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Research Paper

Genetic stability analysis of early maturing pigeonpea genotypes using AMMI and WAASB models

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ABSTRACT

In the present study, 27 early maturing pigeonpea genotypes were evaluated over multi-locations for yielding potential and analyzed using analysis of variance (ANOVA), additive main effect and multiplicative interaction (AMMI) model and Weighted Average of Absolute Scores (WAASB) models. ANOVA displayed significant variation among genotypes, environments and genotype-environment interaction. AMMI models further explained the main and interaction effects with PC1 and PC2 covering 81.8% and 18.2% variance, respectively. AMMI biplots and WAASB matrix denoted ICP 14444, ICP 8817 and ICP 11890 to be potential early maturing, high yielding stable lines across the tested locations. Warangal and Kanpur noted to be the best environments with least and highest discriminating ability respectively. ICP 11543, ICP 16309, ICP 6992 were identified as best-fit cultivars for Patancheru, Warangal and Kanpur, respectively.

Key words: ANOVA, AMMI, Early maturing Pigeonpea, Stability, WAASB

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], commonly known as red gram or tur, is a versatile legume widely cultivated in the arid and semi-arid tropics of the world. They are produced in an area of 6.03 Mha worldwide, yielding 5.3 MT at a productivity of 883.4 kg/ha. India produces 4.3 MT of pigeonpea annually in an area of 5.00 Mha, with a productivity of 861.2 kg/ha, accounting for up to 65% of the world's total production (FAOSTAT 2022). It is mainly consumed as a split dhal or whole seed, primarily for its protein content (22%) while also regarded for its feed, fodder, and fuel. It contributes to crop health through its ameliorative properties and nitrogen fixation (Saxena 2008).

Apart from the utility aspects, its versatility exists in the morphological and phenological characters of the crop (Saxena *et al*. 2023). Majorly four maturity groups have been demarcated based on the maturity duration finding its place across different agroecological zones of the world. They are defined as early (<135 days), mid-early (136-165 days), medium (166-180), and long duration (>180 days) based on their maturity. The majority of the cultivated area is occupied by the medium segment which typically only lasts for one season per year. The current scenario of climate change and the inflating population created the need for rising productivity per unit area and time. This has led to an increase in demand for early maturing pigeonpea genotypes, which could improve overall productivity and expand the crop into new regions and seasons, thereby promoting food security.

To tackle this challenge a concerted effort from breeders is required by developing early-maturing cultivars with high yields and wider adaptability. To select the best-fit cultivars, accurate phenotyping across the locations is crucial. This is accomplished by identifying genotypes that have the closest phenotypic and genotypic values (Crossa 1990). Numerous factors influence the complex understanding of genotype × environment interaction (GEI), necessitating the use of multiple statistical tools for assessing the variation. ANOVA dissects the variation into genotype, environment, and replication yet fails to add the non-additive component, *i.e.,* genotype x environment interaction. Furthermore, by determining the genotype, environment, and GE interaction patterns based on the principal component axes upon singular value decomposition, multivariate stability analysis models like additive main effect and multiplicative interaction (AMMI) gained popularity (Oladosu *et al*. 2017).

The AMMI model makes use of the multiplicative model, which includes the genotype-environment interaction through a PCA, and the additive model of ANOVA, which explains the average genotypic and environmental means. As a result, this strategy is appropriate in cases where both genotypic and GEI are noteworthy (Sabaghnia 2012). The AMMI 1 model plots the GE interaction scores on the y-axis against the genotype and environment main effects on the x-axis. This biplot finds genotypes that are closer to the origin, indicating general adaptability, and closer to the stable environment line, indicating less environmental effects and specific adaptability. The PCA1 and PCA2 scores are plotted in AMMI biplot 2, which further analyses the variation by using a point to represent the values of G, E, and GEI combined (Gauch 1992, Gauch and Zobel 1997). These plots confer in identifying stable high-yielding genotypes for a larger adaptation.

Complex models of analysis increase the gap between the genotypic potential realized and expected. This brings up the utility of best linear unbiased predictions (BLUPs), which shrinks the mean across locations for higher accuracy in predicting genotypic means (Piepho 1994, Olivoto *et al*. 2019). A recent technique that combines BLUPs and the AMMI model to realize the best-fit genotypes in the population is the Weighted Average of Absolute Scores (WAASB). This statistical tool brings along the singular value decomposition of the means generated by BLUPs along with the GEI interaction computed through the AMMI mixed model by plotting into a 2-dimensional graph. This technique further allows for the simultaneous selection of high-yielding and stable genotypes through a WAASB-based superiority index, WAASBY (Olivoto *et al*. 2019). In this regard, 27 early maturing pigeonpea genotypes were tested at multiple locations to identify their yielding potential and stable performance using the additive main effect and multiplicative interaction (AMMI) model and Weighted Average of Absolute Scores (WAASB).

MATERIALS AND METHODS

Plant material and Experimental design

The material under study includes 27 early maturing genotypes, including two checks namely ICPL 87 and ICPL 92016. The lines were obtained from Genebank, ICRISAT, Patancheru, Hyderabad, and single plant selections were carried out for two seasons (*Kharif* 2022; *Summer* 2023) before trialing. The details of the material under study are provided in Supplementary Table 1. The experiment was conducted in alpha lattice design in three locations (environment) namely International Crop Research Institute for the Semi-Arid Tropics, Patancheru (environment 1); Regional Agricultural Research Station, Warangal (environment 2) and Indian Institute of Pulse Research, Kanpur (environment 3) during *Kharif* 2023-24. The lines were selected based on their maturity duration, which is restricted to 135 days. The trial was laid out in 2 replications and 2 rows per genotype, with a row length of 3m and spacing of 75 × 15cm. All necessary agronomic practices were followed. The lines were tested for plot yield (kg/ha) by bulking the seed from the plot.

Statistical analysis

The quantitative traits were subjected to analysis of variance (ANOVA) to estimate the existence of variations among the genotypes, locations, and genotypes by environment using the R software version 4.3.1. The genotypes were treated as fixed variables, while the environments were considered random variables.

Further, to explain the G × E interaction, the multivariate stability analysis was performed graphically based on AMMI biplot using R software version 4.3.1 using agricolae and metan packages. The biplots were based on singular-value partitioning = 2, transformed (transform = 0), environment-centered (centering = 2), and standard deviation-standardized (scaling = 0).

The genotypic stability of each genotype was quantified by the WAASB from the singular value decomposition of the matrix of best linear unbiased predictions for the GEI effects generated by a linear mixed-effect model, estimated as indicated in Equation (2):

$$\mathrm{WAASB}_i = \sum_{k=1}^{p} \left|\mathrm{IPCA}_{ik} \times \mathrm{EP}_k\right| / \sum_{k=1}^{p} \mathrm{EP}_k$$

where WAASB_i is the weighted average of absolute scores of the i^{th} genotype; IPCA_{ik} is the score of the i^{th} genotype in the k^{th} interaction principal component axis (IPCA); and EP_k is the amount of the variance explained by the k^{th} IPCA. The genotype with the lowest WAASB value is considered as the most stable, showing the least deviation from the average performance across the environments. The stability analyses of the multi-environment trial data using WAASB indexes were conducted using the Metan package of the R 4.3.1 software (Olivoto and Lucio 2020)

Simultaneous selection for both plot yield and stability was done using the superiority index WAASBY (lower is better) with the following model (Eqn (2)).

$$\text{WAASBY}_i = [(rGi \times \theta_Y) + (rW_i \times \theta_s)]/(\theta_Y + \theta_s) \quad (2)$$

Where WAASBY_i = ith genotype's superiority index based on mean performance and stability; rG_i = rescaled value (0–100) of the response variable; rW_i = rescaled value (0–100) of WAASB index; θ_Y = weight of response variable; θ_S = weight of WAASB index.

The genotype with the highest WAASBY score would be attributed to first-order rank.

RESULTS AND DISCUSSION

The goal of breeders is to create cultivars that perform better in a wider range of conditions. Although GE interactions are the main obstacle to obtaining precise phenotypic success in selection, several methods have been developed to address the complexity (Sabaghnia 2012). The stability of the genotypes being studied is estimated using both univariate and multivariate statistics.

Combined analysis of variance

The main and interaction effects among the variations were computed through a combined analysis of variation (Table 1). The performance of the genotypes across three locations is utilized to assess the variation. The partition of variation into various parameters showed significant variation among the genotypes, environment, and genotype-environment interaction.

Additive main effect and multiplicative interaction (AMMI) model

AMMI model of analysis decomposes the genotype, environment, and genotype-environment variation into biplots. The IPCA segments PC1 and PC2 covered 81.8% and 18.2% of variance through the biplots. AMMI biplots combined the principal component axis and genotype × environment interaction effects. Biplot 1 gives a combined view of 1st principal component (PC1) and the traits effect respectively. Figure 1 dissected variation among 27 genotypes across three locations. The genotypes placed on the right side of the ordinate axis denoted a high-yielding potential. ICP 16235, ICP 6992, ICP 9236, ICP 11737, ICP 8817, ICP 11890, ICP 16309, ICP 14444, ICP 11543, and ICPL 92016 were the superior genotypes of the panel under study. Among the environment vectors, Environment 2 is found to be the closest to the origin compared to the rest which depicts a better performance of all the genotypes within this location (Warangal). This environment was displayed to be suitable for all the genotypes under study yet a shorter vector length noted the lower dissection ability of variance. Kanpur (Location 3) of all has the longest vector from the origin showing its potential to be the most discriminating environment for the studied genotypes. The environment vectors and genotypes were noted to be placed in different quadrants denoting the diversity among them.

The AMMI biplot 2 dissects the genotype × environment interaction further along the PC1 and PC2 axes (Figure 2). ICP 14832, ICP 11890, ICP 8817, ICP 11627, ICP 15068, ICP 13195, ICP

Table 1. Combined analysis of variance among early maturing pigeonpea genotypes

SOURCE	DF	SUM SQ	MEAN SQ	F VALUE	PR(>F)
ENV	2	2,949,724.029	1,474,862.014	706.422	<0.001***
REP(ENV)	3	2,398.993	799.664	0.383	0.77
GEN	26	7,463,525.202	287,058.662	137.494	<0.001***
GEN: ENV	52	10,583,818.701	203,534.975	97.488	<0.001***
Residuals	78	162,847.733	2,087.791		
CV (%)	13				
MSR+/MSR-	14				
OVmean	342				

[1] Note: * represents p-value < 0.05, ** represents p-value < 0.01, *** represents p-value < 0.001

ANOVA partitioned the variance into genotype, environment, and genotype × environment (GE) variance and confirmed the need of multi-location trailing, yet failed to dissect the GE interaction (Khan *et al.* 2021).

10922, and ICP 14903 were found to be closest to the origin indicating they are less influenced by the environments. The genotypes ICP 11543, ICP 16309, and ICP 6992 were found to be closest to the environment vectors 1,2 and 3 respectively. They show specific adaptation to Patancheru, Warangal, and Kanpur respectively. ICP 6992 was found to be placed on the positive side of the origin showing the high yielding ability and stability owing to its lowest distance from the axis. ICP 9236, ICP 8817, ICP 11890, ICPL 92016, ICP 11737, ICP 8817, ICP 14944, ICP 14936, and ICP 16235 were also found on the positive side of the biplot. Thus, they are high-yielders with lower stability across locations.

ICP 14832, ICP 11890, ICP 8817, ICP 11627, ICP 15068, ICP 13195, ICP 10922, and ICP 14903 were found to be closest to the origin indicating they are stable and provide identical feedback to all the tested environments (biplot 2). A highly adaptable and stable line may display a lower-yielding ability (Oliveira and Godoy, 2006). ICP 14444, ICP 11890, and ICP 8817 were observed to be placed on the right side of the IPCA 1 – PY biplot origin denoting to be stable lines with a higher yielding potential. The genotypes ICP 11543, ICP 16309, and ICP 6992 showed narrow adaptation to Patancheru, Warangal, and Kanpur respectively. These genotypes could be further tested for utility in these particular climatic zones. Warangal (location 2) was found to be the apt environment for superior performance of the genotypes, while Kanpur (location 3) is noted to be the most discriminating. The AMMI technique was utilized in multiple studies in pigeonpea genotypes to identify stable high-yielding genotypes (Muniswamy *et al*. 2018, Singh *et al*. 2018, Gaur *et al*. 2020, Kumar *et al*. 2021, Rao *et al*. 2022, Kumar *et al*. 2023).

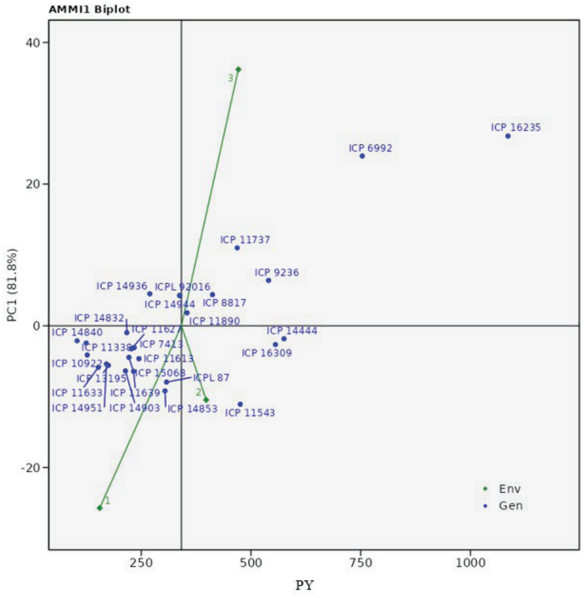

Fig. 1. AMMI biplot 1 depicting the trait main effect and first interaction principal component (PCA1)

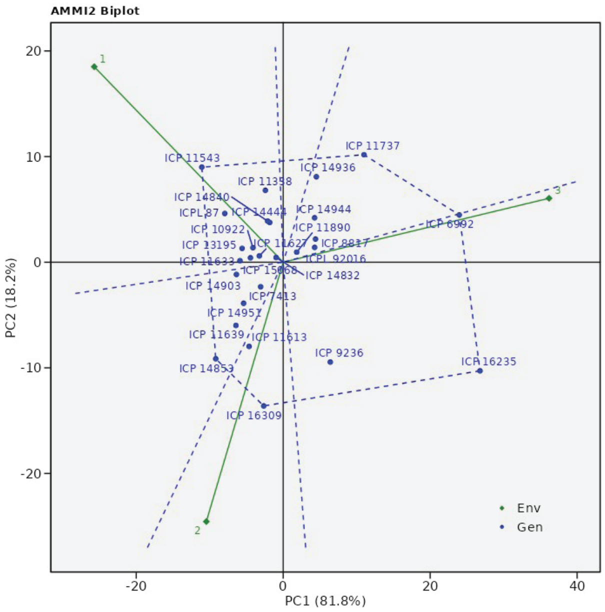

Fig. 2. AMMI biplot 2 depicting the genotype-environment interaction based on principal component axes 1 (PCA1) and 2 (PCA2).

Mean vs Weighted average of the absolute score (WAASB)

Availing genetic gain directly correlates to the precise phenotyping technique. AMMI model characterizes genotypes to be fixed effects and fails to perform as a linear mixed effect model (LMM). BLUPs are recorded to outpace AMMI by using their shrinkage property in assessing the genotypic means across multi-locations (Piepho 1994, Pande *et al*. 2013, Olivoto *et al*. 2019). The Weighted average of the absolute scores (WAASB) converges the BLUPs and AMMI by including the singular value decomposition of the BLUP-generated genotypic means with the GE interactions obtained from the linear mixed effect model (LMM), thus improving the selection efficiency. A 2D representation of the traits mean and the WAASB allows for the selection of genotypes with the highest mean derived from the predictions of BLUPs and the lowest WAASB score.

The results depict the genotypes which are high yielders with stable performance across the

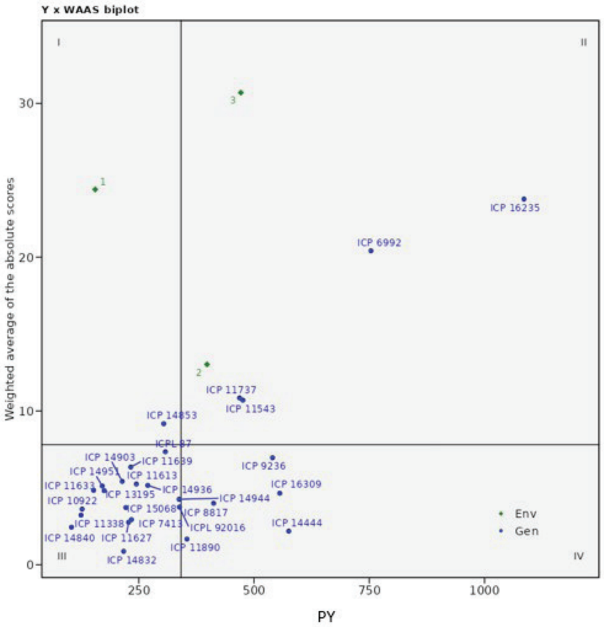

Fig. 3. WAASB biplot for grain yield scores.

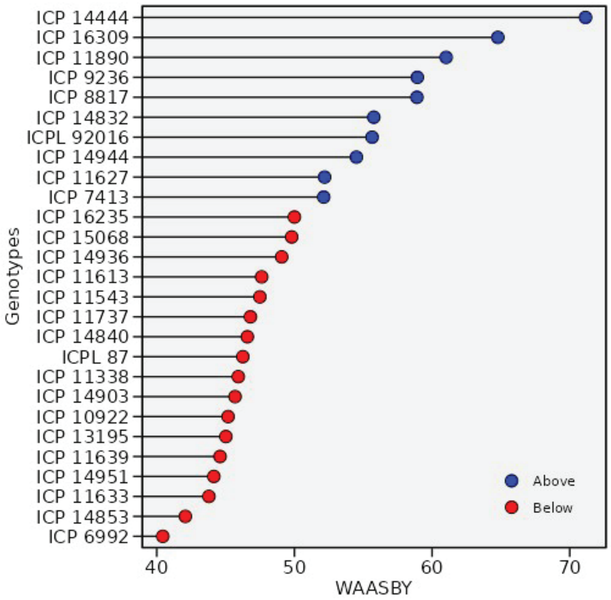

Fig. 4. Genotype performance based on simultaneous selection for grain yield and WAASB score

Table 2. WAASB index for the early maturing genotypes of pigeonpea.

		WAAS Index			
GENOTYPE	**PLOT YIELD**	**WAASB**	**OrWAASB**	**WAASBY**	**OrWAASBY**
ICP 10922	127.13	3.62	8	45.18	21
ICP 11338	124.73	3.23	7	45.93	19
ICP 11543	475.75	10.7	24	47.49	15
ICP 11613	244.8	5.25	18	47.62	14
ICP 11627	228	2.76	5	52.19	9
ICP 11633	152.05	4.84	15	43.79	25
ICP 11639	232.68	6.35	20	44.6	23
ICP 11737	468.61	10.85	25	46.81	16
ICP 11890	354.55	1.67	2	61.02	3
ICP 13195	175.15	4.81	14	45.02	22
ICP 14444	575.27	2.19	3	71.15	1
ICP 14832	217.25	0.88	1	55.77	6
ICP 14840	104.07	2.44	4	46.58	17
ICP 14853	304.15	9.18	23	42.08	26
ICP 14903	214.12	5.42	19	45.69	20
ICP 14936	269.58	5.16	17	49.08	13
ICP 14944	337.4	4.26	12	54.5	8
ICP 14951	170.87	5.12	16	44.15	24
ICP 15068	222.08	3.72	9	49.8	12
ICP 16235	1,085.48	23.78	27	50	11
ICP 16309	555.78	4.65	13	64.78	2
ICP 6992	753.56	20.41	26	40.44	27
ICP 7413	234.17	2.94	6	52.13	10
ICP 8817	412.32	3.99	11	58.9	5
ICP 9236	540.1	6.96	21	58.93	4
ICPL 87	307.75	7.34	22	46.26	18
ICPL 92016	338.15	3.75	10	55.65	7

*WAASB: Weighted average of the absolute score; OrWAASB: Order of genotypes according to WAASB score; WAASBY: Simultaneous selection score based on Plot yield (kg/ha) and WAASB; OrWAASBY: Order of genotypes according to WAASBY score three locations. The biplot mapped based on plot yield and WAASB scores confirmed the Warangal (Location 2) to be the least discriminating based on the smallest vector distance from the origin. ICP 6992, ICP 16235, ICP 9236, ICP 16309, ICP 11737, ICP 11543, ICP 14444, ICP 8817, and ICP 11890 were found to be on the positive side of the vertex along with the check ICPL 92016 (Figure 4). The genotypes ICP 14832 (0.88), ICP 11890 (1.67), ICP 14444 (2.19), ICP 14840 (2.44), ICP 11627 (2.76), ICP 11338 (3.23), ICP 10922 (3.62), and ICP 15068 (3.72) were found to be stable over the check ICPL 92016 (3.75) with the lower WAASB score (Figure 3).

The weighted average of the absolute scores (WAASB) for the plot yield of early maturing pigeonpea lines provides a scoring matrix (WAASBSY) for the simultaneous selection of genotypes for yield and stability. The lowest rank concludes the best-performing genotype with higher stability and yielding potential. In this case, ICP 14444 (71.15) ranked the best followed by ICP 16309 (64.78), ICP 11890 (61.02), ICP 9236 (58.93), ICP 8817 (58.9), and ICP 14382 (55.77) performed better than the check ICPL 92016 (55.65) (Table 2, Figure 4). This technique has been used in multiple crops for assessing the stability of various traits including grain yield, early maturity (Sharifi *et al*. 2020, Nataraj *et al*. 2021, Hassani *et al*. 2023, Lee *et al*. 2023), and stress resistance (Vineeth *et al*. 2022, Danakumara *et al*. 2023). Thus, the biplot analysis of AMMI and WAASBY models combinedly identified ICP 14444, ICP 8817, and ICP 11890 to be the winning cultivars with superior yielders and stability.

CONCLUSION

Pigeonpea is an important crop of the arid and semi-arid tropics serving the protein requirements for millions of people. While there is inflation in demand and consumption, the rise in productivity and production is yet to be par with the need. Hence, early maturing pigeonpea cultivars form a potential solution for expanding the pigeonpea out-turn. In this aspect, 26 pigeonpea lines were assessed for their yielding potential across multi-locations. The selection for high-yielding early maturing and stable lines was performed through AMMI and WAASB analyses. The indices selected identified ICP 14444, ICP 8817, and ICP 11890 to be potential early maturing, high-yielding stable lines across the tested locations. Warangal and Kanpur were noted to be the best environments with the least and highest discriminating ability respectively. ICP 11543, ICP 16309, and ICP 6992 were identified to be the best-fit cultivars for Patancheru, Warangal, and Kanpur respectively. These lines could be further tested in diverse locations to assess their yielding potential, and general and specific adaptability before adding to the breeding pipeline.

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