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GENOTYPE - ENVIRONMENT INTERACTION ANALYSIS FOR YIELD STABILITY AND RESISTANCE TO *ASPERGILLUS FLAVUS* IN GROUNDNUT

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Abstract

This study intended to assess advanced groundnut lines obtained from the cross between the Spanish bunch genotype,GPBD 4, which is resistant to rust and late leaf spot but highly susceptible to *Aspergillus* infection and ICGV 2207, which istolerant to *Aspergillus* infection. 198 advanced lines (F_4-F_6) along with their parents were evaluated for over three consecutive years (2019 to 2021) in Dharwad, Bagalkot and Belagavi comprising five distinct environments. Multivariate stability analysis was conducted to identify high yielding and stable lines with resistance to *Aspergillusflavus* infection. The results revealed highly significant variations ($P<0.001$) with high genotype × environment interaction (GEI) for pod yield and *A.flavus* resistance. AMMI and GGE biplot analyses identified 18 adaptable lines and five stable lines with high yields across the environments.Two lines (Lines 26 and122) showed stability with high yields, while Line 65 showed minimal *A.flavus* infection and is a valuable genetic resource in future resistance breeding programs.

Introduction

Groundnut (*Arachis hypogaea* L.) is a vital oilseed cropworldwide, cultivated on approximately 32.7 million hectares, worldwidewith a production of 53.9 million tons and an average productivity of 1,648 kg/ha. India is the second largest producer of groundnut with an area of 5.50million hectares and production of 7.35million tonnes yielding 1340 kg/ha yield (IPAD 2025). Groundnut seeds are rich in oil (44-56%) and protein (25-34%) and are excellent sources of calcium, iron, and vitamins such as thiamine, riboflavin, and niacin (Habtamu *et al*. 2023).

However, groundnut is highly susceptible to invasion by *Aspergillus flavus*, leading to aflatoxin contamination. Aflatoxins are carcinogenic, teratogenic, and immunosuppressive secondary metabolites produced predominantly by *A. flavus*(Frisvad *et al*. 2019,Amaike and Keller 2011). Strategies to mitigate aflatoxin contamination include biological and chemical control agents(Dornor 2004, Kabak *et al*.2006). Breeding resistant varieties is a promising approach, although limited genetic resources and the complex nature of aflatoxin resistance make this challenging (Banterng*et al*. 2006).

The presence of GEIposes a significantchallenge inthe selection process necessiating the use of multi-environment trials (METs) toassess the performance of genotypes acrosss different environments (Lal *et al*. 2019).GEI indicates that the effect of a genotype and an environment on a phenotype is inconsistent across the test sites, therefore complicating a genotype's potential to exhibit superior performance across environments (Fox *et al*. 1997, Romagosa *et al*. 2009). Numerous methods have been proposed to studyGEI (Becker and Leon 1988, Fox *et al*. 1997, Malosetti*et al*. 2013). However, the additive main effects and multiplicative interaction (AMMI)method and GGE model with biplot display of the first two principal components are widely followed among plant breeders (Yan 2001, Malosetti*et al*.2013). The effect of G × E interaction

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on the performance of genotypes can be minimized by grouping fairly homogeneous locations into mega-environments in order to take advantage of specific adaptations (Gauch and Zobel 1997). This study aimed to conduct multi-environment evaluations of 198 advanced groundnut lines to identify stable, high-yielding lines with resistance to *A. flavus* infection.

Materials and Methods

Multi-environmental trials (MET) were carriedout at three groundnut producingdistrictsin Karnataka (Dharwad, Belagavi and Bagalkot)during the *kharif* and *summer* seasons of 2019-2021.At each site, 198 advanced lines (F_4-F_6)were assessed along with two parents, GPBD 4 (national check for resistance to rust and late leaf spot of groundnut) and ICGV 2207 (resistant/tolerant to *Aspergillusflavus*infection and subsequent contamination) in a randomized block design replicated twice. The experiments were properly maintained with the application of recommended fertilizerstoachieve the best performance. Observationswere recorded on pod yield and shelling percentage for eachline during harvest.

Infection of *A. flavus*wasrecorded on seeds obtained fromtheexperiments at eachlocation and season. A total of 20 healthy seeds from each line were surface sterilized with 0.1% $HgCl_2$ for 3 min and washedthree times with sterile distilled water. Sterilized seeds were further transferred to two sterile petri dishes at the rate of ten seeds per plate. The seeds were infected with a toxigenic strain of *A. flavus*, '*Af* 11-4' spore suspension with the concentration of 10^6 colony forming units/ml. Both Petri dishes were placed in a moist chamber with 100% relative humidity at 28 °C in the dark and were observed seven days after inoculation.The incidence was calculatedusing the following formula:

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

Aspergillus flavus infection severity on seeds was determinedfollowing the rating scale (1-4) suggested by Thakur *et al.* (2000) and the average of two replications is recorded as colonization severity of the respective line.

Analyses of variance (ANOVA)were performed for each experiment and pooled ANOVAwas also worked out using OPSTAT software, a statistical **s**oftware for Agricultural Researchto study the variability among the lines and their interactions.Means of the treatmentswere compared at5% probability of Fisher's least significant difference (LSD) test. Multivariate stability and G ×E interaction analysis was performed by using PBTools software (IRRI 2014, Version1.4, http://bbi.irri.org/products andR CoreTeam2012).

Results and Discussion

Single-site ANOVA revealed highly significant genotypic differences ($p < 0.01$) for both pod yield and *Aspergillus* infection percentage in all test environments (Table 1), indicating sufficient genetic variability among the lines and their potential as a source of diversity for future breeding. Similar findings on the role of diverse environments in causing significant yield variations in groundnut have been reported (Oteng and Dakora 2019, Ajay *et al.* 2020). The combined ANOVA also indicated highly significant variations for pod yield and *Aspergillus* infection percentage, highlighting differential responses of breeding lines across environments. Previous studies have also reported significant GEI effects on groundnut pod yields(Oteng and Dakora 2019,Pobkhunthod*et al.* 2022, Navrood*et al.* 2023). Environmental factors had a more substantial impact on pod yield and *Aspergillus* infection percentage than genotype and GEI. Environment presenting the most significant influence on pod yield variations of groundnut followed by genetics and GEI is evident from the earlier reports (Oteng and Dakora 2019, Pobkhunthod*et al.* 2022).

Table 1. Analysis of variance for yield, shelling percentage (%) and *Aspergillus*infection (%)in different Emvironments.

Seasons	Source of variation	DF	Mean Sum of Squares for different traits		
			Pod yield	Shelling (%)	*Aspergillus*infection (%)
E1	Replication	1	11.701	0.327	16.316
(2019 Summer, Dharwad)	Genotypes	199	3.012**	0.836^{NS}	10.607**
	Error	199	1.000	1.000	1.000
E2	Replication	1	252.756	47.463	386.055
(2020 *Kharif*, Dharwad)	Genotypes	199	4.117**	2.866**	1.522**
	Error	199	1.000	1.000	1.000
E3	Replication	1	0.683	4.791	32.431
(2020 Summer, Dharwad)	Genotypes	199	1.514**	1.231^{NS}	10.975**
	Error	199	1.000	1.000	1.000
E4	Replication	1	0.757	12.645	0.010
(2020 Summer, Belagavi)	Genotypes	199	1.376**	1.068^{NS}	9.058**
	Error	199	1.000	1.000	1.000
E5	Replication	1	12.143	0.014	37.517
(2021 *Kharif*, Bagalkot)	Genotypes	199	1.712**	1.027^{NS}	11.488**
	Error	199	1.000	1.000	1.000
Combined Analysis of Variance for pooled data	Environments	4	196.838*	5,992.58**	5,918.060**
	Replications within seasons	5	55.608	13.048	94.466
	Treatments	199	3.139**	1.730**	16.177**
	Genotype x Environment	796	2.148**	1.325**	6.868**
	Pooled error	995	1.000	1.000	1.000

"*" and "**" representsignificance at 5 and 1% levels, respectively.

The AMMI model identified significant genotype, environment, and GEI effects for pod yield and *Aspergillus* infection percentage. However, for shelling percentage, only genotype and environment effects were significant. Environment and GEI explained 76.5% of the total variation for *Aspergillus* infection percentage and 61.4% for pod yield, indicating their substantial influence. For pod yield, the first two interaction principal component axes (IPCA1 and IPCA2) explained 34.80% and 26.60% of GEI variation, respectively (Table 2). This aligns with earlier findings emphasizing the relevance of the first two IPCA components for stable genotype selection (Gangadhara and Gor 2023, Kona *et al*. 2024). Shelling percentage revealed that the first principal component axis accounted for 38.1% and the second accounted for 24.1% with a total accumulated variance of 62.2%. *Aspergillus*infectionrecorded52.1 and 24.4% contribution from the first and second principal component axes (IPCA)of the interaction of GEI sum ofsquares respectively, with the total accumulated variance of 76.5%.

Fig. 1. presents the AMMI biplot illustrating the main effects of genotype and environment on pod yield, shelling percentage and *Aspergillus* infection percentage.AMMI biplot revealed that genotypes G108and G85have the highest pod yields. Additionally, higherpod yields wereobtainedin the E3, E4, and E5 environments. Similarly, for the *Aspergillus* infection percentage, G38 and G65 lines recorded lowest infection and were stable across the environments. The biplot analysis also indicated that none of the environments had similar means or interaction patterns, reflecting their diverse nature.

Table 2. AMMI model for pod yield, shelling percentage and *Aspergillus* infection among RILs of groundnut.

PC	DF	Pod yield			Shelling Percentage (%)			*Aspergillus* infection (%)		
		Percent variance	Accumu-lated	Mean squares	Percent variance	Accumulated variance	Mean squares	Percent variance	Accumulated variance	Mean squares
PC-1	202	34.8	34.8	2927974**	38.1	38.1	78.406**	52.1	52.1	1012.137**
PC-2	200	26.6	61.4	2258875**	24.1	62.2	50.028**	24.4	76.5	476.961**
PC-3	198	19.5	80.9	1673131**	20.0	82.2	41.854**	17.7	94.2	347.876**
PC-4	196	19.1	100	1660889**	17.8	100	37.721**	5.6	100	116.180**
PC-5	194	0.0	100	0**	0.0	100	0^{NS}	0.0	100	0.0**

"*" and "**"representsignificance at 5 and 1% levels, respectively.

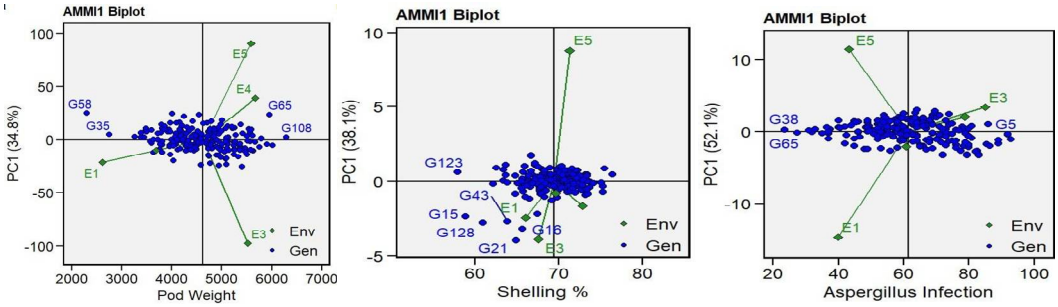
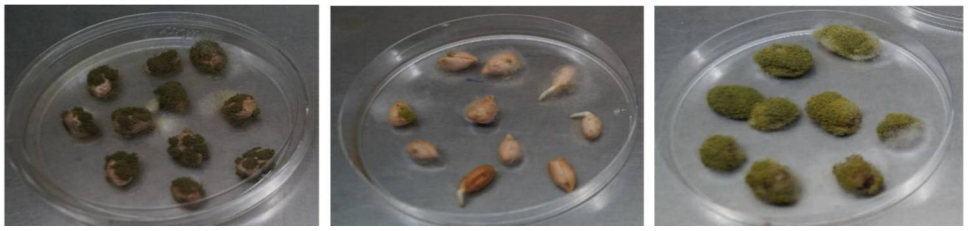


Fig. 1. AMMI biplot for mean pod yield of genotypes and environments (main effects) versus stability (PC1) of 198 lines in five environments.



Susceptible Parent GPBD 4 **Resistant Parent ICGV2207** **Susceptible Check TMV2**

Resistant Line 38 **Resistant Line 35** **ResistantLine 65**

Fig. 2. Screening for resistance to *Aspergillus flavus* in parents and resistant advanced breeding lines.

Table 3. Highly adaptable and stable advanced groundnut lines for pod yield and shelling percentage in five environments.

Sl. No.	Line	Podyield		SP	
		Mean(kg/ha)	bi	Mean(%)	bi
			Highly adaptable		
1	3	3909.02	1.14	70.25	1.15
2	15	2670.50	1.31	60.00	0.71
3	34	3968.35	1.32	71.07	-0.13
4	39	3176.83	1.56	65.61	1.56
5	40	3327.27	1.32	67.80	1.67
6	60	3039.21	1.36	69.92	0.56
7	68	3167.38	1.65	70.20	0.79
8	81	2981.40	1.63	73.11	0.20
9	92	3763.62	1.99	69.02	1.14
10	108	3678.14	1.77	72.53	1.00
11	121	3360.32	1.82	68.01	0.74
12	122	3596.25	1.79	67.83	-0.60
13	125	3488.85	1.30	67.58	-0.39
14	129	3362.87	1.53	70.82	1.14
15	130	3585.09	1.91	69.88	1.77
16	131	3374.62	1.60	65.88	1.90
17	144	2961.08	1.07	70.22	0.85
18	148	2955.02	2.21	67.39	1.42
			Stable		
1	26	3391.67	1.33	67.32	2.03
2	56	2369.45	1.21	70.10	2.00
3	72	2979.36	0.87	72.15	0.86
4	74	2606.95	1.01	72.74	-0.20
5	122	3596.25	1.79	67.83	-0.60
			Bothadaptableandstable		
	122	3596.25	1.79	67.83	-0.60
Promisinglineswithhigh yield and low*Aspergillus flavus* infection					
Sl.No	Line	Mean(kg/ha)	bi	Mean(%)	bi
1	38	2913.43	0.21	23.50	0.44
2	65	4165.08	1.29	27.50	1.01
3	35	1920.47	0.87	27.74	1.18
4	100	2909.52	0.56	32.00	0.87
5	63	2627.73	0.84	33.00	1.17
6	42	2833.53	1.16	35.00	0.57
7	64	2779.88	0.91	36.50	1.07
8	70	3797.45	1.07	36.50	0.50

AMMI analysis identified 18 adaptable lines and 5 stable lines (Table 3). Among the five environments, the highest pod yields were recorded in E4 and E5 (3967.18 and 3910.88 kg/ha, respectively),with favorable conditions enabling consistent performance.Region-specific adaptation was observed for certain lines, such as G138 performing well in E1–E3 and G92 excelling in E4 and E5. Notably, line 65 (Fig. 2) yieldedthe highest (4165.09 kg/ha) with a high shelling percentage (75.5%) and low *Aspergillus* infection percentage (27.5%), suggesting its potential as a genetic resource for breeding improvements.

In summary,the AMMI and GGE models effectively analyzed GEI in multi-environment trials. Genotype 122 emerged as a promising line with high adaptability and stability, while line 65 showed potential for breeding due to its high yield and resistance to *Aspergillus* infection. These findings provide a robust framework for selecting high-yielding and stable genotypes for further varietal development.

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