



RESEARCH ARTICLE

Application of GGE biplot analysis for assessing stability in Recombinant Inbred Lines (RILs) of rice (*Oryza sativa* L.)

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Abstract

This study was conducted to assess the genetic differences among 87 rice (*Oryza sativa* L.) recombinant inbred lines (RILs), identify productive environments, and measure the impacts of genotype by environment interaction (GEI) on yield across four locations. The pooled ANOVA showed significant differences among the environments, genotypes, and GEI for grain yield. A graphical method for analyzing experimental data from multiple environments is to consider genotype as the main effect and genotype x environment interaction (GGE) biplot, which visually assesses the total number of genotypes. The first two primary components cumulatively explained 50.9% of the variation in grain yield. GEI is responsible for more than half of the overall variation for this trait. Peshawar and Charsadda form one mega environment, while Swat and Mansehra constitute another mega environment for grain yield. Recombinant inbred lines, AUP-3 and AUP-30 were identified as the highest-yielding, stable, and ideal genotypes across the environments for grain yield.

Keywords: Genotype × environment interaction, Asian rice, stability analysis, grain yield

Introduction

About 20 wild species and two domesticated species, *Oryza sativa* (an Asian species) and *O. glaberrima* (an African species), constitute the genus *Oryza* (Wanbugu et al. 2013). *O. sativa* is cultivated worldwide, while *O. glaberrima* is primarily grown in West Africa. The two main biotypes or subspecies of *O. sativa*, indica, and japonica, have evolved to adapt to different environmental conditions (Maclean et al. 2002). Rice (*Oryza sativa* L.), a crucial cereal crop globally, has been a stable food since ancient times. Developing new cultivars with high yields and consistent performance in various environments is essential to increase global rice production (Tiwari et al. 2024).

Yield, a complex quantitative trait, is greatly influenced by the environment. Selecting superior genotypes based solely on yield at a single site and in a single year is ineffective, therefore evaluating genotypes for stable performance across diverse environmental conditions and over multiple years has become a crucial aspect of crop improvement programs. Understanding the interaction between genotype and environment helps identify stable genotypes suitable for commercial cultivation (S. C. et al. 2023). Tremendous advancement has been made in analyzing the varietal performance across different environments. The genotype main effects and genotype x environment interaction effects (GGE) model is a popular

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technique (Gauch 2006). GGE biplot analysis simplifies and completes the genotype by environment data analysis, providing insights on long-term fundamental issues and short-term, practical questions (Yan and Tinker 2007).

GGE biplot analysis is a statistical technique employing a multivariate approach that separates GE components into distinct interactions between genes and environmental factors, making it superior to a univariate method (Flores et al. 1998). Currently, GGE biplot analysis is being applied to several crops to find out the stable genotypes (Banik et al. 2024; Kumar et al. 2024). Compared to AMMI analysis, GGE offers a more detailed explanation of the sources of variation in G (genotype) and GE (genotype by environment) (Tiwari et al. 2024). GGE biplot can display genotype average performance and stability, the best genotype and optimal location to maximize yield, the best genotype with the highest yield in a quadrant comprising identical locations (Mega-E), and a specific location (Chandrashekhar et al. 2020; Farshadfar and Sadeghi 2014). Given the importance of GGE biplot analysis, the objectives of the current study were to use GGE biplot to identify generally stable genotypes across different settings; identify broad habitats, and investigate genotypes that perform better at specific sites for desired attributes.

Materials and methods

This research was conducted at four locations in the Khyber Pakhtunkhwa province namely, the University of Agriculture (UOA) Peshawar, Agriculture Research Institute (ARI) Mingora-Swat, Agriculture Research Station (ARS) Bafa-Mansehra and Bacha Khan University (BKU) Charsadda, during the rice growing seasons of 2020 and 2021. These locations represented eight environments in two years such as Peshawar-2020 (E-1), Mingora-2020 (E-2), Mansehra-2020 (E-3), Charsadda-2020 (E-4), Peshawar-2021 (E-5), Mingora-2021 (E-6), Mansehra-2021 (E-7) and Charsadda-2021 (E-8). Recombinant inbred lines (RILs) of rice produced from the F₅ generation as well as the common check cultivars Pakhal, Kashmir-Basmati (K-Bas), and Fakhr-e-Malakand (F-MLD) made up of the genetic material.

The Department of Plant Breeding and Genetics at the University of Agriculture Peshawar’s Rice Breeding Program created the rice RILs. A program of rice hybridization was started in 2010 by crossing several elite Pakistani rice cultivars. The segregating populations have grown substantially up to F₄. Based on superior yield and attributes relevant to yield, single plants from the bulk populations of various cross combinations were chosen for F₅. In F₆, seeds from each selected plant were grown in two-row plots with uniform lines retained and undesirable ones eliminated. These selected lines were then evaluated across the 2020 and 2021 rice growing seasons in the four different locations using an alpha lattice design with three replications. Each replication consisted of six blocks, each containing 15 RILs,

and each genotype was planted in four-row plots with row spacing of 30 cm and a row length of 3 m.

GGE Biplot Analysis

Using the computer program GEA-R, data on days to maturity, and grain yield were analyzed using GGE biplot analysis (Pacheco et al. 2015). The principal components (PCs) utilized in the singular value decomposition (SVD) of the GGE biplot model can be represented as:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \epsilon_{ij}$$

Where;

Y_{ij} is an Observed response variable for an individual in an environment (e.g., yield of genotype in location).

μ is the Overall mean of the response variable.

β_j is a Fixed or random effect associated with environment

λ_1 and λ_2 are factor loadings, which measure the contribution of latent factors.

ξ_{i1} is first latent factor for individual/genotype (e.g., genetic effect).

η_{j1} is the first latent factor for the environment (e.g., environmental factor).

ξ_{i2} is the second latent factor excluding the direct contribution of (possibly a structured residual).

η_{j2} is the second latent factor associated with it.

ϵ_{ij} is a Residual error term accounting for unexplained variability.

Results

Mean performance of grain yield

Highly significant ($p \leq 0.01$) differences among the genotypes, environment, and GEI were observed for grain yield (Table 1). Across environments, grain yield ranged from 2.8 to 4.1 t ha⁻¹ with a mean value of 3.6 t ha⁻¹. Maximum grain yield was observed for AUP-3 and AUP-30 (4.1t ha⁻¹), followed by genotypes AUP-90 and AUP-29 (4.0t ha⁻¹), while minimum grain yield was noticed for AUP-64 (2.8 t ha⁻¹) (Table 2). Within environments, grain yield ranged from 2.6 to 4.8 t ha⁻¹ in E-01; 3.0 to 4.6 t ha⁻¹ in E-02; 3.1 to 5.4 t ha⁻¹ in E-03; 2.1 to 3.9 t ha⁻¹ in E-04; 2.5 to 4.7 t ha⁻¹ in E-05; 3.2 to 4.2 t ha⁻¹ in E-06; 2.7 to 4.9 t ha⁻¹ in E-07 and 1.9 to 3.8 t ha⁻¹ in E-08 (Table 3).

Table 1. Pooled mean squares for grain yield (t ha⁻¹) of 90 genotypes across eight environments during the 2020 and 2021 rice growing seasons

Source of variation	Df	Mean squares
Environments	7	72.5**
Replications (E)	16	0.5
S-Block (Rep (E))	120	0.1
Genotypes	89	0.7**
G × E	623	0.1*
Error	1304	0.1

Table 2. Means of 90 rice genotypes for grain yield (t ha⁻¹) across eight environments

Rice genotypes	Grain yield (t ha ⁻¹)	Rice genotypes	Grain yield (t ha ⁻¹)
AUP-1	3.4	AUP-47	3.4
AUP-2	3.4	AUP-48	3.5
AUP-3	4.2	AUP-49	3.5
AUP-4	3.5	AUP-50	3.4
AUP-5	3.5	AUP-51	3.5
AUP-6	3.4	AUP-52	3.4
AUP-7	3.4	AUP-53	3.4
AUP-8	3.4	AUP-54	3.4
AUP-9	3.5	AUP-55	3.6
AUP-10	3.6	AUP-56	3.4
AUP-11	3.2	AUP-57	3.3
AUP-12	3.4	AUP-58	3.2
AUP-13	3.4	AUP-59	3.3
AUP-14	3.3	AUP-60	3.5
AUP-15	3.3	AUP-61	3.3
AUP-16	3.5	AUP-62	3.4
AUP-17	3.4	AUP-63	3.3
AUP-18	3.4	AUP-64	2.8
AUP-19	3.4	AUP-65	3.4
AUP-20	3.5	AUP-66	3.5
AUP-21	3.4	AUP-67	3.6
AUP-22	3.5	AUP-68	3.5
AUP-23	3.5	AUP-69	3.4
AUP-24	3.2	AUP-70	3.4
AUP-25	3.5	AUP-71	3.4
AUP-26	3.4	AUP-72	3.5
AUP-27	3.5	AUP-73	3.6
AUP-28	3.4	AUP-74	3.3
AUP-29	4.1	AUP-75	3.6
AUP-30	4.2	AUP-76	3.2
AUP-31	3.5	AUP-77	3.5
AUP-32	3.3	AUP-78	3.5
AUP-33	3.6	AUP-79	3.4
AUP-34	3.5	AUP-80	3.4
AUP-35	3.4	AUP-81	3.4
AUP-36	3.4	AUP-82	3.4
AUP-37	3.4	AUP-83	3.5
AUP-38	3.3	AUP-84	3.5

AUP-39	3.3	AUP-85	3.5
AUP-40	3.5	AUP-86	3.4
AUP-41	3.3	AUP-87	3.5
AUP-42	3.3	Pakhal	3.5
AUP-43	3.5	K-Bas	3.4
AUP-44	3.4	F-MLD	3.8
AUP-45	3.5	Mean	3.5
AUP-46	3.4	LSD _(0.05)	0.19

Table 3. Genotypes showing minimum and maximum grain yield (t ha⁻¹) under each environment

Environments	Parameters	Grain yield (t ha ⁻¹)
E-1(Peshawar, 2020)	Minimum	2.6 (AUP- 64)
	Maximum	4.8 (AUP- 3)
	Mean	3.7
E-2(Swat, 2020)	Minimum	3.0 (AUP- 64)
	Maximum	4.6 (AUP- 29)
	Mean	3.7
E-3(Mansehra, 2020)	Minimum	3.1 (AUP- 59)
	Maximum	5.4 (AUP- 30)
	Mean	4.1
E-4(Charsadda, 2020)	Minimum	2.1 (AUP- 7)
	Maximum	3.9 (AUP- 40)
	Mean	2.9
E-5(Peshawar, 2021)	Minimum	2.5 (AUP- 64)
	Maximum	4.7 (AUP- 3)
	Mean	3.7
E-6(Swat, 2021)	Minimum	3.2 (AUP- 40)
	Maximum	4.4 (AUP- 29)
	Mean	3.8
E-7(Mansehra, 2021)	Minimum	2.7 (AUP- 64)
	Maximum	4.9 (AUP- 30)
	Mean	3.8
E-8(Charsadda, 2021)	Minimum	1.9(AUP- 64)
	Maximum	3.8 (AUP- 40)
	Mean	2.8
Mean across allEnvironment	Minimum	2.8 (AUP- 64)
	Maximum	4.1 (AUP- 3)
	Mean	3.6

The 'ordinate' line, which is perpendicular to the Average Environment Axis (AEA) and in green, displays the level of genetic instability. The genotype would be more unstable the higher the projection on the ordinate. According to its reduced projection on coordinate (Fig. 1A), AUP-30 was the genotype for grains panicle⁻¹ which was consistently the most prolific and dependable in all conditions. The enormous projection of genotype AUP-3's coordinates suggests that it had the least stability, although producing an adequate number of grains panicle⁻¹. However, as evidenced by its bigger projection on coordinates, AUP-29, the third-highest grain production, remained rather constant.

Mean performance of genotypes and environments and relationships

GGE biplot analysis was used to examine grain yield data from 90 rice genotypes grown in eight different settings. The variance caused by GE interaction was represented by the first two main components, totaling 63.2% (Fig. 1C). The most variable genotypes across different contexts were AUP-3, AUP-30, AUP-90, and AUP-64, as seen by their greater distances from the origin. Since all environments had smaller vectors, they had the potential to significantly influence genotype performance. AUP-3, AUP-30 and AUP-90 genotypes were identified as stable and widely adapted for grain yield, which aligns with their proximity to the origin. Fig. 1C shows the relationship between test environments. By their smaller angles with one another, environments E-1, E-3, and E-7 can be demonstrated to have close associations. The environments E-2 and E-6

were similar in that they were connected. The angle of environment E-04 with environments E-2 and E-6 is greater than 90 degrees, indicating a negative relationship between these environments and providing contrasting conditions to distinguish genotypes. Environment E-7 had longer vectors based on vector length, allowing for the possibility of genotype selection based on grain.

Mean vs. stability of genotypes

Fig. 1 B shows a biplot of mean vs. stability for grain yield. It can be observed, that AUP--30 was a high-yielding genotype with moderate stability, as it produced a higher mean grain yield with an intermediate projection on the ordinate. AUP-90 was found to be less stable than AUP-30 but comparatively low-yielding. Similarly, AUP-64 had mediocre yields and average stability.

Discussion

Staso et al. (2016) evaluated 20 rice genotypes utilizing six settings for yield and attributes associated with yield and found that the genotypes responded differently for grain yield depending on the environment. The highest grain yield was found to be produced by the rice genotype IR79156A/PK88 (6.11 t ha⁻¹). The development of a high-yielding genotype, which affects the future of the crop, its growers, and the nations, is a crucial component of every plant breeding program. The differential response of 90 rice genotypes across eight settings for grain yield was confirmed by the GGE biplot. Grain yield changes owing to GEI were each explained by the first two primary

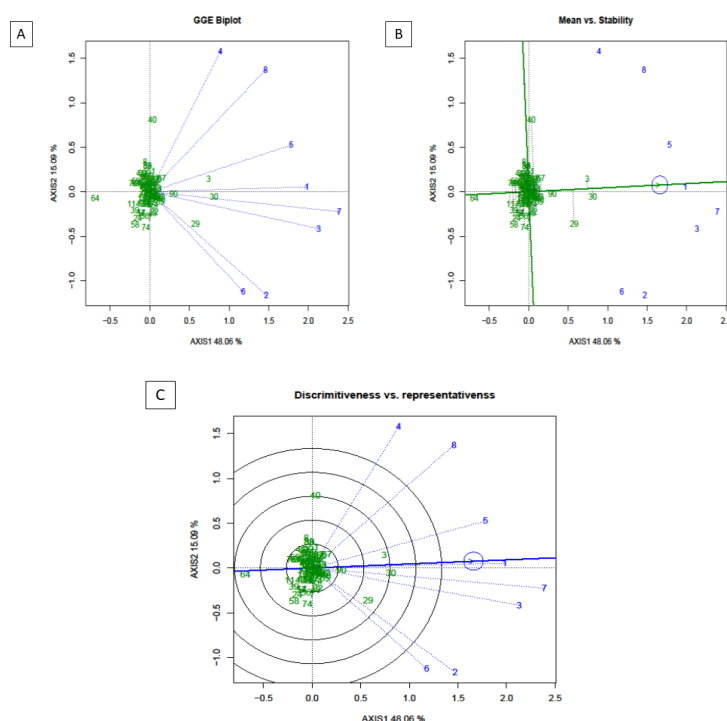


Fig. 1. (A, B and C). GGE Biplots for grains yield including A= GGE biplot, B= Mean vs. Stability and C= Relationship among environment

components to a cumulative extent of 50.9%. The current experiment's findings concur with earlier research by (Lakew et al. 2014 and Luguterh et al. 2016). Using GGE biplot analysis to divide GE interaction, Khatun et al. (2015) found that PC1 and PC2 were responsible for 86.5% and 86.7% of the GGE sum of squares, respectively. In their study of rice, Lakew et al. (2014) found that the first two main components together explained 79.9% of the variance. It can be concluded that superior genotypes AUP-3 and AUP-30 can be successfully adapted for grain yield in Peshawar (E-1 and E-5) and Mansehra (E-3 and E-7).

The AEA line should be observed passing through both the biplot origin and the typical environment. The little circle with the average coordinates of all test environments has been used to represent the average environment. If a test environment has a smaller angle with the AEA, it is more likely to be representative of other test conditions (Khatun et al. 2015 and Satoto et al. 2016). Although the "ideal" genotype may not exist in reality, it can nevertheless be used as a guide when assessing genotypes. Any genotype that is placed nearer to the "ideal" genotype will be regarded favorably (Mitrovia et al. 2012 and Rani et al. 2021). On the graph, the genotype ranking is represented by the so-called genotype "ideal" genotype. A genotype is considered to be excellent if it consistently performs at the highest level across all test settings and has the best yield (Farshadfar et al. 2012; Zewdu et al. 2020).

The settings in Peshawar and Swat were good for grain yield. The cosine of the angle between the vectors, which are lines connecting the environments to the biplot origin, defined the relationship between testers in the relationship among testers. A greater relationship between surroundings and the angle is indicated by (Susanto et al. 2015; Susanto et al. 2015) findings. The close link between the environments E-1, E-3, and E-7 in this instance is indicated by their smaller angles with one another. The environments E-2 and E-6 were similar in this regard. A negative link between these environments, which provide opposing circumstances to separate genotypes, is suggested by the angle of environment E-04 with environments E-2 and E-6 being $>90^\circ$. Longer vectors in environments E-4, E-7, and E-8 made it possible to choose between different genotypes for grain yield.

Authors' contribution

Conceptualization of research (AUR, MA); Designing of the experiments (SMAS); Contribution of experimental materials (AUR, MA); Execution of field/lab experiments and data collection (AUR, MA, SMAS, SMR, WK, MAr); Analysis of data and interpretation (AUR, MA, SMAS, MAS, SK); Preparation of the manuscript (AUR, MA, SMAS, MAS, SK, MS, LMCD).

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