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Multi-environment evaluation of forage maize genotypes for green forage and dry matter yield stability using AMMI and GGE Biplot analysis

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Abstract

The development of high-yielding and genetically stable forage maize genotypes necessitates comprehensive multi-location evaluation across diverse agro-climatic zones. Accordingly, the present study aimed at multi-location testing of twenty-five test entries along with three national checks (J-1006, African Tall and COHM-8) across twenty environments encompassing five agro-climatic zones (Hill, North West, North East, Central and South) of India under the Initial Varietal Trial in Forage Maize (IVTM) of the All India Coordinated Research Project on Forage Crops and Utilization. Analysis of variance revealed the significant impact of genotype (G), environment (E), and their interaction (GE) on both green forage yield (GFY) and dry matter yield (DMY). The environment was the most important source of variation, contributing 67.47% and 73.63% of the total variation for GFY and DMY, respectively, followed by G (9.35% and 7.34%) and GE (23.18% and 19.03%). The first two principal components of the GGE biplot explained 55.57% and 58.51% of the total variation for GFY and DMY, respectively. Environmental evaluation classified the twenty testing locations into distinct mega-environments. Based on the integrated GGE biplot and AMMI analysis, genotype DFH 25-2 exhibited superior performance and stability across multiple locations over the checks for both GFY and DMY traits, followed by JHFM 25-2, PMC-18 and JHFM 25-1. These genotypes possess the capability to address the scarcity of green fodder in the nation and can be subjected to further testing in advanced varietal trials for their potential release.

Keywords: Forage maize, fodder yield, GGE biplot, AMMI, stability, multi-environment

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops globally and serves as an excellent forage crop owing to its high biomass production, palatability, and nutritional quality. In India, maize occupies a pivotal position in the livestock feed sector, contributing significantly to the total fodder availability. The crop is characterized by zero *anti*-nutritional content, higher crude protein, and superior *in vitro* dry matter digestibility (IVDMD) compared to other non-legume fodder crops, making it an ideal candidate for both green feeding and silage preparation (Mahanta *et al.*, 2023) [9].

India is home to the largest and most diverse livestock population globally, with approximately 70% of households depending on the livestock and agriculture sectors for their livelihoods (Ghosh *et al.*, 2016) [7]. Despite this, the country faces a significant deficit of 35.6% in green fodder, 10.5% in dry fodder, and 44% in concentrates (Anonymous, 2015) [1]. This perennial fodder scarcity has been identified as a major constraint limiting livestock productivity and, consequently, the livelihoods of millions of small and marginal farmers across the country. The development of high-yielding forage maize varieties adapted to diverse agro-climatic conditions offers a promising strategy to bridge this deficit.

Maize, being a cross-pollinated crop, offers immense potential for harnessing heterosis, and both composite varieties and single-cross hybrids have been developed for forage purposes (Singh *et al.*, 2021; Das *et al.*, 2021) [19, 3]. However, the presence of genotype $\times$ environment interaction (GEI) necessitates the measurement of both performance and stability for any genotypes being evaluated in breeding programs (Devi *et al.*, 2019; Sah *et al.*, 2016; Pour-Aboughadareh *et al.*, 2022) [5, 18, 13]. This allows for a comprehensive assessment of the impact of GEI on phenotypic and genotypic values, avoiding potential biases in estimating heritability and predicting genetic advances.

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Two commonly used biplots for comprehending GEI analysis are the AMMI biplot (Gauch, 1992) [6] and the genotype main effect plus genotype-environment interaction (GGE) biplot (Yan *et al.*, 2000; Yan and Kang, 2003) [23, 21]. The AMMI model partitions the GEI into multiplicative interaction principal component axes (IPCAs), while the GGE biplot eliminates the environment component and integrates the genotype with the GEI effect. The key distinction between the two lies in the fact that the GGE biplot focuses on the genotype and GE interaction, which are the most relevant for genotype evaluation, whereas the AMMI biplot separates the additive main effects from the multiplicative interaction effects (Yan *et al.*, 2007) [24].

The All India Coordinated Research Project (AICRP) on Forage Crops and Utilization conducts systematic multi-location testing of forage maize genotypes across the country through a structured pipeline: Initial Varietal Trial in Maize (IVTM), followed by Advanced Varietal Trial in Maize-1 (AVTM-1), and then AVTM-2. These coordinated trials provide an excellent framework for evaluating genotypic stability across diverse environments. Consequently, this study examined the performance of forage maize genotypes tested under IVTM with the specified objectives: (i) to estimate the GEI of forage maize

genotypes for green forage yield and dry matter yield, (ii) to identify high-yielding and stable genotypes, and (iii) to classify the testing locations into megaenvironments.

Materials and Methods

Plant materials and experimentation: The investigation involved the evaluation of 25 forage maize test entries along with three national checks, viz., J-1006, African Tall and COHM-8, across 20 locations during the designated cropping season under the AICRP on Forage Crops and Utilization. These 20 locations represented five distinct agro-climatic zones as defined by the AICRP framework: Hill Zone (Palampur, Srinagar, Rajouri and Almora), North West Zone (Ludhiana, Hisar and Pantnagar), North East Zone (Bhubaneswar, Ranchi, Pusa, Ayodhya and Imphal), Central Zone (Raipur, Jabalpur, Rahuri, Urulikanchan and Jhansi), and South Zone (Hyderabad, Coimbatore and Mandya). The multi-location testing represented a wide range of maize production ecosystems, distinguished by variations in latitude, altitude, soil type, and macro-climatic conditions. All locations adhered to the standard recommended practices for crop management as outlined by the AICRP.

Table 1: Details of genotypes used in the study

S. No.	Genotype Code	Type
1	JHFM 25-2	Test Entry
2	DFH 25-2	Test Entry
3	GK 33-10	Test Entry
4	IFH-11-248	Test Entry
5	KDFM-12	Test Entry
6	OUFM 25-1	Test Entry
7	IFH-11-249	Test Entry
8	HPFM-14	Test Entry
9	DFH 25-3	Test Entry
10	AH-4732	Test Entry
11	TNFMH 2315	Test Entry
12	PH-1534	Test Entry
13	NT-009	Test Entry
14	JHFM 25-3	Test Entry
15	HPFM-13	Test Entry
16	TNFMH 2335	Test Entry
17	MAH 22-32	Test Entry
18	JH 20209	Test Entry
19	BAIF Maize-9	Test Entry
20	JHFM 25-1	Test Entry
21	IFH-11-250	Test Entry
22	DFH 25-1	Test Entry
23	PH-421266	Test Entry
24	PFM-27	Test Entry
25	PMC-18	Test Entry
26	J-1006 (NC)	National Check
27	African Tall (NC)	National Check
28	COHM-8 (NC)	National Check

Recording of observations: The 28 forage maize genotypes were evaluated for two primary traits, viz., green forage yield (GFY; q/ha) and dry matter yield (DMY; q/ha). The green forage was harvested at 50% flowering stage, and the fresh weight was recorded for computing GFY. Representative samples were oven-dried at 65°C until constant weight for determination of dry matter content and subsequent calculation of DMY.

Data Analysis: Analysis of variance (ANOVA) was performed to analyse genotype (G), environment (E), and their interactions (GEI) effects. The proportion of variation attributable to G, E, and GEI was computed from the respective sum of squares. Additive main effects and multiplicative interaction (AMMI) analysis was conducted following Gauch (1992) [6], and genotype main effect plus genotype-environment interaction (GGE) biplot analysis

was performed following Yan *et al.* (2000) [23] and Yan and Tinker (2006) [22]. In the analysis, no scaling was applied (scaling = 0) to generate a tester-centered (centering = 2) GGE biplot. For genotype evaluation, genotype-focused singular value partitioning (SVP = 1) was applied using the Mean versus Stability option, while environment-focused singular value partitioning (SVP=2) was employed for environmental evaluation. The Which-Won-Where option was utilized to identify the winning genotype within a given set of environments. The AMMI Stability Value (ASV) was computed following Purchase *et al.* (2000) [14], and the Genotype Selection Index (GSI) was calculated as the sum of ranks for mean yield and ASV. All analyses were performed using the metan package (Olivoto and Lúcio, 2020) [12] in R statistical software (R Studio, 2020) [17].

Results and Discussion

Analysis of Variance: The multi-location testing selected for the present experiment reflected diverse forage maize production ecosystems across India, characterized by differences in latitude, altitude, and macro-climatic conditions spanning five agro-climatic zones. The analysis of variance (ANOVA) indicated the significant influence of G, E, and GE for both traits studied in the experiment (Table 2). The analysis revealed that the environmental factor accounted for a substantial proportion of the observed variation in the dataset: 67.47% for GFY and 73.63% for

DMY, while the contributions of genotype (9.35% and 7.34%) and GEI (23.18% and 19.03%) were relatively lower (Table 2). Notably, the contribution of G outweighed the contribution of GEI for DMY, while for GFY the GEI component was substantially higher than the genotypic component, suggesting that genotype performance for forage yield was considerably influenced by environmental interaction. The significant GEI component underscored the necessity for stability analysis to identify genotypes with consistent performance across environments.

The predominance of environmental effects is consistent with earlier reports in maize (Mohammadi *et al.*, 2009; Li *et al.*, 2023; Bocianowski *et al.*, 2024; Yathish *et al.*, 2024) [10, 8, 2, 26] and sorghum (Rakshit *et al.*, 2012) [16]. However, the higher GEI proportion observed in the present study (19-23%) compared to Yathish *et al.* (2024) [26] who reported 1-3% GEI in baby corn, could be attributed to the wider environmental spread (20 locations across 5 zones vs. 4 locations across 3 zones) and the greater genetic diversity (28 genotypes vs. 13) tested in the present investigation. When considering location-wise means, the highest GFY was observed in Coimbatore (510.28 q/ha) followed by Pantnagar (484.12 q/ha), while the lowest was recorded in Srinagar (139.18 q/ha). For DMY, Urulikanchan (109.89 q/ha) recorded the highest mean, while Pantnagar recorded the lowest (Table 2).

Table 2: ANOVA and proportion of variation (G+E+GE) explained by genotype (G), environment (E), and GE interaction for GFY and DMY across locations

Traits	MS value and% proportion	G	E	GE	Grand Mean
GFY	MS	30744.47***	315388.84***	4012.84***	330.65
	% of G+E+GE	9.35	67.47	23.18	
DMY	MS	1461.21***	20817.08***	199.23***	69.00
	% of G+E+GE	7.34	73.63	19.03	

*** $p < 0.001$

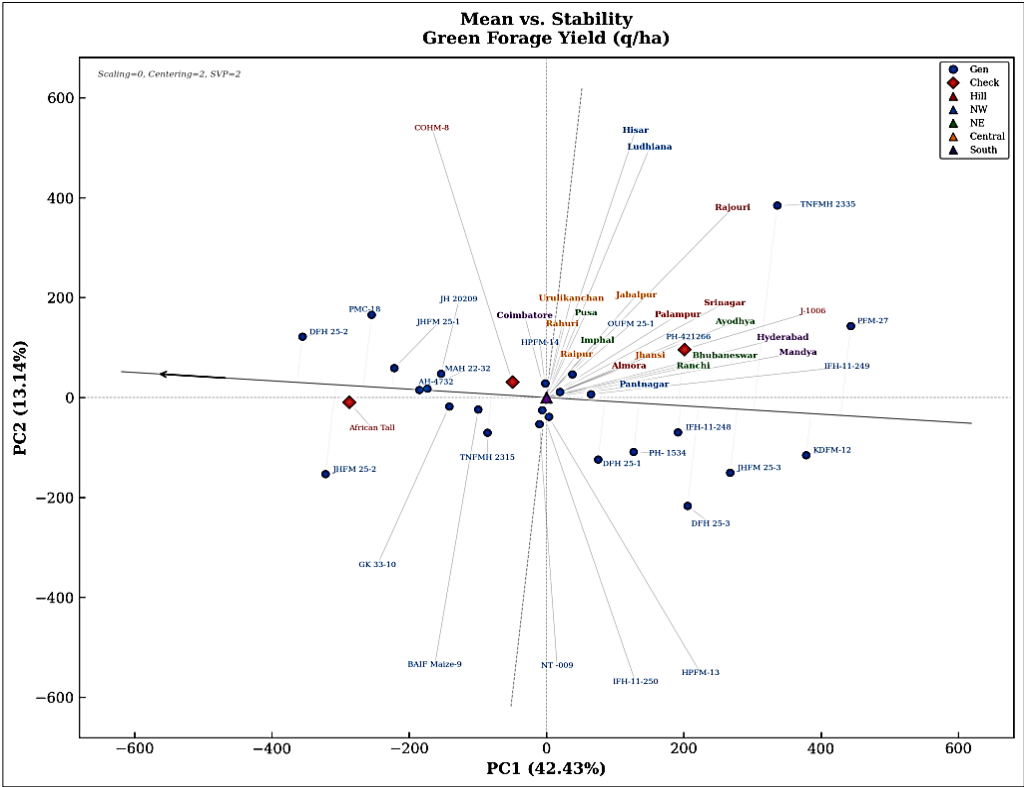


Fig 1a: Mean vs. stability (Green Forage Yield)

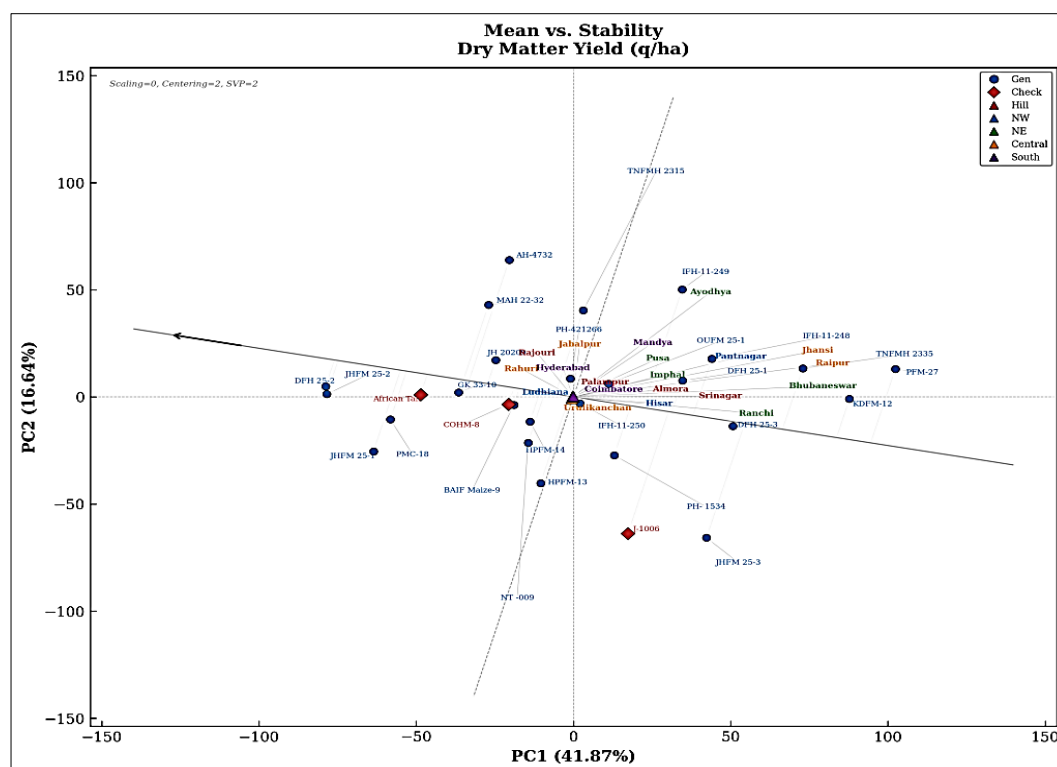


Fig 1b: Mean vs. stability (Dry Matter Yield)

Fig 1: Mean performance and stability of genotypes for GFY and DMY

Mean performance and stability of the genotypes across locations:

The intricate interaction between genotypes and the environment was effectively simplified and visualized using GGE biplot analysis, which represents the data graphically through principal components (Yan and Tinker, 2006) [22]. The first two PCs explained 55.57% (PC1 = 42.43%, PC2 = 13.14%) and 58.51% (PC1 = 41.87%, PC2 = 16.64%) of the total variation for GFY and DMY, respectively, validating the GGE biplots as reliable graphical representations of the multi-location data. A cumulative variation of >60% (PC1 and PC2) was considered to provide a reliable approximation of the $G \times E$ variation (Yang *et al.*, 2009) [25].

In the mean vs. stability graph (Fig. 1a-b), the line characterized by a single arrowhead represented the AEC abscissa, which intersected the biplot origin and served as a marker for the average environment, progressing towards higher mean values. The AEC ordinate, perpendicular to the AEC abscissa, reflected genotype stability, with longer projections indicating lesser stability. It was evident from the mean vs. stability graph that genotype DFH 25-2 outperformed all check genotypes, displaying the highest mean values for both GFY (414.24 q/ha) and DMY (86.73 q/ha). This genotype exhibited 10.00% superiority over the best check (African Tall) for GFY and 12.54% for DMY. JHFM 25-2 (GFY: 380.64 q/ha) and PMC-18 (GFY: 380.62 q/ha) were the next best performers. Among the checks, African Tall (376.59 q/ha) outperformed COHM-8 (350.63 q/ha) and J-1006 (288.26 q/ha).

Genotypic and environmental evaluation: One major advantage of the graphical presentation of GEI is the facile identification of genotypes that are closer to the ideal genotype (Yan and Tinker, 2006) [22]. The ideal genotype, characterized by high yield and greater stability, is represented by the longest vector length of the highest-yielding genotype with zero GEI, located at the center of the concentric circles (Fig. 2a-b). Based on this study, it could be concluded that genotype DFH 25-2 most closely approached the ideal genotype for both GFY and DMY traits. JHFM 25-2, PMC-18, and JHFM 25-1 were the next closest to the ideal, all outperforming the national checks in terms of combined mean performance and stability.

The graphical representation of biplots also facilitated the interpretation of the interaction between testing environments through the angle between their vectors (Fig. 3a-b). The cosine of the angle between two environment vectors indicates their correlation (Yan and Tinker, 2006) [22]. Analysis of both GFY and DMY traits revealed that environments within the same agro-climatic zone generally displayed acute vector angles, indicating positive correlations. For instance, the Hill Zone locations (Palampur, Srinagar, Rajouri, Almora) formed a tight cluster, as did the Central Zone locations (Raipur, Jabalpur, Urulikanchan). In contrast, nearly right or obtuse angles were observed between locations from different zones, such as between Hill and South Zone environments, revealing that genotypes performing well in one zone might exhibit different rankings in another. This crossover GEI justified the classification of testing locations into multiple mega-environments.

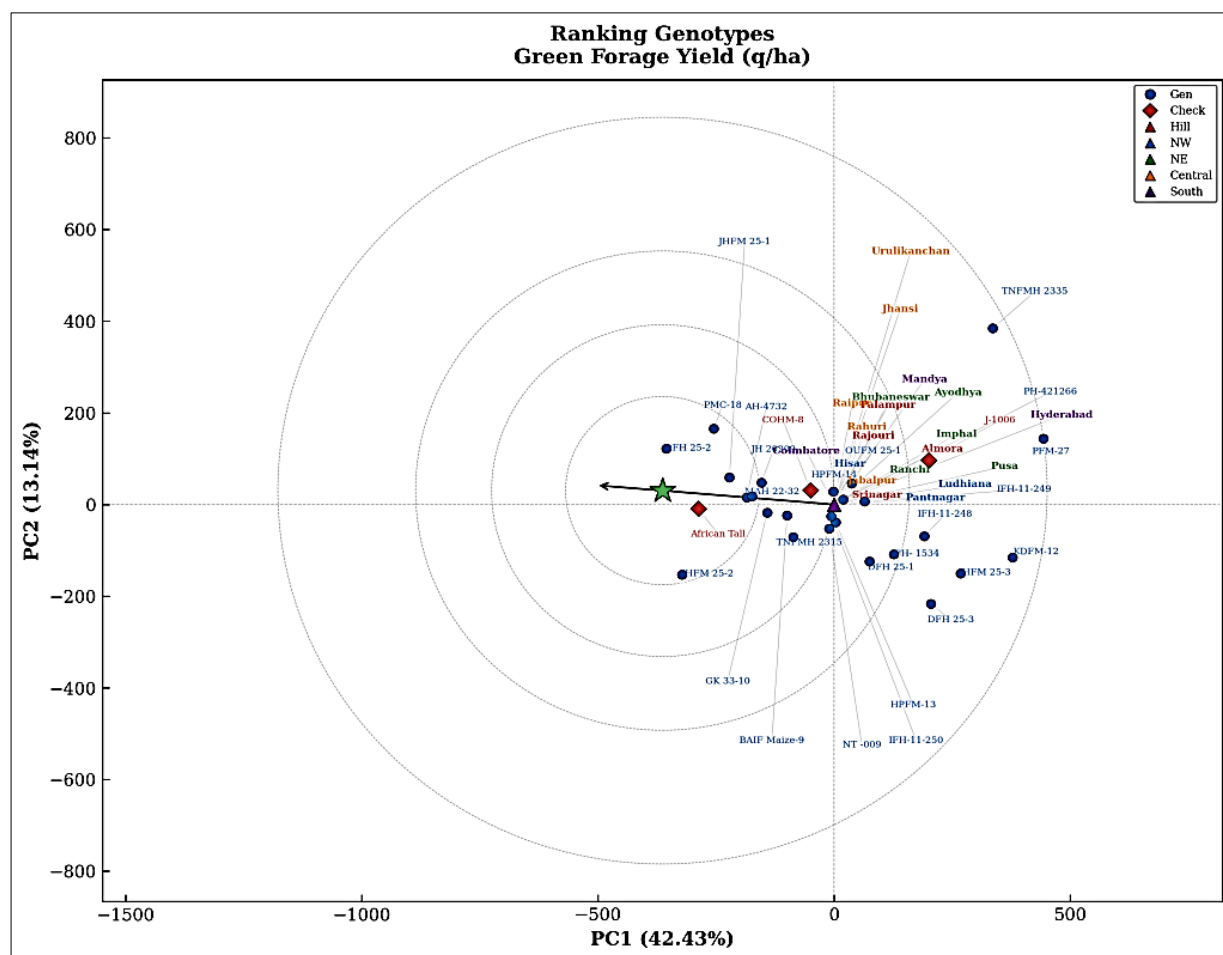


Fig 2a: Ideal genotype (Green Forage Yield)

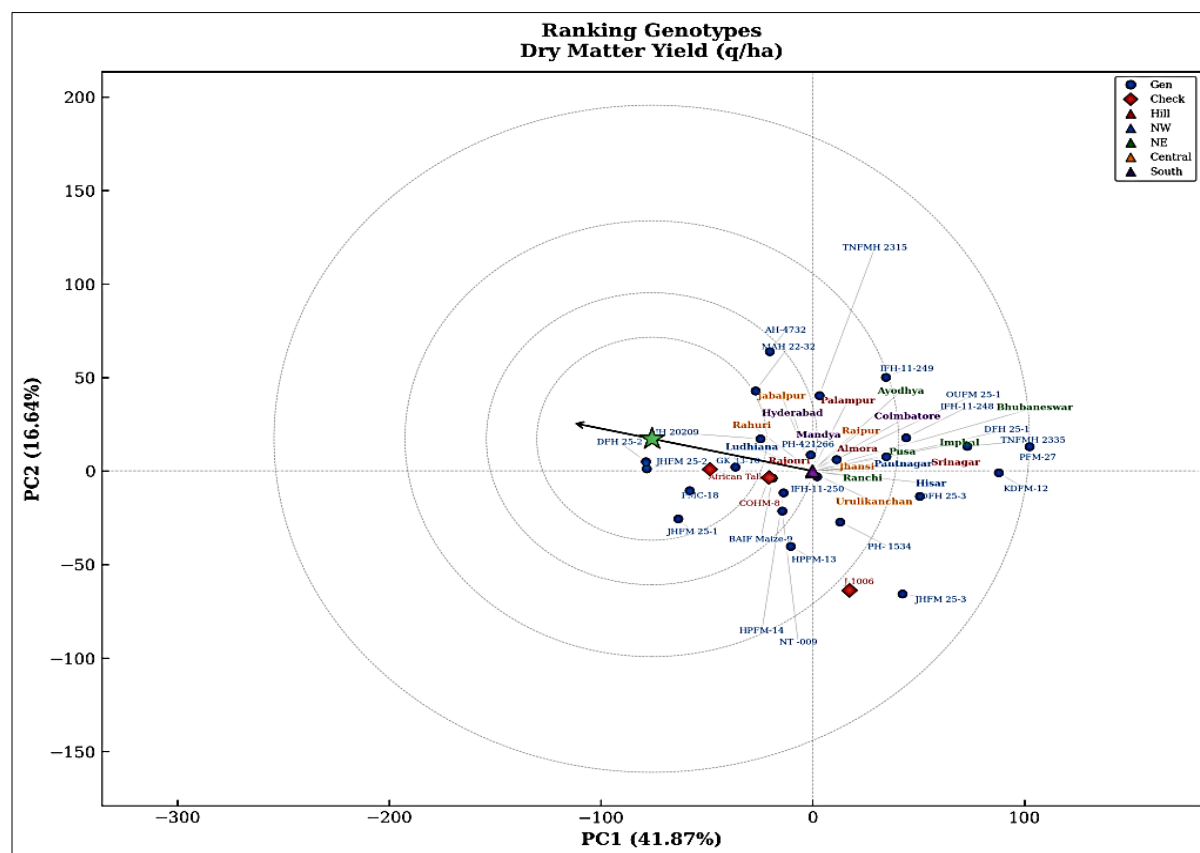


Fig 2b: Ideal genotype (Dry Matter Yield)

Fig 2: Genotypic evaluation for ranking of genotypes and identification of ideal genotypes

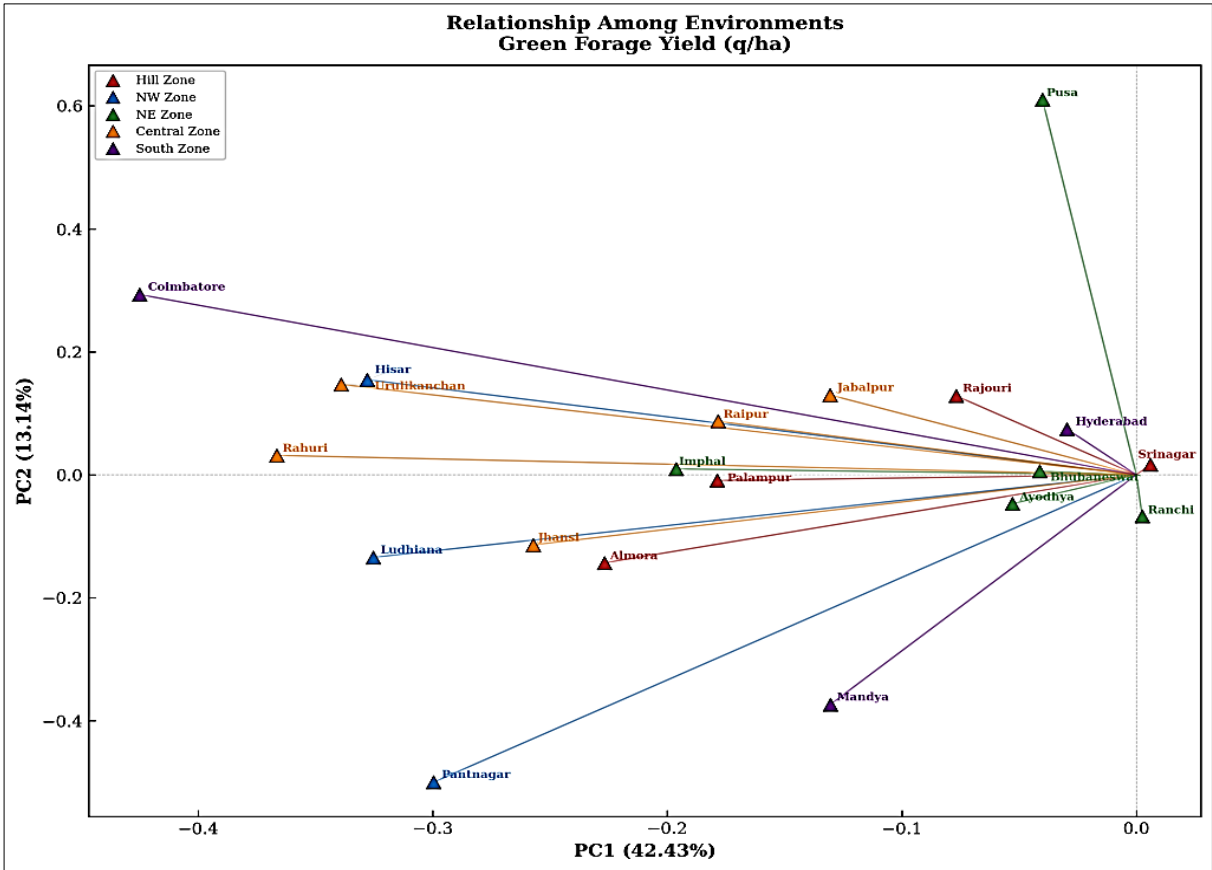


Fig 3a: Environmental relation (Green Forage Yield)

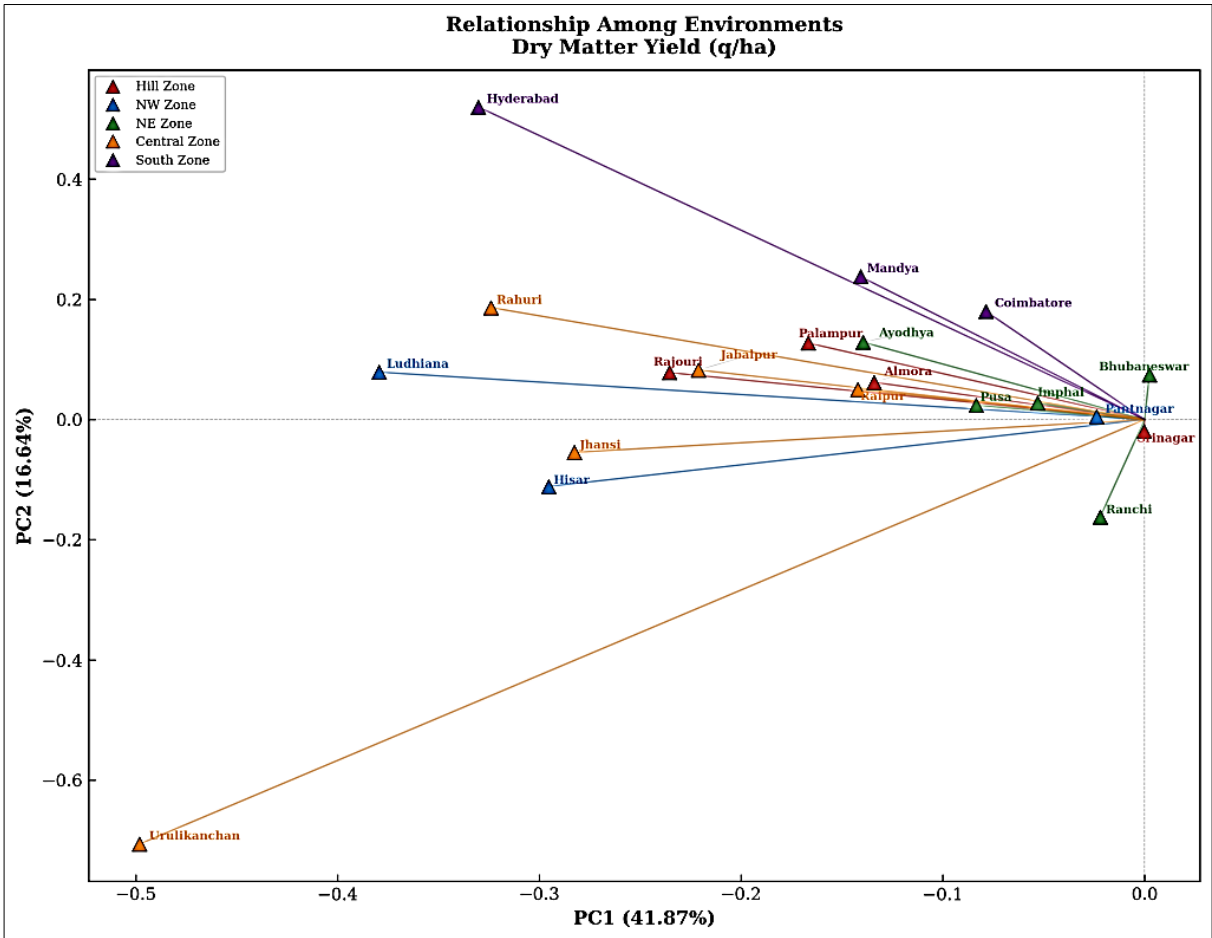


Fig 3b: Environmental relation (Dry Matter Yield)

Fig 3: Relationship among environments based on environmental evaluation

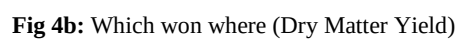


Fig 4: Which won where and mega environment identification for green forage and dry matter yields

Fig 5a: AMMI I biplot (Green Forage Yield)

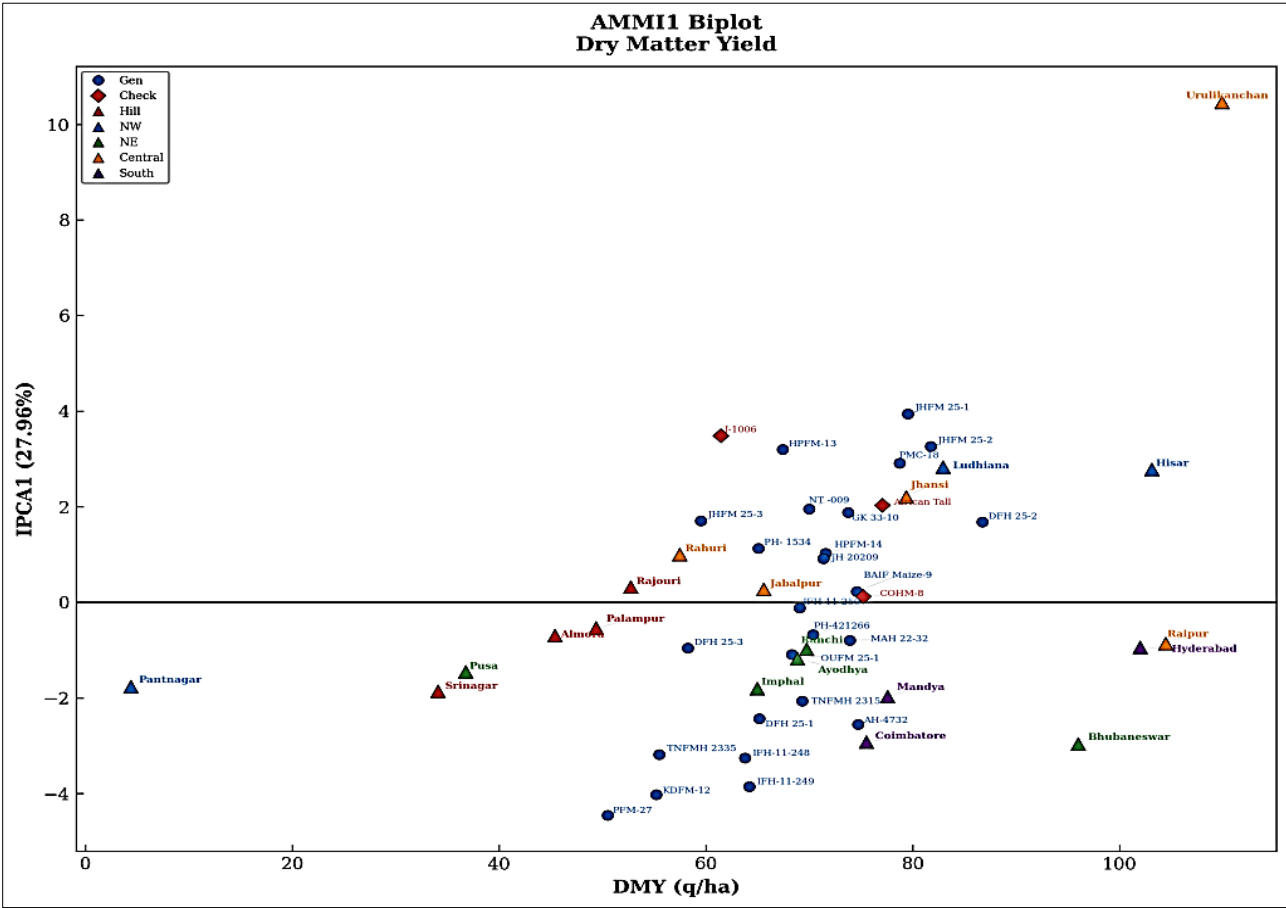


Fig 5b: AMMI I biplot (Dry Matter Yield)

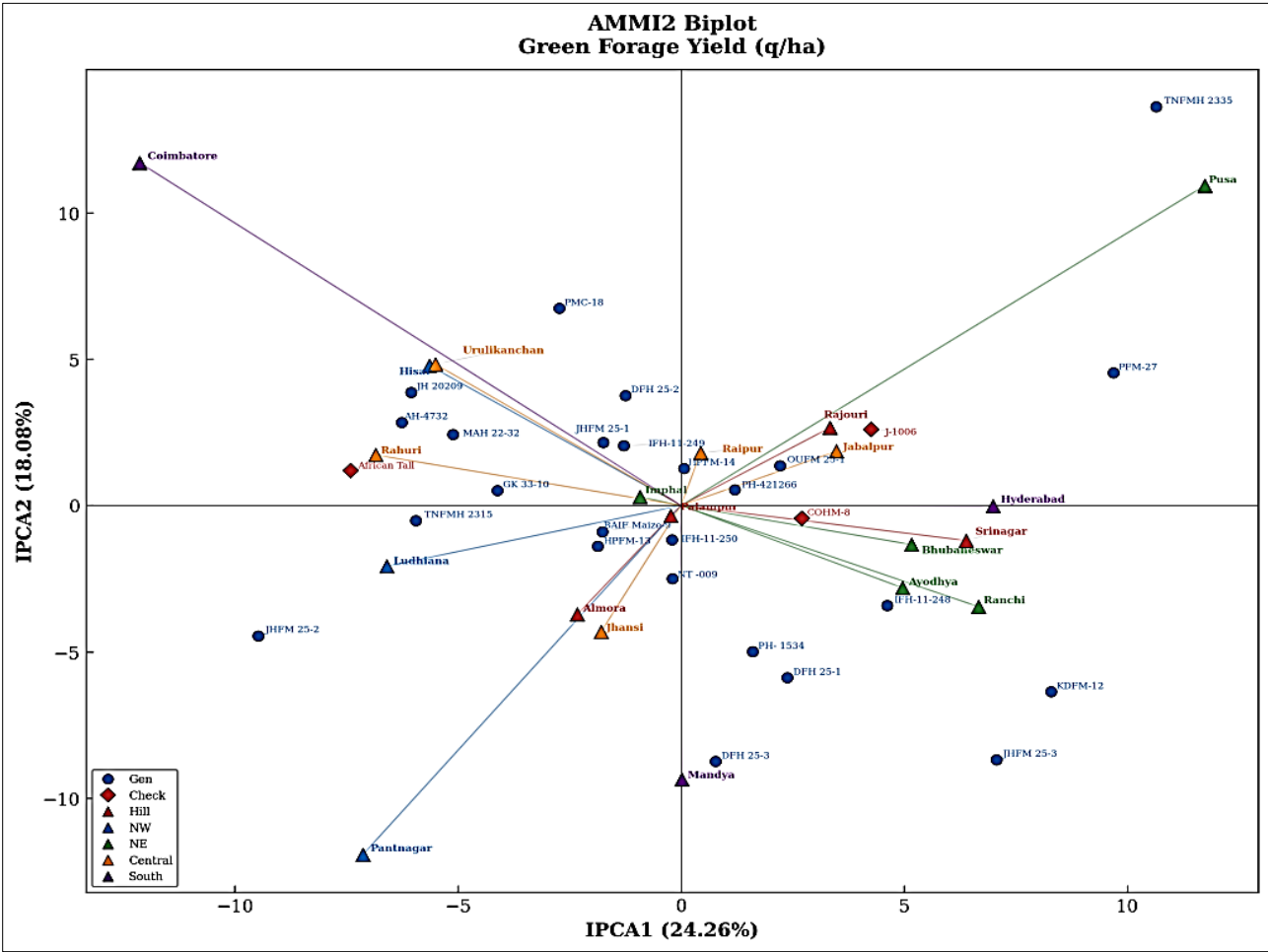


Fig 6a: AMMI II biplot (Green Forage Yield)

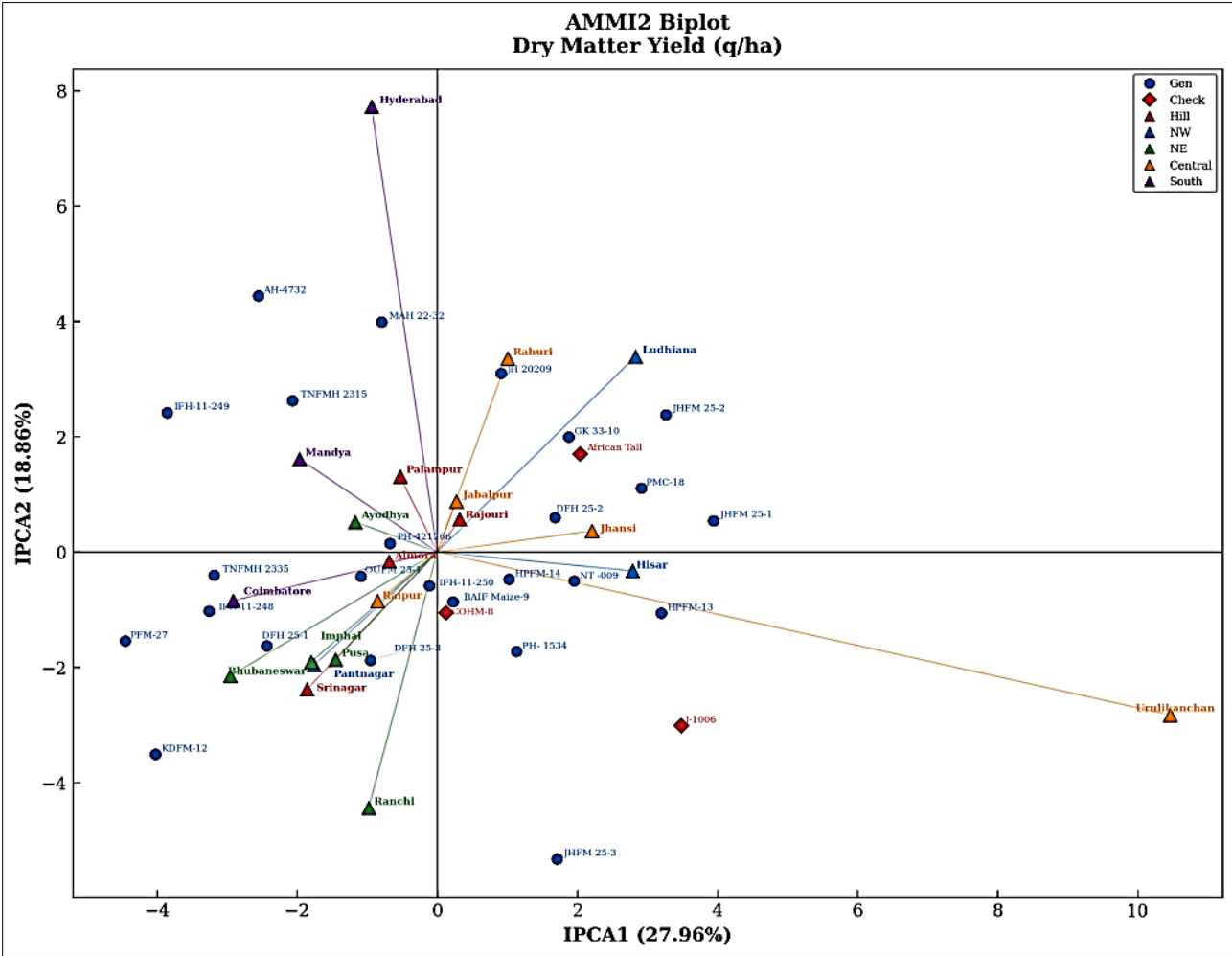


Fig 6b: AMMI II biplot (Dry Matter Yield)

Fig 5-6: AMMI I and AMMI II biplot analysis for green forage and dry matter yields

Stability indices and genotype selection: The AMMI Stability Value (ASV) and Genotype Selection Index (GSI) were computed to integrate yield performance with stability (Table 3). The GSI, calculated as the sum of ranks for mean yield and ASV, provides a balanced criterion for selecting genotypes that are both high-yielding and stable. Genotypes DFH 25-2 and JHFM 25-1 had the lowest GSI values (13

each), indicating the best combination of high yield and stability for GFY. DFH 25-2 ranked 2nd for mean GFY (414.24 q/ha) with an ASV of 4.12 (rank 11), while JHFM 25-1 ranked 5th for mean (379.90 q/ha) with an ASV of 3.18 (rank 8). For DMY, DFH 25-2 (GSI = 11), BAIF Maize-9 (GSI = 11), and COHM-8 (GSI = 11) showed the best combination of yield and stability.

Table 3: Stability indices for top 10 genotypes (GFY, q/ha) sorted by GSI

Genotype	Mean	Rank (Mean)	ASV	Rank (ASV)	GSI	bi
DFH 25-2	414.24	2	4.12	11	13	1.15
JHFM 25-1	379.90	5	3.18	8	13	1.12
BAIF Maize-9	350.01	11	2.54	5	16	1.13
PH-421266	333.45	14	1.69	3	17	0.82
IFH-11-250	331.51	17	1.21	1	18	0.94
HPFM-14	332.23	16	1.27	2	18	0.99
NT-009	332.81	15	2.51	4	19	1.04
COHM-8 (NC)	350.63	10	3.65	10	20	1.15
PMC-18	380.62	4	7.67	18	22	1.20
GK 33-10	354.86	9	5.55	13	22	0.93

The Finlay-Wilkinson regression coefficient (*bi*) provides additional insight into genotype responsiveness. A *bi* close to 1.0 indicates average responsiveness, *bi* > 1.0 indicates above-average response to favourable environments, and *bi* < 1.0 indicates better adaptation to unfavourable environments. DFH 25-2 displayed a *bi* of 1.15 for GFY,

indicating slightly above-average responsiveness to favourable environments while maintaining high yield across all environments. In contrast, PH-421266 (*bi* = 0.82) showed better adaptation to stress environments, making it a candidate for low-input farming systems.

Conclusion

According to the integrated GGE biplot and AMMI analysis, it was inferred that the environmental assessment could categorize the 20 testing locations across five agro-climatic zones into distinct mega-environments. Genotype DFH 25-2 exhibited superior performance and stability across multiple locations for both GFY (414.24 q/ha, 10% superiority over best check) and DMY (86.73 q/ha, 12.54% superiority) traits, followed by JHFM 25-2, PMC-18, and JHFM 25-1.

The substantial GEI observed (19 to 23 percent of total variation) emphasized the importance of multi-location testing for reliable genotype evaluation. These promising genotypes possess the capability to address the scarcity of green fodder in the nation and can be advanced to AVTM-1 trials for further evaluation and potential release. Furthermore, the parental lines of these superior genotypes can be employed in breeding programs, utilizing different mating designs with other elite germplasm to develop improved forage maize cultivars.

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