



## **Evaluation of Phenotypic Stability for Grain Yield in Rice (*Oryza sativa* L.) Genotypes Using AMMI and GGE Biplot Models**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author BSC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KRD, NL and KRP managed the analyses of the study. Author YH managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

The experiments were carried out under four seasons with 9 rice genotypes at Regional Agricultural Research Station, Warangal, Telangana State, India during *kharif* season (July to November) 2019 (E1), *rabi* season (December to April) 2019-20 (E2), *kharif* season (July to November) 2020 (E3) and *rabi* season (December to April) 2020-21 (E4). The objective of the study was to assess the stability and adaptability of 9 rice genotypes over four seasons. Analysis of variance clearly showed that environments contributed the highest (69.66%) in the total sum of squares followed by genotypes $\times$ environments (12.66%) indicating a very greater role played by environments and their interactions in realizing final grain yield. AMMI 1 analysis revealed that rice genotypes *viz.*, G4

(WGL 1367), G3 (WGL 1362), and G6 (WGL 1370) recorded higher mean grain yield with positive IPCA1 scores. AMMI 2 revealed that the genotypes, G6 (WGL 1370) and G3 (WGL 1362) were plotted near to zero IPCA1 axis indicating that these genotypes are relatively more stable across locations. GGE bi-plot genotype view depicts that the genotypes G4 (WGL 1367) and G3 (WGL 1362) were fell in the second concentric circle and found to be more stable across environments. GGE bi-plot environment view showed that *rabi* season of 2020-21 (E4) was the most ideal environment. However, *rabi* season of 2019-20 (E2) and *kharif* season 2020 (E3) were poor and most discriminating. What-won-where biplot indicated that four environments fell into two mega environments. Hence the genotype G4 (WGL 1367) was the winning genotype in the mega environment 1 viz., *rabi* season of 2019-20 (E2), *rabi* season of 2020-21 (E4) and *kharif* season of 2019 (E1). Whereas the genotype G9 (KNM 118) was the winner in the mega environment 2 i.e., *Kharif*, season of 2020 (E3).

**Keywords:** *G x E interaction; AMMI; GGE-biplot; polygon; yield; rice; stability.*

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the globally recognized staple food crops that feed the hunger and calorie need of millions [1]. It is cultivated worldwide in an area of 164.19 million ha, with 756.74 million tons of paddy production and 4.61 t ha<sup>-1</sup> productivity [2]. In India, rice is grown in an area of 45.77 million ha with a production of 124.37 million tons [3]. India is the second largest rice-growing country in the world; however, its productivity per unit area is low i.e., 2717 kg ha<sup>-1</sup>. Whereas in Telangana state, rice is cultivated in an area of 3.18 million ha with a production of 10.22 million tons and an average productivity of 3206 kg ha<sup>-1</sup> [4]. "Although more than 900 rice varieties have been released in India, many of them were no longer cultivated within a few years due to inconsistent performance in diverse environments and only a few varieties with stable performance continue under cultivation after 15 to 20 years of their release" [5]. "Nevertheless, there is still a large gap between production and demand. To meet this challenge, there is a need to develop rice varieties with higher stability. Grain yield is the most important trait in any crop. Grain yield, being a quantitative trait is highly influenced by the environment. So a breeder should identify a variety that is less influenced by environments i.e., a stable one" [6]. "Plant breeders conduct multi environment trials (MET) primarily to identify the superior cultivars for a target region and secondarily to determine if the target region can be subdivided into different mega environments" [7-8]. "Identification of superior genotypes through GEI became complicated for a range of environments to determine their true genetic potential" [9]. "Breeders must therefore use tools to efficiently and accurately measure the response of the lines in multiple test

environments" [10]. "There are several biometric models proposed to analyze the GEI and explore adaptability and stability. Various statistical models such as additive main effects and multiplicative interaction (AMMI)" [11] and "genotype main effects in addition to genotype by environment interaction (GGE) biplots have been used" [7]. "AMMI and GGE biplots are the most effective and commonly used multivariate models for the analyses of stability, adaptability and ranking of genotypes and for selecting suitable mega environments" [11-13]. "Both models integrate principal component analysis (PCA) and biplot for the explanation of genotype by environment interaction (GEI). The AMMI model combines ANOVA for the genotype and environment main effects with principal components analysis of GEI" [14,15]. Keeping in view of the above, the present investigation was undertaken to assess the extent of G x E interaction and to select the stable rice genotypes for grain yield over and combined across the growing seasons by using AMMI and GGE biplot models.

## 2. MATERIALS AND METHODS

The experiments were carried out under four seasons with 9 genotypes during *kharif* season (July to November) of 2019 (E1), *rabi* season (December to April) of 2019-20 (E2), *kharif* season (July to November) of 2020 (E3) and *rabi* season (December to April) of 2020-21 (E4) at Regional Agricultural Research Station, Warangal, Telangana. The details of the experimental material and environments are presented in Table 1. The farm is geographically situated at 18°01' N Latitude, 79°60' E Longitude and an elevation of 270 m AMSL. The experiments were carried out using a randomized complete block design (RCBD) with

three replications in four environments. Twentyfive days old seedlings were transplanted at the main field under irrigated ecosystem at all four environments. The experimental plots size of 10 m<sup>2</sup> with row-to-row and plant-to-plant distances was kept at 20 cm and 15 cm, respectively in all the environments. Recommended package of practices was followed to raise the crop. Grain yield was recorded in each plot and expressed as kg ha<sup>-1</sup>. Trial in each season was conducted as one environment for the multi–environment analysis. Data obtained from each season was analyzed separately by running a single analysis of variance and thereafter data from all four seasons was pooled for analysis of variance to perform the combined analysis of advanced lines across the seasons to test the presence of significant genotype, environment and genotype–environment variation.

## 2.1 Statistical Analysis

“The grain yield data were subjected to combined ANOVA and AMMI analysis to understand the pattern of genotype performance across the four environments. ANOVA was used to partition genotype deviations, environment deviations, and G×E deviations from the grand mean. Subsequently, multiplication effect analysis (AMMI) was used to partition GE deviations into different interaction principal component axes (IPCA). The GGE biplot graphically represents G and GEI effect present in the multi-location trial data using environment centred data. This methodology uses a biplot to show the factors (G and GE) that are important in genotype evaluation and that are also sources of variation in GEI analysis of multilocation trial data” [7,12,16]. GGE biplots were used to identify (i) the mega-environment using which-won-where pattern, to recommend the genotypes for specific mega-environments (ii) the stable genotypes that can be recommended across the environments (iii) the target environments for different genotypes under study. ANOVA and stability analysis for yield trait was carried out by using the AMMI and GGE bi-plot models R–packages 1.5, PB Tools 1.4 version IRRI.

The AMMI model used for the stability analysis is as follows:

$$Y_{ij} = \mu + g_i + e_j + \sum \lambda_k a_{ik} \gamma_{jk} + \varepsilon_{ij}$$

where  $Y_{ij}$  = mean of a trait of  $i^{\text{th}}$  genotype in  $j^{\text{th}}$  environment;

$\mu$  = the grand mean;  
 $g_i$  = genotypic effect;  
 $e_j$  = environmental effect;  
 $\lambda_k$  = eigenvalue of Interaction Principal Components Axes (IPCA)  $k$ ;  
 $a_{ik}$  = eigenvector of genotype  $i$  for PC  $k$ ;  
 $\gamma_{jk}$  = eigenvector for environment  $j$  for PC  $k$ ;  
 $\varepsilon_{ij}$  = error associated with genotype  $i$  in environment  $j$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Analysis of Variance

Analysis of variance clearly showed that grain yield was significantly different among genotypes, environments, and genotypes×environment interactions depicting the presence of significant variability among genotypes, considerable influence of environments and interaction of genotypes with environments in the expression of the trait (Table 2). Further, environments contributed the highest (69.66%) in the total sum of squares followed by genotypes × environments (12.66%) and genotypes (11.49%) indicating that the environments were diverse, with large differences among environments causing most of the variation for grain yield. Mohan et al. [17] reported a highly significant difference in grain yield in rice hybrids by genotype (7.50%), environment (65.47%), and their interaction (21.19%). Further Akter et al. [18], Islam et al. [19] and Chavan et al. [20] also reported similar results in rice production.

The grain yield over environments ranged from 4697 kg ha<sup>-1</sup> in *kharif*, 2020 (E3) to 8059 kg ha<sup>-1</sup> in *rabi*, 2020-21 (E4). The Genotypic grain yield ranged from 6125 kg ha<sup>-1</sup> G7 (RNR 15048) to 7690 kg ha<sup>-1</sup> G4 (WGL 1367) (Table 3). GE interaction was a crossover type with different yield rankings of genotypes across environments. The significant interaction in genotype and environment for yield validated the need to take more care while selecting promising genotypes by considering stability and adaptability. Significant differences across years were also observed by Dwivedi et al. [6] in basmati rice genotypes using AMMI model.

### 3.2 AMMI Analysis

“The significant G×E interactions were further partitioned by PCA [21] into three interaction principal component axes (IPCA) explaining 68.3, 22.1 and 9.6% of GEI sum of squares,

respectively (Table 2)". "The first two IPCA axes, IPCA1 and IPCA2 together contributed 90.4% of the total interaction variance. Earlier reports confirmed that in most of cases the maximum genotype and environment interaction" could be explained by using the first two PCAs by Akter et al. [22], Chavan et al. [20], Dwivedi et al. [6], Kesh et al. [23], Mohan et al. [17]. Rao et al. [24] reported similar reports in Pigeon pea genotypes from AMMI analysis. Therefore, IPCA1 and IPCA2 were used for the construction of AMMI 1 and AMMI 2 biplots.

"The mean grain yield and IPCA1 (interaction effects) were plotted on the x and y axis, respectively for the construction of the AMMI1 biplot (Fig. 1). The four quadrants (Q) of the bi-plot corresponded to a higher mean (Q I, II), lower mean (QIII, IV), positive IPCA1 score (QI, IV) and negative IPCA1 score (QII, III) and a genotype falling in the same quadrant denote positive interaction and vice-versa. A genotype with IPCA1 score near to zero is considered to be more stable across environments. The IPCA score of a genotype in the AMMI analysis is an indication of the adaptability over environments and the association between genotypes and environments" [15, 25]. Conversely, "a genotype with high IPCA1 score is highly variable among environments" [6,24].

### 3.2.1 AMMI 1 biplot

Accordingly, the rice genotype, G6 (WGL 1370), G3 (WGL 1362) and G4 (WGL 1367) were recorded with higher grain yield with positive IPCA1 scores (Fig. 1). The genotype G9 (KNM 118) recorded high mean grain yield with negative IPCA score and close to the origin implying a poor yield with wider adaptability. However, G6 (WGL 1370) and G8 (MTU 1010) were plotted near to zero IPCA1 axis indicating that these hybrids are relatively more stable across locations. The genotype, G4 (WGL 1367), was found superior among all the genotypes as well as over the checks and across all the environments under study. These findings were in agreement with Rukmini Devi et al. [26], Kesh et al. [23], Mohan et al. [17], Siddi et al. [27]. The remaining genotypes had less than the mean grain yield and found specific adaptation to few tested environments. Likewise, Jain et al. [28] reported that "genotypes with PC1 scores close to zero are usually widely adapted and they were considered more stable in their performance across test environments".

### 3.2.2 AMMI 2 biplot

"In AMMI 2 biplot (Fig. 2) depicts the magnitude of genotype-environment interaction. The genotypes and environments that are the furthest away from the origin are the least stable. When genotypes and environments are in the same sector, they interact positively; when they are in opposite sectors, they interact negatively" [29]. Furthermore, "when IPCA1 was plotted against IPCA2, Purchase [30] observed that the genotypes that scored closest to the center of the biplot (Fig. 2) were the most stable". In this study, most stable genotypes G6 (WGL 1370) and G3 (WGL 1362) were having higher yields and their positions were closest to the origin and PC1 axes of AMMI biplot 1, and hence they are less interactive to environmental differences on grain yield. Out of these genotypes, G3 (WGL 1362) was positioned to closest to the centre of origin in comparison with the all-other genotypes, this genotype showed the least variation and was considered as the most stable genotype. In contrast, genotypes located far from the center, have specific adaption [31-33], in rice introduced stable genotypes with specific adaptability to different environments using AMMI model and biplot results. Some corner genotypes G5 (WGL 1369), G4 (WGL 1367), G9 (KNM 118) and G1 (WGL 1191) are the most responsive ones that can be visually determined. These were either the best or the poorest genotypes at some or all the test seasons and would be utilized to determine the mega-environments. Since genotypes G1 (WGL 1191) and G9 (KNM 118) located far from the axis center, they had no suitable general adaptability. However, they were found to be suitable specific adaptability with environments E1 (*kharif*, 2019) and E3 (*Kharif*, 2020) respectively having below average mean yield. Accordingly, proximity of genotype G5 (WGL 1369) to the environment E4 (*rabi*, 2020-21) indicates their specific adaptability to the above said environment with above average mean yield. Based on the mean yield vs stability, G6 and G3 were found to be stable genotypes over the seasons.

### 3.3 GGE Bi-plot Model Analysis

"GGE bi-plots provide an effective evaluation of genotypes and allow for a comprehensive understanding of the target and test environments through various IPCAs. The genotype x environment interactions were partitioned into four significant interaction PCAs and 85.7% variance was explained by the first two IPCAs together (Table 4)". These findings

agree with the results reported by Zewdu et al. [34], Mohan et al. [17] and Siddi et al. [27].

“Among the environments, *rabi* season 2020-21 (E4) was found to be the most suitable environment for the potential expression of grain yield and the most ideal environment (Fig. 3) for testing general adoption as it made a small angle with the Average Environment Axis (AEA) and large PC1 score and small PC2 score, and representative of all the four environments. This season will help in selecting cultivars that are widely adopted and bear general adoption. It was observed that grain yield was significantly higher in the dry season (*rabi*) than wet season (*khariif*) under irrigated rice production in tropical conditions and the variation was observed for the ideotype suitability for different seasons” [35]. On the other hand, *rabi* season 2019-20 (E2) and *khariif* season (E3) had the longest vectors and indicating that they were the poor and most discriminating and desirable testing season for examining special adoption for grain yield. Correspondingly, Zewdu et al. [34] reported that “E6, E1, E3, and E2 environments were ideal with short vectors, while E4 and E5 had long spokes and indicated a high discriminating ability of these environments”. Similarly, Mohan et al. [17] reported that “the most powerful interpretive tool for AMMI models is Bi-plot analysis and identified that environment E4 had short vectors and they did not exert strong

interactive forces while E6 and E1 with long vectors were more differentiating environments”.

### 3.4 Mean Performance and Stability of Genotypes

“The magnitude of interaction can be visualized for each genotype and each environment using IPCA vs. mean yield and IPCA1 vs. IPCA2 biplot model” [36]. “An ideal genotype is one with large PC1 scores representing the high yielding ability and small PC2 scores representing high stability” [7]. “The concentric circles help to rank the genotypes based on their distances to the ideal genotype, and the genotypes evaluated in multi-environmental trials, shifts in the relative ranking of genotype by environment interactions often occur” [37,38,17,27]. Thus, Fig. 4 revealed that genotype G4 (WGL 1367) was identified as ideal genotype followed by G3 (WGL 1362) with higher mean yield and good stability whereas, G1 (WGL 1191) and G7 (RNR 15048) were found to be most unstable. Similarly, among environments, *rabi* season of 2020-21 (E4) was identified as the best location for realizing higher grain yields. Further, the genotype G6 (WGL 1370) was identified as highly stable with the least dispersion from AEA axis and also recorded reasonably good mean grain yield. These results are in close correspondence with the results reported by Mohan et al. [17] and Siddi et al. [27].

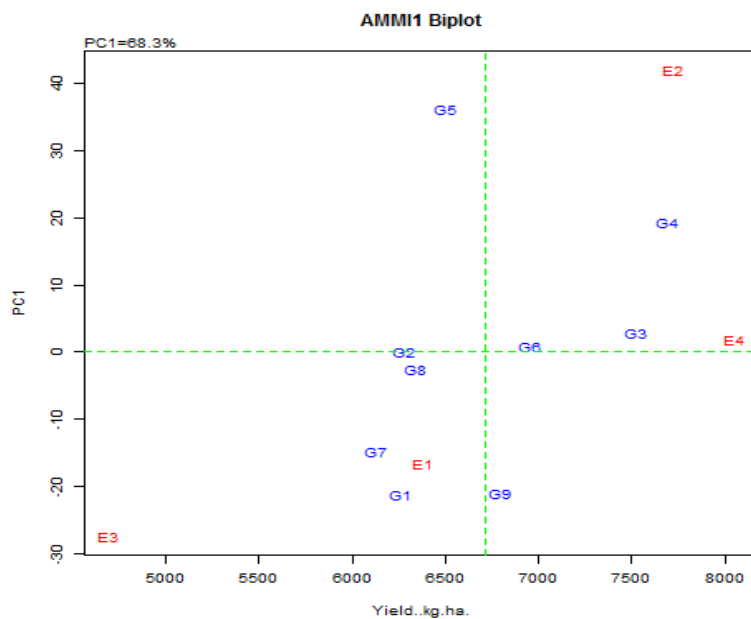


Fig. 1. AMMI biplot 1 for grain yield showing the means of genotypes (G) and environments (E) against their respective IPCA1 scores in rice

**Table 1. Genotype code and designation of rice genotypes for four seasons**

S. No.	Genotype code	Designation	Pedigree	Source	Environment code	Environment
1	G1	WGL-1296	KMP 150 / JGL 17025	RARS, Warangal	E1	<i>Kharif</i> , 2019
2	G2	WGL-1191	RP 4092-115-08-5-3 / KMP 150	RARS, Warangal	E2	<i>Rabi</i> , 2019-20
3	G3	WGL-1362	BPT 5204 / JGL 17653	RARS, Warangal	E3	<i>Kharif</i> , 2020
4	G4	WGL-1367	WGL 32100 / NR 6226	RARS, Warangal	E4	<i>Rabi</i> , 2020-21
5	G5	WGL-1369	NLR 34449 / NR 6226	RARS, Warangal		
6	G6	WGL-1370	NLR 34449 / NR 6226	RARS, Warangal		
7	G7	MTU-1010	Variety check	RARS, Maruteru		
8	G8	RNR15048	Variety check	RRC, Rajendranagar		
9	G9	KNM-118	Variety check	ARS, Kunaram		

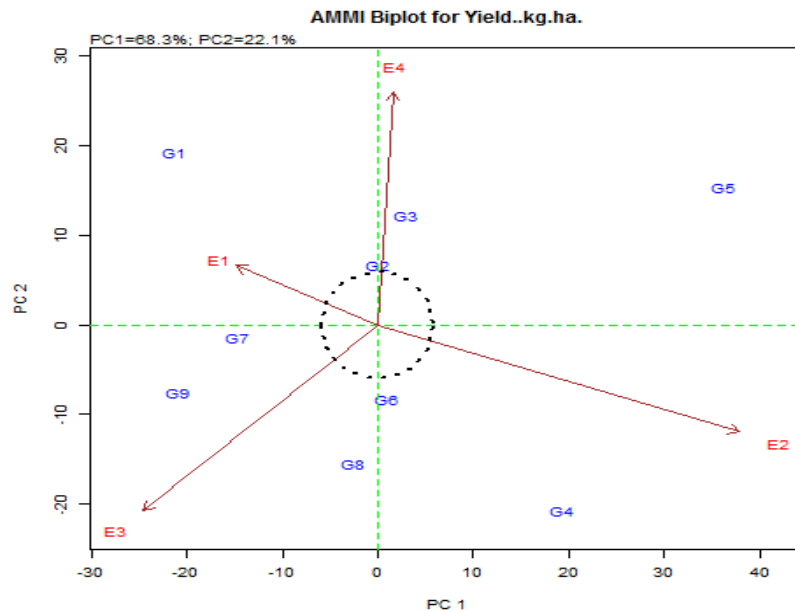
**Table 2. AMMI analysis of variance for yield (kg ha<sup>-1</sup>) of nine rice genotypes combined across four environments**

Source of variation	DF	SS	MS	% Explained SS
Genotypes (G)	8	31273617	3909202**	11.49
Environments (E)	3	189455781	63151927**	69.66
Genotypes xEnvironments (G xE)	24	34432631	1434692**	12.66
IPCA1	10	23530449	2353044	68.3
IPCA2	8	7598065	949758	22.1
IPCA3	6	3305445	550907	9.6
<b>Error</b>	64	13735691	214620	
<b>Total</b>	99	271960848	2747079	

\*\**P*<0.01, *DF*: Degrees of freedom; *MS*: Mean sum of square; *SS*: Sum of square

**Table 3. Mean yield (kg ha<sup>-1</sup>) of the rice genotypes combined across four environments**

Genotype	Genotype Code	E1	E2	E3	E4	Genotype by mean	PC1	PC2	PC3
WGL 1191	G1	6524	6135	4331	8058	6262	-21.21	19.21	-3.90
WGL 1296	G2	6634	7264	3783	7420	6275	0.06	6.60	-25.14
WGL 1362	G3	6896	8470	5316	9432	7529	2.95	12.17	13.49
WGL 1367	G4	6968	9795	5575	8420	7690	19.27	-	-3.05
								20.65	
WGL 1369	G5	5552	8822	3205	8437	6504	36.19	15.42	5.41
WGL 1370	G6	6699	8129	5017	7959	6951	0.96	-8.33	-6.05
RNR 15048	G7	5767	6513	4667	7551	6125	-14.68	-1.48	10.21
MTU 1010	G8	5820	7435	4802	7300	6339	-2.62	-	4.72
								15.45	
KNM 118	G9	6651	7019	5576	7950	6799	-20.91	-7.50	4.31
<b>Environment mean</b>		6390	7731	4697	8059	GM=6719			



**Fig. 2. AMMI 2 Biplot for grain yield (kg ha<sup>-1</sup>) showing the interaction of IPCA2 against IPCA1 scores of 9 rice genotypes (G) in four environments (E)**

**Table 4. GGE analysis of variance for yield (kg ha<sup>-1</sup>) of nine rice genotypes across four environments**

Source of variation	DF	SS	MS	% Explained SS
Genotypes (G)	8	31273617	3909202**	11.49
Environments (E)	3	189455781	63151927**	69.66
Genotypes x Environments (G xE)	24	34432631	1434692**	12.66
IPCA1	10	38695608	3869560.8	58.9
IPCA2	8	17578533	2197316.6	26.8
IPCA3	6	6372607	1062101.2	9.7
IPCA4	4	3058088	764522.1	4.7
<b>Error</b>	64	13735691	214620	
<b>Total</b>	99	271960848	2747079	

\*\*P<0.01, DF: Degrees of freedom; MS: Mean sum of square; SS: Sum of square;

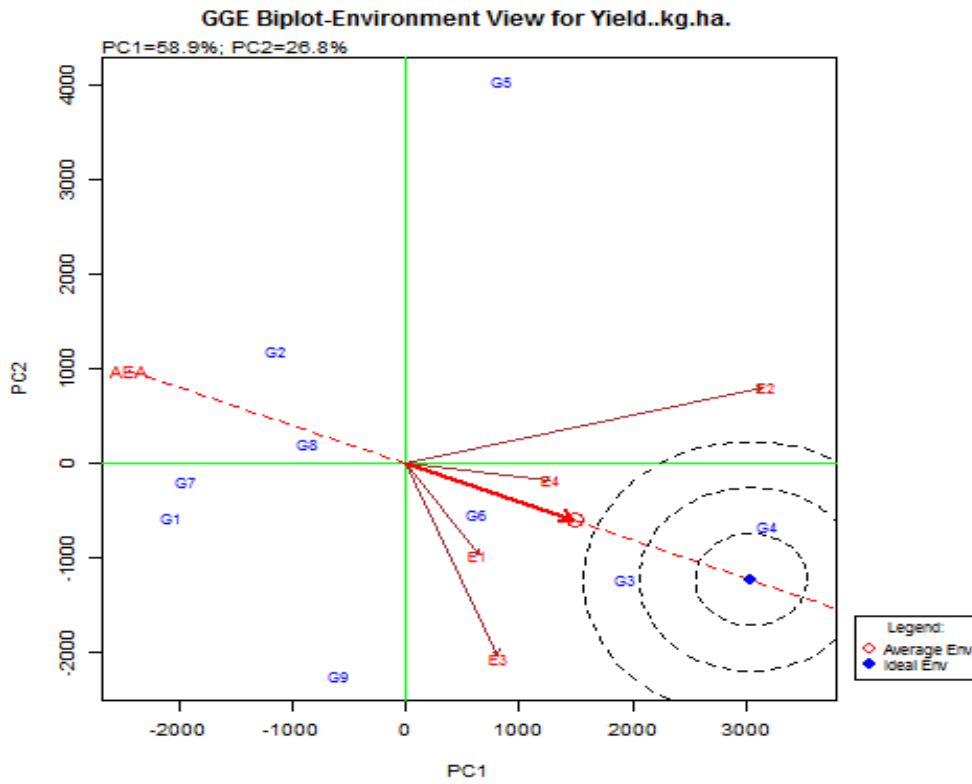


Fig. 3. GGE Biplot–environment view for yield (kg ha<sup>-1</sup>)

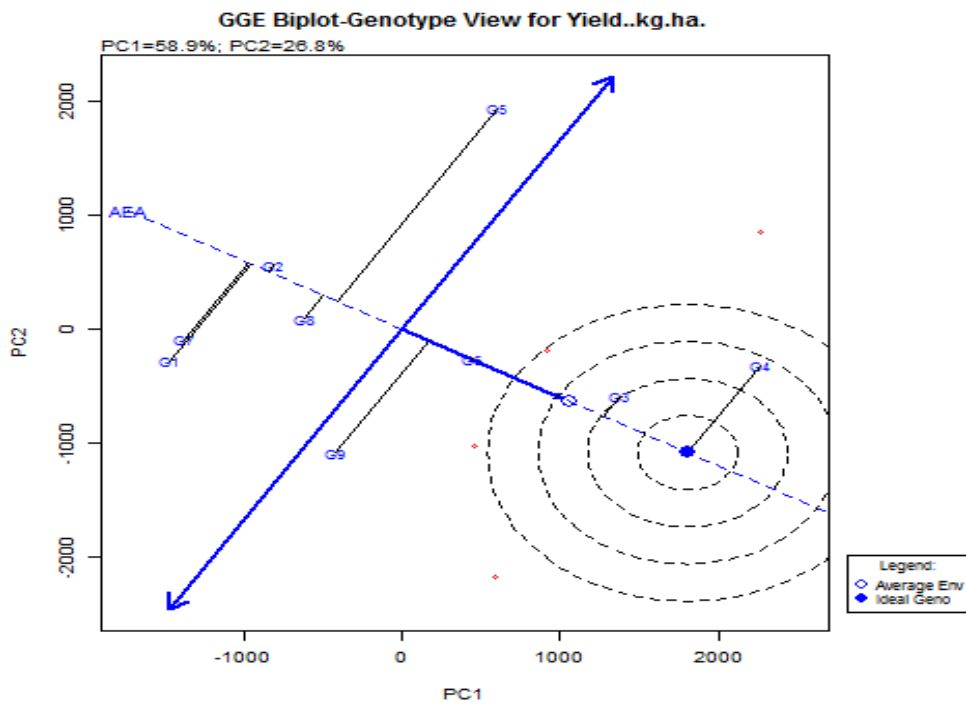


Fig. 4. GGE Biplot of stability and mean performance of genotypes across average environments



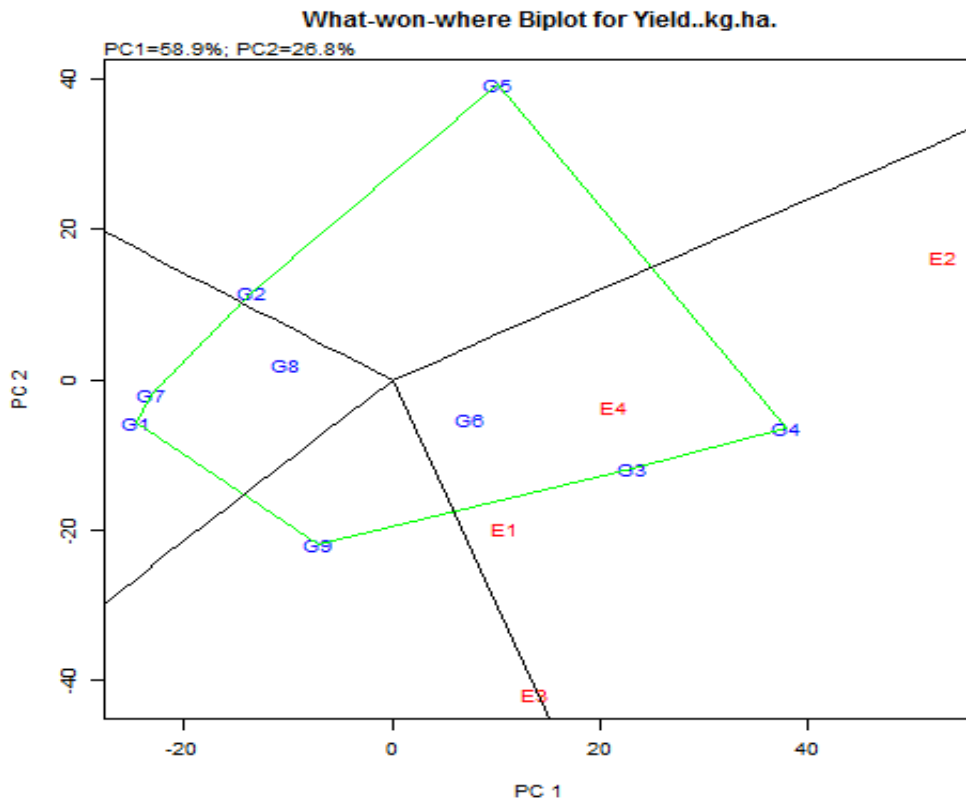


Fig. 5. What-won-where GGE biplot for yield

### 3.5 What-Won-Where Bi-Plot

"The what-won-where view of the GGE bi-plot [7] is the best model for multi-environment trial data for grouping the environments and also identifying best performing genotype in each". "Many researchers found this biplot intriguing, as it graphically addresses important concepts such as crossover GE, mega environment differentiation, and specific adaptation. A polygon is first drawn on genotypes that are furthest from the biplot origin so that all other genotypes are contained within the polygon. The perpendicular lines to each side of the polygon are drawn, starting from the biplot origin" [13]. It divided the biplot into four sections and four environments fall into two mega environments viz., E2 and E3 for grain yield (Fig. 5). Genotypes located on the vertices of the polygon performs either the best or the poorest in one or more environments. Vertex genotype G4 (WGL 1367) was the winning genotype in mega environment 1 consisting of *rabi* season of 2019-20 (E2), *rabi* season of 2020-21 (E4) and *kharif* season of 2019 (E1) While the genotype G9 (KNM 118) was the winner in mega environment 2 *i.e.* *Kharif*,

season of 2020 (E3). Similarly, genotype G6 (WGL 1370) was better in *rabi* season of 2020-21 (E4) environment. It concludes that "different cultivars should be selected and deployed for each different environment". Similar results were reported by the rice workers viz., Akter et al. [22], Rukmini Devi et al. [26], Lingaiah et al. [39], Mohan et al. [17] and Siddi et al. [27]. Whereas other vertex genotypes G5 (WGL 1369), G7 (RNR 15048) and G1 (WGL 1191) fall in separate groups with poor performance in all the environments.

### 4. CONCLUSION

Based on AMMI and GGE bi plot models it was concluded that rice genotypes G3 (WGL 1362), G6 (WGL 1370) and G4 (WGL 1367) were more stable across locations with higher grain yields. GGE bi-plot environment view confirmed that *rabi* season of 2020-21 (E4) as the most ideal environment to obtain higher grain yields. Vertex genotype G4 (WGL 1367) was the winning genotype in mega environment 1. While the genotype G9 (KNM 118) was the winner in mega environment 2 *i.e.*, *Kharif*, season of 2020 (E3).

These two methods can be effectively utilized for the identification of the suitable genotypes for suitable environments.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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