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Multivariate stability analysis to select elite rice (*Oryza sativa* L.) genotypes for grain yield, zinc and Iron

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The present study was conducted to evaluate 30 rice genotypes at three different locations in eastern Uttar Pradesh during the Wet- 2020–21 and determine the impact of GEI on grain yield (tha^{-1}), days to 50% flowering, grain Fe content (PPM), and grain Zn content (PPM). The study also aimed to identify the genotypes that displayed the best performance according to the multi-trait stability index (MTSI), multi-trait genotype-ideotype distance index (MGIDI), and factor analysis and ideotype-design (FAI-BLUP) index. AMMI analysis demonstrated significant variation for environment (E), genotype (G), and genotype-by-environment interaction (GEI) ($P < 0.01$) for all the studied traits. The AMMI1 biplot showed that PC1 explained the majority of the variation for GY (77.6%), DTF (90.5%), Fe (73.5%), and Zn (86.8%), helping to identify stable and high-performing genotypes. AMMI2 biplot further resolved complex GEI patterns, highlighting genotypes with specific adaptability to individual environments. The GGE biplot revealed clear "which-won-where" patterns for GY, DTF, Fe, and Zn, explaining 94.37%, 99.71%, 83.49%, and 96.93% of GEI variation, respectively. BLUP analysis using a linear mixed model revealed significant GEI effects for GY, DTF, Fe, and Zn across 30 rice genotypes in three environments. Low heritability was observed for Fe (28.2%) and moderate for GY (54.4%) and Zn (56.4%), while DTF showed high heritability with strong genotypic accuracy. Genotype G7 was identified as stable, early, high-yielding, and rich in Fe based on HMGV, RPGV, and HMRPGV indices. The MTSI, MGIDI and FAI-BLUP analysis revealed that BHU-SKS-1 (G15) and IR105696 -1–2-3–1-1–1 -B (G9) were the most stable and best mean performer for high grain yield and high grain Fe & Zn content, while IR 108,195–3-1–1-2 (G7) was the most stable and best mean performer for high grain yield and high grain Fe content with early flowering.

Keywords AMMI, Biofortification, Ideotype indices, Rice, WAASB

Rice is an important staple food in the human diet, is the second most important cereal crop and provides over one-fifth of the calories consumed globally[1]. Hidden hunger, also known as micronutrient deficiency, affects nearly 2 billion people worldwide[2]. Iron (Fe), and zinc (Zn) are crucial nutrients for human health. Sufficient micronutrient intake is crucial for various physiological functions within the human body. Fe is a vital component of hemoglobin and myoglobin and its deficiency is strongly associated with anemia. Iron deficiency also causes impaired development, and heightened vulnerability to malaria, HIV/AIDS, and tuberculosis[3]. Approximately 17.3% of the global population is at risk of Zn deficiency[4]. In Asia rice constitutes a primary source of Zn intake. Specifically, rice contributes significantly to dietary Zn intake, accounting for 49% of Zn intake among children and 69% among women[5]. Through HarvestPlus Project, 14 conventionally bred rice varieties with high grain Zn and yield have been released in seven countries[6]. The development of high Zn rice through biofortification breeding is viable solution for elevating hidden hunger and tackling malnutrition in India[2,3,6]. The zinc content in grains has been observed to be influenced by various environmental factors, including soil zinc status and temperature[7]. Additionally, genotype stability and the application of fertilizers[8,9] play significant roles in determining the zinc content in grains. Grain yield, Zn and Fe are quantitative trait governed by many genes and their expression

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varies in varying environment[10,11]. The efficiency of a genotype in utilizing Zn and Fe is strongly affected by their translocation from the roots to the shoots and the overall uptake by the plant[12–14]. Hence, understanding of G x E interaction not only helps in identifying stable genotypes for grain yield, Zn and Fe but also help plant breeders in devising suitable breeding strategies for developing biofortified rice varieties with enhanced grain Zn and Fe content[2,15]. To identify widely adaptable high-yielding, high Zn & Fe genotypes, a series of multi-environment trials (METs) are carried out in the advanced selection phase[16,17]. Each genotype should be evaluated in different environment to record genotypic responses and identify a superior and stable genotype(s) for cultivation in target areas[16,17]. METs are crucial for obtaining reliable results by cultivating genotypes in diverse environments. The AMMI model is a widely used method for analyzing information obtained from METs, which combines analysis of variance and principal components analysis. The Weighted Average Absolute Scores (WAAS) index is a recent addition to the AMMI-based indices and considers all IPCs to draw a biplot of average performance against the first PC of GEI, including the variance of GEI in the selection process[18]. The AMMI model and the GGE biplot graphical model are often used together to identify stable genotypes and winning genotypes within each environment. The GGE biplot model is based on PC analysis and has been proposed for breeders to evaluate genotype stability and the combination of stability with yield across different environments through graphical representation of General Combining Ability (GCA)[19]. Additionally to AMMI and GGE biplot, Best linear unbiased prediction (BLUP) method has been used to analyze METs. BLUP estimates the mean yield of genotypes in mixed models with high efficiency[20]. To utilize the advantages of both AMMI and BLUP, an index called Weighted Average Absolute Scores of BLUPs (WAASB) was introduced[21], which is integration of AMMI and BLUP. WAASB is derived through the singular value decomposition of the BLUP matrix, which is obtained from a LMM capturing GEI effects. This model characterizes ideal genotypes by incorporating a balance of both stability and yield performance considerations[22]. As breeders consider both stability and yield characteristics at the same time to identify and introduce new cultivars, the WAASBY index was introduced, which takes into account both stability and yield characteristics at the same time, using the WAASB index and yield (Y). Recently multivariate analysis methods MTSI, in addition to AMMI, WAASB and GGE biplot are being used to identify stable genotypes possessing different combination of desirable traits[23,24]. For simultaneous selection of stability and higher mean performance for multiple traits Olivoto and Nardino[25] recently introduced the Multi-Trait Genotype-Ideotype Distance Index (MGIDI), a method for selecting high-yielding and stable genotypes in METs using multiple traits and considering both fixed and random effects models. The MGIDI and MTSI both rely on a common theoretical framework that includes the rescaling of traits, conducting factor analysis, and assessing the distance from each genotype to the ideotype. The primary distinction between the two approaches is found in the re-scaled matrix employed for factor analysis in MGIDI. This matrix is derived through BLUP for genotype mean performance, as opposed to the WAASBY, which is used in MTSI for both mean performance and stability assessment. This index offers the possibility of selecting stable genotypes with a positive selection differential for traits aimed at increasing and a negative selection differential for traits aimed at decreasing. Furthermore, the MGIDI is useful for breeders seeking to select for both average performance and stability by considering multiple traits, as it provides an easy-to-interpret selection process. As far as we know, there are only few studies reported use of MTSI, MGIDI, and FAI-BLUP analysis to select the genotypes possessing high yield along with higher grain Zn or higher grain Fe content in rice. Thus, the present study aimed to (i) assess 30 rice genotypes across three distinct locations in eastern Uttar Pradesh and analyze the influence of GEI on flowering, grain yield, Zn, and Fe content; (ii) pinpoint the stability traits contributing to the overall performance of these genotypes; and (iii) determine the genotypes exhibiting optimal performance based on the MTSI, MGIDI, and FAIBLUP indices, along with the highest stability as per the WAASB model when cultivated in three diverse environmental conditions.

Result and discussion

Mean and additive main effects and multiplicative interaction (AMMI) analysis of variance

Trait mean values in each environment for the traits grain yield (tha^{-1}) (GY) (Fig. 1A), days to 50% flowering (DTF) (Fig. 1B), grain Fe (PPM) (Fig. 1C), and grain Zn (PPM) (Fig. 1D), evaluated on 30 rice genotypes across three environments during Wet-2020 has been represented in Fig. 1 and Table-S1. AMMI analysis assumes homoscedasticity (equal variances across environments). In the present study, Levene's test was performed to assess the equality of variances across environments. Among the studied traits, Fe ($F = 14.099$, $p < 0.001$) and Zn ($F = 3.780$, $p < 0.05$) exhibited significant differences across environments, whereas DTF ($F = 0.020$), PHT ($F = 1.887$), and GY ($F = 1.518$) showed non-significant variation. Given that, Levene's test confirmed unequal variances across environments for Fe and Zn ($p < 0.05$) and hence, data were log-transformed to stabilize variance, and AMMI analysis was performed on the transformed data for the traits Zn and Fe. This approach ensured valid interpretation of G × E interactions. The AMMI analysis of variance was performed for GY, DTF, Fe, and Zn in Rice (Table 1). The 30 rice genotypes tested at three locations in eastern U.P. demonstrated that the mean square value for all the studied traits (GY, DTF, Zn, Fe) showed significant variation for environment (E), genotype (G), and genotype-by-environment interaction (GEI) ($P < 0.01$). The differences within the replications of the environment were non-significant for traits DTF and Zn. However, traits GY and Fe revealed significant difference among replication within environment ($P < 0.05$) (Table 1). Explained SS (%) indicated that the effect of environment was lower than the genotype effect for all the traits studied. The environment effect was highest for trait Fe (23.87%), followed by GY (12.03%), Zn (3.57%) and lowest for DTF (2.52%). The GEI effect was highest for trait Fe (31.29%), followed by GY (24.64%), Zn (21.04%) and lowest for DTF (3.06%). The presence of significant GEI effect pose problem in selection of genotypes with desirable breeding value as their performance differ in changing environmental conditions. Through studying the GEI effect breeder will be able to identify stable genotype with superior genotypic performance in given environment. This has been supported by several previous studies where the workers evaluated the genotypes in different environment over

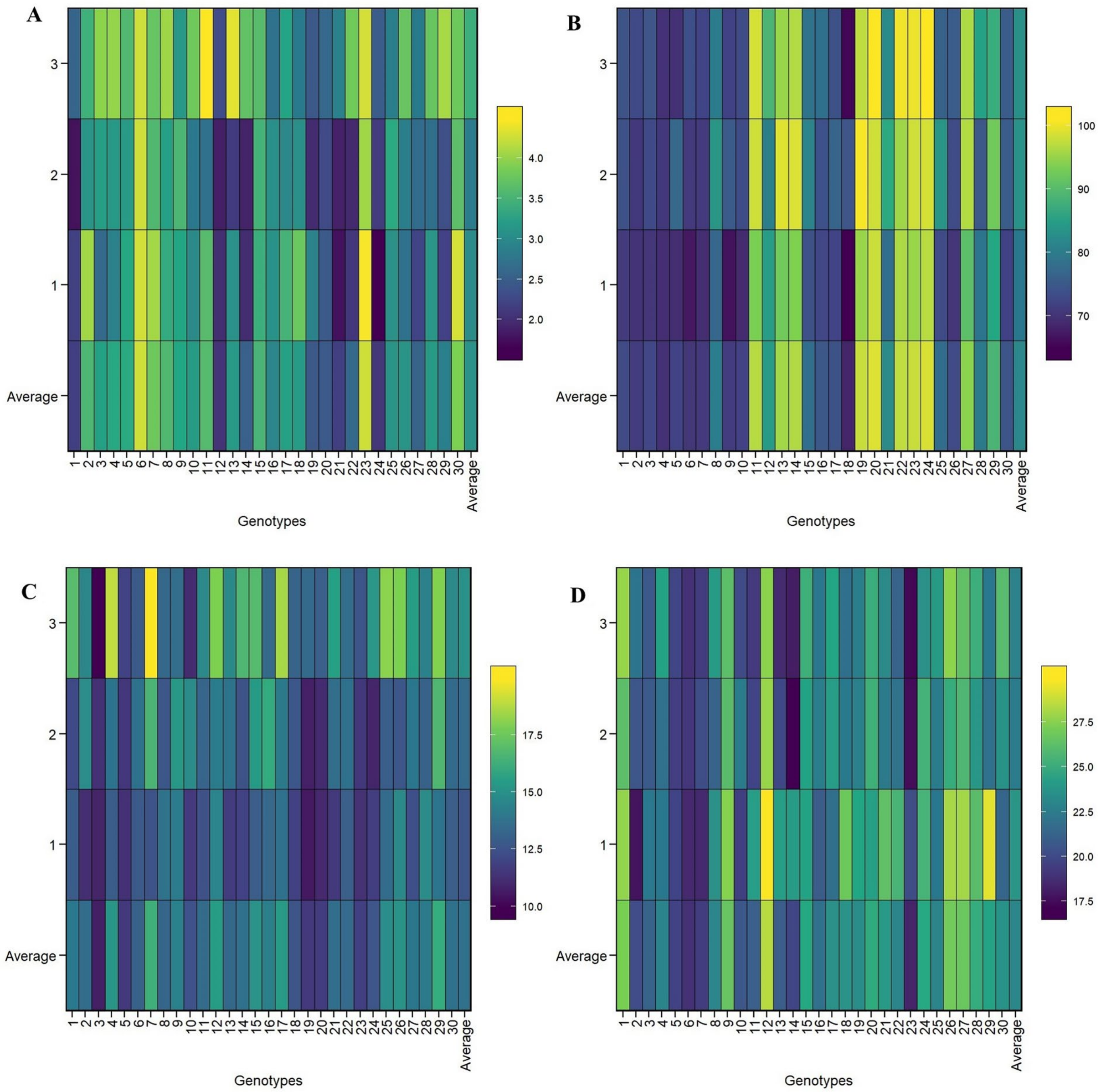

Fig. 1. Heatmap of the trait variation among the evaluated 30 rice genotypes across three environments during Wet-2020. [**A**] Grain yield (tha^{-1}), [**B**] Days to 50% flowering, [**C**] Grain Fe content (PPM) & [**D**] Grain Zn content (PPM).

the years and identified stable high yield, Zn and Fe genotypes[16,26,27]. To understand the underlying factors of GEI, advanced multivariate techniques that consider additive main effects of G and E along with multiplicative effects of interactivity as a source of variation[28] can be employed. The GEI can be further partitioned into two Interaction Principal Components (PC). For trait GY, significant contributions from PC1 and PC2 (77.6% and 22.4%, respectively) were observed. Similarly, PC1 and PC2 contributed 90.5% and 9.5%, respectively for trait DTF, 73.5% and 26.5% for trait Fe, respectively while, 86.8% and 13.2% for trait Zn, respectively to the total variation. According to Gauch and Zobel[28] the first two PCs are sufficient for precise analysis of AMMI model. Madhusudhana et al.,[29] also found that first two PCs are significant for grain Zn and Fe content in sorghum crop and accounting for 100% GEI. Similarly, in rice for grain yield the first two PCs accounted for 63.11% and 36.89% of the GEI[30].

AMMI biplot

The principal components (PC1 and PC2) are shown in the biplot, with the abscissa and ordinate axes indicating the AMMI 1 for the PC1 term and trait main effects (Figure-S1). The scores for both the environments and genotypes were plotted against the GY, DTF, Fe and Zn for the environments and genotypes (Figure S1: A, B, C, D). The testing of 30 accessions in three locations enabled us to analyze the GEI for the studied traits using AMMI. Similarities and dissimilarities among the 30 accessions were observed based on the GEI study. AMMI

		GY		DTF		Fe		Zn	
Source	**df**	**MSS**	**Explained SS (%)**	**MSS**	**Explained SS (%)**	**MSS**	**Explained SS (%)**	**MSS**	**Explained SS (%)**
ENV	2	9.09[c]	12.03	438	2.52	14[b]	23.87	45.2[b]	3.57
REP(ENV)	6	0.13[a]		1.04		5.27[a]		2.31	
GEN	29	3.3[c]	63.33	1130[c]	94.42	18.4[c]	44.84	65.8[c]	75.39
GEN:ENV	58	0.642[c]	24.64	18.3[c]	3.06	6.42[c]	31.29	9.18[c]	21.04
PC1	30	0.962	77.60	32.1	90.50	9.13	73.50	15.4	86.80
PC2	28	0.298	22.40	3.61	9.50	3.52	26.50	2.52	13.20
Residuals	174	0.0498		0.727		2.23		2.71	
Total	327	0.604		109		6.06		10.8	

Table 1. Combined ANOVA for studied traits towards total variation among 30 rice genotypes tested at three locations in Wet-2020. a,b and c significance at $p < 0.05$, $p < 0.01$and $p < 0.001$, respectively, df = degrees of freedom, DTF = Days to 50% flowering, GY = Grain yield (tha^{-1}), Fe = grain Fe content in PPM, Zn = grain Zn content in PPM .

biplot demonstrated 51% goodness of fit for YLD and 67.1% for Zn. The PC1 values for the traits observed were 77.6%, 90.5%, 73.5%, and 86.8%. It is apparent that for grain yield (A), genotypes G28, G10, G5 and G17 were closer to the origin, indicating their stability for GY. Although, genotypes G23, G6, and G30 were higher yielder still due to their distant proximity to origin they are highly unstable for GY. For DTF (B), genotypes G8, and G15 had low scores on the first PC1 and were close to the origin and hence they are stable for DTF. Meanwhile, genotypes G18, and G5 were far from the origin. For grain Fe (C), genotypes G27 with higher grain Fe content had low scores on the PC1 and were nearly close to the origin indicating its stability for trait Fe. Meanwhile, genotypes G7, G29, and G17 with higher grain Fe content were placed far from the origin indicating their instability. For the grain Zn (D), genotypes G8, and G19 had low scores on the first PC1 and were closer to the origin. Meanwhile, genotypes G12, G26, G1, G9 and G29 were far from the origin although they have higher grain Zn content. In terms of performance, genotypes on the same parallel line in AMMI 1 had similar performance, while those on the right side of the center of the axis had higher performance than those on the left-hand side. In Fig. 1A, genotypes G6, G23, G30, and G7 located on the right side of the center of the axis had higher yields than genotypes G12, G24, G1, and G21 located on the left-hand side. The AMMI1 biplot has been commonly employed to assess stability[31–34]. Despite its advantages in stability analysis, the AMMI1 biplot may be limited when only one or two IPCAs are used, as it may not fully capture the complex interaction patterns unless these scores are low for the genotype in the first two IPCAs[35].

AMMI2 biplot

The Ammi 2 biplot of 30 rice genotypes evaluated at three different locations is presented in Figure S2. The PC1 scores for both the environments and genotypes were plotted against the PC2 scores for the environments and genotypes, compared to Figure S1 where PC1 was plotted against studied variables. The AMMI 2 revealed the importance of PC2 scores along the PC1 scores in explaining the complexity of GEI, involving significant multi-environments and identifying the adaptation of genotypes. The environmental and genotypic scores of PC1 and PC2 were then used to generate the biplot. In this study, the PC2 values for the traits observed were 22.4%, 9.5%, 26.5%, and 13.2% for traits GY, DTF, Fe and Zn, respectively. Additionally, it was observed that the first two PC interactions accounted for 100% of the G + G × E interaction variation for all the four agronomic traits used for the AMMI 2 biplot. For the trait GY, genotypes G12, G8, G5, and G20 were close to the origin, while G13, G18, G19, G25, G3, G29 and G2 were vertex genotypes. For the trait DTF, genotypes G30, G7, and G10 were close to the origin, while G28, G12, G4, G18, G25, and G9 were vertex genotypes. For trait Fe the genotypes G19, G30, G11, and G20 were near the origin, while G28, G26, G4, G7, and G10 were vertex genotypes. Similarly, for trait Zn, genotypes G9, G5, and G3 were near the origin, while G30, G2, G10, G29, and G14 were vertex genotypes. The genotype positioned at the vertex of the polygon, where an environmental marker also placed, is indicated to yield better and exhibit superior performance in that specific environment. Conversely, a genotype associated with the polygon vertex where no environmental marker is present suggests poorer performance across various environments. Genotypes within the polygon are considered less stable in the environment compared to those at the corners of the polygon[36]. For the trait GY, G18, G2, and G19 had specific adaptability with ENV1, G25 had specific adaptation with ENV2, and G22 had specific adaptation with ENV3. For grain Fe, G3 and G28 showed specific adaptability with ENV1, G10 with ENV2, while, G4 and G7 for ENV3. For grain Zn G29, G14 showed specific adaptability with ENV1, G10 with ENV2 while, G2, G30, G4 and G8 with ENV3.

GGE biplots

Which-won-where and what?

In the GGE biplot, the 'Which-won-where' pattern is evident for the traits GY, DTF, Fe and Zn, with the sum of the first and second PCA axes accounting for 94.37%, 99.71%, 83.49% and 96.93%, respectively of the total GEI variation, as shown in Fig. 2. The genotypes with the vertex designation were found to be the most responsive, as they had the longest distance from the origin in their respective direction, as stated by Yan and Tinker[36]. Whereas, the genotypes positioned inside the polygon closer to the origin are considered to be less responsive to environmental variations[37]. The vertex genotypes, which are the genotypes that are farthest away from the

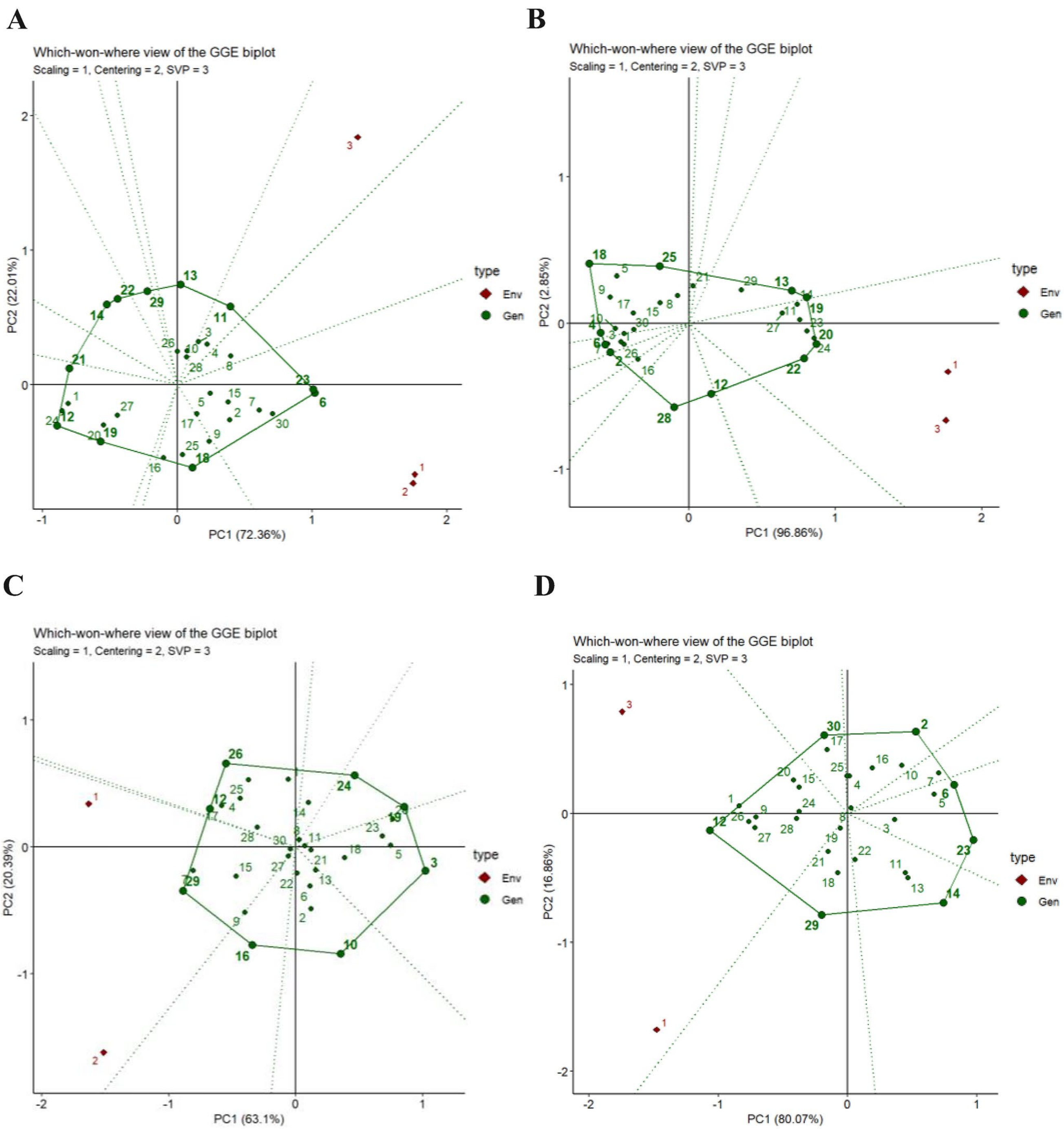

Fig. 2. Which-won-where view of 30 rice genotypes (Green) and three environments (Red) for [**A**.=] Grain yield (tha^{-1}), [**B**] Days to 50% flowering, [**C**] Grain Fe content (PPM) & [**D**] Grain Zn content (PPM) evaluated during Wet- 2020. The ranking genotypes were based on scaling = 1, centering = 2, and singular value partitioning (SVP) = 3.

origin in the direction of the PC1 and PC2 axes, are the most responsive genotypes to the environment in their direction. For trait GY, vertex genotypes include G11, G13, G18, G6, G23, G12, G20, G19, G17, G21, G24, and G22. Vertex genotypes that are not near any environmental indicators are the poorly performing ones. For example, G11 and G13 were the most responsive genotypes in ENV3, while G18, G6, and G23 were the most responsive genotypes in ENV1 and ENV2. In ENV1 and ENV3, the genotypes G20, G12, and G22 showed the greatest response to trait DTF, while for grain Fe, G12 and G26 were the most responsive genotypes in ENV1 and G16 and G29 were the most responsive in ENV2. Similarly, for grain Zn, G12 and G29 were the most responsive genotypes in ENV1, and G30 was the most responsive in ENV3.

Best linear unbiased prediction-based stability and adaptability

Overall performance, variance components, and predicted means

BLUPs values are estimated for the GEI effects generated by an LMM method for the studied traits variation present in 30 rice genotypes evaluated across three environments in Wet-2020 (Figure-S3). The estimated variance components and genetic parameters are mentioned in Table 2. Low estimates of broad-sense heritability were observed for trait Fe (28.2), GY (54.4%) followed by Zn (56.4%) whereas, for trait DTF high broad sense heritability was observed. The genotypic accuracy of selection (As), which measures the correlation between predicted and observed values was of 0.89, 0.99, 0.81, 0.93 for the traits GY, DTF, Fe and Zn, respectively. There is high significant correlation between genotypic values across environments (r_{ge}) for trait DTF (0.89) followed by GY (0.789) and grain Zn (0.44) while, trait Fe exhibited lowest r_{ge} (0.385). This has further demonstrated by high σ_{ge}/σ_g ratio (1.053) for trait Fe (Table 2).

The HMGV, RPGV, and HMRPGV (Table S2), were evaluated using REML/BLUP-derived values for the traits GY (Table S2a), DTF (Table S2b), Fe (Table S2c) and Zn (Table S2d) to identify stable genotypes for these traits. These methods are effective in identifying genotypic effects and enable the ranking of genotypes based on their performance, providing valuable insights into their stability across different conditions in Wheat[31], Finger millet[32], Winter Lentils[33], Pearl millet[38], and Common bean[39],. According to the stability indices, such as HMGV, RPGV, and HMRPGV, the genotype G7 was identified as being very stable, early, high-yielding and high Fe genotype.

Simultaneous selection for mean performance and stability in 30 rice genotypes

Y × WAASB biplot

Olivoto et al.[18] proposed the Y × WAASB biplot, which represents four quadrants, for interpreting mean and stability. Genotypes or environments falling within quadrant I are characterized as unstable with high discrimination ability but exhibit low productivity below the grand mean. Quadrant II encompasses unstable genotypes with productivity surpassing the grand mean, requiring special attention due to their high response variable values, strong discrimination ability, and high productivity. Quadrant III includes stable genotypes with low productivity attributed to low WAASB values; a lower WAASB value signifies greater stability. Environments in this quadrant are regarded as poorly productive with low discrimination ability. Genotypes within quadrant IV are deemed highly productive and broadly adapted, showcasing both a high magnitude of the response variable and superior stability performance indicated by lower WAASB values. According to the Y × WAASB biplot (Fig. 3), for trait GY, 4 genotypes, ENV1 and ENV2 are in quandrant1 indicating greater instability of genotypes with low productivity, whereas 7 genotypes including ENV2 are in the second quadrant featured with poor stability and higher seed yield which advised for paying extra attention to the environment in order to increase seed yield. While, 7 genotypes present in quadrant III indicating low productivity and greater stability. The quadrant IV contains 12 genotypes among these G23, G6, G7, G15, G5, and G8 demonstrated higher yield with higher stability due to lower WAASB value. For trait DTF, early flowering with higher stability is desired. The 13 genotypes present in quadrant III demonstrated early flowering and higher stability. For trait grain Fe, 5 genotypes (G27, G28, G15, G12, G29) demonstrated to have high Fe and higher stability. For trait grain Zn, 11 genotypes (G19, G20, G21, G15, G24, G28, G27, G9, G26, G1, G12) present in quadrant IV exhibited lower WAASB value indicating their higher stability for high grain Zn. Y × WAASB biplot were used by Madhusudhana et al.,[29] to identify stable high Zn sorghum lines and by Memon et al.,[34] to select stable high yielding castor genotypes. The main benefit of the WAASB biplot over the AMMI biplot lies in its ability to incorporate all IPCA

Parameters	GY	DTF	Fe	Zn
σ^2_g	0.295 (54.4%)	123 (94.6)	1.33 (26.8%)	6.29 (56.2%)
σ^2_{ge}	0.197 (36.3%)	5.87(4.5)	1.4(28.2%)	2.16 (19.3%)
σ^2_e	0.0498 (9.2%)	0.727 (0.56%)	2.23 (45%)	2.71 (24.2%)
Phenotypic variance	0.542	130	4.96	11.2
Heritability (broad-sense)	0.544	0.949	0.268	0.564
R^2_{gei}	0.364	0.0453	0.282	0.194
h^2_{mg}	0.805	0.984	0.65	0.86
As	0.897	0.992	0.807	0.928
r_{ge}	0.798	0.89	0.385	0.444
CV_g (%)	17.5	13.5	8.41	10.8
CV_e (%)	7.19	1.04	10.9	7.12
CV_g / CV_e ratio	2.43	13	0.771	1.52
σ_{ge}/σ_g ratio	0.668	0.048	1.053	0.343

Table 2. Estimated variance components and genetic parameters for studied traits of 30 rice genotypes evaluated in 3 environments in Wet-2020. $\boldsymbol{\sigma^2_g}$: genotypic variance; $\boldsymbol{\sigma^2_{ge}}$, variance of G × E interaction; $\boldsymbol{\sigma^2_e}$,residual variance; R^2_{gei}, coefficient of determination for the genotype-vs-environment interaction effects; h^2_{mg}, heritability of the genotypic mean; As, accuracy of genotype selection; r_{ge}, correlation between genotypic values across environments; CVg (%), genotypic coefficient of variation; CVe (%), residual coefficient of variation.

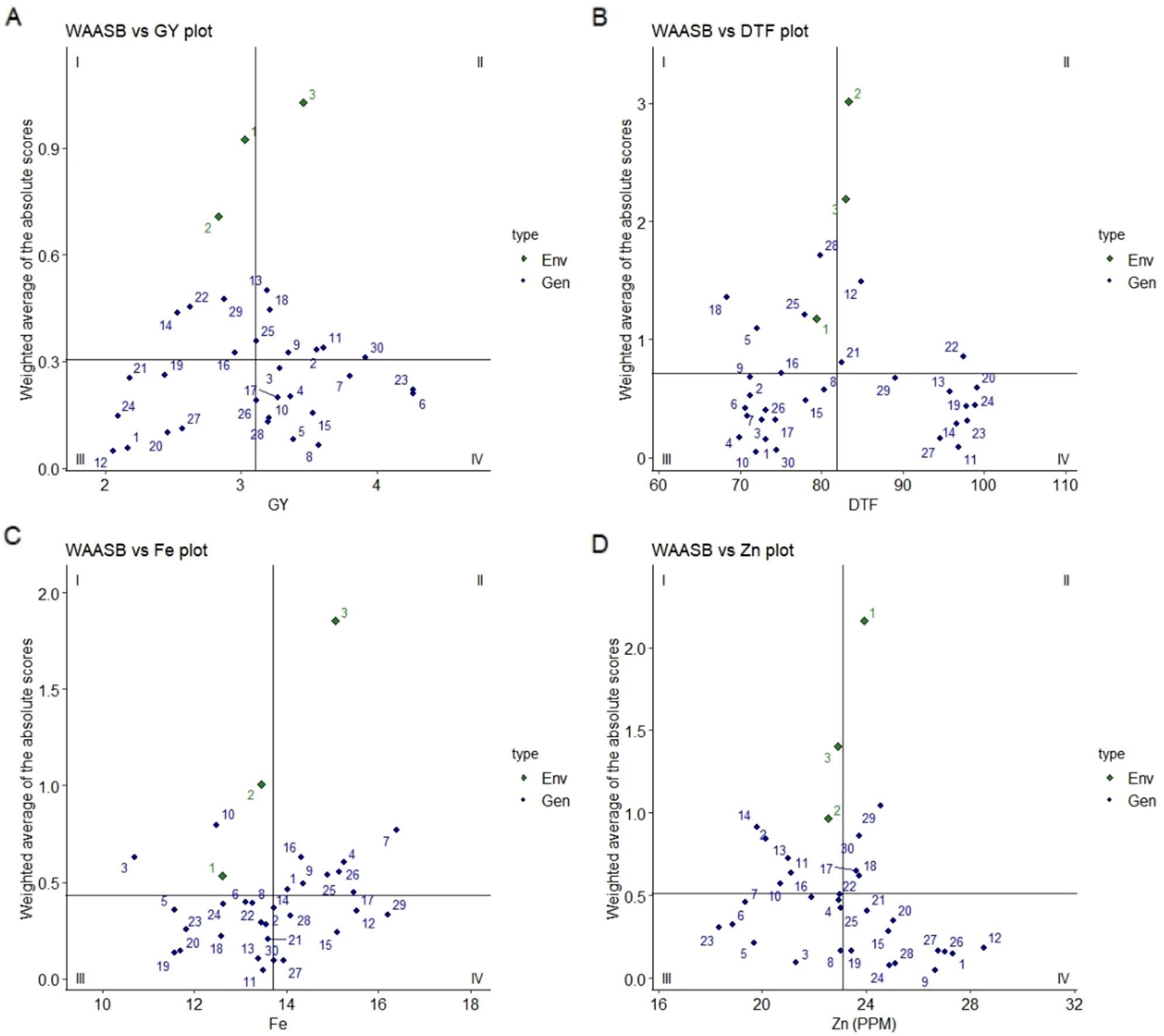

Fig. 3. Biplot of the grain yield vs. weighted average of absolute scores for the best linear unbiased predictions of the GEI (WAASB) of 30 rice genotypes evaluated in 3 environments in WET-2020.

axes, enabling the consideration of GEI patterns beyond just IPCA1 when ranking genotypes[21,34]. Additionally, WAASB offers more reliable outcomes by minimizing the contribution of deviations to the later axes[21].

WAASBY index

The performance of 30 rice genotypes was evaluated based on estimated values of WAASBY, as shown in Figure S4. The genotypes in green circles were the top performers, while the red circles represented those with below average performance. The bottom genotypes were the poorest performers (Figure-S4). For trait GY, G8 had the highest mean performance among the tested genotypes in the three locations, followed by G6, G23, G5, and G15, and had higher estimated values of WAASBY. Similarly, for grain Fe, G29 had the highest mean performance, followed by G15, G27, G11, and G30. For grain Zn, G12 had the highest mean performance, followed by G9, G1, G26, and G27, while G14 had the poorest performance. G15, G28, and G27 were the top performers for grain yield, grain Fe, and grain Zn. The genotype with the highest WAASBY values would be considered as highest yielding and stable performance[21]. The WAASB × Y biplot (Figure S4) effectively combines stability and productivity in a two-dimensional plot while accounting for all model IPCAs. This approach shows promise for identifying high-yielding genotypes and for genotype adaptation in future research[21,30].

Multi trait selection indices (MTSI, MGIDI, and FAI-BLUP)

Simultaneous selection of genotypes possessing multiple desirable traits based on MTSI, MGIDI and FAI BLUP was carried out for traits GY, DTF, Fe and Zn. The four principal components were categorized into PC1, PC2, PC3 and PC4 with respective eigen value and the represented variation by calculated through MTSI, MGIDI

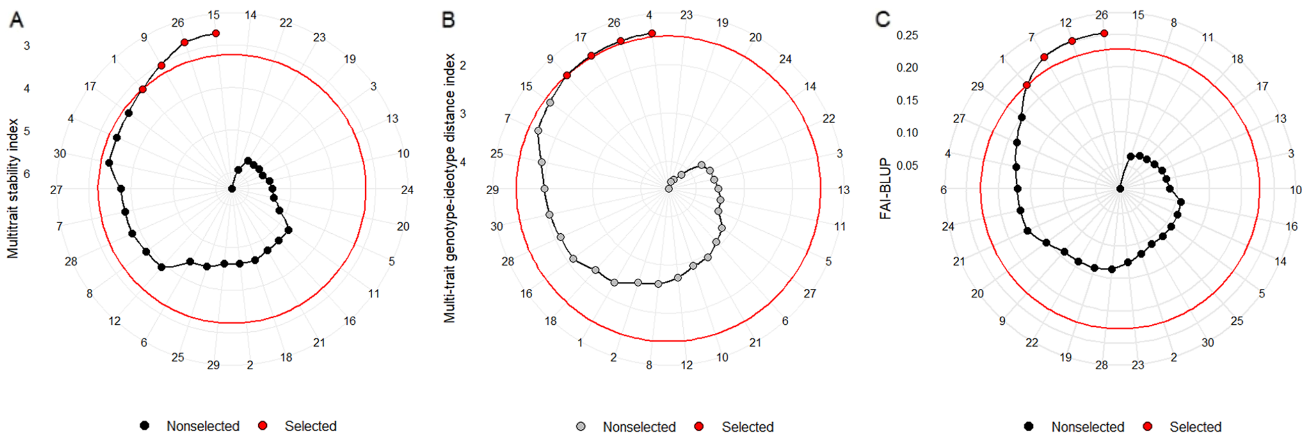

Fig. 4. Genotype ranking and selected genotypes for the MTSI index [**A**], MGIDI [**B**], and FAI BLUP [**C**] considering a selection intensity of 20%.

VAR	Goal	MTSI				MGIDI				FAIBLUP			
		Xo	Xs	SD(%)	SG(%)	Xo	Xs	SD(%)	SG(%)	Xo	Xs	SD(%)	SG(%)
GY	increase	3.10	3.13	0.86	0.69	3.10	3.34	7.73	6.23	3.10	2.83	-8.84	-7.01
DTF	decrease	81.90	73.30	-10.50	-10.30	81.90	73.10	-10.80	-10.57	81.90	80.90	-1.13	-1.20
Fe	increase	13.70	14.90	8.66	5.63	13.70	14.70	7.50	4.74	13.70	14.70	7.13	4.74
Zn	increase	23.10	25.40	9.92	8.53	23.10	23.90	3.60	2.98	23.10	25.20	9.21	7.82

Table 3. Estimation of selection differential, selection gain and heritability based on MTSI, MGIDI, and FAI-BLUP for selected traits in Rice. GY = Grain yield (tha^{-1}); DTF = Days to 50% flowering; Fe = Grain Fe content (PPM); Zn = Grain Zn content (PPM); Xo = mean of genotypes; Xs = mean of selected genotypes (G01, G09, G26, and G15); SD = selection differential; SG = selection gain.

and FAI-BLUP analysis were mentioned in supplementary Table-S3. The MTSI values were computed in which lowest MTSI value exhibited by genotype G15 (2.71) and highest value was recorded by G14 (6.38). The four genotypes of rice were identified according to their lowest MTSI values that included G15 (2.71), G26 (2.77), G9 (3.04), and G1(3.23) (Fig. 4). The MGIDI values were computed and low MGIDI value is obtained for genotype G4 (1.33) and highest for G23 (4.57). The selected genotype as per MGIDI value were G4 (1.33), G26 (1.36), G17 (1.38), G9 (1.41). Through FAI-BLUP analysis the selected genotypes were G26, G12, G7 and G1 (Fig. 4). The mean of all the genotypes (Xo), mean of all the selected genotypes (Xs), selection differential (SD %), selection gain (SG %) were computed and mentioned in (Table 3). The results demonstrated that the highest positive SD (%) and SG (%) were recorded for trait Zn followed by Fe and lowest for trait GY for MTSI index. SG was negative for DTF as the early maturing rice variety is targeted for selection. For MGIDI index highest positive GY followed by Fe and Zn. However, for FAI_BLUP analysis negative selection gain reported for GY (Table 3). According to Olivoto and Nardino[25] MGIDI is more powerful in selection of superior genotype based on multi-environment data. Based on the coincidence index, only one genotype (DRR Dhan 45) was found stable with high GY, Fe &Zn (Figure-S5). Genotype DRR Dhan 45 is semidwarf, medium maturity high zinc rice variety released through All India Coordinated Research Project on Rice(AICRPR)[40]. The analysis of present study recommended for use of DRR Dhan 45 as donor for high Zn and yield in further breeding program. While genotype (G15, G9, G7, G4) common between MTSI & MGIDI, G1 common between MTSI &FAI-BLUP, G7 common between MGIDI &FAI-BLUP have been nominated in AICRPR trials during Wet-2023 for all India testing and may be used as parent in future breeding program. Phenotypic performance of selected genotypes is shown in Fig. 5.

Materials & methods
Plant materials
The experimental material consisted 30 entries including 22 advanced breeding lines, 3 yield checks, 3 Zn checks and 2 local checks received under Harvest-Plus Project supported by IFPRI (Washington D.C.) & CIAT (Columbia) running in collaboration with IRRI, Philippines. The list of genotypes used in the study is provided in Table 4.

Experimental site
The field experiment was conducted during Wet-2020 at three locations in Eastern Uttar Pradesh to evaluate 30 rice genotypes and understand the genotype by environment interaction playing role in expression of days to

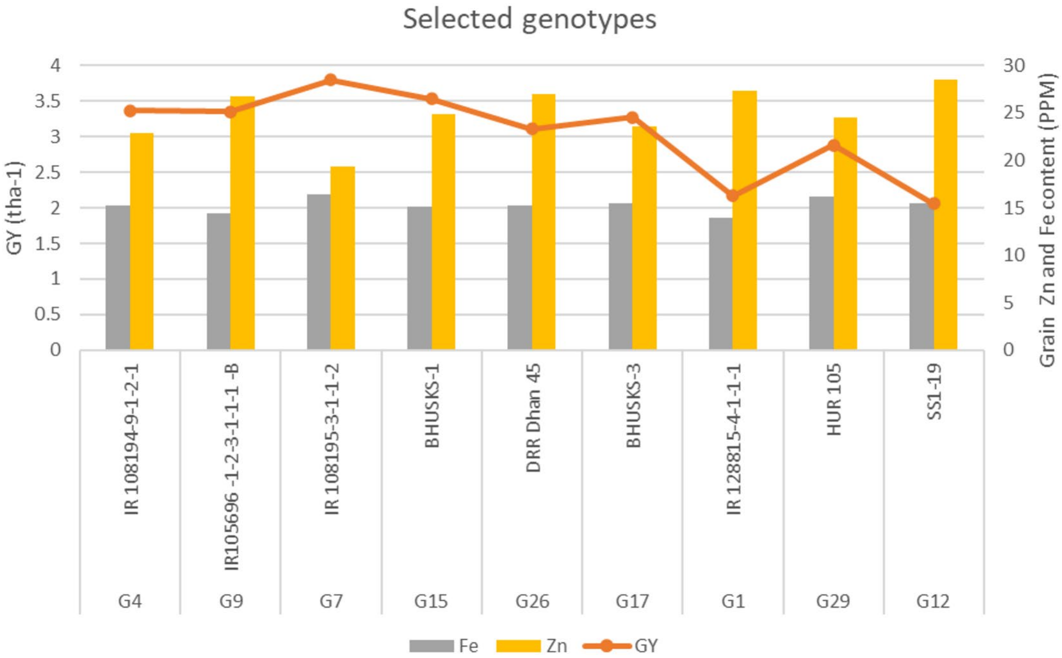

Fig. 5. Phenotypic performance of Selected genotypes as per simultaneous selection indices considering a selection intensity of 20%.

50% maturity, grain yield, grain Zn and grain Fe. The three locations consisted of 1) Agricultural Research Farm, Banaras Hindu University, Varanasi (ENV1), 2) farmer's field at Bhikharipur, Varanasi (ENV2) and 3) farmer's field at Rampur, Chunar, Mirzapur (ENV3). Soil Fe and Zn content of ENV 1 were 49.54 PPM and 1.4 PPM, respectively while, ENV2 were 56.26 PPM and 3.24 PPM, respectively and ENV3 were 26.5 PPM and 1.92 PPM, respectively. The experiment was laid out in Randomized complete block design with 2 replications. The plot size of 3 m^2 was maintained for each accession with spacing of 15 X 20 cm between plant to plant and row to row, respectively. Recommended agronomic practices were adopted to raise a healthy crop. The recommended dose of fertilizers was applied at the rate of 120 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare. Intercultural practice like weeding was done at regular intervals with one spray of weedicide and twice hand weeding. The field trial experiment on evaluation of Rice genotypes at three different locations in Eastern Uttar Pradesh, India were conducted in compliance with our institutional, national, and international guidelines and legislation.

Variables measured

The genotypes were evaluated for 4 different traits viz., days to 50% flowering (DTF), grain yield in tha^{-1}(GY), grain Fe in PPM (Fe) and grain Zinc in PPM (Zn)). For trait DTF observations were recorded on plot basis for each replication and environment. For trait GY, the observations were recorded on 5 randomly chosen plants from each replication and environment as per the standard procedure. For estimation of grain Zn and Fe, 10 g brown rice grain sample from each replication of each environment were taken in replicate and analyzed with energy dispersive X-ray fluorescence spectrophotometer (ED—XRF) instrument available at IRRI, South Asia Hub, Hyderabad. The data were recorded and subjected to statistical analysis using mean values.

Statistical analysis

The data is subjected to statistical analysis using statistical software R Studio version 4.1.1[41]. The variance components and genetic parameters related to genotype-by-environment (G × E) interactions were estimated using a linear mixed model (LMM)[18]. These parameters included the coefficient of determination (R^2_{gei}) for G × E effects, heritability of the genotypic mean ($h2_{mg}$), selection accuracy (As), correlation of genotypic values across environments (r_{ge}), genotypic coefficient of variation (CVg (%)), and residual coefficient of variation (CVe (%))[42].

AMMI analysis

The Additive Main Effects and Multiplicative Interaction (AMMI) model integrates Analysis of Variance (ANOVA) and Principal Component Analysis (PCA) to evaluate genotype-by-environment interactions in multi-environment trials (METs). This approach helps in understanding both the main effects (genotypic and environmental) and their interaction patterns. The AMMI analysis was conducted by applying the following model to the studied traits, as described by Gauch et al. (1996)[28].

ENTRY	GENOTYPE	Designation	Type
1	G1	IR 128,815–4-1–1-1	Advanced breeding line
2	G2	IR 128,764–236-1–1-1 -B	Advanced breeding line
3	G3	IR105695 -1–1-1-B-1	Advanced breeding line
4	G4	IR 108,194–9-1–2-1	Advanced breeding line
5	G5	IR105692 -1–1-3-B-1	Advanced breeding line
6	G6	IR 108,195–3-1–4	Advanced breeding line
7	G7	IR 108,195–3-1–1-2	Advanced breeding line
8	G8	IR 108,198–1-1–1-2	Advanced breeding line
9	G9	IR105696 -1–2-3–1-1–1 -B	Advanced breeding line
10	G10	IR 108,194–9-1–1-2	Advanced breeding line
11	G11	SS1-12	Advanced breeding line
12	G12	SS1-19	Advanced breeding line
13	G13	SS1-21	Advanced breeding line
14	G14	SS1-4	Advanced breeding line
15	G15	BHUSKS-1	Advanced breeding line
16	G16	BHUSKS-2	Advanced breeding line
17	G17	BHUSKS-3	Advanced breeding line
18	G18	BHUSKS-4	Advanced breeding line
19	G19	SM-1	Advanced breeding line
20	G20	SM-2	Advanced breeding line
21	G21	SM-3	Advanced breeding line
22	G22	SM-4	Advanced breeding line
23	G23	Swarna Sub1	Yield check
24	G24	Samba Mahsuri	Yield check
25	G25	MTU1010	Yield check
26	G26	DRR Dhan 45	Zinc check
27	G27	DRR Dhan 48	Zinc check
28	G28	BRR Dhan 64	Zinc check
29	G29	HUR 105	Local check
30	G30	HUR 1309	Local check

Table 4. List of Genotypes evaluated in Wet-2020.

$$Y_{ij} = \mu + g_i + e_j + \sum_{(k=1)}^{n} \lambda_k a_{ik} \gamma_{jk} + \theta_{ij}$$

where Y_{ij} = mean yield of the genotype i (i = 1, 2, …, 30) in the environment j (j = 1, 2, 3);

μ = general mean, g_i = i^{th} genotypic effect; e_j = j^{th} environment effect; λ_k = eigenvalue of the PC axis k; α_{ik} and γ_{jk} = i^{th} genotype in j^{th} environment PCA scores for the PCA axis k; θij = residual.

GGE biplot

The Site Regression (SREG) genotype-environment interaction (GGEI) biplot model is a powerful tool for analyzing multi-environment trial (MET) data in plant breeding[19]. This approach effectively visualizes genotype performance and stability across different environments. The GGE biplot was generated by plotting the first two principal components (PC1 and PC2), which capture the majority of the variation in the dataset. These biplots were constructed using environment-centered data and the symmetric singular value partitioning (SVP) method, following the model as per below:

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

where, Y_{ij} = mean of i^{th} genotype in j^{th} environment, μ = Grand mean, β_j = main effect of j^{th} environment, λ_1 and λ_2 = special quantities for PC1 and PC2, respectively, ξ_{i1} and ξ_{i2} = special vectors of genotypes, and η_{j1} and η_{j2} = environmental vectors of PC1 and PC2, respectively, and ε_{ij} = residual for the i^{th} genotype in j^{th} environment.

The which-won-where pattern of GGE biplot is constructed to analyze the genotypes capacity to adapt to specific environments across testing locations[33,34].

Estimation of BLUP and BLUP-based stability indices

Best Linear Unbiased Prediction (BLUP) is a statistical approach used to estimate random effects in mixed models while minimizing the mean squared error. The BLUP value has been estimated by using the formula provided in the study of Piepho & Möhring (2007)[43]

$$BLUPi = \mu + \hat{g}_i$$

where,

$$Genotypic\,effect(\hat{g}_i) = h_g^2(\overline{Y}_i - \overline{Y})$$

where $h^2_g = (\sigma^2_{ge} + e\ \sigma^2_g) / (\sigma^2_{ge} + \sigma^2_e + e\ \sigma^2_g)$ is the shrinkage effect for the genotype effect.

σ^2_g : genotypic variance; σ^2_{ge}, variance of G × E interaction; σ^2_e, residual variance.

Colombari Filho et al. (2013)[44] demonstrated the application of three BLUP-based indices for selecting genotypes that combine high performance with stability. These indices include the Harmonic Mean of Genotypic Values (HMGV), Relative Performance of Genotypic Values (RPGV), and Harmonic Mean of Relative Performance of Genotypic Values (HMRPGV). Each of these indices is derived from BLUP estimates and provides valuable insights into genotype performance. Genotypes with higher HMGV and HMRPGV values are considered superior, showing high yield potential and stability across environments[44]. The computation of HMGV, RPGV, and HMRPGV indices are carried out using below equations:

$$\mathrm{HMGV_i} = \frac{n}{\sum_{j=1}^{n}(\frac{1}{\mathrm{GV}_{ij}})}$$

$$\mathrm{RPGV_i} = \frac{1}{n}[\frac{(\sum_{j=1}^{n}\mathrm{GV}_{ij})}{\mathrm{M}_j}]$$

$$\mathrm{HMRPGV_i} = \frac{n}{\sum_{j=1}^{n}(\frac{1}{\mathrm{RPGV}_{ij}})}$$

where, n is the number of environment (n = 3); GV_{ij} is the genetic value of i^{th} genotype in j^{th} environment and calculated using the formula.

$$\mathrm{GV_{ij}} = \mu_\mathrm{j} + \mathrm{g_i} + \mathrm{ge_{ij}}$$

Where, μ_j is the average of j^{th} environment, g_i is the BLUP value of i^{th} genotype, and ge_{ij} is the BLUP value of the interaction between i^{th} genotype and j^{th} environment; M_j is the mean grain yield in the j^{th} environment[44].

WAASB (weighted average of absolute scores from the singular value decomposition of the matrix of best linear unbiased predictors)

The Weighted Average of Absolute Scores of Biplots (WAASB) index, proposed by Olivoto et al. (2019)[21], was used to assess genotype stability. This index quantifies the deviation of a genotype from the overall mean performance across multiple environments, with lower WAASB values indicating greater stability. It is computed as per below equation, ensuring that genotypes with minimal variation across environments are prioritized for stability.

$$\mathrm{WAAS}B_\mathrm{i} = \frac{\sum_{\mathrm{k=1}}^{\mathrm{P}}|\mathrm{PC_{ik}} \times \mathrm{EP_k}|}{\sum_{\mathrm{k=1}}^{\mathrm{P}}\mathrm{EP_k}}$$

where $WAASB_i$ is the weighted average of absolute scores of the i^{th} genotype or environment; PC_{ik} = absolute score of the i^{th} genotype or environment in the k^{th} IPC; and EP_k = magnitude of the variance explained by the k^{th} IPC.

To simultaneously select for both yield and stability, the Weighted Average of Absolute Scores of Biplots for Yield (WAASBY) index was applied, following below mentioned equation (Olivoto et al., 2019)[21]. WAASBY allows for the allocation of weights to yield and stability according to their relative importance in selection. Unlike WAASB, where lower values indicate stability, WAASBY prioritizes genotypes with higher scores, ranking them based on their combined yield potential and stability.

$$\mathrm{WAASBY}_i = \frac{(\mathrm{rG}_i \times \theta_Y) + (\mathrm{rW}_i \times \theta_s)}{\theta_Y + \theta_s}\mathrm{EQ} - 8$$

where, θ_Y and θ_S = response variable's weights; rW_i and rG_i = rescaled values of 0–100 for WAASB and response variable, respectively.

Multi-trait stability index (MTSI)

The Multi-Trait Stability Index (MTSI) was calculated using Euclidean distance from an ideal genotype based on factor analysis (FA) (Olivoto et al., 2019)[21]. The lowest MTSI score indicates the most stable and high-performing genotype. A 15% selection intensity was applied, and MTSI scores were plotted to differentiate selected and non-selected genotypes.

$$\mathrm{MTSI} = \left[\sum_{j=1}^{f} (\mathrm{M}_{ij} - \mathrm{M}_{j})^{2}\right]^{0.5}$$

where MTSI = multi-trait selection Index M_j = ideotype's j^{th} score; M_{ij} = i^{th} genotype's j^{th} score.

Multi-trait genotypic-ideotype distance index (MGIDI)
The Multi-Trait Genotypic-Ideotype Distance Index (MGIDI) ranks genotypes based on their proximity to an ideal ideotype, integrating multiple traits using factor analysis (FA)[25]. Genotypes with lower MGIDI scores are closer to the ideotype, making them ideal selections[25]. The MGIDI score for each genotype is computed as:

$$MGIDI_i = \left[\sum_{j=1}^{f} (\gamma_{ij} - \gamma_j)^2\right]^{0.5}$$

where, MGIDI_i = multi-trait genotype-ideotype distance index for the i^{th} treatment; γ_{ij} = score of the i^{th} treatment in the j^{th} factor ($i = 1,2,\ldots,t$; $j = 1,2,\ldots,f$), being t and f the number of treatments and factors, respectively; and γ_j = j^{th} score of the ideal treatment.

Multi-trait index based on factor analysis and genotype-ideotype distance (FAI-BLUP index)
The FAI-BLUP (Factor Analysis and Ideotype-Based BLUP) Index ranks genotypes based on their distance from an ideotype, converting this distance into a spatial probability to facilitate selection[45]. This approach integrates Factor Analysis (FA) and Best Linear Unbiased Prediction (BLUP) to enhance genotype ranking and computed using following equation:

$$P_{ij} = \frac{\frac{1}{d_{ij}}}{\sum_{i=1;j=1}^{i=n;j=m} \frac{1}{d_{ij}}}$$

where P_{ij} = probability of the i^{th} genotype ($i = 1, 2, \ldots, n$) to be similar to the j^{th} ideotype ($j = 1, 2, \ldots, m$), and d^{ij} = genotype-ideotype distance from the i^{th} genotype to the j^{th} ideotype—based on standardized average Euclidean distance.

All the analysis were carried out using METAN package version 1.18.0 [42] in R Studio.

Conclusion

The primary objective of this comprehensive multi-location study is to assess rice (*Oryza sativa* L.) genotypes based on their mean performance across a diverse range of environments, aiming to identify superior early genotypes for higher grain yield, Zn and Fe. The rice genotypes subjected to a MET offer valuable insights into genotype adaptability and stability under varying environmental conditions. It is essential to evaluate genotype susceptibility to GEI before proposing a genotype for AICRIP nomination and release as variety. With the help of multivariate statistical analyses, we have reported identification of ideal superior genotype with higher grain yield coupled with higher grain Zn and/or Fe. Genotype G7 exhibited early flowering (70.9 days) with higher mean yield (3.8 tha^{-1}) and higher grain Fe content (16.4 PPM) whereas, Genotype G15 recorded early DTF (78 days) with higher mean yield (3.53 tha^{-1}), higher grain Fe content (15.1 PPM) and higher grain Zn content (24.3 PPM). Both these genotypes exhibited higher WAASBY values. Stability indices, such as HMGV, RPGV, and HMRPGV also demonstrated good ranking for both these genotypes indicating their stability for higher grain yield, Zn and Fe. Coincidence index among MTSI and MGIDI also identified G15 as common genotype while Coincidence index among MGIDI and FAI-BLUP reported G7 to be highly stable for these traits. Hence, among the investigated genotypes, G7, G9 and G15 consistently performed well across all test locations, emerging as the top choices due to their favorable mean performance, stability, high grain yield, grain Zn and Fe content. The findings suggest that targeted breeding efforts can enhance genetic gain for these traits, and the ideal ideotype genotypes identified in this study could be recommended for AICRIP nominations and also for further use as parent in biofortification breeding programme.

Data availability

All data supporting the findings in this study can be obtained from the corresponding author upon reasonable request.

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Author contributions
DKS, SKS, VKS, and AK designed the study. DKS, AS and SKS participated in the experiment. AS drafted the original manuscript. SKS, VKS, and AK provided constructive suggestions. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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