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## AMMI and GGE biplot analysis for grain zinc and iron content and yield stability among selected rice genotypes (*Oryza sativa* L.)

**Vinay Premi, Devidas Thombare, Hemant Sahu, and Ajit Kumar Mannade**

### Abstract

The study aimed to investigate the genotype-environment interaction (GEI) among 22 rice genotypes across three environments (Raipur, Bilaspur, and Bhatapara districts of Chhattisgarh) during the Kharif season of 2019, utilizing GGE bi-plot analysis. The analysis revealed that the major source of variation was the interaction between genotype and management, followed by the environment. The first two principal components (PCs) of the GGE bi-plot accounted for the majority of the observed variation. The AMMI analysis identified specific environments as the most discriminating for different traits. E1 and E2 were found to be the most discriminating environments for yield, while E3 showed high discrimination for zinc content, and E1 exhibited the greatest discrimination for iron content. Additionally, certain genotypes were identified as stable performers for specific traits. G2 and G10 were found to be stable lines for zinc content, while G18 emerged as the most stable line for iron content. The "which-won-where" analysis revealed the presence of three mega-environments (ME) among the test locations for yield. Each ME represented a single environment, with specific genotypes identified as winners. For zinc content, all environments fell within a single ME, and the winning genotype was G2. In the case of iron content, two MEs were identified: one comprising E2 and E3, with G4 and G2 as the winning genotype respectively, and another consisting of E1, with G18 as the winning genotype. These findings provide valuable insights into the performance and stability of genotypes across different environments for yield, zinc content, and iron content. Understanding the specific environments where genotypes perform well and identifying stable genotypes for targeted traits can guide breeding efforts to develop rice varieties with improved performance and desirable micronutrient characteristics.

**Keywords:** GEI, GGE bi-plot, AMMI, ME

### 1. Introduction

Multi-environment trials (MET) serve as crucial evaluations conducted by plant breeders to assess new and improved genotypes across various test environments, encompassing multiple locations. These trials aim to determine the extent of genotype-environment (GE) interaction, which involves assessing how different genotypes perform across diverse environments. The evaluation of GE interaction, as highlighted by researchers such as Annicchiarico (2002) [5], Karimizadeh *et al.* (2012), and Yan *et al.* (2007) [30], plays a significant role in MET. It involves analyzing the varying rankings of genotypes across different environments, offering valuable insights to guide the selection process and aid in recommending suitable genotypes for specific target environments. By considering GE interaction, breeders can better tailor their recommendations and enhance the production of genotypes that cater to the needs of growers. This approach is supported by scholars like Ebdon and Gauch (2002) [8] and Gauch (2006) [10]. Genotype-environment interaction has been a research focus among the breeders and geneticists which would help to get the information on the adaptability and stability performance and may complement the selection process and recommendation of a genotype for a target environment (Ebdon and Gauch, 2002; Gauch, 2006; Ahmadi *et al.*, 2012; Jeberson *et al.*, 2017) [8, 12, 10, 2, 12]. Breeders must therefore use tools to efficiently and accurately measure the response of the lines in multiple test environments (Yan *et al.*, 2007) [30]. There are several biometric models proposed to analyze the GEI and explore adaptability and stability. However, multiplicative models that look at the response of genotypes to specific environments or to different environments have more accurate criteria to analyze this phenomenon in different crops (Goncalves *et al.*, 2020) [11].

Various statistical models such as AMMI (Gauch, 2006) [10] and GGE bi-plot models (Yan *et al.*, 2007) [30] are widely used across the seasons to assess their stability and to quantify the effect of genotype x environment (GXE) interaction on the yield of genotypes (Balakrishnan *et al.*, 2016; Rasul *et al.*, 2017) [6, 22]. Rice is a staple food consumed by a significant portion of the global population, with over half of the world relying on it as a dietary staple (Rao *et al.*, 2016; Nili *et al.*, 2017; Sharifi *et al.*, 2017; Poli *et al.*, 2018; Suman *et al.*, 2021) [21, 17, 23, 19, 25]. Asian countries, in particular, contribute approximately 80% of the world's rice production. India, among the prominent rice-growing nations, cultivates rice on a vast scale, covering an area of about 44.1 million hectares and producing 165.3 million tons; however, the productivity per unit area remains low at 3.78 t/ha (Kesh *et al.*, 2021) [13]. With a growing population, diminishing arable land, and the impact of climate change, the need for increased rice productivity has become a pressing concern worldwide. The sustainability of rice production depends on the development of new rice cultivars with high yields and stable performance across diverse environments (Akter *et al.*, 2014) [4]. It is therefore essential to apply new approaches to increase rice yield in already cultivated areas (Khush, 2005) [14].

Micronutrient deficiency is a significant nutritional issue affecting more than two billion people in developing countries, emphasizing the need to enhance the concentration and bioavailability of essential micronutrients like iron (Fe) and zinc (Zn) in rice grains to improve their nutritional quality. Zinc is particularly crucial for the proper functioning of over 300 enzymes involved in carbohydrate, lipid, protein, and nucleic acid metabolism, essential for the normal growth and development of plants and animals.

In a study conducted by Martinez *et al.* 2008 [16], brown rice was found to contain 10-11 ppm Fe and 20-25 ppm Zn, while milled rice had 2-3 ppm Fe and 16-17 ppm Zn. Adequate Zn supply is vital for maintaining a healthy and productive life, with an average daily requirement of 7 to 13 mg for adults (Department of Health (UK), 1991; Institute of Medicine Food and Nutrition Board IMFNB, 2001). Various approaches, such as dietary changes to include Zn-rich foods and preventive supplementation through Zn-fortified foods, are employed to combat human Zn deficiency-related problems. Apart from agronomic practices, selecting genotypes with high efficiency in accumulating Fe and Zn in the endosperm and enhancing their bioavailability from existing germplasm collections can be an effective and reliable method to provide Fe nutrition benefits to farmers and the local population. Identifying donor parents carrying the target traits is necessary for breeding staple food crops with higher Fe and Zn content.

The main objective of our study was to evaluate the performance of high-yielding rice varieties with enhanced grain Fe/Zn content across different environments in Chhattisgarh. We aimed to assess genotype by environment (GXE) interactions for agronomic and micronutrient traits and identify stable varieties with high yield and grain Zn content that consistently perform well across diverse environments. For achieving these objectives, we aimed to facilitate the selection of high-performing rice varieties that exhibit broad adaptability, high-yielding and Zn-rich rice varieties with improved nutritional value.

## 2. Materials and Methods

The experiment was carried out under three location *i.e.* Raipur, Bilaspur and Bhatapara of Chhattisgarh with 22 grain Fe/Zn rich rice varieties along with yield and micronutrient check (IR-64 & Kalanamak) during kharif 2019 under MLT conducted by department of PMBB, IGKV Raipur, Chhattisgarh. The experiment was conducted in Randomized Block Design (RBD) with three replications having a plot size of 10 m<sup>2</sup> in each replication each environment. Standard agronomic practices and appropriate control measures for weeds, insect pests and diseases were followed. Fertilizer was applied at the recommended dose of 120:80:60 (N: P: K) at each experimental location. Yield data was recorded at physiological maturity and plot data harvested was converted to kg/ha using the plot size as factor. For the analysis of grain Fe/Zn content, 100 g of paddy samples was processed to rice by using Zaccaria rice dehusker. Around 50 g polished sample was washed and cleaned with autoclaved distilled water and each sample was analyzed in duplicate using X-ray fluorescence spectrometer (XRF), Oxford X-Supreme 8000 at IRRI, Hyderabad

**Table 1:** Details of 22 Fe/Zn rich rice genotypes along with yield and micronutrient check

G. No.	Name of Genotypes	G. No.	Name of Genotypes
G1	R-56	G12	R-RHZ-LI-23
G2	R-RHP-MI-30	G13	MI-127
G3	R-RHZ-IB-80	G14	IR-64 (Yield check)
G4	CGZR-1	G15	IET-26383
G5	Kalanamak (Micronutrients check)	G16	CGZR-2
G6	IET-24780	G17	R-RHZ-IH-82
G7	R-RHZ-SM-14	G18	DRR Dhan-49
G8	MI-156	G19	Chandrasahini
G9	Samba Mahsuri	G20	R-RHZ-MI-93
G10	ZincoRice MS	G21	R-RHZ-SD-94
G11	DRR Dhan-45	G22	CR Dhan-311

**Table 2:** Pooled analysis of variance over different environments for different traits in rice varieties (Eberhart and Russell, 1966 model)

S. N.	Source	d.f	Mean same square (MSS)		
			GY (kg/h)	Fe (ppm)	Zn (ppm)
1	Replication	6	835409	3.56	6.251
2	Genotypes	21	108975**	2.277**	29.02**
3	ENV+ GXE	44	104671*	9.783**	1.696**
4	ENV(linear)	1	667101*	344.8**	34.20**
5	G X E(linear)	21	187513*	0.071*	1.794*
6	POOLED DEV	22	33.67	3.827**	0.124*
7	ERROR(pooled)	126	306290	1.116	1.979

\*, \*\*Significant at 5% and 1% respectively

### 2.1 Statistical analysis

The data obtained from the experiment was analyzed using R Studio software (R Core Team 2012) and Plant Breeding Tools software (Version 1.3, <http://bbi.irri.org/products>). To assess genotype by environment interaction (GEI), a GGE bi-plot was constructed using R software (R Core Team 2012) and Plant Breeding Tools. The bi-plot graph depicted the relationship between yield means and the scores of the first principal component of interaction (IPCA1).

Furthermore, the data were analyzed to determine the discriminativeness versus representativeness ranking of environments, as well as the ranking of genotypes relative to the ideal environment. Additionally, the ranking of environments based on the ideal genotype was performed. To identify mega-environments and winning genotypes within the given set of environments, the "which-won-where" option was utilized. These analyses provided valuable insights into the performance of rice genotypes across different environments, enabling the identification of winning genotypes and the characterization of mega-environments. The use of these analytical tools facilitated a comprehensive understanding of genotype-environment interactions, aiding in the selection of high-performing rice varieties with broad adaptability and stability across diverse environments.

### 3. Results and Discussion

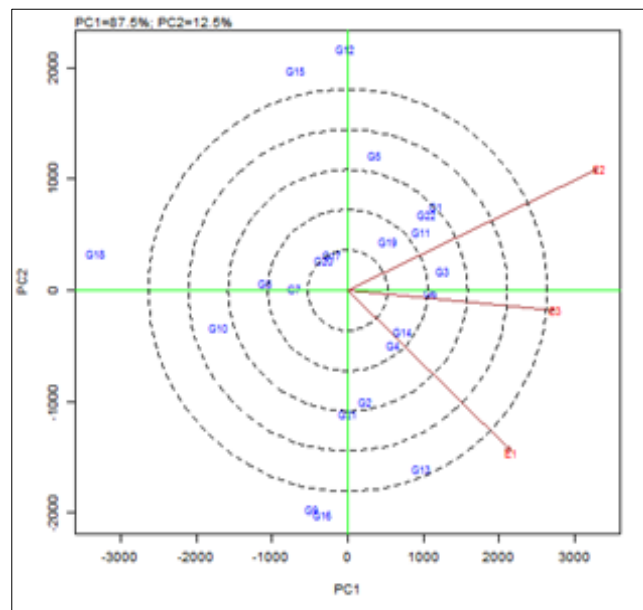
The presence of GEI was clearly demonstrated by AMMI model and the interaction was partitioned among the first two interaction principal component axis (IPCA), while the cumulative variance was 100% for PCAI and PCAII thereby, demonstrating that genotypes may be selected for adaptation to specific environments. These results are in harmony with the findings of Aina *et al.*, (2009) [3] and XuFei-fei *et al.*, (2014) [29] in G x E interactions effects. The environmental variance was also found to be significant which indicates that the environments under study were different from each other. The model was additive and the results of AMMI analysis were represented in the form of graphs called bi-plots (Gauch and Zobel, 1996). Further Gauch (1988) [9] recommended that the most accurate model for AMMI can be predicted by using first two principal component analysis. Admassu *et al.*, (2008) [1], in accordance with Zobel *et al.*, (1988), proposed that two interaction principal component axes for the AMMI model were sufficient for a predictive model. Thus, the interaction of 22 rice genotypes with three environments was predicted by the first two components of genotypes and environments (Sivapalan *et al.*, 2000) [24].

Stable genotypes were identified by graphical representation (GGE bi-plot) which uses genotype and GxE components and identifies GxE interaction pattern of multi-environment data and clearly shows which variety performs best in which environment (Lakew *et al.*, 2014) [15]. Vijay kumar *et al.*, (2001) [28] detailed that these bi-plots help in visual interpretation of GE patterns and identify genotypes or environment that exhibit low, medium or high interaction effects.

#### 3.1 Combined GGE bi-plot analysis of genotype

It is environment view of GGE bi-plot for yield, zinc and iron. The bi-plot explained 100% total variation of environment centered G x E. the cosine of the angle between the vectors of two environment approximate the correlation between them. All the environment combination (Fig. 1) *viz.* E1, E2 and E1, E3 and E2 E3 were positively correlated this exhibit weak GXE interaction. The distance between two environments measures their dissimilarity in discriminating the genotype. Thus the three environments fell in to two apparent groups. E1 and E3 formed one group and E2 formed another group. The close association between environment revealed that the same set of information could be generated from few test environment hence it may potentially reduced the testing cost and any one of the location could be dropped. This will help

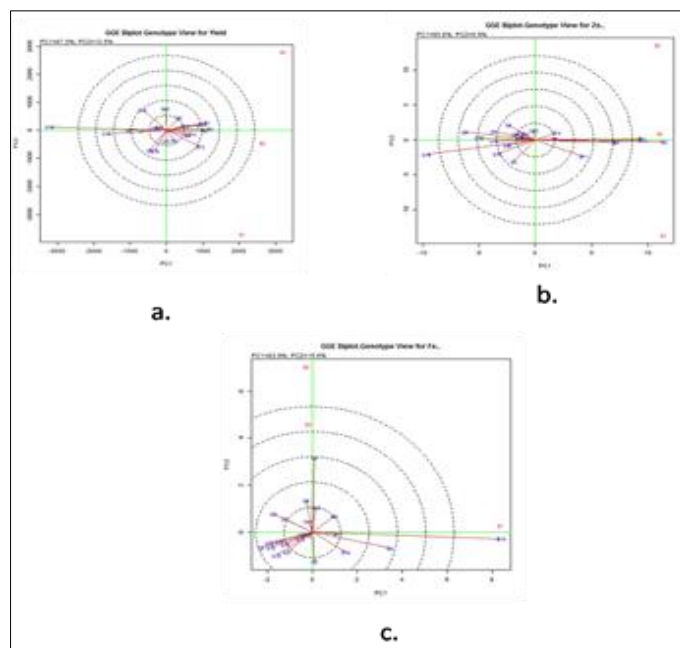
in optimum allocation of limited resources during multi-location trials. The concentric circle which are proportional to representative environment and is a measure of discriminating ability of environment. Hence E1 and E2 are the most discriminating and E3 is least discriminating. Least/Non discriminating environment provide little information on genotype hence cannot be used as test environment.



**Fig 1:** Ranking of environments based on discriminating ability and representatives

#### 3.2 Identification of stable genotypes with highest mean performance

In GGE bi-plot the complex GEI are partitioned in different principal components (PCs) and the data obtained are presented graphically against PCs to depict the GGE bi-plot abridgment mean performance and stability of different genotypes. The stability of genotype is analyzed by the absolute length of the projection of a genotype. G18 and G3, G12 and G21 (Fig. 2) are very different, whereas, G3 and G6 are quite similar. The dissimilarity can be due to difference in mean yield genotype and/or in interaction with environment. G2, G21, G10 and G5 are virtual genotypes that assume average value in each environment and average genotype has zero contribution to both G and GXE. Genotype located near bi-plot origin has little contribution to both G and GXE, and genotype with longest vector. G10 and G18 are poorest yielder and unstable genotype. The greater the absolute length of projection of a genotype, the less stable it is (Jay Laxami *et al.*, 2017). The angle between two genotypes indicates similarity in response to environment. An acute angle between G3 and G1 and other genotype means that these genotypes responded similarly and difference between them was proportion in all environments. G12 and G13 and other genotype which exhibit obtuse angle exhibit inverse response between genotype. Wherever the first genotype performed well the other genotype will perform poorly, while angle between G13 and G16 indicates that they response to environment independently. In the obtuse angle and acute angle cases the difference between the genotypes contributed more to G than to GXE. In the right angle case the difference contributed mostly to GXE.



**Fig 2:** The genotype-vector view of the GGE bi-plot to show similarities among genotypes in individual environment for a. yield, b. Zn and c. Fe

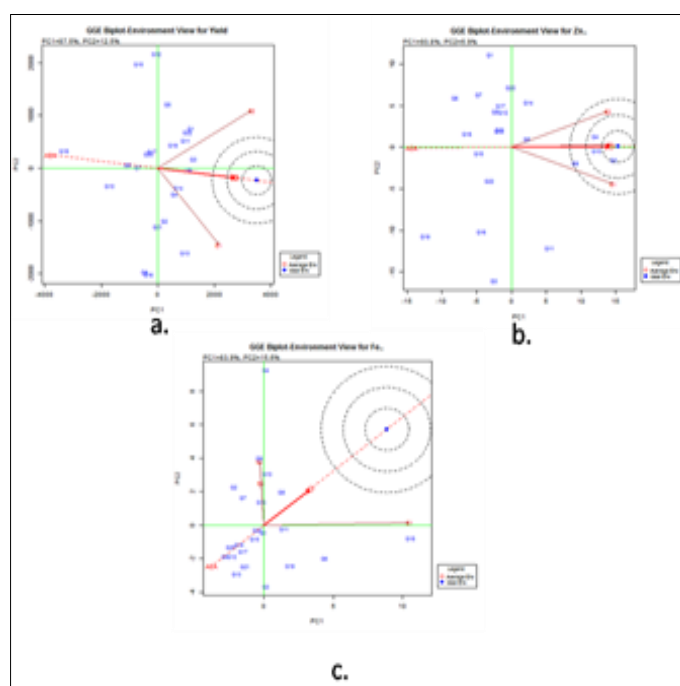
G2, G4, G9 and G10 (Fig. 2.b) are very similar to each other and differ to G19. This Dissimilarity is due to G and/or interaction with environment G14, G15, G20 and G12 are virtual genotypes and has zero contribution to both G and GXE. G19 is the poorest yielder and unstable and G2 and G10 are higher yielder and stable for zinc. Acute angle was reported between G10, G9, G4 and G11 shows genotype responded similarly and difference between them was proportional in all environments. Obtuse angle between G11 and G1 exhibit inverse relationship between genotype in environment. Right angle between G2 and G3 indicate that these two genotypes responded independently in all environments.

G18, G6, G19 and G1 (Fig. 2.c) are similar performance genotypes for Iron content, whereas, G2 and G18 are very

different genotypes across the environment. For iron content genotypes G1, G8, G16, G14, G18 and G17 are close to bi-plot origin and exhibit very little contribution to G+ GXE. G18 has longest vector and high iron content in environment E1

### 3.3 Environment evaluation

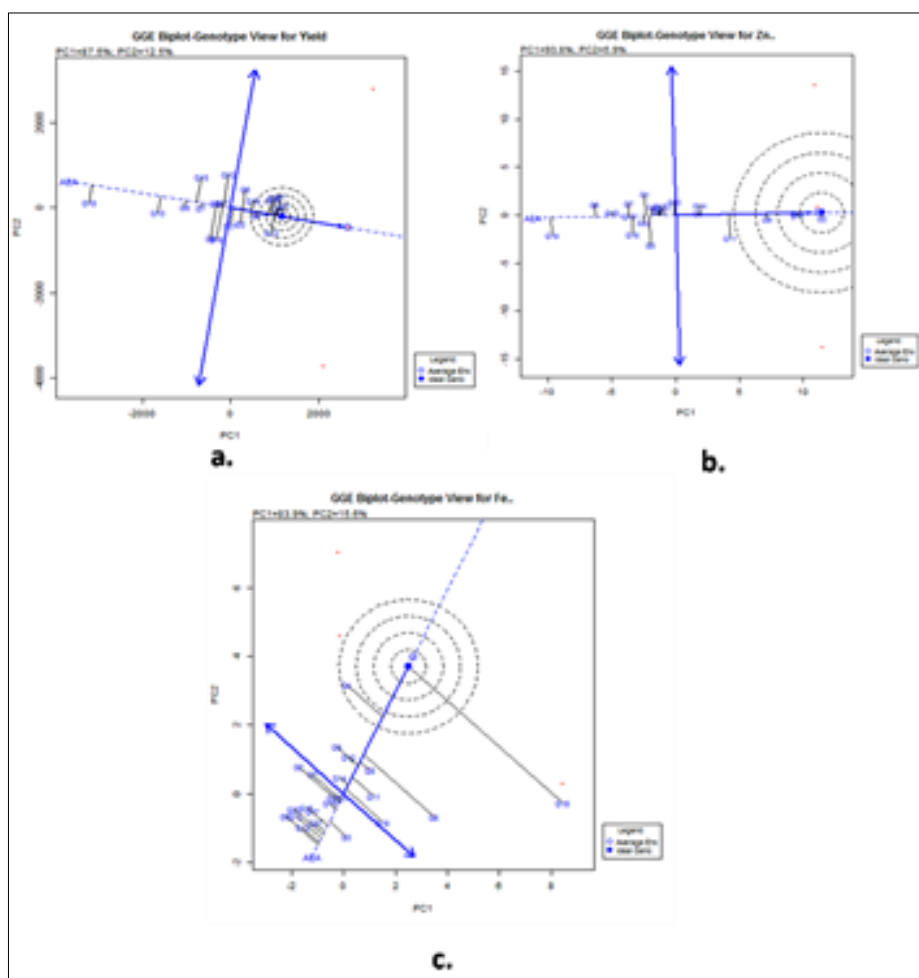
Angles between environment vectors in bi-plots indicate their relationship as the cosine of the vector angles is indicative of their correlation. Acute angle between two environment vectors indicates positive correlation while an obtuse angle indicates negative correlation and right angle suggests no relation. Environments show complex relationship among themselves. The ideal environment is denoted by a small circle at the center of the concentric rings.



**Fig 3:** GGE Bi-plot environment view

Environment vector length has the discriminating power, viz. longer the vector length more the discriminating ability between genotype and small angle between environment vector and AEA line make it the best representative environment for discriminating the genotype. Here in our study (Fig.3) E3 has the shortest vector length and E2 has the longest vector length for yield. E2 exhibit minimum angle between environment vector and AEA line, hence, it may be

the best representative environment for discriminating genotype for yield. Similarly, for Zn content all the three environment had almost equal vector length, but E3 exhibit minimum angle with AEA line hence it is most discriminating environment for zinc. For iron content E1 had longest environment vector length followed by E2 and E3. E1 exhibit minimum angle with AEA line compare to E2 and E3 hence it is the most discrimination environment for iron content.



**Fig 4:** Mean performance and stability of genotype for a. yield, b. Zn and c. Fe

The mean performance and stability of the genotype should be evaluated across environments. Ideal genotypes should have the highest mean performance and be absolutely stable across environments (Yan and Kang 2007) [30]. For this purpose Average environmental co-ordinate (AEC) view (Fig. 3) of the GGE bi-plot is used. The double-headed line is the Average Environment Co-ordinate (ACE) and shows greater variability (less stability) moving in both the directions. An ideal genotype is a genotype which lies on the AEA line, genotype located closer to the ideal genotype are more stable than others. Thus the genotype G15 is highly stable performing across all environments consistently followed by genotype G14 and G8 for yield. For Zn content, G5 and G10 exhibit stable performance followed by genotype G2, G9 and G18. G15 were the most stable for Fe content followed by G14. This meant that "stable" genotypes were only desirable if they had high mean performance. The relative contributions of stability and high mean seed yield to the identification of desirable genotypes in this study were similar to those found

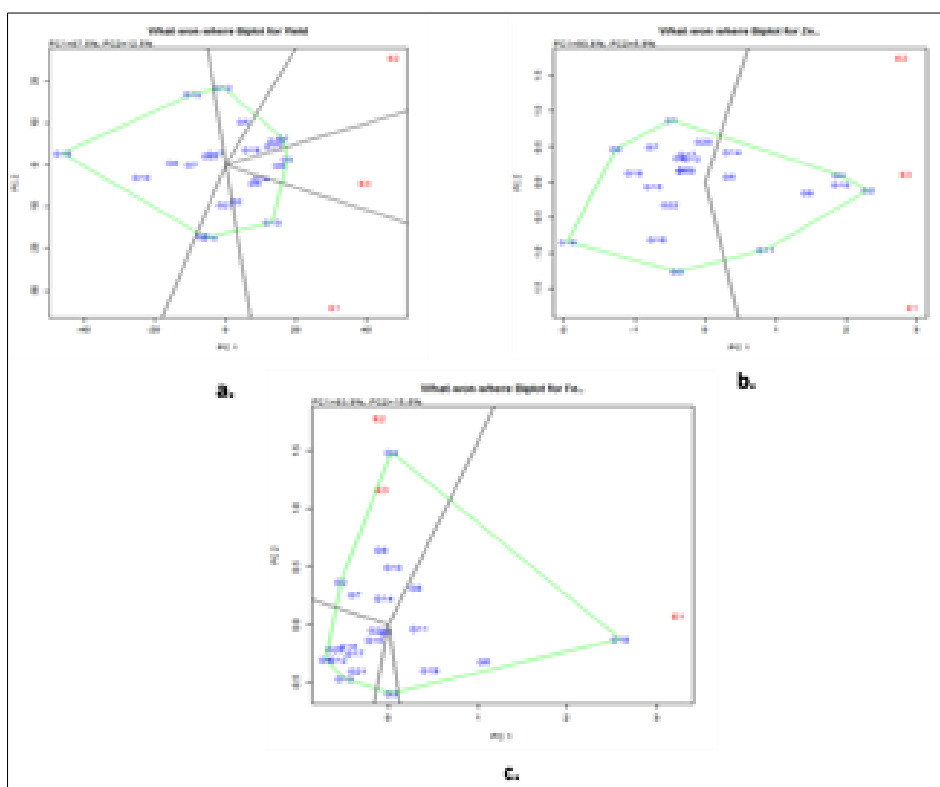
in other crop stability studies using the ideal genotypes procedure of the GGE bi-plot.

### 3.4 “Which-won-where” and mega environment identification

“Which-won-where” analyses involving GEI, mega-environment differentiation, specific adaptation of genotypes etc. are graphically addressed. The twenty two rice genotype tested in three locations generates a bi-plot which is divided into hexagon sections (Fig. 5) for yield with genotype G1, G3, G13, G12, G18 and G16, for Zn with genotype G2, G1, G6, G19, G3 and G11 and for Fe with genotype G4, G18, G3, G13, G20 and G2 respectively, at vertices. The equality lines divided the bi-plot into six sectors effectively for yield, two sectors effectively for Zn and four sectors effectively for Fe. Three testing environments were spread within the bi-plot, for yield, three environments falls in one sector each and for Zn, the entire environment falls in one sector and for Fe, two environments fall in one sector and one environment in

another section. Testing environment could be partitioned into mega-environment (ME). The sectors, which do not have any single environment and hence it did not reflect any separate ME and could be merged into nearest MEs. Three MEs was reported for yield study with G13, G1 and G3 as winning genotype respectively. Only one ME with all the three environments was reported for Zn with G2, G4 and G11 as winning genotype. ME1 with E2 and E3 environments with G4 and G2 as winning genotype and ME2 with E1 only have G18 as winning genotype. Thus, this study established the effectiveness of GGE bi-plot analysis in identifying stable and superior genotypes. If a genotype is placed in a sector where no environment marker falls then this genotype is considered as poorly performing in all the environments. The genotype

which falls on the vertex of the polygon where an environment marker drops such genotype is suggested to provide better yield and perform better in that particular environment. On the other hand, the genotype linked to the polygon vertex where no environment marker drops in the sector indicates that such genotype performs poorly across the environments. The genotypes falling within the polygon are less stable in the environment than the corner genotypes (Yan and Tinker, 2007) [30]. Similar findings and interpretation have been made by Islam *et al.*, (2014) [4]. Several authors used AMMI to evaluate multi-environment experiments to distinguish the effects of the genotype and the environment and then assess the G x E interaction in a reduced dimensional space with minimum error.



**Fig 5:** which-won –where view of GGE Bi-plot for a. yield, b. Zn and c. Fe

Similar kind of results was obtained earlier reported by Oikeh *et al.*, (2004) [18], Velu *et al.*, (2012) [27], BishawZ and Van Gastel in 2009 [7], Suwanto and Nasrullah (2011) [26]. GGE bi-plots are very useful in selected lines with target environments and were utilized in MLT Trials and in coordinated variety testing programme. Prasad *et al.*, (2001) [20] reported stability and yield performance of mega varieties

#### 4. Conclusions

Based on the analysis, genotypes G11 and G1 have demonstrated potential as donors for high grain yield, while genotypes G10 and G18 exhibit promise for zinc content improvement, and genotypes G14 and G12 show potential for enhancing iron content. Considering the current situation, a crossing program between these genotypes holds promise as a prospective approach for developing high-yielding varieties with enhanced micronutrient content. This approach aligns with the increasing demand for such varieties while requiring minimal inputs. To validate and confirm the performance of these winning genotypes in terms of yield and micronutrient

content, further testing is necessary. Multi-location and multi-environmental trials are recommended to evaluate their stability and adaptability across diverse growing conditions. These trials will provide crucial information to assess the suitability and performance of these genotypes in different regions, ensuring the development of robust and widely adaptable varieties with improved grain yield and desirable micronutrient characteristics.

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