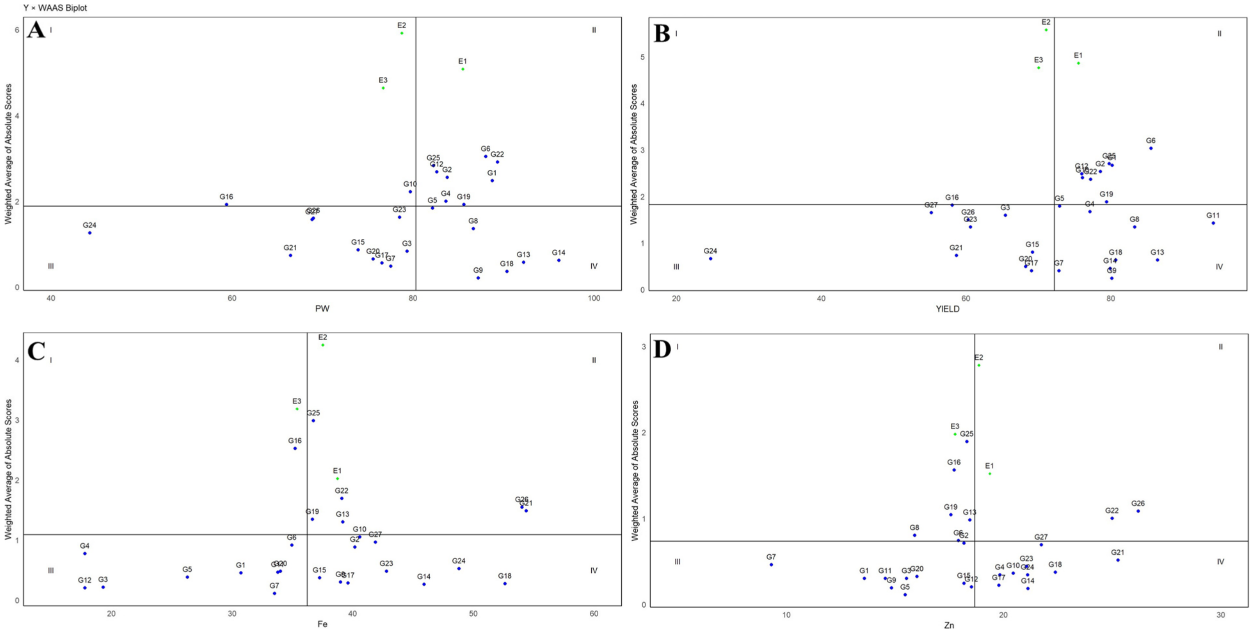

Fig. 8 Y × WAASB biplot for 27 red sorghum genotypes (blue dots) evaluated in three environments (green dots) during the summer of 2024. **A** Panicle weight, **B** single-plant yield, **C** iron content, **D** zinc content

on mean performance, regression, and deviation from regression parameters may fail to indicate what truly happens about both mean performance and trait stability regarding the selection of the most stable genotypes. Sharifi, et al. [52] and Koundinya, et al. et al. [53] noted that the MTSI is highly beneficial for plant breeders in selecting superior genotypes across multiple traits using data from diverse environments. The MTSI method, which is designed to overcome such limitations, represents a step ahead in quantitative genetics for the utilization and identification of favorable variants across multiple traits in different crops.

In further studies, all of these characteristics used in the MTSI computation exhibited a significant level of importance in terms of the GEI in the combined ANOVA. The WAASBY was found by Karl Pearson's association matrix, through which support was available to identify the occurrence of a highly desired association that was merged into one single factor. In this research, this exploratory factor analysis used data for thirteen traits and resulted in four principal components, which explained 79.04% of the cumulative variance (Table 3). The communality or shared variance ranged from 0.396 for PL to 0.919 for YIELD, with the average for the thirteen traits being 0.791 after varimax rotation. Specific variance refers to the fraction of the variability not held in common with other traits and was greater for PL and greater for YIELD (Table 3). The traits were then grouped into four factors, and the corresponding WAASBY values were extracted for each factor (Table 4). PL, PWI, PW, TGW, and YIELD were grouped under FA1; DTF, LLH, and SG were grouped under FA2; LFW, NL and PH were grouped under FA3; and the Fe and Zn contents were grouped under FA4. The mean performance selection was selected depending on the nature of the traits; thus, DTF was negatively selected, whereas the remaining traits were chosen in a positive direction. Selection of the WAASBY value was performed by assigning equal weights to both the mean performance and the stability. The selection differential for DTF improved the antithetical direction since early-maturing genotypes were selected. Conversely, the traits LLH, NL, SG, PWI, PH, PW, TGW, YIELD and Fe presented positive selection differences, indicating that the genotypes have thus far been selected for YIELD and its contributing characteristics. Furthermore, PL was found to be negatively associated with PW in the selected genotypes, although a counterselection differential due to the characteristic associations was recorded. Mean performance and stability, as revealed from WAASBY, were found to be positively selected only for PW. The selection included in Fig. 9 was used to compute the average of the chosen genotypes. In general, the results were in accordance with expectations, except for grain Fe and Zn, which tended to be negatively related to grain yield. Singamsetti, et al. [54] adopted a similar approach in the assessment of the effect of moisture stress on maize.

Table 3 Factorial loadings after varimax rotation, communalities, uniqueness, Eigenvalues and explained variance, procured in the factor analysis of the significant GEI traits studied in 27 red sorghum genotypes across three environments during the summer of 2024

Traits	FA1	FA2	FA3	FA4	Communality	Uniqueness
DTF	−0.222	−0.894	0.003	−0.013	0.848	0.152
LLH	0.166	−0.572	−0.321	−0.296	0.545	0.455
LFW	−0.159	−0.372	−0.809	0.143	0.838	0.162
NL	−0.455	−0.073	−0.793	0.051	0.845	0.155
PL	−0.453	−0.200	−0.309	0.236	0.396	0.604
SG	−0.407	−0.616	−0.519	0.189	0.850	0.150
PWI	−0.629	−0.618	−0.243	0.136	0.855	0.145
PH	−0.154	−0.058	−0.936	0.045	0.906	0.094
PW	−0.886	−0.170	−0.053	0.166	0.845	0.155
TGW	−0.800	0.043	−0.307	0.034	0.737	0.263
YIELD	−0.927	−0.154	−0.180	0.067	0.919	0.081
Fe	−0.269	0.124	−0.321	0.789	0.814	0.186
Zn	−0.029	−0.037	0.039	0.935	0.879	0.121
Eigen values	5.810	1.867	1.467	1.132	0.790[a]	
Variance (%)	44.689	14.359	11.288	8.707		
Accumulated (%)	44.689	59.049	70.337	79.044		

FA factor analysis, *DTF* days to 50% flowering, *LLH* leaf length, *LFW* leaf width, *PL* panicle length, *NL* number of leaves per plant, *SG* stem girth, *PWI* panicle width, *PH* plant height, *PW* panicle weight, *TSW* thousand-seed weight, *YIELD* single-plant yield, *Fe* iron content, *Zn* zinc content

[a] Average of the communality

Table 4 WAASBY index and selection differential for the means of the 13 traits of 27 red sorghum genotypes across three environments during the summer of 2024

		Mean Performance			**WAASBY**		
TRAITS	**FACTOR**	**Overall (X0)**	**Selected Genotype (Xs)**	**SD (%)**	**Overall (X0)**	**Selected Genotype (Xs)**	**SD (%)**
DTF	FA2	90.907	78.700	−12.207	1.004	0.390	−0.613
LLH	FA2	75.985	76.875	0.890	1.266	0.540	−0.727
LFW	FA3	8.337	8.300	−0.037	0.491	0.140	−0.351
NL	FA3	10.733	11.450	0.717	0.558	0.287	−0.271
PL	FA1	24.381	22.625	−1.756	0.710	0.186	−0.525
SG	FA2	3.533	4.100	0.567	0.266	0.094	−0.172
PWI	FA1	7.678	10.425	2.747	0.455	0.290	−0.165
PH	FA3	253.267	257.100	3.833	2.390	0.993	−1.397
PW	FA1	80.319	90.175	9.856	1.514	0.649	−0.865
TGW	FA1	31.370	32.100	0.730	0.940	0.415	−0.525
YIELD	FA1	72.204	80.775	8.571	1.465	0.629	−0.836
Fe	FA4	37.22	39.58	2.352	0.855	0.326	−0.530
Zn	FA4	18.67	17.57	−1.092	0.580	0.225	−0.355

FA factor analysis, *DTF* days to 50% flowering, *LLH* leaf length *LFW* leaf width, *NL* number of leaves per plant, *PL* panicle length, *SG* stem girth, *PWI* panicle width, *PH* plant height, *PW* panicle weight, *TSW* thousand-seed weight, *YIELD* single-plant yield, *Fe* grain iron level, *Zn* grain zinc level, *SD* selection differential and *WAASBY* weighted average of absolute scores of stability with yield, *Xo* average of all genotypes, *Xs* average of selected genotypes

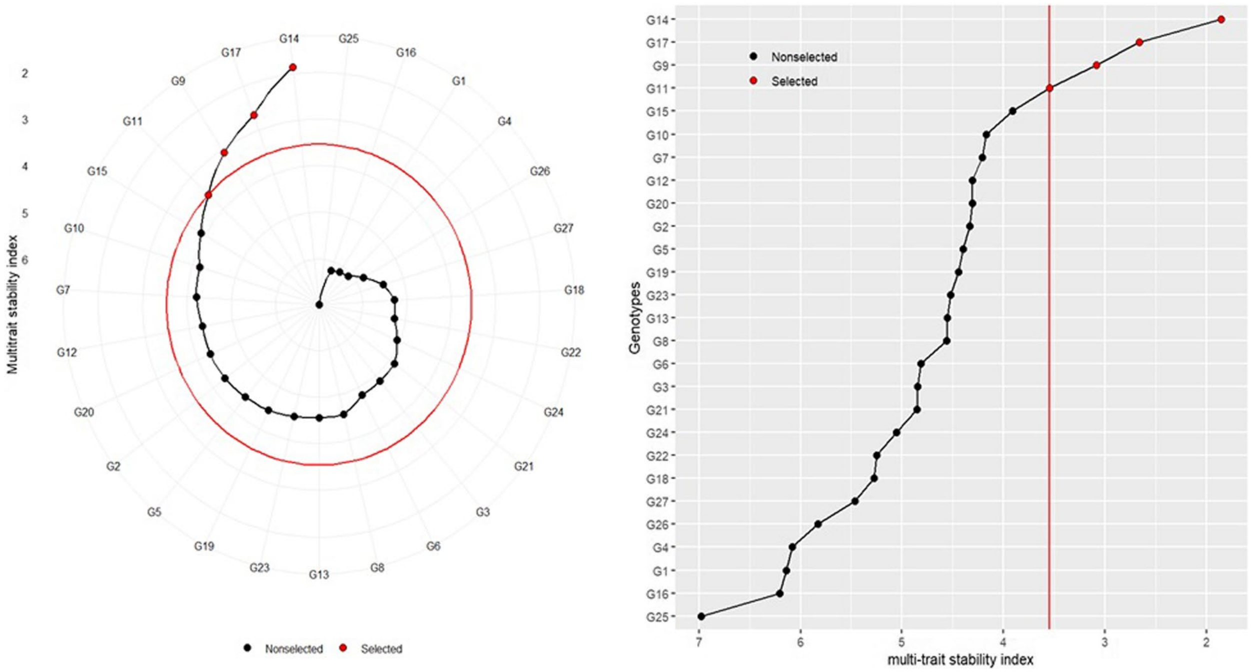

Fig. 9 Genotype ranking and selected genotypes among 27 red sorghum genotypes for multitrait stability index (MTSI) view considering 15% selection intensity

The most significant goal of stability studies is to identify stable genotypes with enhanced mean outcomes across numerous traits. Exploratory factor analysis was conducted via Euclidean distance-based scores between genotypes and ideotypes. Ideotype estimates and this analysis provided the scores for 27 genotypes for the notable first four factors (Table S9).

The MTSI allows the selection of highly stable genotypes with superior mean performance for important interacting traits. Memon et al. used the MTSI to select castor accessions for optimum values of six yield-contributing and biochemical traits. The selection is based on lower MTSI values and 15% intensity. Among these, G14 (MTSI = 1.854), G17 (MTSI = 2.663), G9 (MTSI = 3.084), and G15 (MTSI = 3.912) were selected for the present study. These genes presented high stability with strong mean performance for the analyzed traits (Fig. 9 and Table S9). The cutoff point at an MTSI value of 3.543 is indicated by a red circle (Table S9 and Fig. 9). G25 presented an MTSI value as high as 6.979, followed by G16 with an MTSI value of 6.207, G1 with an MTSI value of 6.143, and G4 with an MTSI value of 6.084 (Table S9), which means that all these genotypes were unstable and performed poorly in terms of the investigated traits. Koundinya, et al. [53] selected highly stable genotypes of cassava to improve important traits, such as starch yield per plant, dry matter, the leaf area index, the harvest index and yield per plant. Behera, et al. [17] used the WAASBY-based MTSI approach to select genotypes G90 (424B) and G80 (382B) as superior genotypes across five environments for high forage yields and other key traits in sorghum breeding. Similarly, Patanè, et al. [55] used similar methods to study the impact of drought and salinity stresses on sweet sorghum seed germination, whereas Murillo-Amador, et al. [56] conducted studies on cowpea via similar methods. Zuffo, et al. [14] utilized MTSI to identify soybean genotypes that exhibit stability under conditions of drought and salinity stress. Rajabi, et al. [57] identified five sugar beet genotypes as stable performers under field conditions affected by rhizomania disease, based on the MTSI results.

The MTSI facilitates the selection of those genotypes that have the best mean performance for numerous traits with high stability. In the present study, the genotypes G11, G9, G17, and G14 were the best performers for the majority of traits evaluated. Additionally, G14 was placed in the Y × WAAS biplot's fourth quadrant for yield, panicle weight, iron content, and zinc content, indicating its very good performance with high stability (Fig. 8, Table 2 and S7). G14 also appeared to be the best genotype for optimizing panicle weight along with grain yield. G11 exhibited moderate stability and good performance, especially for the YIELD and PW. Similarly, on the basis of the biplot of Mean vs. Stability, G18 has exceptional sorghum grain Fe and Zn contents across all regions. From the Which-Won-Where and What biplot for panicle weight, G14 was the best performer for the mega environments E1 and E3, in addition to adding the advantage of stability for the YIELD across all environments. G14 also moderately affected the Fe and Zn contents. Similarly, Memon, et al. [8], while presenting their castor study, also utilized this kind of approach in genotype selection. In fact, these selected genotypes hold great promise for more stable genetic underpinnings in future large-scale breeding programs.

Genotype selection via various multitrait stability analyses

This study enables the selection of superior genotypes by applying positive selection differentials for traits targeted for improvement and negative selection differentials for traits intended to decrease. This approach is beneficial for breeders and agronomists focused on simultaneous selection for multiple traits and average performance, as it offers a straightforward selection process that accounts for the correlation structure among traits [12]. Four of the 27 red sorghum genotypes were selected with a selection intensity of 15%. The genotype rankings, determined by MGIDI scores, are shown in Figure S2, with genotypes G22, G14, G18, and G25 selected for their MGIDI scores. The results of the MGIDI factor analysis for 13 adaptive traits in these 27 genotypes are detailed in Table S10. Four factors with eigenvalues greater than one were retained, explaining 78.59% of the total variance, on the basis of the WAASBY values of the BLUP estimates. The average communality after varimax rotation was 0.78, with a maximum of 0.94 for the panicle weight and a minimum of 0.47 for the thousand-grain weight (Table S10). The traits were categorized into four factors: FA1 included PWI, PW, and SPY; FA2 included LFW, NL, PH, and TGW; FA3 included LLH; and FA4 included DTF, PL, SG, Fe, and Zn (Table S11). The estimated genetic gains and selection differentials for all 13 traits are presented in Table S11, where beneficial selection differentials were observed for all traits except stem girth (−0.19), which showed an undesired negative selection differential.

The effects of each component on the MGIDI index were categorized into two groups: less significant contributors and more significant contributors. The more significant contributors were positioned toward the center, whereas the less significant contributors were placed closer to the edges. Figure S2 illustrates the genotype strengths and weaknesses selected among the 27 red sorghum genotypes on the basis of their MGIDI scores. For FA1, genotypes G14 and G18 were the major contributors (Figure S2). In FA2, G22, G14, and G25 were weaker contributors, exhibiting stability and proximity to the ideotype, making them ideal candidates for breeding programs aimed at improving the traits in FA2 (Figure S2). For FA3 (panicle length), G18, G25, and G14 were weaker contributors to MGIDI than the others were, indicating their better stability and closeness to the ideotype (Figure S2). In FA4, G18 and G14 were also identified as weaker contributors (Figure S2).

The genotypes were selected via various multitrait stability indices, with a 15% selection intensity (Table 5).

When different multitrait stability models were compared, a higher coincidence index was observed between models with more common genotypes. The coincidence index values and common genotypes are presented in Table 6, along with a Venn diagram in Fig. 10. The highest coincidence index with three common genotypes was found between SMITH and FAI-BLUP, MGIDI and FAI-BLUP, and FAI-BLUP and MTMPS (70.59%, 3 genotypes), followed by MGIDI and SMITH (41.18%, 2 genotypes). The lowest values were noted between MGIDI and MTMPS (11.76%, 1 genotype) and between SMITH and MTMPS (11.76%, 1 genotype). The Venn diagram (Fig. 10) illustrates that the genotypes selected by all indices exhibit broad adaptability and demonstrate strong performance across various environments. Genotype G14 was common across all the models (Fig. 10), including MTSI, making it highly desirable and appealing. As a result, these genotypes were included in ideotype-based breeding programs. Yue, et al. [58] reported analogous outcomes with maize hybrids, whereas Ambrósio, et al. [16], Olivoto and Nardino [15] and Benakanahalli, et al. [59] reported comparable results in wheat, black bean and guar, respectively (Fig. 11).

Experimental precision was evaluated by analyzing selective accuracy estimates. This parameter indicates the efficiency of the data and methods used in predicting genetic values [16]. Selective accuracy, which is closely associated with selection accuracy, measures the correlation between individuals' predicted genetic values and their true genetic values, as outlined by Pimentel [60]. As reported by MAVd Resende, et al. [61], moderate to high accuracy values were noted, indicating reliable precision in identifying superior individuals. The majority of the genotypes selected by the indexes were advanced breeding lines. It can therefore be concluded that the selected genotypes possess the genetic potential to develop into new cultivars, exhibiting desirable traits that set them apart from those already available on the market. In a study on Avena sativa L., Olivoto, et al. [12] applied a 15% selection intensity, and the three selected genotypes fell within the cutoff point (red circle) for this intensity. Consequently, future research could explore the performance of genotypes situated near the cutoff point.

Overall, the MGIDI index (Table S12) demonstrated satisfactory gains and effectively selected genotypes aligning closely with the ideotype, characterized by high gain by panicle weight, yield and in micronutrient content. A simulation study assessed the performance of the MGIDI index in comparison to the classic Smith-Hazel (SH) index and the modern FAI-BLUP index in terms of successful feature selection with desired gains. The MGIDI index consistently outperformed both the FAI-BLUP and SH indexes across all simulation scenarios, with its advantages being particularly pronounced in datasets with low trait correlations (Table S12). Overall, MGIDI achieves a success rate of 71.7% in selecting traits with the desired gains [15]. When comparing the Smith-Hazel index to the FAI-BLUP index, MTSI, and MGIDI, the total selection differentials for SH indicate positive gains (45.5%) (Table S12). The overall gains from SH were similar to those achieved with the MGIDI index for grain yield and its components. However, the SH index produced lower gains compared to FAI-BLUP (Table S12).

Numerous studies have highlighted the effectiveness of multivariate selection indexes for simultaneous selection in plant breeding. Examples include identifying soybean

Table 5 Genotype selection via various multitrait stability models

Stability Model	Selected Genotypes	Figures
MGIDI	G22, G14, G18, G25	Figure S2
FAI-BLUP	G18, G14, G22, G23	Figure S3
MTMPS	G14, G9, G17, G11	Figure S4
SMITH	G14, G18, G26, G23	Figure S5

MGIDI—multitrait genotype–ideotype distance index, FAI–BLUP—multitrait index based on factor analysis and genotype–ideotype distance, MTMPS—multitrait mean performance and stability index and SMITH—Smith–Hazel index

Table 6 Genotypes shared between various stability models via the coincidence index

Method-1	Method-2	Coincidence index	No.of.common genotypes	Common Genotypes	Common genotype across all models
MGIDI	SMITH	41.18	2	G14, G18	G14
MGIDI	FAI – BLUP	70.59	3	G22, G14, G18	
MGIDI	MTMPS	11.76	1	G14	
SMITH	FAI – BLUP	70.59	3	G18, G14, G23	
SMITH	MTMPS	11.76	1	G14	
FAI – BLUP	MTMPS	70.59	3	G14, G18, G23	

MGIDI—multitrait genotype–ideotype distance index, FAI–BLUP—multitrait index based on factor analysis and genotype–ideotype distance, MTMPS—multitrait mean performance and stability index and SMITH—Smith–Hazel index

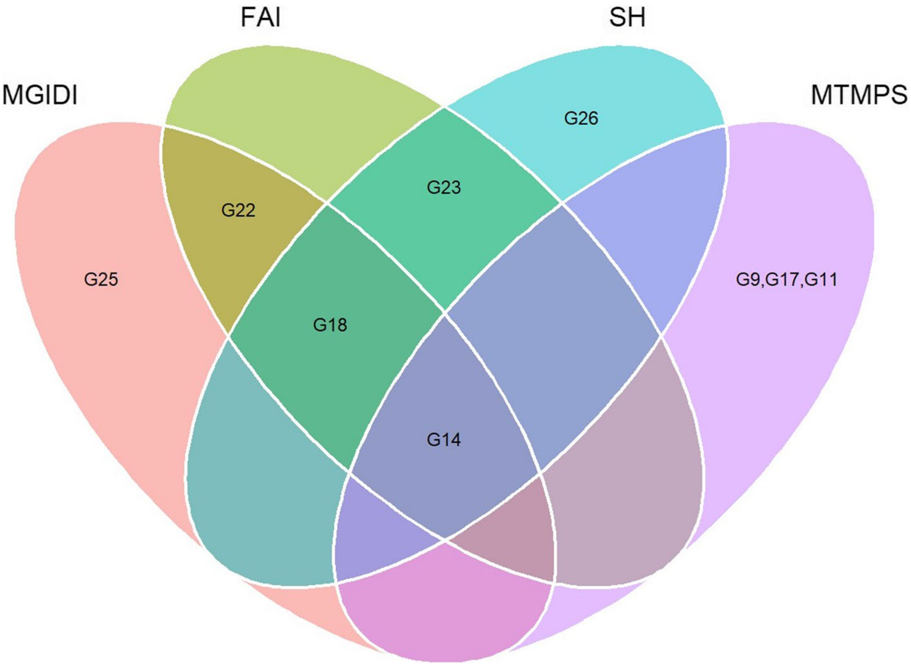

Fig. 10 Venn diagram of common genotypes selected across various stability selection analyses

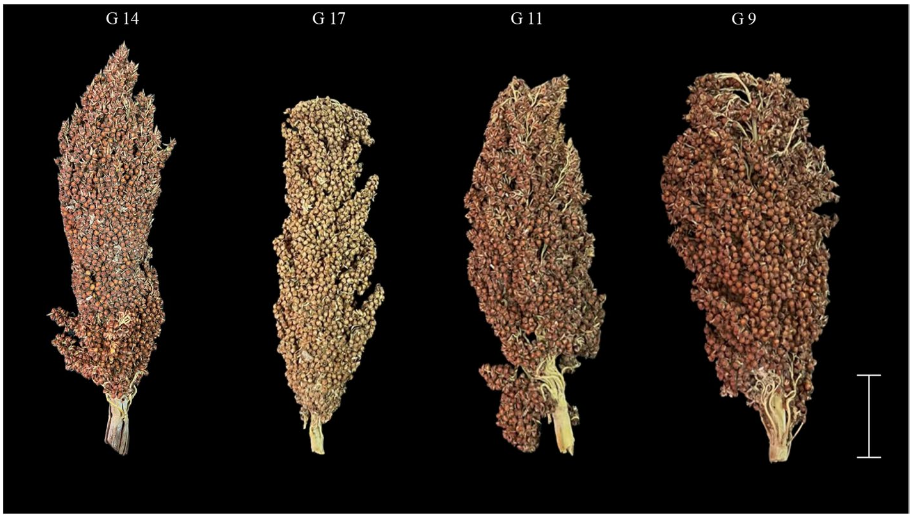

Fig. 11 High-yield stable red sorghum genotypes (scale bar: 5 cm)

genotypes resistant to drought and salinity [14], developing bread wheat ideotypes suited for early sowing conditions [62], selecting millet strains resistant to shoot fly infestations [63], and breeding chickpea genotypes with improved drought tolerance [64].

In summary, the present study revealed that the genotypes namely G11 for E1, E3 and G6 for E2 were suitable for the particular environment and the genotypes viz., G9 and G14 were highly suitable for all the three environments. Genotype G14, identified as stable in the stability analysis, demonstrated superior micronutrient content compared to the check and consistently ranked as a top performer across all multi-trait stability evaluation methods. The genetic potential of the genotypes, along with environmental variability such as differences in weather conditions, soil types, and diverse ecologies across test locations, likely influenced their performance and stability. The study highlighted the stability of grain yield performance across environments, suggesting that these genotypes hold significant potential as parental or pre-breeding material for future red sorghum breeding programs aimed at developing high-yielding varieties with enhanced micronutrient content.

Conclusions

The biological yield of red sorghum is strongly affected by genotypic factors and changing conditions. In these findings, the evaluated genotypes demonstrated significant variability across all locations for most traits. Strong genotype–environment interactions (GEIs) were detected for many of the biometric and quality characteristics. Pearson's correlation revealed a positive and significant relationship among the agronomic traits, whereas the Fe and Zn levels were negatively correlated. These trade-offs highlight the complexity of breeding for multi-trait improvement. Future research could focus on marker-assisted selection to address these challenges, ensuring simultaneous gains in yield and nutritional content. G21 and G26 were identified as superior genotypes for red sorghum grain Fe and Zn content via AMMI and GGE biplot analysis. G11 stood out as the genotype with the highest grain yield. On the basis of the discriminativeness and representativeness biplots, G17 and G18, as well as G2 and G15, were selected as ideal genotypes for YIELD and micronutrient content, respectively. Across all the examined locations, G14, G17, and G18 were identified as highly stable genotypes with consistently significant Fe and Zn levels, considering the results of the mean vs. stability analysis. Similarly, genotypes G14, G17, G9, and G11 were selected through the multitrait stability index (MTSI) as superior genotypes for stability and performance in agronomic traits across all locations. The Y × WAAS biplot further supported the selection of G14 as a stable, high-yielding genotype with greater panicle weight and mineral content. Various multitrait selection methods consistently identified G14 as the most trustworthy genotype across all studied traits. Given its high yield, stability, and performance, G14 is recommended for cultivation in different environmental zones of Tamil Nadu, contributing to food, feed and nutritional security for the growing population.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

A.G., L.N.S., S.E., and M.M.L.: Conceptualization and methodology; R.K.S.,: Conducted the field trial and data collection; R.K.S., A.G., L.N.S., S.E., and M.M.L.: Data curation and analysis, software, writing and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data are contained within this article and the Supplementary Information.

Declarations

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Consent for publication

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Competing interests

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