



Evaluation of Diverse Greengram Genotypes for Seed Yield Stability under Acidic Soils by AMMI and GGE Biplots Analysis

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ABSTRACT

Background: The objective of this study was to identify potential greengram genotypes for breeding in the acidic soils of the North East Hill Region (NEHR) by quantifying the effects of genotype $\times$ environment interaction (GEI) and to determine seed yield stability among the genotypes.

Methods: Thirty-two greengram genotypes were tested under three acidic soil locations for five seasons, using randomized block design with three replications. The data collected was analyzed for variability among the genotypes for the traits plant height, days to maturity, number of pods per plant, pod length, seeds per pod and seed yield per plant followed by stability analysis for seed yield per plant using biplot analysis of genotype plus genotype $\times$ environment interaction (GGE) and additive main effects and multiplicative interaction (AMMI) models.

Result: Variability was observed among the genotypes for seed yield and its component traits. Significant genotype, environment and $G \times E$ interaction was found in all the 32 genotypes seed yield per plant in pooled and AMMI anova. Thirty-two genotypes mean seed yield ranged from 2.52 gm Pusa 1431 (G21) to 4.66 gm Pusa 1031 (G3) across the environments. Genotype (Pusa 1031 (G3) exhibited seed yield (4.66 gm) higher than mean seed yield of (3.51 gm) with adaptability for the environment E1 (ICAR NEH farm), E4(NBPGR farm) and E5 (CPGS-AS farm). The NBPGR farm (E4) was shown to be the most suitable site for the potential expression of higher seed yield in the genotypes. The genotypes Pusa 1031 (G3) and Ganga-1 (G28) are stable and high yielding across the locations according to the stability analysis results.

Key words: GEI, Greengram, Seed yield, Stability.

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek]] is highly adaptable grain legume and have been farmed on the minimal-fertility ground with low productivity and limited input in India (Ali *et al.*, 2024). For people who live in Asia and Africa, it offers an affordable supply of vegetable protein (24-26%), carbs (51%), minerals (4%) and vitamins (3%) (Karthikeyan *et al.*, 2019). Ninety percent of the world's mungbean crop is produced in Asia, with India being the top producer (Wilbur, 2023). The North East Hill Region (NEHR) of India is distinguished by its physiographic location, climate, crop suitability and market accessibility having potential of pulse production (Pahraj and Singh 2019). Still, the development rate is slow because of several complex, interconnected elements, ranging from limitations caused by soil acidity, aluminium toxicity and climate issues (Bhadana *et al.*, 2013). Greengram also known as mungbean, a short-duration pulse crop, being a potential pulse in NEH region, it is important to identify genotypes suitable in acidic soil of NEHR.

Grain yield is a complicated quantitative character with strong environmental interaction; developing varieties or choosing parental materials for breeding programmes can be accomplished by selecting genotypes based only on how well they perform in a particular environment. Genotype $\times$ environment interaction ($G \times E$) is a major barrier for crop to attain the full genetic advantages (Gruneberg *et al.*, 2005).

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Before plant breeders can firmly decide on a breeding target, they must have a comprehensive understanding of the $G \times E$ interaction in mungbean. The Additive Main Effects and Multiplicative Interaction (AMMI) model and the Genotype, Genotype $\times$ Environment Interaction Effects (GGE) model are widely used for depiction of $G \times E$ interaction and genotype ranking among the environments. Additionally, GGE and AMMI have proven to be especially helpful visualizing $G \times E$ effects in graphical representation. Hence a study was conducted with an objective to determine stable

performing greengram genotypes with high yield under acidic soils of Meghalaya using AMMI and GGE biplot analysis.

MATERIALS AND METHODS

Study material

A panel of 32 greengram genotypes obtained from the Indian Agricultural Research Institute (IARI), New Delhi were used as the germplasm for this investigation (Table 1).

Experimental sites and design

During the 2021-2022 and 2022-2023 cropping seasons, the field trials were carried out at three carefully chosen

locations in Umiam, Meghalaya *i.e.*, the ICAR NEH farm, NBPGR and Institutional farm CPGS-AS, CAU(Imphal). Because of the soil type and site location vary, every season and site combination provided different environmental circumstances. Consequently, five environments were identified for the genotype evaluation due to location×season pairings. In 2021, the ICAR NEH farm (Soil pH 4.80) and NBPGR farm (Soil pH 5.12) were designated as Environment 1 (E1) and Environment 2 (E2). In 2022, the ICAR NEH farm and NBPGR farm were categorized as Environment 3 (E3) and Environment 4 (E4), respectively, while in 2023, the institutional farm CPGS-AS, CAU (Imphal) (Soil pH 5.05) was assigned as

Table 1: Average seed yield per plant values of thirty-two genotypes of greengram, IPCA1 and IPCA2 scores.

Genotype and environment names	Genotype and environment code	Seed yield per plant (gms)	IPCA1	IPCA2
PM-5	G1	3.22	-0.13	-0.72
Pusa baisakhi	G2	3.26	0.34	0.19
Pusa 1031	G3	4.66	-0.38	0.11
Pusa 1033	G4	3.98	-0.42	-0.27
Pusa 871	G5	3.41	-0.80	0.11
P 9531	G6	3.86	-0.81	-0.01
P 672	G7	3.6	0.03	0.28
Pusa vishal	G8	4.07	0.78	-0.18
Meha	G9	3.6	0.64	0.08
Shikha (IPM 410-3)	G10	3.93	-0.98	0.24
Samarat	G11	2.85	0.80	-0.20
IPM-2-3	G12	3.88	0.13	0.41
IPM-2-14	G13	3.02	0.70	0.41
IPM-2-15	G14	3.5	0.09	-0.06
IPM-2-19	G15	3.36	-0.60	-0.01
MH-215	G16	3.19	0.39	-0.26
MH-318	G17	3.81	-0.25	0.08
MH-421	G18	3.34	-0.10	-0.58
MH-565	G19	3.33	0.31	-0.09
PM-4	G20	3.89	0.50	-0.12
P-1431	G21	2.66	-0.24	-0.07
HUM-1	G22	3.56	-0.45	0.13
HUM-6	G23	3.66	-0.05	-0.19
HUM-12	G24	3.48	-0.21	-0.11
HUM-16	G25	2.87	0.02	0.15
SM-11-75	G26	3.32	0.58	-0.16
SML-668	G27	4.27	0.34	0.34
Ganga-1	G28	4.44	-0.02	0.33
Porbandar mung	G29	3.94	0.15	0.23
MH-125	G30	2.69	-0.40	-0.45
Virat	G31	3.14	0.47	0.22
Pusa ratna	G32	2.52	0.22	0.20
ICAR NEH farm (2021)	E1	3.63	-1.17	0.01
NBPGR farm (2021)	E2	3.4	1.42	-0.29
ICAR NEH farm (2022)	E3	3.21	1.47	0.22
NBPGR farm (2022)	E4	3.21	-0.79	1.08
CPGS-AS farm (2023)	E5	4.1	-0.93	-1.02

Environment 5 (E5). Every experiment had three replications and was carried out using a randomized block design.

Statistical analysis

The average data of five randomly selected plants per genotype for the traits plant height, days to maturity, number of pods per plant and seed yield per plant were utilised to ascertain the range and overall mean for each environment. To investigate the genotype×environment interaction of the genotypes in all environments, seed yield per plant data were submitted to combined analysis of variance. The G×E interaction and genotype yield stability were analysed sequentially using the AMMI model and GGE biplot models. AMMI, GGE biplot analysis were performed in R studio using a multi-environment trial analysis package 'metan' version 1.18.0 (Olivoto and Lucio 2020). Following Gauch Jr. (1988), the partitioning of genotype and genotype×environment interaction effects was carried out. By determining each genotype's AMMI IPCA values in accordance with Purchase *et al.*, (2000), the genotypes stability across sites was examined. The GGE biplot, which is based on the model as stated by Yan *et al.*, (2000), was used to further graphically demonstrate the correlation between genotypes and environments.

RESULTS AND DISCUSSION

The variation in performance of thirty-two genotypes for yield and yield attributing traits are presented in Table 2. Days to maturity ranged from 63 days in E3 to 91 days in E1. The mean of plant height ranged from 15.2 (E2) to 37.8 in (E5). The character number of pods per plant varied from 3.2 (E1) to 20 (E2). The environment E1 expressed maximum pod length whereas E2 showed minimum mean of 4.64. The average number of seeds per pod ranged from 15.4 (E5) to 6 (E1). Seed yield per plant ranged from a maximum mean of 6.99 in E1 to a minimum mean of 1.3 (E1). Genotypes exhibiting maximum and minimum mean values for seed yield and its contributing traits in all the environments have been displayed in Table 2. Hence in the present study considerable trait variation existed in 32 genotypes under study and similar variations in seed yield

and its component traits among 52 greengram genotypes have been observed by Samyuktha *et al.* (2020).

Significant variations between genotypes and environments, as well as significant genotype×environment interaction, were found in the combined analysis of variance for seed yield per plant (gm). The strong genotype-environment interaction effect highlighted how the environment affected the seed yield per plant of greengram genotypes. The mean seed yield per plant for the 32 genotypes ranged from 2.52 gm (G21) to 4.66 gm (G3), with a mean yield of 3.51 gm. Out of the five environments evaluated, environments E5 (4.10 gm) and E3 (3.20) had the highest and lowest mean seed yield per plant (Table 1). Researchers have assessed in similar fashion of the current study results, how well different environments affect the genotypic performance of chickpea Rao *et al.* (2023) rice, Xing *et al.* (2021), blackgram Kumawat *et al.* (2023) and pigeon pea Yohane *et al.* (2021). The significant impact of G X E interaction on seed yield per plant would confound selection of superior genotypes for yield improvement.

AMMI analysis of variance for seed yield per plant (gm) of 32 greengram genotypes tested across five environments showed that the main effects of genotypes (G) and environments (E) accounted for 34.40% and 15.22% of the total sum of squares (Table 3). A contribution 50.38% of the total sum of squares was by G×E interaction, which facilitates selecting a genotype that is appropriate for a given environment or set of environments (Table 3). This interaction clearly illustrates the diversity among the genotypes and their varying responses due to environmental conditions. Similar results of genotypes showing highest degree of genotype environment interaction (GEI) were observed in the studies by Singamsetti *et al.* (2021), Sriwichai *et al.* (2021); Rao *et al.* (2023) and Yohane *et al.*, (2021), in maize, winged bean, chickpea and pigeon pea implying that the genotypes selection should be based on broader adaptation. The considerable proportion of variance in yield is contributed by the environment as per the AMMI, when the interaction was divided among the first four interaction principal component axes (IPCA) because they were significant in the prognostic evaluation. Each interaction PCA captured 82.20%, 9.10%, 6.90% and 1.80% of the overall variation in

Table 2: Details on performance of seed yield and its component traits in 32 greengram genotypes.

Parameters	PH	DM	PP	PL	SP	SYP
Mean	24.7	76.54	9.79	7.43	9.47	3.51
SE	0.18	0.36	0.15	0.04	0.06	0.05
SD	4.02	7.83	3.32	0.88	1.37	1.07
CV	16.3	10.24	33.94	11.91	14.47	30.48
Min	15.2 (G18 in E3)	63 (G8 in E3)	3.2 (G17 in E1)	4.64 (G21 in E1)	6 (G21 in E1)	1.3 (G19 in E1)
Max	37.8 (G28 in E5)	91 (G27 in E1)	20 (G3 in E2)	9.9 (G17 in E2)	15.4 (G22 in E5)	6.99 (G3 in E1)
MinENV	E4 (23.2)	E5 (69.66)	E4 (8.89)	E4 (6.97)	E4 (9.18)	E3 (3.21)
MaxENV	E2 (25.73)	E1 (87.35)	E2 (10.88)	E2 (7.8)	E5 (9.84)	E5 (4.1)
MinGEN	G9 (19.44)	G4 (73.27)	G17 (6.47)	G24 (6.17)	G18 (7.57)	G32 (2.52)
MaxGEN	G3 (32.2)	G27 (78.8)	G3 (17.23)	G15 (8.68)	G22 (11.37)	G3 (4.66)

the G×E interaction sum of squares, respectively (Table 3). These PCAs axes combinedly accounted for were 91.3% of the G×E interaction, which was highly significant and declaring the usage of GGE biplot to describe variance brought on by G+E+GEI across the environments was deemed to be effective. The first two PCAs described the largest GEI, according to prior reports from Saxena *et al.* (2020); Zewdu *et al.* (2020) and Rao *et al.* (2023). As a result, the first two main components of genotypes and environments accounted for the majority of the GEI (91.3%) among the 32 greengram genotypes examined in five different environments. According to AMMI anova, environment (15.22%) in the current study contributes significantly to yield variance, demonstrating its diversity. Prior studies conducted by Biswas *et al.* (2019) and Rao *et al.* (2023) verified that the top two PCAs could account for the largest GEI in the majority of situations. As a result, AMMI1 and AMMI2 biplots were generated using IPCA1 and IPCA2 scores.

AMMI-1 biplot analysis

AMMI analysis results further clarified the relative contribution of the first two IPCA axes to the interaction effects, as shown in Fig 1a. The PCA1 scores and mean

performances for genotypes, as well as the environments were used to construct the biplots. Environments in the biplot are designated by letter 'E' with suffix 1 to 5 whereas the numbers ranging from 1 to 32 denote the genotypes with prefix 'G'. The graph quadrants stand for the following: greater mean for QI and QII, lower mean for QIII and QIV, +ve IPCA1 scores for QI and QIV and -ve IPCA1 scores for QII and QIII. A genotype and the environment are said to interact positively when their signs on the PCA1 axis are similar; when they are opposite, they are said to interact negatively. As a result, a genotype with a PCA1 score close to zero indicated that it had little interaction effect and was therefore regarded as stable across all environments. Conversely, it was believed that genotypes with high mean yield and high PCA scores were obviously suited to particular environment (Abdi and Williams, 2010; Askari *et al.*, 2017; Mustapha and Bakari, 2014).

In the current investigation, genotype G3 (4.66 gm) among greengram genotypes produced the highest yield, with a negative IPCA 1 score; genotypes G27 (4.27 gm), G8 (4.07) and G10 (3.99) showed high yield and positive IPCA scores (Fig 1a). IPCA 1 showed nearly zero scores for genotypes G3 (-0.38) and G5 (-0.8) indicating minimal interaction with the environment in which genotype G3

Table 3: AMMI analysis of variance (ANOVA) for seed yield per plant of 32 greengram genotypes tested across five environments for G×E interaction.

Source	Df	Sum Sq.	Mean Sq.	F value	% Explained SS
Genotypes (G)	31	122.33	3.95***	16.49	34.40
Environment (E)	4	54.12	13.53***	1.15	15.22
Genotypes×Environment (G×E)	124	179.12	1.44***	6.04	50.38
PC1	34	147.15	4.33***	18.09	82.20
PC2	32	16.36	0.51***	2.14	9.10
PC3	30	12.42	0.41	1.73	6.90
PC4	28	3.18	0.11	0.47	1.80
Residuals	310	74.17	0.24		
Total	603	727.00	1.21		

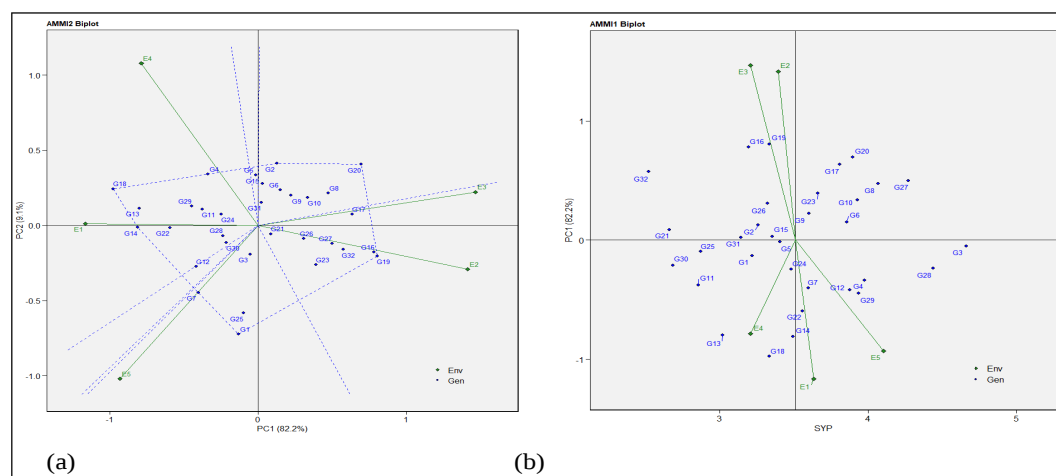


Fig 1: (a) AMMI 1 biplot showing mean yield vs PCA1 scores (b) AMMI 2 biplot displaying PCA1 scores vs PCA2 scores of genotypes and environments.

showed above average in yield (Table 1 and Fig 1a). Most of the genotype stability was attributed by environments E4 (-0.79) and E5 (-0.93), which had the lowest IPCA1 scores (Table 1 and Fig 1a). With respect to GEI contribution, environments E3 (1.47) and E2 (1.42) exhibited the highest levels of contribution, as indicated by their high IPCA1 scores (Table 1 and Fig 1a). Environmental conditions E5 (4.1 gms), E1 (3.63 gms) and E2 (3.4 gms) yielded the highest mean, while E3 (3.21 gms) had the lowest mean seed yield per plant (Table1).

AMMI-2 biplot analysis for association between genotypes and environments

AMMI 2 biplot focuses solely on the $G \times E$ interaction. The magnitude of the $G \times E$ interaction was illustrated by plotting first principal component axis (IPCA1) scores vs second principal component axis (IPCA2) scores of genotypes and environments. The vector length of each environment can be used to indicate the ability of each environment to discriminate between the genotypes. AMMI 2 biplot depicts that genotypes present near the origin shows minimum interaction with the environment and can be considered as stable. In similar fashion genotypes which are distant from the origin having lengthy vector are thought to be showing maximum interaction with the environment.

AMMI-2 biplot (Fig 1b), depicted E4 as the environment where the vector was the shortest and it could discriminate

between the genotypes. E2 and E3 are the least discriminating environments which had longer vector length. Genotypes G21, G26, G27, G32, G16 and G19 have been associated with the vector E2 and G4, G24 and G5 were correlated with the E4. Additionally, environment E5 had association with G3, G7, G30 and G12. The vector E1 was associated with genotypes 14, 13 and 22. On the other hand, E3 was associated with genotype G17. Genotypes linked to a specific environment have primarily adapted to that environment. Being plotted close to the origin revealed the genotypes G3, G21 and G31 to be the stable genotypes. The AMMI 2 biplot demonstrated a varied response of genotypes to distinct environmental situations, underscoring the need of multi-environment trials in plant breeding for identifying appropriate genotypes for selection.

GGE Bi-plot analysis

Environment-view GGE biplot for yield

The environment pattern is estimated using an environment-centered GGE biplot (Fig 2a). For comparing the association between environments, lines were constructed as environment vectors connecting the test environments to the biplot origin. According to Yan and Tinker (2006), a large obtuse angle across environments indicates a high level of genotype crossover and environment interaction and indicates negative interaction between environments. The degree of connection between

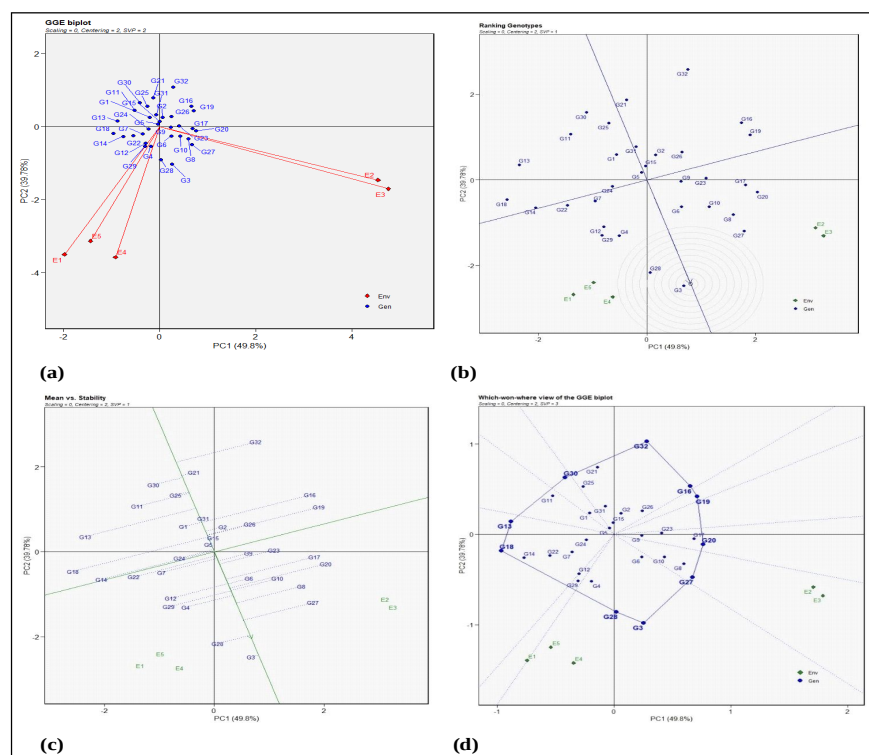


Fig 2: (a) The GGE-biplot's environment vector view illustrates the connections between the five environments (b): GGE biplot representing Ranking genotypes relative to ideal genotype (c): GGE biplot showing Mean performance vs. stability of the genotypes. (d): GGE biplot for "Which-Won-Where" analysis of thirty-two greengram genotypes.

two environments can be explained using the angle cosine between them (Dehghani *et al.*, 2006) and shows positive relation between environments. In current study an acute angle of positive correlation between environments E2 and E3 and an obtuse angle *i.e.* a negative correlation, between the environments E2 and E1 (Fig 2a) is observed.

GGE biplot for ranking genotypes relative to ideal genotype

A genotype is deemed to be perfect in a GGE biplot if it is situated at the centre of the concentric circles pointing in the positive direction of the average environmental axis (AEA). Higher mean performance and great stability in all investigated environments are characteristics of an ideal genotype. As a result, genotypes that are closer to the ideal genotype are more desirable than those that are farther away. In the Fig 2b, GGE biplot concentric circles displays genotype G3 is closer to inner circle, hence considered as ideal genotype for seed yield per plant having higher mean and stability across environments. Genotype G28 was closer to G3 hence can be considered as desirable genotype with high mean. Genotype G32 had shown poorer performance across all environments as it is located farthest from the ideal genotype.

Biplot of stability and mean performance of genotypes across average environments

Using the average environment axis (AEC), genotype yield performance and stability were illustrated. The AEC is represented by the line (single arrow) that passes through the biplot origin and the average environment. The mean genotype yield is represented by genotypic marker projections to AEA. Genotypes are arranged along the ordinate, line that divides the genotypes into two groups *i.e.* those above average and below average *i.e.* perpendicular to the average environment axis (AEA).

The seed yield per plant (Fig 2c) of genotypes G3, G28, G27, G8, G4, G29, G6, G9 and G7 has been above average. The yield for genotypes G14, G24, G19, G16, G13, G11, G21 and G32 has been below average. More stability is indicated by greater projection into the AEA ordinate in any direction (Yan *et al.*, 2007). In Fig 2c, with shorter projections, the genotypes G3, G28, G6, G5, G15, G31, G25 and G21 remain stable across environments. G3 and G28 are stable and high yielding genotypes among them. In contrast, the low seed yielding and stable genotypes were as follows *i.e.*, G5, G15, G31, G25 and G21. A genotype that performs consistently in all environments and expresses its full genetic potential is known as ideal genotype (Kaya *et al.*, 2006; Yan and Tinker, 2006). The environment (E4) is believed to be the optimum environment for determining the stability of the genotypes since it is closest to the axis. The findings of Akinyosoye (2022), Rao *et al.* (2023) and Tiwari *et al.* (2022) also showed that the environment nearest to the axis/concentric point is optimal for stable genotype selection. The longest projectiles from the axis were recorded in Environment 3 (E3), suggesting that this is the optimal environment for

choosing high yielding genotypes. However, this does not imply that genotypes selected in this environment are stable; rather, high yielding genotypes selected from this environment can be used for developing high yielding varieties. The most effective genotypes for stability and adaptability might be chosen as progenitors in yield enhancement programs to create new cultivars.

“Which-Won-Where” pattern analysis

“Which-Won-Where” pattern analysis and “ranking of genotypes in individual environments” utilising GGE Biplot were used to identify genotypes adaptative ability for a particular environment or group of environments. It also exactly determines the best G×E interaction (Yan and Kang, 2003). As per Fig. 2d, the environments under study were split up into two mega environments, denoted as M1 and M2. Environments E4 and E5 are closely related and fall under the mega Environment M1. E3 is closely related with E1 so it falls under mega environment M2. Genotypes G29, G4, G28, G3 are winning genotypes of Mega environment M1. Genotype G27 is the winning genotype in mega environment M2. The ideal genotype or genotypes for each environment are provided by the polygon viewpoint of the GGE bi-plot. In terms of response to environment, the vertex genotypes (G3, G28, G18, G13, G30, G32, G16, G19, G20 and 27) have the longest vectors in each direction. In the environments under each sector, vertex genotypes produced the highest seed yield per plant. In the environments E4, E2 and E3, the genotype with the highest yield is G3. G28 yields well in environments E4, E5 and E1. Since the genotypes G18, G30, G13, G32, G16 and G19 lack environment on their vertex they are considered as poorest in all environments. The results of this study are in consistent with Rao *et al.* (2023); Yohane *et al.* (2021), Akinyosoye (2022) and Ebadi *et al.* (2010) who demonstrated that in every environmental sector, genotypes could be cultivated in the location where they showed an edge in seed production. Cultivating genotypes in any of the environments under study that didn't fit into any of the environment sectors is improper. There is a necessity to include molecular approaches to improve selection efficiency and bypass the significant environmental constraints. The existence of crossover effects of GEI on greengram seed yield per plant suggested the necessity to breed for a particular adaption.

CONCLUSION

A substantial amount of variation was observed for yield and its component traits among 32 genotypes under study. Environment and genotypes had a considerable impact on yield stability. These factors also showed that major interaction effects could be defined by the first two main components in the AMMI model. The genotypes G3 (4.66 gm), G28 (4.44 gm) and G27 (4.27 gm) produced above average seed yield per plant of all genotypes, whereas the genotype G32 (2.52 gm) produced the least amount of

seed yield. G3 and G28 are the stable and high-yielding genotypes, respectively. Genotype G3 demonstrated greater adaptability for environments E1 (ICAR NEH farm), E4 (NBPGR farm) and E5 (CPGS-AS farm) with a higher seed yield per plant (4.66 gm) than the mean seed yield (3.51 gm). It was determined that the NBPGR farm (E4) was most suited for the possible expression of seed yield. Of all the environments, E4 is the most representative, while E2 and E3 are the least representative. According to the results, the CPGS-AS institutional farm (E5), had the highest mean grain yield (4.1 gms) and was determined to be the most ideal environment for seed yield expression. In the North East Hill Region (NEH) region, the genotype Pusa 1031 (G3) can be a potential source of breeding material for future breeding programmes because it demonstrated high seed yield and stability across all environments with desirable mean performance.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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