



## Genotype-by-environment interaction studies for yield and *Aspergillus* resistance in groundnut (*Arachis hypogaea*) using AMMI and GGE biplots

ARATI YADAWAD<sup>1\*</sup> and RAMESH BHAT<sup>2</sup>

University of Agricultural Sciences, Dharwad, Karnataka 580 005, India

Received: 16 August 2024; Accepted: 21 January 2026

### ABSTRACT

The present study was carried out during rainy (*kharif*) and summer seasons of 2019, 2020 and 2021 at three test locations of Dharwad, Belagavi and Bagalkot districts of Karnataka intended to assess the genotype by environment interaction (GEI) effects to identify high yielding and stable lines with *Aspergillus* resistance in groundnut (*Arachis hypogaea* L.) through AMMI and GGE biplots. Seventy-four advanced groundnut lines (F<sub>4</sub>–F<sub>6</sub>) derived from the cross between, GPBD 4, Spanish bunch variety resistant to rust and LLS but highly susceptible to *Aspergillus* infection and ICGV 2266, tolerant to *Aspergillus* infection were field evaluated. Multi environmental trials were conducted at three locations namely Dharwad, Bagalkot and Belagavi districts of Karnataka following randomised complete block design (RCBD) with two replications. Significant variability was revealed for pod yield, shelling percentage and *Aspergillus* infection percentage at individual locations and seasons. Pooled analysis of variance revealed highly significant ( $p < 0.001$ ) influences of Genotype (21.70%) and GEI (37.37%) on pod yield. In contrast, shelling percentage and *Aspergillus* infection percentage revealed high environmental influence of 93.24% and 55.04%, respectively with limited impacts of genotype and GEI. AMMI and GGE biplot models identified seven adaptable lines for high pod yields across the environments. G19 line was identified as potential high yielding line (4165.23 kg/ha) with low *Aspergillus* infection and two lines (66 and 43) showed high pod yields along with low *Aspergillus* infection percentage indicating the potential of these lines for development of promising groundnut varieties with high yields and tolerance to *Aspergillus* infection. Line 59 showed least *Aspergillus* infection percentage (29%) and can serve as a valuable genetic resource in future *Aspergillus flavus* resistance breeding programmes.

**Keywords:** AMMI, *Aspergillus flavus*, GGE biplot, Groundnut, Yield

Groundnut (*Arachis hypogaea* L.) is a major crop grown for its high oil and protein content. It is cultivated in an area of 32.7 million hectares with a production of 53.9 million tonnes and productivity of 1,648 kg/ha (FAOSTAT 2023). Groundnut cultivation in India is on an area of 5.97 million hectares yielding about 10.2 million tonnes with the yield of 1,716 kg/ha (FAOSTAT 2023). Genotype × environment interactions (GEI) represents an important challenge to the plant breeders leading to inconsistency in performance of genotypes across the cropping environments. It is imperative that breeders have to test their breeding material in multi-environments for selection of best performing varieties across the test environments. Powerful methods for examining multi-environment data in breeding programmes include the AMMI and GGE biplot models. Additive main effect and multiplicative interaction (AMMI) is a most widely

used for analysis of MET data that involves combination of principal component analysis (PCA) with analysis of variance with additive parameters are combined into a single analysis to arrive with the interaction of a genotype and the environment. AMMI analysis enables effective visualization of GEI using biplots that is graphical representation of the interpretation of genotype and environment interactions. GGE biplot (Yan 2001) is a versatile method with the ability to analyze a range of data types using a two-way structure and it combines the additive main effects from the ANOVA and the multiplicative effects from the principal component (PC) analysis of GEI (Gauch 2006, Gauch and Zobel 1997). Both AMMI and GGE approaches complement each other and help to analyse the GEI effects, the best genotypes and the suitable environments.

Aflatoxin contamination by the fungi, *Aspergillus flavus* is a serious food quality problem associated with groundnut and its products during pre and post-harvest periods of groundnut cultivation. Intake of aflatoxins contaminated products leads to suppressed immune systems and subsequently cause several diseases both in humans and animals. Many strategies have been implemented to prevent

<sup>1</sup>Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka; <sup>2</sup>University of Agricultural Sciences, Dharwad, Karnataka. \*Corresponding author email: aratiyadawad@uahs.edu.in

aflatoxin contamination (Kabak *et al.* 2006) including the adoption of good agricultural practices. However, breeding for aflatoxin contamination resistance is most challenging due to limited availability of improved germplasm (Soni *et al.* 2020) that restricts the range of genetic variability making it difficult to identify suitable genetic resources for breeding. Additionally, significant genotype-environment interaction for aflatoxin contamination poses difficulty in selection of stable resistant genotypes across the environments (Yu *et al.* 2019).

Current study aimed to examine the influence of genotype by environment interactions and identify potential high yielding lines with stable performance over a wide range of environments by using AMMI and GGE biplot models. Another objective of this study was to identify the high-yielding lines with resistance to *Aspergillus* infection to use as genetic resource for future *Aspergillus* infection resistance breeding programmes.

#### MATERIALS AND METHODS

The experiments were carried out at three test locations of Dharwad, Belagavi and Bagalkot districts of Karnataka during the rainy (*kharif*) and summer seasons of 2019 to 2021 comprising of five environments namely E<sub>1</sub> (Summer season of 2019 at Dharwad), E<sub>2</sub> (*Kharif* season of 2020 at Dharwad), E<sub>3</sub> (Summer season of 2020 at Dharwad), E<sub>4</sub> (Summer season of 2020 at Belagavi) and E<sub>5</sub> (*Kharif* season of 2021 at Bagalkot). Seventy-four advanced groundnut lines and two parents, GPBD 4 (national check for resistance to rust and late leaf spot of groundnut) and ICGV 2266 (resistant/tolerant to *Aspergillus flavus* infection and subsequent contamination) were evaluated in multi-environmental trials (MET). Each trial was laid out in a randomized complete block design (RCBD) with two replications to measure inherent experimental variability and to distinguish true effects. The trials were managed to reach the best yield performance with recommended package of practices. Observations were recorded on yield parameters at harvest.

Field screening methods for *Aspergillus* infection are not much reliable and consistent in effective selection and breeding. Screening for *in vitro* seed colonization for *Aspergillus flavus* was adopted for screening of 74 lines and parents following completely randomised design (CRD) with 2 replicates. Each replicate comprised of 10 healthy kernels. 20 healthy seeds were randomly selected from 76 lines and were subjected for surface sterilization with 0.1% HgCl<sub>2</sub> for 3 min and further rinsed with sterile distilled water thrice. Surface sterilized seeds were then transferred to sterile Petri dishes 10 seeds/plate. The seeds were inoculated with *A. flavus* strain 'AF 11-4' spore suspension at the rate of 10<sup>6</sup> colony forming units/ml. Petri dishes with *A. flavus* inoculated seeds were maintained at 100% relative humidity and 28°C temperature in a moist chamber. *A. flavus* colonization of peanuts was recorded as percentage of mycelial growth and coverage on the peanut kernels of each line from all the five environments. The

incidence was recorded:

$$\text{Incidence (\%)} = \frac{\text{Number of seeds showing pathogen colonization}}{\text{Total number of seeds}} \times 100$$

The severity of the incidence was rated following severity scale (1–4) of Thakur *et al.* (2000). Mean colonization severity of each line was recorded.

The data recorded for all traits was analysed for Analysis of Variance (ANOVA) using OPSTAT software to study the variability among the lines and their interactions. Individual and pooled ANOVA across the five environments was carried out. Means of the treatments were tested using Fisher's protected least significant difference (LSD) with 5% probability. To study the interaction effects of genotype and the environments, PB Tools software (PB Tools, version 1.4, 2014 <http://bbi.irri.org/products>) with multivariate stability analysis and R (R Core Team 2015), a graphical tool developed at IRRI were used. Data collected from advanced breeding lines was subjected for analysis using the multivariate models of AMMI and GGE Biplot (Yan and Kang 2003). AMMI (Gauch and Zobel 1997) analysis was used to measure multiplicative effects of the GE interaction by the principal component analysis and the main or additive genotype and environmental effects by analysis of variance. The GGE biplot analysis (Crossa and Cornelius 1997, Crossa *et al.* 2002) was used for graphical representation. IPCA<sub>1</sub> and IPCA<sub>2</sub> scores were used to maximize the useful effects of GEI and increase the accuracy of genotype recommendations.

#### RESULTS AND DISCUSSION

*Analysis of variance:* Pod yield exhibited highly significant variations ( $p < 0.01$ ) (Table 1) due to genotypes in four test environments except E<sub>4</sub>. This indicated the presence of high variability among the lines. High variability among the groundnut breeding lines (Oteng and Dakora 2019, Ajay *et al.* 2020) would help in selection of promising groundnut genotypes. Similarly, shelling percentage also revealed highly significant differences in three test environments (E<sub>1</sub>, E<sub>2</sub> and E<sub>5</sub>). *Aspergillus* infection percentage recorded highly significant variations in all the test locations except E<sub>2</sub>. The combined analysis of variance for pod yield revealed highly significant variations of genotype and genotype × environment while environmental influence was not significant for the expression of pod yield. These results revealed differential performance of lines that were inconsistent across the environments. Similar trends of higher contributions of GEI on pod yields of groundnut has been reported by Oteng and Dakora (2019), Navrood *et al.* (2023) and Pobkhunthod *et al.* (2022). Shelling percentage and *Aspergillus* infection percentage revealed that all the three sources of variation namely environment, genotype and genotype × environment were highly significant. Mean and range of advanced groundnut lines for all three traits in five environments is presented in Table 1. Highest pod yields were observed in E<sub>5</sub> with an average of 3483.15 kg/ha

Table 1 Analysis of variance and performance of advanced groundnut lines for pod yield, shelling percentage and *Aspergillus* infection percentage.

Seasons	Source of variation	DF	Mean sum of squares			Parameter	Mean performance of groundnut lines		
			Pod yield	Shelling percentage	<i>Aspergillus</i> infection percentage		Pod yield	Shelling percentage	<i>Aspergillus</i> infection percentage
E <sub>1</sub> (2019 summer, Dharwad)	Replication	1	5.016	7.573	0.306	Mean	2166.25	66.89	41.35
	Genotypes	75	6.428**	3.656**	6.355**	Maximum	4273.85	75.92	95.00
	Error	75	1.000	1.000	1.000	Minimum	667.80	49.40	5.00
E <sub>2</sub> (2020 kharif, Dharwad)	Replication	1	169.931	9.625	3.357	Mean	2069.81	71.70	61.51
	Genotypes	75	5.054**	1.736**	1.261 <sup>NS</sup>	Maximum	5273.34	81.59	90.00
	Error	75	1.000	1.000	1.000	Minimum	583.34	44.04	30.00
E <sub>3</sub> (2020 summer, Dharwad)	Replication	1	3.658	0.364	13.015	Mean	3285.75	71.26	80.33
	Genotypes	75	2.121**	1.257 <sup>NS</sup>	11.635**	Maximum	5541.67	80.00	100.00
	Error	75	1.000	1.000	1.000	Minimum	1446.67	47.00	25.00
E <sub>4</sub> (2020 summer, Belagavi)	Replication	1	7.820	0.671	8.142	Mean	3327.66	70.99	67.83
	Genotypes	75	1.380 <sup>NS</sup>	1.259 <sup>NS</sup>	21.061**	Maximum	5250.00	81.00	100.00
	Error	75	1.000	1.000	1.000	Minimum	1687.78	57.00	15.00
E <sub>5</sub> (2021 kharif, Bagalkot)	Replication	1	5.363	42.968	27.581	Mean	3483.15	72.88	60.72
	Genotypes	75	6.225**	7.659**	10.245**	Maximum	6349.24	80.50	100.00
	Error	75	1.000	1.000	1.000	Minimum	1892.57	50.00	25.00
Combined analysis of variance for pooled data	Environments	4	133.661 <sup>NS</sup>	5,530.607**	1,291.751**	Mean	2,962.02	70.65	62.05
	Genotypes	75	7.792**	5.203**	13.645**	Maximum	4,165.24	77.32	85.00
	Genotype × Environment	300	3.354**	2.591**	9.228**	Minimum	1,764.65	61.96	29.00

NS, Non-significant; \* and \*\* represent significance at 5% and 1% levels, respectively.

followed by E<sub>4</sub> and E<sub>3</sub> (3327.66 kg/ha and 3285.75 kg/ha), respectively. In these environments, all the accessions performed well as the favourable conditions were prevailed. Conversely, the lowest pod yields were recorded in E<sub>1</sub> (2166.25 kg/ha) and E<sub>2</sub> (2069.81 kg/ha) indicating that these environments were not favourable for the expression of pod yield. Similarly for shelling percentage, E<sub>5</sub> environment recorded higher mean (72.88%) followed by E<sub>2</sub> (71.70%). For *Aspergillus* infection percentage, very low mean infection % and wide range with minimum infection of 5.00% was recorded in E<sub>1</sub> environment indicating that this environment was effective for expression of this trait.

The magnitude of environmental influence on pod yield, shelling percentage and *Aspergillus* infection percentage was greater as compared to genotype and G × E variations. Similar findings of significant environment and G × E effects has been reported for pod yield/ha in groundnut (Chaudhari *et al.* 2019, Oteng and Dakora 2019). For pod yield, environmental influence accounted for the highest proportion of the total variation (59.07%) in the phenotype with GEI and genotype contributing for only 37.37% and 21.70% of the total mean sum of squares, respectively. Similar trends of environment as the main source of variation with G and GEI having smaller influence in yield trials across locations has been evident from the reports of Yan

and Kang (2003). Zerihun (2011) evidenced that in most cases of multi-environment yield trials, environment (E) contributes about 80% or higher of the total yield variation while genotype (G) and GEI accounting for about 10% each. For shelling percentage and *Aspergillus* infection percentage, environments recorded highest proportion of variation (>90%) with very less proportion of variation accounted through G and GEI. These results indicated the predominance of environmental influence and less contribution of genotypic differences on the expression of these traits. Interplay of G, E and GEI interactions has complicated the selection of promising lines in breeding programmes. Hence, multi-environmental trials were widely used (Golkari *et al.* 2016, Chen *et al.* 2017, Ajay *et al.* 2020) to select environment specific stable genotypes.

*Genotype × environment interaction*: Plant productivity is a direct consequence of adoptability of a genotype to the surrounding environment. The differential response of genotypes to environmental variation is genotype by environment interaction that has significant impact on the genotype's adaptation and evaluation. Greater gain from selection can be achieved with greater ability to predict the performance of specific genotype-environment combinations. For examining G × E in genotype evaluation, stability analyses, additive main effects and multiplicative

Table 2 AMMI and GGE model of stability for pod yield, shelling percentage and *Aspergillus* infection percentage among advanced lines of groundnut

AMMI Model										
PC	DF	Pod yield			Shelling percentage			<i>Aspergillus</i> infection percentage		
		Percent variance	Accumulated	Mean squares	Percent variance	Accumulated	Mean squares	Percent variance	Accumulated	Mean squares
PC 1	78	38.6	38.6	2799326	31.5	31.5	45.895**	51.4	51.4	1031.85
PC 2	76	34.6	73.2	75611	28.4	59.9	42.449**	25.7	77.1	529.489
PC 3	74	14.0	87.2	1072779	22.6	82.5	34.674**	19.4	96.5	410.075
PC 4	72	12.9	100	1010899	17.6	100	27.869**	3.6	100	78.508
PC 5	70	0.0	100	0	0.0	100	0 <sup>NS</sup>	0.0	100	0.0

GGE Model										
PC	DF	Pod yield			Shelling percentage			<i>Aspergillus</i> infection percentage		
		Percent variance	Accumulated	Mean squares	Percent variance	Accumulated	Mean squares	Percent variance	Accumulated	Mean squares
PC 1	78	38.8	38.8	4490861	39.2	39.2	89.023**	41.0	41.0	1221.74
PC 2	76	23.6	62.4	2807974	18.8	58.0	43.963**	27.0	68.0	825.46
PC 3	74	21.3	83.7	2599869	16.2	74.2	38.908**	16.5	84.5	18.81
PC 4	72	8.8	92.5	1102447	14.4	88.6	35.487**	13.1	97.6	421.30
PC 5	70	7.5	100	961447	11.3	99.9	28.664**	2.4	100	79.08

\* and \*\* represent significance at 5% and 1% levels, respectively.

interaction (AMMI) and the genotype, genotype by environment (GGE) biplot technique have been widely used. Stability analysis model is solely an univariate statistics or linear regressions rather than being comprehensive multivariate methods like AMMI and GGE biplots. GGE biplots are highly effective for visualizing GEI for identification of stable, high-yielding genotypes.

*Analysis of G × E interaction using AMMI model:* In the Additive Main Effects and Multiplicative Interaction (AMMI) model, the first two Interaction Principal Component Axes (IPCA<sub>1</sub> and IPCA<sub>2</sub>) are essential as they explain large proportion of the GEI sum of squares that is critical component for accurate stability and prediction of genotype performance. The AMMI ANOVA for pod yield revealed contribution of IPCA<sub>1</sub> and IPCA<sub>2</sub> to the extent of 38.6% and 34.6% of the GEI sum of squares, respectively (Table 2) accounting for 73.2% of the total GEI. High GEI of 99% as revealed by AMMI analysis has been reported for pod yield per plant in previous studies (Gangadhara and Gor 2023). For shelling percentage, first principal component axis accounted for 31.5% and the second accounted for 28.4% of the interaction together accounting for 59.9% of total GEI. *Aspergillus* infection% revealed 51.4% and 25.7% contribution from the first and second principal component axes accounting for 77.1% of the total GEI. First two principal components of AMMI model adequately serving as the basis for selection of stable groundnut genotypes has been reported by Kona *et al.* (2024). Fig. 1 represents the AMMI biplot with pod yield on the X-axis and IPCA scores on the Y-axis. The vertical line at the middle represents the grand mean of the pod yield while the horizontal line at the middle is the PC<sub>1</sub> value of

zero. Lines located farthest on the X-axis are high yielders whereas on the Y-axis genotypes whose IPCA<sub>1</sub> scores are closer to zero are more stable. AMMI<sub>1</sub> biplot depicted that all the five environments were extremely different as revealed by their differential positions placed away from the horizontal and vertical lines on the biplot. Two of the five environments, E<sub>1</sub> and E<sub>2</sub> were having low main effects and were poor environments. While E<sub>3</sub>, E<sub>4</sub> and E<sub>5</sub> had the highest main effects and were favourable environments for

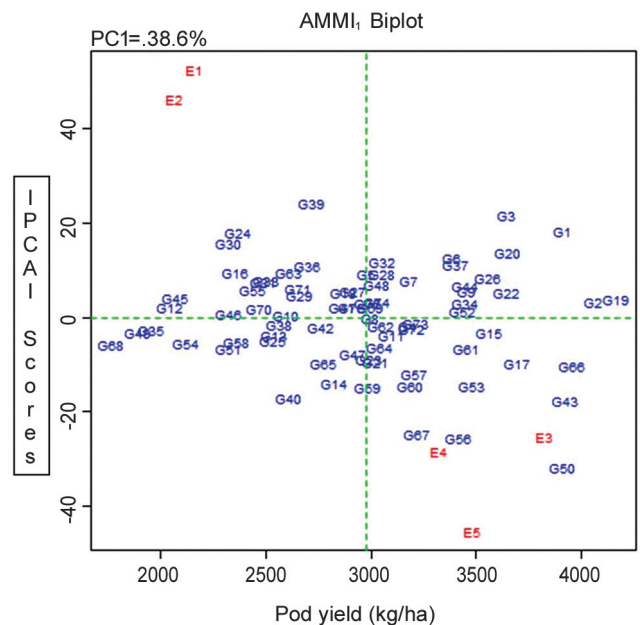


Fig. 1 AMMI Biplot showing distribution of lines for pod yield and environments (main effects) versus stability (PC1) in five environments pod yield (kg/ha)

the performance of most of the groundnut lines. Considering the genotypes G<sub>2</sub>, G<sub>19</sub>, G<sub>22</sub>, G<sub>15</sub> and G<sub>52</sub> were stable lines across the environments indicating the potentiality of these lines for further evaluation for selection of high yielding and stable genotypes.

**GGE biplot model:** The contribution of environment and GEI component to the total variance was highest for *Aspergillus* infection% (68%) followed by shelling percentage (58.0%) and pod yield/hectare (62.4) (Table 2). GGE biplots of response for pod weight, shelling percentage and *Aspergillus* infection percentage revealed significant cross over interaction among the lines in all the five test environments. High cross over interaction was recorded among high yielding lines for pod yield in E<sub>2</sub> and E<sub>4</sub> environments. Whereas, for low yielding genotypes interaction was more in E<sub>4</sub> and E<sub>5</sub> environments. Shelling percentage and *Aspergillus* infection percentage exhibited lower cross over interactions among the lines in all the five test environments..

**Mega environment evaluation for pod yield:** GGE biplot model facilitate which-won-where analysis for determining the discriminating ability and representativeness of the environments in addition to the genotypic evaluation for assessing the mean performance and stability over different environments. For pod yield, two mega-environments were identified (Fig. 2). One mega-environment comprised of two environments (E<sub>1</sub> and E<sub>2</sub>) with G<sub>19</sub>, G<sub>1</sub> and G<sub>3</sub> lines situated at the corners of the polygon while the second mega-environment with E<sub>3</sub>, E<sub>4</sub> and E<sub>5</sub> comprising of G<sub>50</sub>, G<sub>56</sub> and G<sub>43</sub> lines situated at the vertex of the polygon were winning genotypes in these mega-environments. The detection of different winners in the mega-environments indicated the presence of crossovers due to their differential yield ranking and stability across the test environments. Similar

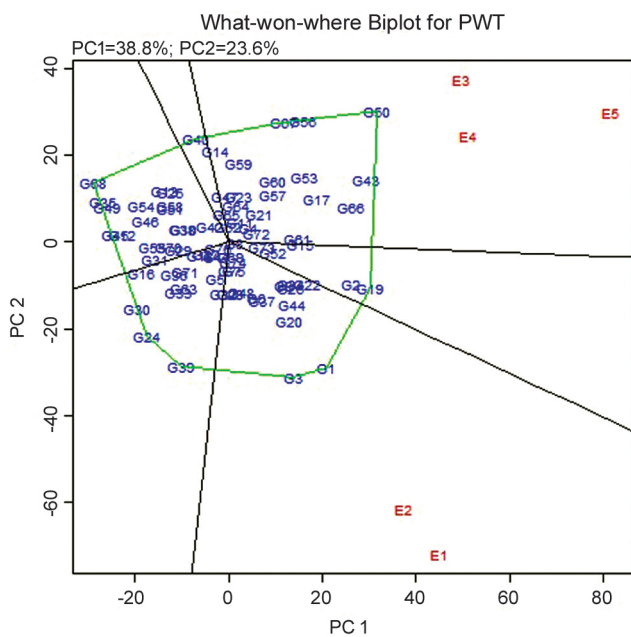


Fig. 2 Which–Won–Where biplot for pod yield in groundnut.

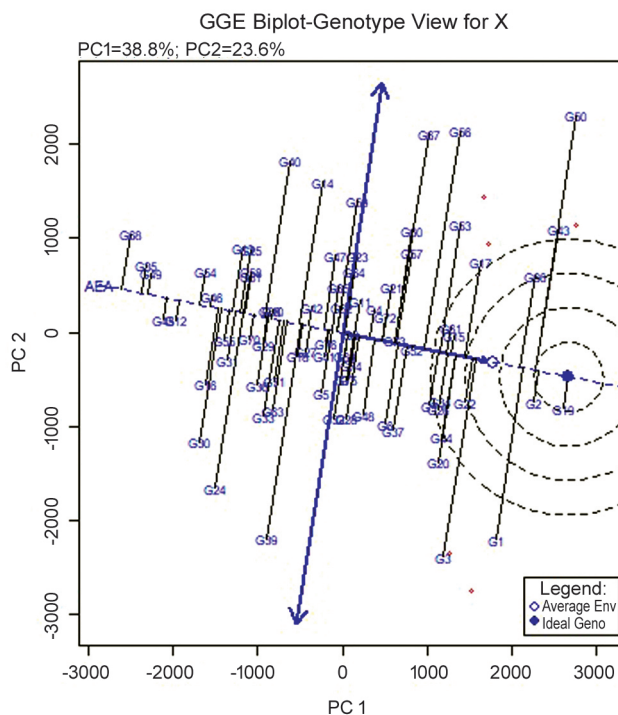


Fig. 3 Mean versus stability based on PC1 and PC2 of the GGE–biplot showing ranking of groundnut lines and stability for pod yield in five environments.

findings of differential yield ranking of genotypes across the locations for pod yield and related traits in groundnut have been reported (Kumar *et al.* 2019, Gangadhara and Gor 2023, Kona *et al.* 2024).

**Mean versus stability for pod yield:** Graphical representation of mean versus stability based on PC1 and PC2 of the GGE biplot with ranking of groundnut lines and stability for pod yield in five environments is presented in Fig. 3. The AEA ordinate serves as an indicator for genotypic contribution to the G × E interaction. Close proximity of the genotype to AEA abscissa indicates high average yield and stability of lines across various test environments. Shorter projection of lines onto the AEA abscissa represents greater stability. G<sub>19</sub> line ranked first with higher mean pod yield in addition to high stability. Two lines namely, G<sub>1</sub> and G<sub>50</sub> lines recorded higher mean pod yield across the environments but found to be relatively unstable due to their longer projection from the AEA abscissa. G<sub>43</sub> and G<sub>66</sub> were moderately stable lines with higher mean pod yield. Similar studies of GGE biplot analysis for identification of stable genotypes in different crops with discriminating ability have been reported by Zerehgar *et al.* (2023).

**Performance of promising lines for pod yield across the environments:** Mean performance of promising and highly adoptable lines across the environments (Table 3) revealed that 7 lines were promising lines with higher pod yield/ha and highly adoptable across the test environments. Line 19 recorded highest pod yield (4165.23 kg/ha) followed by Line 66 (3957.07 kg/ha) and Line 43 (3921.04 kg/ha) across the five environments. These best performing

Table 3 Highly adaptable and stable advanced groundnut lines with high pod yield, shelling percentage and *Aspergillus* infection percentage across the environments

S. No.	Line no.	Pod weight (kg/ha)	Shelling percentage	<i>Aspergillus</i> infection percentage
Highly adoptable and promising lines for pod yield				
1	66	3957.07	76.13	44
2	43	3921.04	70.39	33
3	50	3911.20	66.34	71
4	17	3697.11	74.61	58
5	22	3642.01	77.31	65
6	53	3475.79	68.10	67
7	56	3415.26	67.72	82
Highly adoptable and promising lines for shelling percentage				
1	18	2868.60	73.33	57
2	60	3187.52	68.72	55
3	65	2776.50	63.41	66
4	73	2909.67	72.74	78
Promising lines with low <i>Aspergillus</i> infection percentage				
1	19	4165.23	69.91	48
2	22	3642.01	70.14	35
3	3	3642.59	72.02	49
4	32	3053.31	72.14	48
5	59	2983.08	70.65	29

lines can be included in the varietal evaluation trials for identification and development of stable and high yielding genotypes. Line 66 and 76 recorded high shelling percentage (76.13% and 76.06%). G<sub>59</sub> recorded very low *Aspergillus* infection% (29%) followed by G<sub>44</sub> (33.5%). G<sub>43</sub> line that was found to be promising for pod yield and recorded relatively low *Aspergillus* infection percentage of 33.0% indicating the potential of this line for further evaluation and development of promising variety with high yield and tolerance to *Aspergillus* infection.

This study highlights the importance of multi-environmental trials in groundnut breeding programs for identification of potential lines for development of climate-resilient varieties with higher yield potential and stability for complex trait like *Aspergillus* infection percentage. High contribution of environment and G × E interaction components of variations as revealed through AMMI and GGE biplot models indicated that environmental factors influence significantly to the observed variation for pod yield, shelling percentage and *Aspergillus* infection percentage. AMMI and GGE biplot models provided a better understanding of GEI through analysis of variance and specificity of genotypic fitness to specific environments. G<sub>19</sub>, G<sub>66</sub> and G<sub>43</sub> lines were found to be high yielding and

highly adoptable at multi locations. G<sub>59</sub> recorded very low *Aspergillus* infection percentage (29%) and proved to be an ideal candidate for further evaluation and use as a genetic resource in future *Aspergillus* infection resistance breeding programmes.

#### ACKNOWLEDGEMENTS

This study was supported by Women Scientist-A project (WoS-A) of Department of Science and Technology (DST). Author is grateful to the DST for a competitive national post-doctoral fellowship award.

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