



Analysis of Genotype by Environment Interactions using AMMI and GGE Biplot Methods for Soybean Germplasm Lines

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ABSTRACT

Background: Soybean breeding program face challenges due to genotype-by-environment interaction (GEI), where genotypes exhibit varying performance across different environments, complicating the identification of stable, high-yield cultivars. This study aimed to elucidate GEI's impact on soybean yield and identify genotypes with superior adaptation and yield stability across diverse environments.

Methods: Fifty genotypes were evaluated over three years in a randomized block design with three replications per environment, assessing yield components and other agronomic traits. Stability performance was analyzed using general linear methods, GGE biplot, AMMI analysis and ASV rank analysis.

Result: The GGE biplot explained 75.10% of the total variation (PC1: 30.93%, PC2: 44.8%), while the AMMI model's first two interaction principal component axes (IPCA1: 59.3%, IPCA2: 40.7%) explained the GEI variation. AMMI analysis identified G26 (JS 22-88) and G39 (JS 22-101) as the most stable, high-performing genotypes, whereas GGE biplot analysis identified G9 (JS 22-71).

Key words: AMMI, G × E Interaction, GGE, Stability.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill], a self-pollinating leguminous crop of significant global cultivation, is a crucial source of food, feed, fuel and oil, valued highly in commercial markets. Approximately 5000 years ago, soybean (*Glycine max*) was domesticated from its wild precursor, *G. soja*, in China and subsequently spread globally (Du *et al.*, 2023). Belonging to the Fabaceae family, this legume, often hailed as the “king of beans,” is esteemed worldwide for its versatility, thriving on every continent (Hossain *et al.*, 2024). Soybean, known for its role as a supplement in livestock feed, is a photoperiod-sensitive legume crop that forms a symbiotic relationship with N-fixing rhizobia, making it a typical pod crop. Soybeans are rich in protein, healthy oils and flavones special compounds (Zhong *et al.*, 2024). Soybean seeds, when dry, contain approximately 40% protein and 20% oil (Gunjan *et al.*, 2016; Jin *et al.*, 2023). The increasing demand for vegetable oils and biodiesel has led to a significant rise in soybean oil production, driven by the high oil content in soybean seeds. This has underscored the importance of improving seed composition, particularly in light of growing awareness of health concerns related to dietary fats. Soybean plants are versatile crops utilized in various applications, including soy food production, animal feed, alleviating malnutrition, improving soil fertility and serving as raw material in the processing sector.

In Multi-Environment Trials (MET) of soybean, understanding the influence of environment (E) and genotype-environment interactions (GEI) is crucial for effective genotype selection. Strong genotype-environment

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interactions can complicate the identification of superior genotypes for new environments, posing challenges for breeders and farmers who prefer high-yielding genotypes that lack adaptability and stability within specific environments. Therefore, developing versatile soybean varieties suitable for diverse agricultural settings requires a thorough understanding of environmental influences and genotype-environment interactions. Researchers and breeders have extensively studied and reported grain yield stability across multiple seasons using various soybean genotypes throughout the year (Happ *et al.*, 2021). Regression techniques, like those developed by Eberhart and Russell, (1966), have been foundational in understanding genotype-environment interactions (GEI) patterns. However, AMMI and GGE methods have proven

significantly more effective in interpreting GEI data and are widely preferred over other stability analysis methods (Ngalamu *et al.*, 2020; Khan *et al.*, 2021; Ngalamu *et al.*, 2023). The AMMI method integrates ANOVA and principal component analysis to generate a biplot that crucially presents genotype means and their correlation with the first PCA component. The GGE biplot, valued for its ability to elucidate complex genotype-environment interactions in multi-environment breeding trials and agronomic studies, is widely utilized in soybean researches for its precision and adaptability (Kumar *et al.*, 2020; Silva *et al.*, 2021). In 2013, Hagos and Abay proposed using PCA scores and the AMMI stability value (ASV), derived from AMMI analysis, to identify stable genotypes across different locations. The ASV is calculated based on the interaction principal components axis 1 and 2 (IPCA1 and IPCA2) scores for each genotype in the AMMI model (Pramanik *et al.*, 2024). To assess the consistent performance of genotypes, varieties, or cultivars across different seasons, it is crucial to analyze genotype interactions with various environments through GEI analysis. Genotypes with lower ASVs are generally considered broadly adapted. Additionally, genotypes with IPCA2 scores near zero exhibit greater stability, whereas those with higher IPCA2 scores are more sensitive and less stable. The primary objective of the current study was to evaluate the genotype-environment

relationship, focusing on yield and trait stability, to identify lines that are broadly or specifically adapted. This research is pertinent for future breeding programs and farmer adoption, achieved through the application of AMMI and GGE biplot analyses.

MATERIALS AND METHODS

The 50 advanced soybean breeding lines, derived from diverse crosses (Table 1), represent elite, high-yielding varieties developed at the seed breeding farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. These lines were chosen based on prior multi-season performance, desirable agronomic traits and genetic diversity to ensure a comprehensive evaluation of genotype-by-environment interactions (GEI). The three generations (F7, F8 and F9), of these lines were evaluated during the *Kharif* seasons of 2019 and 2020 and the Rabi season of 2021, designated as trials E1, E2 and E3 respectively. The experiment was carried in a randomized complete block design (RCBD) with three replications at seed breeding farm, JNKVV, Jabalpur. The experimental layout maintained a spacing of 45 cm between rows and 7 cm between plants within a row, with each row measuring 3.0 meters in length. For the assessment of quantitative traits, five competitive plants were randomly selected and tagged from each plot in every replication. The JNKVV, located at an altitude of 411.78 m

Table 1: Details of the material with pedigree and sources.

Genotypes	Pedigree	Genotypes	Pedigree
G1	JS 20-30 × JS 95-60	G26	JS 20-63 × JS 20-35
G2	JS 97-52 × JS 95-60-5-12-1	G27	JS 20-29 × JS 93-05
G3	JS 20-53 × JS 20-34	G28	JS 20-82 × JS 95-60
G4	JS 20-71 × JS 20-22	G29	JS 20-74 × JS 20-22
G5	JS 20-53 × JS 20-34	G30	JS 20-88 × JSM 196
G6	JS 20-71 × JS 20-22	G31	JS 20-63 × JS 95-60
G7	JS 20-88 × JS 20-34	G32	JS 20-53 × JS 20-34
G8	SL 738 × JS 95-60	G33	JS 20-98 × JS 20-34
G9	JS 20-53 × JS 20-34	G34	JS 20-53 × JS 20-34
G10	JS 20-98 × JS 20-34	G35	JS 20-29 × JSM 275
G11	JS 20-29 × JSM 275	G36	JSM 226 × JS 20-34
G12	JS 20-63 × JS 20-35	G37	JS 97-52 × JS (IS) 90-5-12-1
G13	JS 20-75 × JS 20-14	G38	JS 20-29 × JS 93-05
G14	JS 20-79 × JS 335	G39	SL 738 × JS 95-60
G15	JS 20-53 × JS 20-34	G40	JS 20-69 × JS 335
G16	JS 20-63 × JS 20-35	G41	JS 20-88 × JSM196
G17	20-09 × PS1475	G42	JS 20-53 × JS 20-34
G18	20-82 × JS 95-60	G43	JS 20-79 × JS 335
G19	20-29 × JS 20-22	G44	JS 20-71 × JS 20-22
G20	20-63 × JS 96-60	G45	JS 20-29 × JS 20-22
G21	20-29 × JS 20-22	G46	JS 20-09 × JSM 258
G22	NRC 86 × JS 20-34	G47	JS 20-29 × JSM 275
G23	JS 20-71 × JS 20-22	G48	SL 738 × JS 20-88
G24	JS 20-63 × JS 20-35	G49	JS 20-98(c)
G25	JS 20-29 × JS 93-05	G50	JS 20-34(c)

Sources: Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

above sea level between 22°49' to 20°80' North latitude and 78°21' to 80°58' East longitude. Traits, five competitive plants were randomly selected and tagged from each plot in every replication.

To ascertain the genetic variance, a combined ANOVA was performed along with AMMI and GGE model, used to analyse genotype-environment interactions in soybean yield data, captures interaction components effectively through R software version 4.2. The genotype selection index (GSI) was calculated as per Farshadfar *et al.* (2012).

To assess variability in yield performance, a combined analysis of variance (ANOVA) was conducted across the three environments. Genotype-environment interactions were further analyzed using both the additive main effects and multiplicative interaction (AMMI) model and the genotype and genotype-by-environment (GGE) biplot method using R software version 4.2. AMMI was employed to partition and interpret the main effects (genotype and environment) and their interactions using principal component analysis, while GGE biplot focused on visualizing both genotype performance and stability across environments. Together, these models offer complementary insights-AMMI excels in separating interaction effects, whereas GGE provides a more intuitive graphical representation for selecting stable and high-yielding genotypes. Additionally, the genotype selection index (GSI) was calculated as per the method proposed by Farshadfar *et al.* (2012), combining mean yield and stability metrics to facilitate genotype ranking.

RESULTS AND DISCUSSION

AMMI analysis

AMMI Analysis of Variance (ANOVA) revealed that there were significant differences ($P < 0.001$) in the genotype, environment and $G \times E$ interactions (Table 2). The AMMI analysis of variance exhibited that 16.96% of the total sum of squares was attributable to environmental, 28.31% to genotypic and 54.66% to $G \times E$ effects (Table 2). The magnitude of the $G \times E$ interaction sum of squares was 1.93 times higher than that for genotypes, indicating that there were substantial differences in genotypic response across environments. In agreement with these results Amogne *et al.* (2024) and Dharshini *et al.* (2024) reported that the genotype by environment interaction effect accounted for the largest total sum of square, followed by genotype and environment. However, this was contrary with the findings from Silva *et al.* (2021) and Habtegebriel *et al.* (2023) who reported that the environment is the most contributing, followed by the genotype by environment interaction effect and the genotype effect. The GEIs were further divided into two significant interaction principal components (IPCA1). The first (IPCA1) and second (IPCA2) interaction principal components were highly significant (Table 2) and they explained a total of 59.3% and 40.7% of the $G \times E$ variation respectively. Since both IPCA1 and IPCA2 were significant, best performing and stable genotypes across the three

environments could be determined using ASV and GSI scores. These results were in line with the Amogne *et al.* (2024) and Dharshini *et al.* (2024). Based on the mean seed yield (SY), the genotypes G3 (JS 22-65), G9 (JS 22-71), G39 (JS 22-101), G32 (JS 22-94) and G2 (JS 22-64) recorded mean seed yields of 14.58, 13.22, 12.90, 12.33 and 11.83, respectively, across environments. AMMI stability parameters such as the AMMI stability value (ASV) and Genotype selection index (GSI) provide additional information on genotype variation. Genotypes with ASV values close to zero are considered stable (Table 3). The range of ASV value from 0.14 (G26) to 2.94 (G3) were observed for seed yield. According to ASV, genotypes G26 (0.14), G50 (0.22) and G49 (0.23), were identified as most stable for having lower ASV values, whereas genotypes G3 (2.94), G2 (2) and G25 (1.92) were identified as being most unstable (Table 3). To clearly define more stable genotypes, the ASV parameter was used as an ancillary. According to Yan and Kang, (2002) GSI method incorporates both stability and high performance into a single index to identify ideal genotypes and removing the problem of selecting lines solely based on yield stability, taking into account that the most stable genotypes do not always have the best yield performance. Accordingly, the GSI parameter associated with genotype classification is based on the ASV and the ranking of genotypes. Genotypes with the lowest GSI scores represent the more desirable genotypes. The most stable lines according to the GSI for seed yield across the environments were G39 (16), G26 (20) and G19 (25) showed a lower GSI value, indicating it is the most stable genotype with a high mean yield (Table 3).

This finding aligns with previous studies by Mushoriwa *et al.* (2022) and Wodebo *et al.* (2023) which identified stable, high-yield genotypes through the genotype selection index based on rankings of mean yield and AMMI stability value. The first two PCs exhibited 100% of the total variation, in which PC1 contribute 59.3% and PC2 contribute 40.7%. Therefore, AMMI1 (IPCA1 vs additive main effects) biplots were generated to illustrate the genotype and environment effects simultaneously (Fig 1). The AMMI1 biplot indicated that the environment E3 was high yielding followed by E2 and E3. The genotypes G26, G12, G34, G38, G8 and G25 were located near to the environment E3

Table 2: AMMI analysis of variance for soybean genotypes.

Source of variation	DF	SS	MS	% TSS
Environment	2	645.28	322.64***	16.96
Genotypes	49	1077.00	21.97***	28.31
$G \times E$	98	2079.21	21.21***	54.66
PC1	50	1232.54	24.65***	59.3
PC2	48	846.68	17.63***	40.7
Error	147	82.10	0.55	

$G \times E$ = Genotype by environment interaction, PC = Principal components, *** Significant at $P < 0.001$, MS = Mean squares, SS = Sum of squares, DF = Degrees of freedom.

Table 3: Mean seed yield of 50 soybean genotypes across three environments and AMMI stability estimates.

GEN	S Y	Rank	ASV	RASV	PC1	PC2	SIPC	GSI
G1	6.53	48	0.63	16	4.04	0.88	0.67	64
G2	11.83	5	2.39	49	2.93	-8.95	2.25	54
G3	14.58	3	2.94	50	-13.81	-1.41	2.47	51
G4	10.58	12	1.26	32	-3.82	-3.27	1.53	44
G5	9.73	15	0.83	21	-1.55	-2.46	0.92	36
G6	9.25	20	1.29	33	1.18	-4.3	1.57	53
G7	11.4	7	0.81	20	-1.31	-4.99	0.98	27
G8	9.38	18	0.93	23	-2.59	2.03	0.84	41
G9	13.22	2	1.04	26	-7.07	-3.63	1.09	28
G10	10.87	11	1.47	40	2.09	-4.85	1.1	51
G11	8.03	32	1.58	41	5.22	0.85	1.77	73
G12	8.83	25	1.05	27	-2.58	1.66	0.92	52
G13	9.48	17	1.47	39	0.83	-5.11	1.78	56
G14	11.82	6	1.81	46	-3.21	2.72	2.1	52
G15	7.35	45	0.84	22	-0.45	2.7	0.8	67
G16	9.23	21	1.45	38	3	-4.24	1.53	59
G17	11.05	9	1.6	43	0.69	0.48	1.92	52
G18	6.83	47	0.26	4	3.11	2.15	0.26	51
G19	9.72	16	0.49	9	-1.03	-1.81	0.52	25
G20	7.48	42	0.51	11	3.1	1.33	0.53	53
G21	9.95	14	1.6	42	3.48	-3.78	1.15	56
G22	7.4	43	0.32	7	2.2	0.76	0.39	50
G23	8	33.5	0.51	10	2.24	1.67	0.62	43.5
G24	7.38	44	0.56	14	0.35	3	0.44	58
G25	9.2	22	1.92	48	-5.35	3.07	1.5	70
G26	9.28	19	0.14	1	-0.49	-0.22	0.16	20
G27	8.53	28	0.29	5	1.19	-0.25	0.32	33
G28	8	33.5	0.38	8	0.47	2.35	0.44	41.5
G29	7.98	35	1.04	25	-1.59	3.27	0.76	60
G30	7.53	40	1.12	28	4.66	0.98	1.17	68
G31	7.65	39	1.41	36	5.43	-0.1	1.31	75
G32	12.23	4	1.43	37	-0.59	-7.61	1.63	41
G33	11.3	8	1.82	47	-5.62	3.3	2.07	55
G34	8.97	24	1.74	44	-4.63	2.09	1.63	68
G35	8.65	27	1.16	30	-2.35	3.4	1.07	57
G36	7.52	41	1.19	31	4.48	2.21	1.42	72
G37	6.9	46	0.54	12	2.77	0.57	0.66	58
G38	8.98	23	0.31	6	0.02	-0.7	0.32	29
G39	12.9	3	0.56	13	-5.24	-2.45	0.55	16
G40	8.82	26	1.37	34	3.79	0.33	1.57	60
G41	10.95	10	1.39	35	-5.06	-2.75	1.66	45
G42	7.8	37	0.59	15	0.84	3.27	0.71	52
G43	10.45	13	1.15	29	-1.83	2.21	1.36	42
G44	8.18	31	0.78	18	3.03	-0.68	0.6	49
G45	6.47	49	0.66	17	4.31	1.02	0.64	66
G46	8.35	29	0.98	24	-0.34	3.97	1.19	53
G47	8.28	30	1.77	45	-3.87	4.39	1.27	75
G48	7.88	36	0.78	19	3.38	0.9	0.84	55
G49	7.68	38	0.23	3	2.02	1.66	0.28	41
G50	5.6	50	0.22	2	3.54	4.37	0.27	52

GEN- Genotypes, SY- Seed yield, ASV- AMMI stability value, PC- Principal component, GSI- Genotype stability index, IPC- Interaction principal components, SIPC- Sum of the value of the IPC scores.

showed high yielding for that particular environment, genotypes such as G27, G44, G16, G14, G6 and G40 which were located near to the environment E2 were high yielding genotypes and the genotypes G23, G11, G31, G30 and G36 high yielding for the environment E1 (Fig 1). The AMMI model, which integrates Principal Component Analysis (PCA) and analysis of variance, enables a thorough examination of genotype and environment interactions, helping to identify interaction patterns (Gupta *et al.*, 2023). These results align with previous research by Nowosad *et al.* (2016) and Mossie *et al.* (2024).

GGE biplot analysis

Fig 2-6 present GGE biplots for seed yield of 50 soybean genotypes, highlighting top-performing genotypes, stable genotypes across environments and representative mega-

environments (Silva *et al.*, 2022). This method simplifies complex $G \times E$ interactions into principal components, with PC1 representing average genotype performance and PC2 capturing $G \times E$ interaction and genotype instability (Khan *et al.*, 2021; Kumar *et al.*, 2023). In this study, PC1 and PC2 of the GGE biplot together accounted for 75.10% of the $G \times E$ variation in seed yield (Fig 2), which is significantly lower than the 95% reported in previous research. The “which-won-where” polygon view (Fig 2) illustrates the top-performing soybean genotypes in each environment. The GGE biplot analysis of 50 genotypes for seed yield explained 75.10% of the total variation, with PC1 and PC2 accounting for 30.93% and 44.8% of the variation, respectively. The GGE biplot's polygon view highlights top-performing genotypes by connecting those farthest from the origin, with sectors dividing mega-environments. Genotypes at

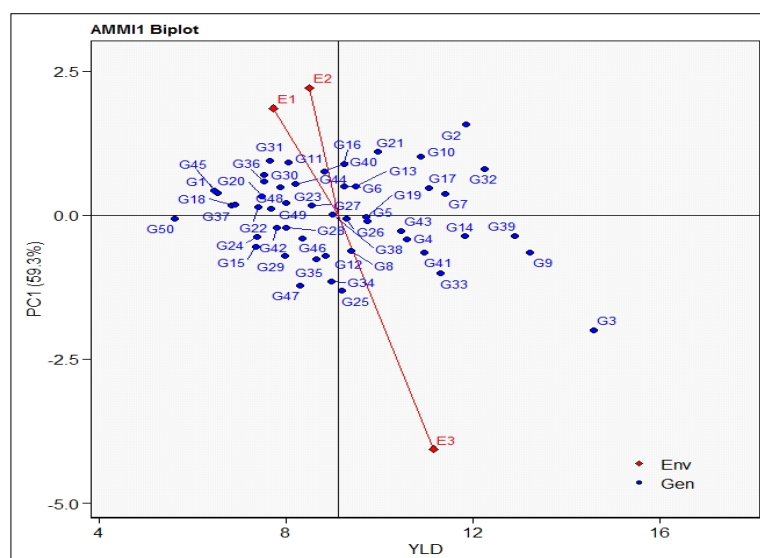


Fig 1: AMMI 1 biplot for soybean genotypes.

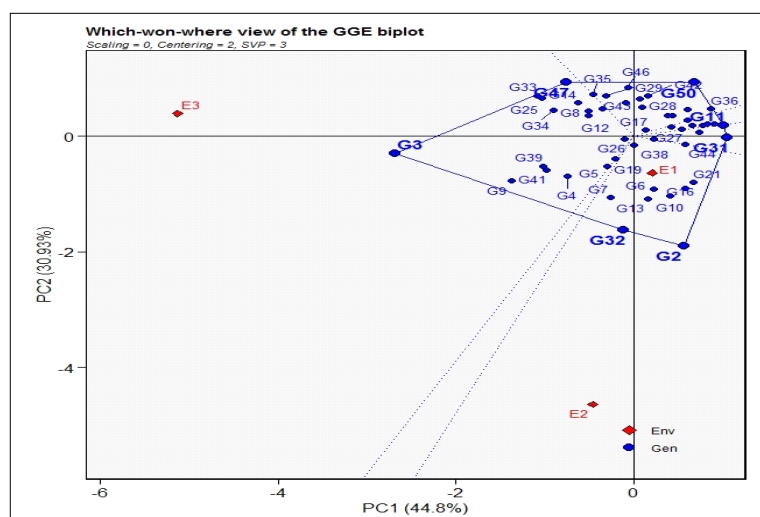


Fig 2: GGE biplot polygon view showing the genotype main effect and GEI of 50 soybean genotypes across three environments for seed yield (Centering=2, SVP=3, Scaling=0).

the polygon vertices, including G2, G3, G47, G50, G11, G32 and G31, represent the best performers in their respective environments, while genotypes closer to the origin are considered more stable across environments. These genotypes showed the highest responsiveness in their environments, with G3 achieving the highest seed yield in E3. Similarly, Habtegegbriel *et al.* (2023) found that the polygon view of the biplot grouped test environments into three sectors and genotypes into four, with three sectors lacking test environments. Fig 3 shows the ranking of genotypes based on mean performance and stability. In a GGE biplot, PC1 reflects genotype main effects, while PC2 represents $G \times E$ effects, serving as a measure of genotype instability. The average environment coordinate (AEC) axis,

extending from the biplot origin to the average environment circle, is determined by the average scores of PC1 and PC2 across all environments, with the arrow pointing to the highest yield value. In our study, the AEC method, using average principal components, assessed genotype yield stability. The GGE biplot revealed that 75.10% of yield variation was due to genotype and $G \times E$ interaction (Fig 3). A line from the biplot origin to the average environment indicates stronger genotype main effects. Moving away from this line, either towards or away from the origin, signifies increased $G \times E$ interaction effects. The length of genotype projections onto the AEA (dotted lines) estimates their contribution to $G \times E$ interaction, with longer projections indicating greater instability and lower yield stability across

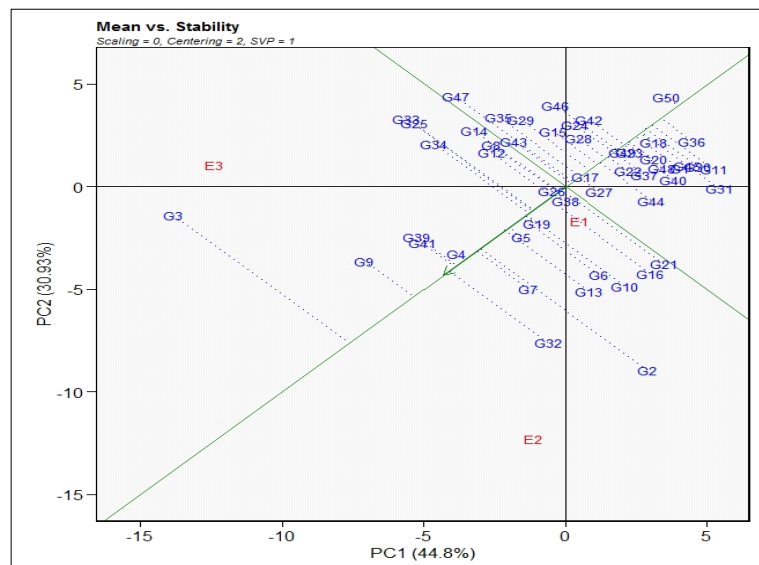


Fig 3: The 'Mean vs. stability' pattern of the GGE biplot showing the interaction effect of 50 soybean genotypes across three environments for seed yield (Centering=2, SVP=1, Scaling=0).

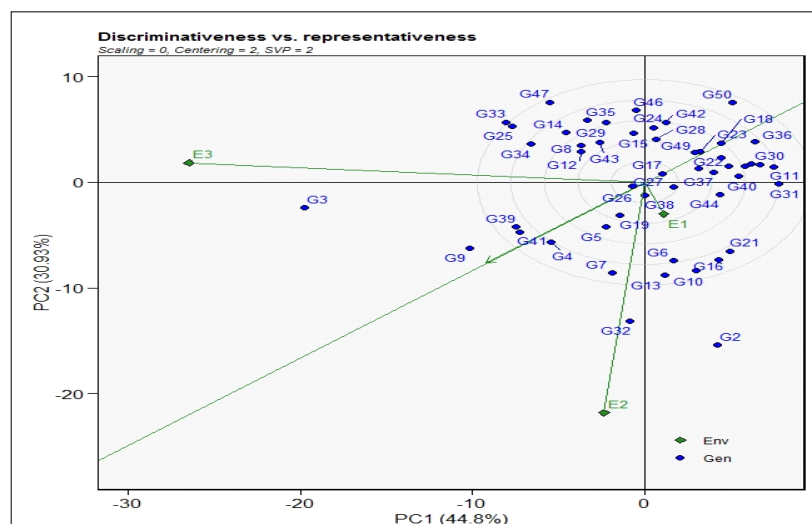


Fig 4: The GGE biplot 'Discriminativeness vs. Representativeness' pattern comparing genotypes with the ideal genotype, showing $G + G \times E$ interaction of 50 soybean genotypes across two years and three environments for seed yield (Centering= 2, SVP= 2, Scaling= 0).

environments. The line divides genotypes into below-average and above-average performers. Genotypes G3 (14.58), G9 (13.22), G39 (12.9), G32 (12.23), G41 (10.95) and G4 (10.58) and showed higher yield performance than the average (Fig 3), a finding also reported by Silva *et al.* (2021, 2022) and Habtegebriel *et al.* (2023). The GGE biplot effectively evaluates a test environment's ability to differentiate genotypes and represent the broader mega-environment. In this study, E3 and E2 had the longest vector lengths (Fig 4), identifying them as the most discriminating environments. E1 had the shortest vector length, indicating it is the least discriminating environment, providing limited information on genotype differences. Similar findings were reported in soybean studies by Habtegebriel *et al.* (2023). The ideal genotype is the one that with the highest mean performance and absolutely stable (Yan and Kang, 2002).

This is assumed to be in the centre of the concentric circles is an ideal genotype across the tested environment. It is more desirable for a genotype to be located closer to the ideal genotype. Hence, the GGE biplots (Fig 5) shows that genotype G9 was ideal in terms of higher-yielding ability and stability as compared to the other genotypes across the environment. The GGE biplot identifies ideal environments as those near the center of concentric circles with longer vectors, indicating high discriminating power and representativeness of average target growing conditions. Therefore, based on the GGE biplot, E2 followed by E1 might be ideal environments in this study, reflecting Yan and Kang, (2002) emphasis on discriminating power and target environment representativeness. Likewise, Arega *et al.* (2018) also observed similar result by using GGE biplot analysis.

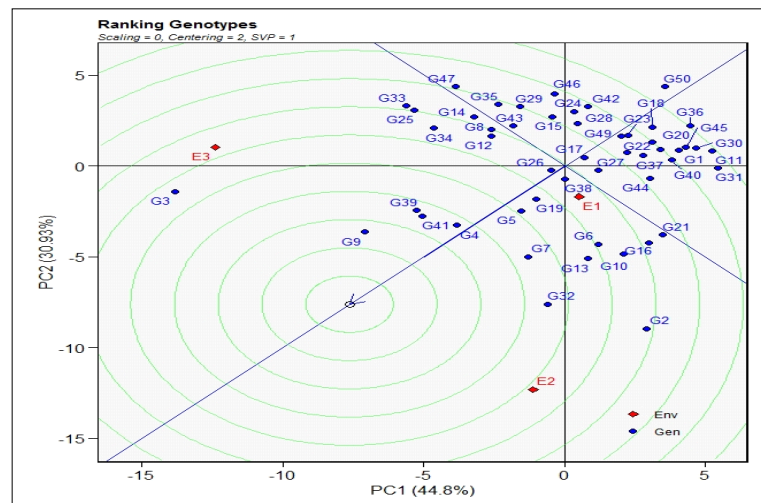


Fig 5: The GGE biplot 'Genotype ranking' pattern comparing environments with the ideal environment, showing $G + G \times E$ interaction of 50 soybean genotypes across two years (3 environments) for seed yield per hectare (Centering=2, SVP=1, Scaling=0).

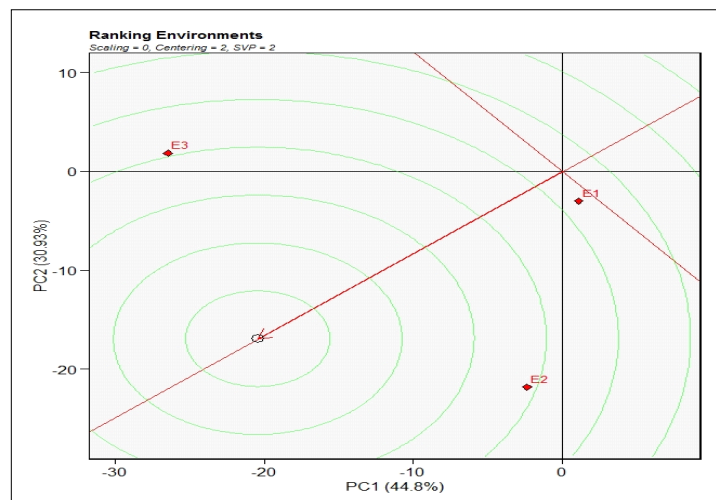


Fig 6: The GGE biplot 'Environment ranking' pattern comparing environments with the ideal environment, showing $G + G \times E$ interaction of 50 soybean genotypes across three environments for seed yield per hectare (Centering= 0, SVP= 2, Scaling= 0).

CONCLUSION

The grain yield of soybean varieties varied significantly due to environmental factors, variety differences and their interactions (GEI). Environmental factors had the largest impact on yield variability, with notable genotype-by-environment interactions. AMMI analysis identified genotypes G26 (JS 22-88) with 20 and G39 (JS 22-101) with 16 as the most stable and high-yielding genotypes across different environments. And GGE biplot analysis highlighted G9 (JS 22-71) as the most productive and consistent genotype.

Conflict of interest

The authors say that there is no conflict of interest.

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