Genetic gain and selection of stable genotypes in high zinc rice using AMMI and BLUP based stability methods

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ABSTRACT

Rice is the staple food of almost half of the world's population, impacting nutrition especially in children, pregnant women, and nursing mothers. Because the traits were quantitatively inherited, they are affected by changes in location and year. A RBD with three replications was used to identify superior and stable high-zinc rice genotypes in Uttar Pradesh, India. Grain zinc content (GZC) is negatively correlated with grain yield using genetic association study. There was a significant G × E interaction (GEI) and V16 and V21 for GYP and V9, V2 and V10 for GZC were identified as stable based on the AMMI model and bi-plot. V11, V5, V21 for grain yield per plant (GYP) and for GZC, V14, and V10 are found to be stable and common in all AMMI stability parameters. V6, V13 and V5 for GYP and V10, V8 and V2 for GZC were identified as stable based on the mean vs. WAASB bi-plot. V21 for GYP and V4 for GZC was the highest yielder and widely adaptable based on WAASBY scores. V13 for GYP and V1 for GZC were all-time winners. V13 and V1 have the highest predicted mean for GYP and GZC, respectively, based on BLUP. V6, V21and V13 were identified as stable and selected based on the multi-trait stability index (MTSI). These selected genotypes selected through BLUP-based stability methods, MTSI, and strength and weakness plots make it easier to evaluate and select genotypes for varietal recommendations and future Zn-fortified rice breeding studies.

Keywords: GEI; High zinc rice; MTSI; Stable genotype

INTRODUCTION

Rice (Oryza sativa L.) is the one of the leading cereal food grain crop in the world and supplying two-thirds of total calories for more than 3 billion people and one-third of daily calories for roughly 1.5 billion people in Latin America and Africa (Anon., 2018). Rice is linked with life for more than half of the human race and has influenced the cultures, cuisines, and economics of hundreds of millions of people worldwide. Rice is abundant in carbs (70–80%), proteins (7–10%), lipids and fats (1%) (Yang et al., 2019), key micronutrients and vitamins (thiamin, niacin, riboflavin, pantothenic acid and vitamin B6), and minerals (calcium, magnesium, zinc, iron, phosphorus, potassium, and manganese) (USDA, 1950). Rice might be genetically modified to include more essential nutrients and bioactive chemicals, improving human health and preventing chronic diseases. Rice is a great target cereal crop for hidden hunger because it can be bio-fortified to increase nutritional density, which is sustainable for individuals who eat rice and have limited access to other meals. Over 40 crores children (below five years) die each year as a result of zinc deficiency, which affects about 18% of the global population (Hefferon, 2019). These are the consequences of Zn's different actions. An adequate quantity of Zn (together with iron) is essential to regulate Fe absorption in the intestines and to cure IDA (Graham et al., 2012). Human growth, immunological, reproductive, neurobehavioral, and sensory functioning all need Zn. As the only element involved in all six enzyme groups, Zn is required for activating over 300 proteins and enzymes (such as zinc finger proteins) (Levenson and Morris, 2011). Rice may help fight hidden hunger by increasing its zinc content via traditional breeding or

genetic modification. Conversely, genotype x environment interactions (GEI) is a major component in plant sciences and therefore, the introduction of new stable crop varieties. According to Dixon and Nukenine (1997) and Manimaran and Prakash (2018), individual genotypes of rice cultivars are sensitive to changes in soil, climate (rainfall, wind, light, humidity, temperature, etc.), and biotic factors. When evaluating adaptation, the GEI must be taken into account. It takes a lot of study to increase production because yield is a complex variable influenced by many genes and environmental factors (Nyadanu and Dikera, 2014). In a number of circumstances, using stable cultivars of rice may increase yield and mineral content. Plant breeders would benefit from discovering the consistently highest yielding crop types and zinc-rich rice varieties for a particular environment because yield estimations based just on genotype (G) and environment (E) impacts are low.

The two methods for studying GEI are regression analysis (Finlay and Wilkinson, 1963) and the linear regression model (Eberhart and Russell, 1966). According to Eberhart and Russell (1966) and Perkins and Jinks (1968), the regression model has been widely employed because of its range of applications and simplicity. Since linear regression uses E means, which are often poor predictors of the environment, the fitted lines often only show a small part of the whole GEI (Zobel et al., 1988). The principal component analysis (PCA) is a multiplicative method that lacks sources of variation for additive G or E major effects and fails to appropriately evaluate interactions. ANOVA (analysis of variance) is a model that quickly divides the entire sum of squares into the environment main effect, genotype main effect, and the GEI effect; therefore, it is sometimes employed even when there is sufficient knowledge about the structure of the GEI (Shinde et al., 2002). A genotype's stability to environmental or seasonal variation may be estimated using GEI effects. AMMI is likely the most contemporary and widely utilised approach to GEI analysis to detect stable variations and the overall ranking of crop genotypes across places and seasons. It calculates the net GEI effect of individual crop genotypes and divides the GEI into environment-related interaction effects (Vijayakumar et al., 2001). So that genotypes with higher yield potential may be selected more effectively, the AMMI technique combines traditional ANOVA for additive effects with PCA for multiplicative structures within interactions. In multi-location genotype yield investigations, this strategy is preferable (Gauch, 1993). The AMMI-mediated research may help choose cultivars, especially via mega-environment assessment, which determines the most suitable cultivar for each sub-region. The results of the AMMI study are bi-plot graphs that show

markers from both environments and genotypes (Ebdon and Gauch, 2002). This feature allows you to see E and G on the same scatter plot, enabling you to make inferences about their relationships.

Even though AMMI and GGE bi-plots are the best tools for comparing average grain yield and genotype stability, they lack precise numerical data. BLUP is a technique for computing the simultaneous selection index (SSI), which helps choose high-yielding and stable crops simultaneously. The WAASB (weighted average of absolute scores from singular value decomposition of the matrix of BLUPs) is a quantitative stability index based on a linear mixed model. It was developed to analyse GEI using bi-plots and identify stable genetic variants (Olivoto and Lúcio, 2020; Olivoto et al., 2019a). The MTSI Index is a selection approach that enables simultaneous selection based on the performance outcomes of multiple traits and crop stability (Olivoto et al., 2019b). If the investigation comprises many attributes, the MTSI may be determined using either average performance or stability alone. This score helps breeders identify genotypes that are superior in many ways. MTSI measures the distance between an ideotype and a genotype using component analysis scores, making it excellent for ideotype breeding. Considering the above facts, the current study concentrated on the multiple environment-based stability analysis for the selection of stable high-grain zinc rice by using the AMMI, WAASB, BLUP, and MTSI approaches.

MATERIALS AND METHODS

Experimental unit, location and material
Twenty-one high zinc rice genotypes, comprising four checks (Swarna, Samba Mahsuri, MTU 1010, and IR 64) and one local check (HUR3022), with fourteen yield and grain quality attributes, were employed in this investigation (Supplementary Table 1). The genotype lines were collected from the IRRI South Asia Hub, Hyderabad, India. The experiment was conducted during *Kharif*, 2017 at five locations in Uttar Pradesh, India *viz.,* BHU Agriculture Research farm I & II, BHIARIPUR, KARSADA, and RAMPUR. The description of each individual environment was given in Table 1 and Supplementary Fig. 1.

Experimental design
The experiment followed a completely randomised block design with three replications at five locations in irrigated ecosystems and medium-upland areas with transplanted nursery. Each location under study had a net plot size of 2.4 m × 2.4 m. The seedlings were transplanted with the spacing of 15 cm x 20 cm. The trials involved the implementation of need-based recommended cultural

practises and plant protection measures throughout the entire crop growth cycle.

Soil and weather condition

The soil condition was inceptisol type (Typicustochrept) and sandy clay loam texture for all the trials. The weather conditions throughout the evaluation period from June to November, 2017 nearly normal and favourable for crop growth in each trial (Supplementary Fig. 2).

Observations and estimation

In each replication, five competing plants were selected randomly in each genotype and observed for all yield and related traits. The attributes used to evaluate cultivar performance described by Biodiversity International (IPGRI and WARDA, 2007) were mentioned in the Table 2. Zinc concentration of grain samples was evaluated by an atomic absorption spectrophotometer at 213.86 nm at the Indian Institute of Rice Research, Hyderabad, following Sahrawat et al. (2002).

Statistical analysis

Additive multiplicative mean interaction model (AMMI)

AMMI analysis was performed using R studio to understand the genotype and environment interaction. The G×E interaction was evaluated by considering the first two PCA. The AMMI model (Gauch, 1988). Total five AMMI based stability parameters were computed such as, AMMI stability value (ASV) (Purchase et al., 2000); Sums of the absolute value of the IPC scores (SIPC) (Sneller et al., 1997); Averages of the squared eigenvector values (EV) (Zobel,1994); Absolute value of the relative contribution of IPCs to the interaction (Z_a) (Zali et al., 2012). The lowest values of ASV, SIPC and WAASB indicate the greatest stability of tested genotypes over environments.

Weighted average absolute scores based on BLUP (WAASB)

BLUP shows the predicted means for each genotype and environment combination. The likelihood ratio test was used to determine the significance of random effects (genotype and GxE)(Dempster et al., 1977).The calculation of the weighted average of absolute scores takes into account all IPCA derived from the singular value decomposition (SVD) of the matrix representing genotype-environment interaction (GEI) effects. This calculation is performed using a linear mixed-effect model, as described by Olivoto et al. (2019a). WAASBY index for simultaneous selection based on grain yield (Y) and stability (WAASB) was obtained by equation suggested by Olivoto et al., (2019a).

Multi trait stability index (MTSI)

Simultaneous selection is a method of selecting multiple candidates at the same time and the MTSI was calculated by formula given by Olivoto et al., (2019b).

The statistical analyses were conducted using R open-source software version 4.0.5 and R Studio (Team R, 2019). The 'metan' package developed by Olivoto and Lúcio (2020) was utilised for these analyses. The data visualisation utilised various bi-plots and heat maps through the ggplot2 package (Wickham et al., 2016).

RESULTS

Mean grain yield and Zinc content

Grain yield performance and zinc content of all the genotypes in five locations were measured and summarised in the Supplementary Fig. 3. Among the five locations, E1, E2 and E3 had favourable environments for all

Table 1: Description of environments used in the experiment

Environment Code	Location Name	Latitude	Longitude	Altitude	Land Type	Soil Type
E1	BHU Agriculture Research farm –I	25.18° N	80.30° E	81M	Up land	Inceptisol
E2	BHU Agriculture Research farm –II	25.18° N	80.30° E	81M	Low land	Inceptisol
E3	Bhikaripur	25.26° N	82.83° E	87M	Low land	Inceptisol
E4	Karsada	25.22° N	82.90° E	85M	Up land	Inceptisol
E5	Rampur	25.23°N	82.89°E	80M	Up land	Inceptisol

Table 2: Genotypes selected for various conditions based on Mean x WAASB bi-plot

Sl. No.	Trait	Category	Genotypes and Environments
1	GYP	Highly productive and stable genotypes	V7, V21, V14, V16, V6, V13, V18, V5
		Stable but poorly productive genotypes	V17, V8, V19, V9, V11, V4, V10
		Productive but unstable genotypes	V3, V20, V12, E1, E2, E3
		Poorly productive and unstable genotypes	V15, V1, V2, E4, E5
2	GZC	Highly productive and stable genotypes	V3, V2, V8, V9, V10, V12, V11
		Stable but poorly productive genotypes	V17, V21, V15, V18, V16, V5, V13, V14
		Productive but unstable genotypes	V1, V4, V7, E1, E2, E3, E5
		Poorly productive and unstable genotypes	V20, V19, V6, E4

the genotypes, and E4 and E5 had negative effects on genotype performance. Different genotypes gave the highest yields in different locations. In BHU Agriculture Research Farm-I and BHU Agriculture Research Farm-II, the genotypes V13 and V6 gave the highest yield, and in Bhikaripur, the genotypes V20 and V13 gave the highest yield. The genotypes V3 and V5 in Karsada and V13 and V5 in E5 had high grain yields. The zinc content of the rice genotypes was lower in the environment (E4). It may be due to the lower soil zinc content of the location. Different genotypes had the highest zinc content in different locations. However, in four environments other than E4, genotype V1 had the highest zinc content. After V1, the genotypes V7, followed by V2, had high zinc content in three environments.

Genetic association of quantitative traits focusing mainly on GYP and GZC

Correlation among all the studied characters showed various degrees of association among themselves (Fig. 1). Day to 50% flowering (50% DF) and day to maturity (DM) had a perfect positive relationship with days to first flowering (1st DF). The traits number of grains per panicle (NGP), grain weight per panicle (GWP), and grain yield per plant (GYP) had a significant positive correlation with the number of seeds per panicle (NSP). Significant positive correlations were found for GYP with GWP, for grain Length/Breadth ratio (GLBR) with total effective tiller number (ENT) and for weight of 1000 Seed weight (1000SW) with plant height (PH). The trait ENT had a significant negative correlation with panicle length (PL), NSP, NGP, GWP and GYP. PH
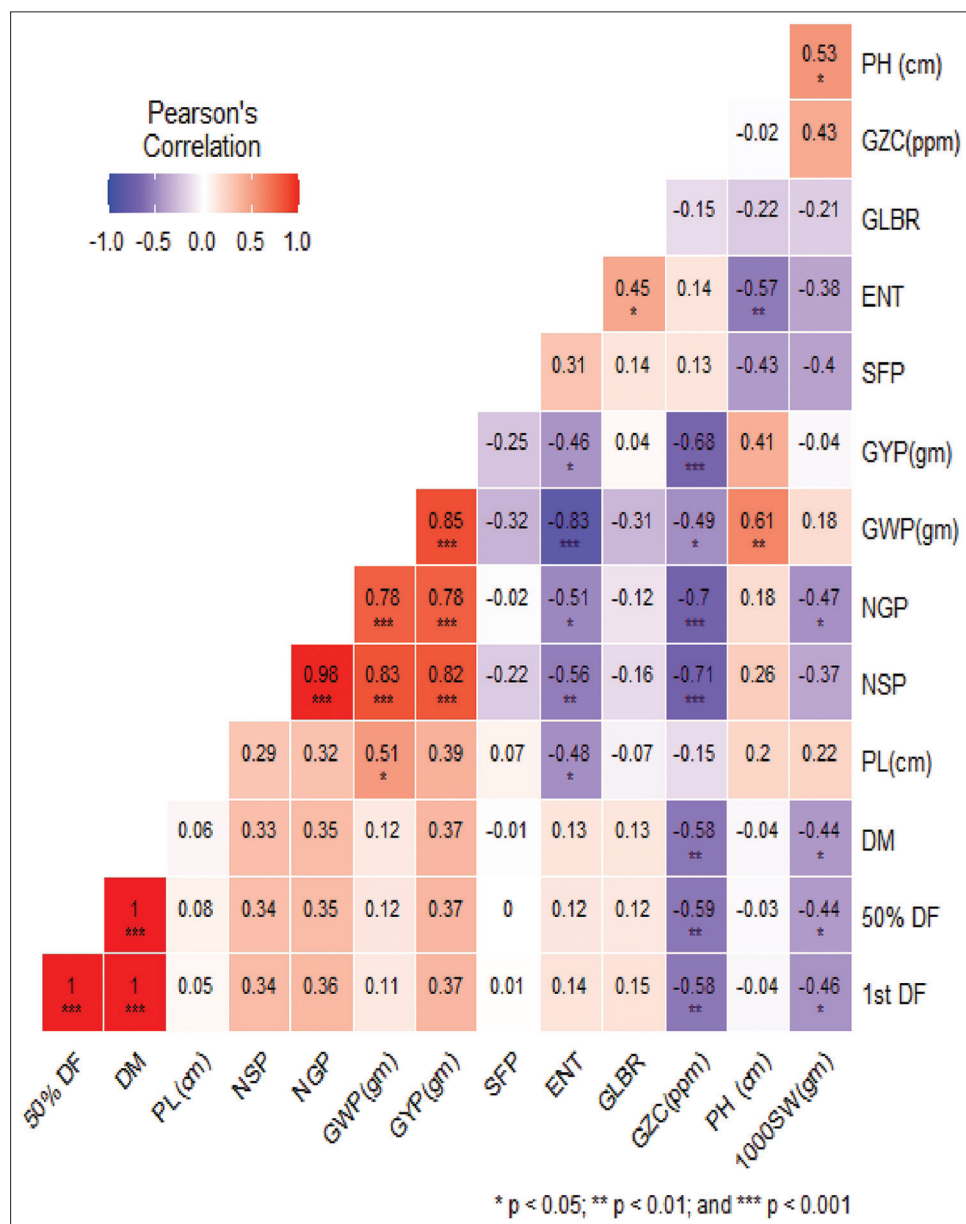

Fig 1. Genotypic correlations among 14 yield traits in the 21 high zinc rice genotypes.

had a significant negative association with ENT (-0.57). But it had non-significant correlations with DM and GYP. The most important trait, i.e., GZC, expressed a significant negative correlation with seven other traits, namely, 1st DF, 50% DF, DM, NSP, NGP, GYP, and GWP. The trait 1000SW also had a significant negative association with four traits, namely 1st DF, 50% DF, DM, and NGP. The rest of the trait combinations had non-significant positive and negative correlations among themselves.

Evaluation and selection of rice genotypes using AMMI based stability method

ANOVA using AMMI analysis of high zinc rice genotypes under multi environment

A combined analysis of variance was performed and presented in Table 3. The mean square of genotypes, environments and genotype x environment interactions showed significant differences (p ≤ 0.01, ≤ 0.05) for the grain yield and zinc content of rice genotypes. The first two principal components had high significant variation and accounted for cumulative variance was about 78.4% (PC1 = 52.8% and PC2 = 25.6%) for grain yield and the first three PCs were highly significant and accounted for 91.4% (PC1 = 53.1%, PC2 = 24.4%, and PC3 = 13.9%) of the interaction sum of squares for grain zinc content.

Ranking of the rice genotypes according to AMMI based stability measures

Various AMMI stability indices for grain yield and GZC were estimated and presented in the Supplementary Table 3. The mean grain yield was observed to be highest for V13, followed by V6 and V5. The least ASV was expressed by V11, V5, V21, V9 and higher values by V20, followed by V3 and V1. Low SIPC scores indicate more stable genotypes, so V11, V21, and V5 were stable in nature, while V12, 220, and V19 were the least stable. Low EV values are also associated with great stability, like SIPC. V11, V21, and V5 had lower EV values, and V12 had the highest EV value, followed by V20. The relative contribution of IPCs to the interaction was computed as ZA values. ZA identified V11, followed by V21 and V5, as stable ones, whereas V20, V12, and V1 would be the least stable genotypes.

According to WAASB, genotypes V11, V21, and V5 were highly stable genotypes. So, the genotypes V5 (IR 97443-11-2-1-1-B) and V21 (HUR3022) have shown high yield with good stability. A correlation analysis among AMMI-based stability indices was performed and is represented in the supplementary Fig. 4. All the indices have shown significant and strong positive correlations among themselves. The mean grain zinc content was observed to be highest for V1, followed by V4 and V7. The least ASV was expressed by V14, V11 and V5. Low SIPC scores indicate more stable genotypes, so genotypes V14 and V10 were stable at high zinc content. The genotype V14, followed by V10 and V12, had lower EV values. ZA identified V14, followed by V10 and V11 as stable ones. According to WAASB, genotypes V14, V10, and V11 were highly stable genotypes.

AMMI bi-plots

The AMMI stability, indicating the association between genotypes under investigation and test locations, was depicted in grain yield/plant (GYP) vs. PC1 bi-plot and grain zinc content (GZC) vs. PC1 bi-plot (Fig. 2). In the grain yield/plant (GYP) vs. PC1 bi-plot, environments E3 and E4 were farthest from the bi-plot origin, explaining the great interaction forces, while environments E1, E2 and E5 were closer to the origin of the bi-plot with shorter vectors, indicating the weak interaction forces. Environments that cluster together influence genotypes in a similar way. As the bi-plot indicated, V13, followed by V6, had the maximum yield performance. While, some genotypes, namely V17, V11, V4, and V15, had poor yield performance. Therefore, genotypes V4, V11, and V9 exhibited great stability, while genotypes V16 and V21 were considered the most desirable because they had good yield and stability. As per the zinc content vs. PC1 bi-plot (Fig. 2), environments E4 and E5 were farthest from the bi-plot origin, indicating the great interaction forces. Environments E1, E2 and E3 were clustered together, showing their similar influence on the GZC of the rice genotypes. The genotypes V1, V4 and V7 had high zinc content, while V14, V11 and V17 showed great stability for GZC as they were near the bi-plot origin. However, genotype V2, followed by V10, had a good amount of GZC with high stability.

Table 3: The AMMI analysis of variance of grain yield and zinc content in rice genotypes

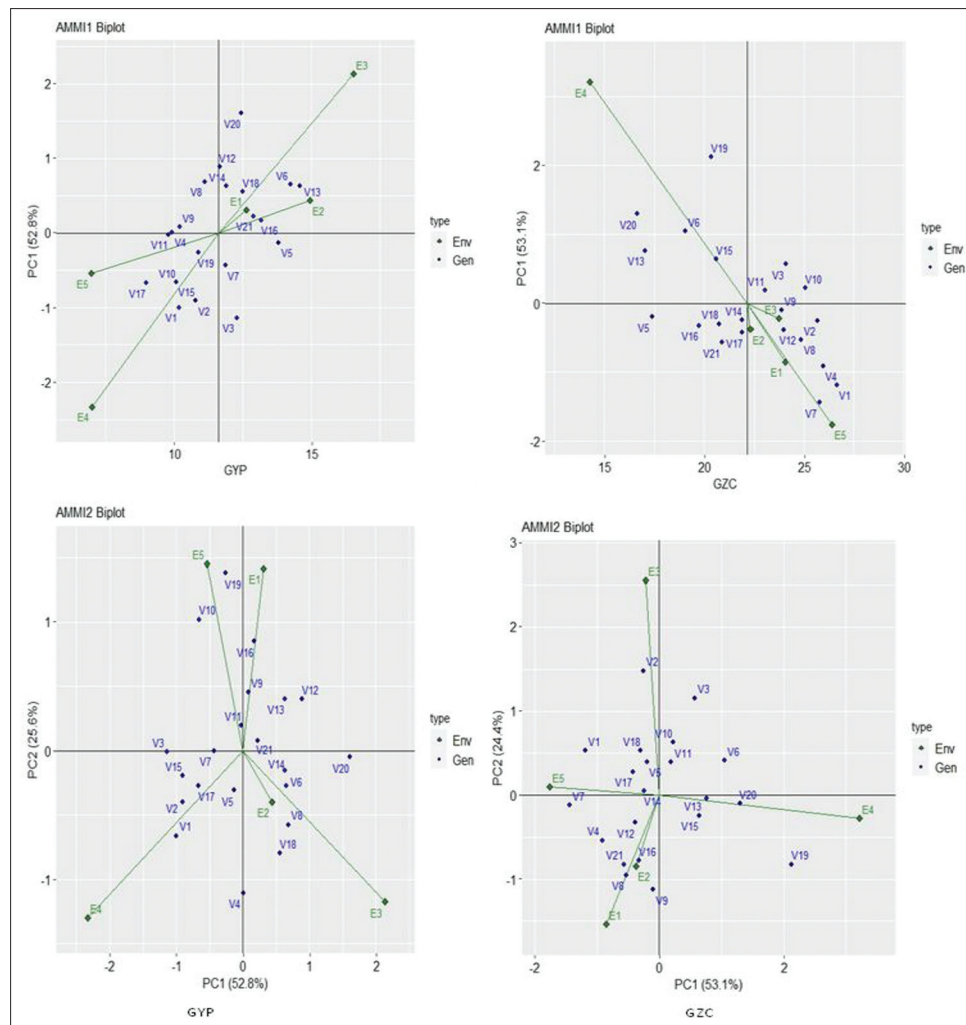| Source	DF	Grain Yield per Plant (GYP)			Grain Zinc Content (GZC)		
		Mean Sq	% Explained	% Accumulated	Mean Sq	% Explained	% Accumulated
Environments	4	1242.509**			1362.36**		
Replication (within environment)	10	8.152**			2.97[NS]		
Genotypes	20	36.303**			143.28**		
GEI	80	7.907**			14.45**		
PC1	23	14.535**	52.8	52.8	26.69**	53.1	53.1
PC2	21	7.698**	25.6	78.4	13.41**	24.4	77.5
PC3	19	4.045*	12.1	90.6	8.46**	13.9	91.4
PC4	17	3.516[NS]	9.4	100	5.86[NS]	8.6	100
Residuals	200	2.404			3.91		

Fig 2. AMMI 1 and AMMI 2 bi-plot for GYP and GZC in the 21 rice genotypes.

All five studied environments were diverse in nature, as represented by the AMMI bi-plots of PC1 vs. PC2 for grain yield and grain zinc (Fig. 2). The AMMI bi-plot for grain yields indicated that PC1 (52.8%) and PC2 (25.6%) together explained 784.4% of the total variation by genotype and GEI (Fig. 2). The results indicated that genotype V19 exhibited the highest grain yield in environments E1 and E5, while genotype V1 performed best in E4 and genotype V20 in E3. The genotypes V3 and V4 exhibited low yields across various environments, suggesting that they were not high-yielding genotypes in any environment. Genotypes located closer to the origin of the plot, such as V11, V21, V7, and V5, exhibited greater adaptation to low-yielding locations. Based on the bi-plot (Fig. 2) depicting PC1 vs. PC2, genotypes V19, V1, and V20 exhibited high stability and yield. According to the PC1 vs. grain yield AMMI bi-plot, genotypes V20 and V13 exhibited high yields and relatively low genotype-by-environment interaction (GEI), making them stable varieties. The AMMI 2 bi-plot for grain zinc concentration showed that PC1 (53.1% of the variance) and PC2 (24.4% of the variance) explained

77.5% of the difference between genotype and GEI. The figure indicated that genotype V8 had the highest grain zinc concentration in E1 and E2, followed by V2 in E3, V19 in E4, and V7 in E5. V1, V3, and V9 were not high zinc genotypes, suggesting inadequate zinc concentration in some or all environments.

Evaluation and selection of rice genotypes using WAASB based stability method
WAASB based stability analysis
The WAAS bi-plot is also known as the Mean vs. WAASB bi-plot. It was constructed by considering the x-axis as mean values and the y-axis as the WAASB scores of the genotypes (Fig. 3). The mean grain yield per plant (g), grain zinc content (ppm) and WAASB scores of 21 high zinc rice genotypes are presented in the Supplementary Table 3. The genotypes identified for various conditions based on the mean vs. WAASB bi-plot are presented in Table 2. From the WAASB-based stability scores of GYP and GZC, the most stable genotypes were V3 (0.11) and V6 (0.16), respectively. A WAASB bi-plot is used for simultaneous analysis of
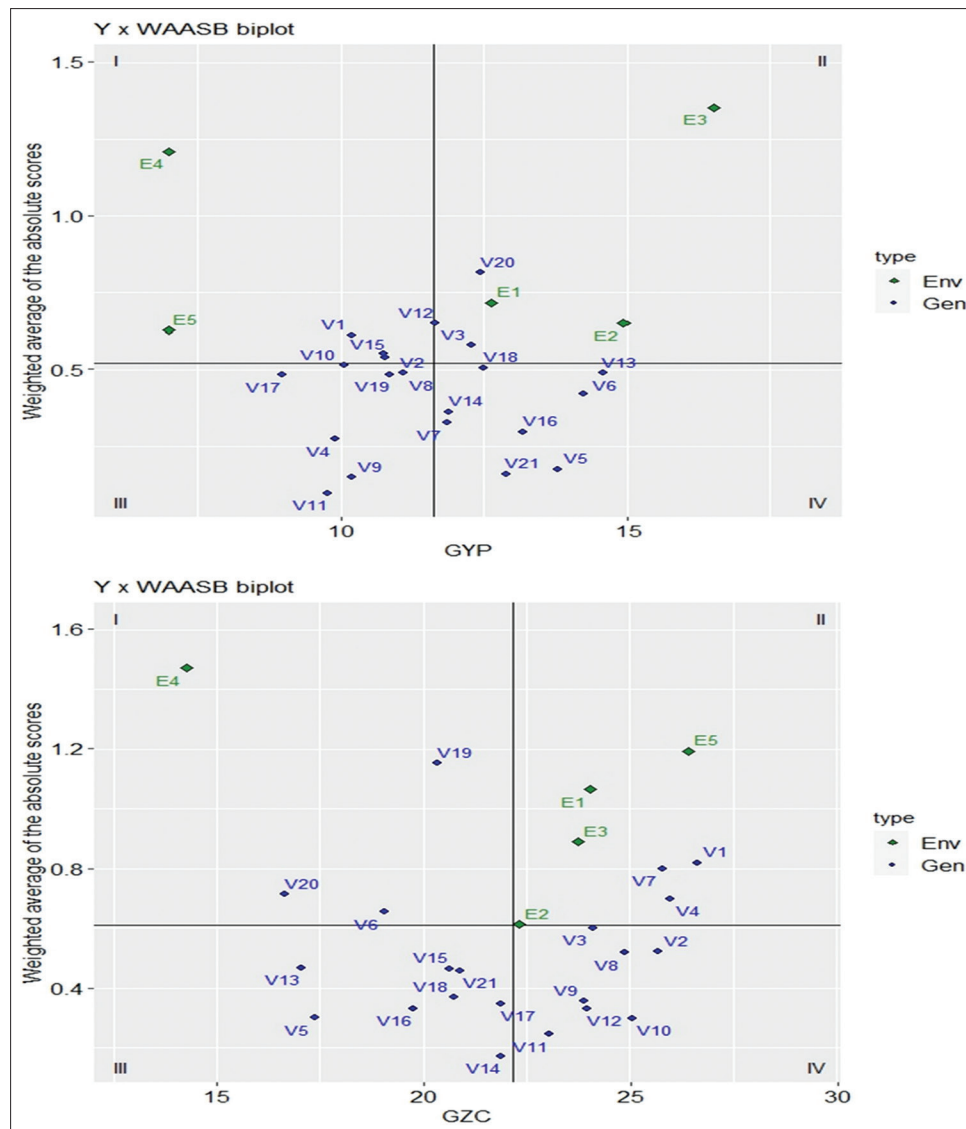
Fig 3. Mean vs. WAASB bi-plot for GYP and GZC in high zinc rice genotypes.

productivity, stability, and environment, with quadrants containing four different genotype and environment classes (Fig. 3).

Highly productive and stable genotypes whose means were greater than the grand mean with low WAASB scores were present in the fourth quadrant. The genotypes in this quadrant were V7, V21, V14, V16, V6, V13, V18 and V5 for GYP and, for GZC, V3, V2, V8, V9, V10, V12 and V11. The genotypes in the third quadrant were stable but poorly productive, i.e., stable genotypes whose means were lower than the grand mean with low WAASB scores. The genotypes present in the third quadrant were V17, V8, V19, V9, V11, V4 and V10 for GYP and, for GZC, V17, V21, V15, V18, V16, V5, V13 and V14. Productive but unstable genotypes were present in the second quadrant, with the mean being greater than the grand mean. The unstable genotypes listed for GYP are V3, V20 and V12, and for

GZC they are V1 V4 and V7. The environments found to be effective for discriminating against were E3, E1 and E2 for GYP, and for GZC, they were E5, E1, E3 and E2. In the first quadrant, poorly productive and unstable genotypes and highly discriminating environments were present. This quadrant includes a mean that is less than the grand mean and the genotypes present in this quadrant for GYP are V15, V1, and V2 and for GZC they are V20, V19 and V6. In the first quadrant, environments E4 and E5 for GYP and GZC were discriminatory.

Supplementary Fig. 5 and 6 indicate the ranks of 21 high zinc rice genotypes depending upon the number of retained IPCA used in WAASB estimation for GYP and GZC. In the present study, four axes were considered. From Supplementary Fig. 5 and 6, it was evident that the genotype ranks for GYP and GZC changed according to the extent to which IPCAs were incorporated in the

WAASB assessment. This indicates the benefit of using the WAASB index to calculate stability.

Genotype ranking based on WAASBY index

The mean GYP, GZC, and WAASBBY scores of 21 high-zinc rice genotypes are presented in the Supplementary Table 3. In the present study, WAASBY was used for selection for grain yield and stability at the same time, with weights of 50 and 50 for yielding and stability, respectively. The WAASBY for 21 high-zinc rice genotypes for GYP and GZC tested in five environments is shown in Supplementary Table 3 and Fig. 4. The genotypes with the highest WAASBY scores for GYP were V21 (82.24), V3 (79.61), and V6 (79.23), with the lowest being V4 (19.98), V1 (24.37), and V11 (29.89). Genotypes with the highest WAASBY scores for GZC were V4 (88.66), V2 (87.88), V8 (83.19), V3 (82.60) and V10 (82.33), while the lowest were reported for V13 (23.79), V11 (32.02) and V20 (32.73).

Supplementary Fig. 4 depicts the evolution of 21 high-zinc rice genotype rankings based on the weight of stability (WAASB) and yield. The ranking of genotypes based only on the WAASB index in the first column on the left side indicated that V3, V21 for GYP, and V6, V21 for GZC were the most stable genotypes. The genotype rankings in the last column on the right side were exclusively based on GYP and GZC, with V13 and V1, respectively, being the most superior genotypes.

Supplementary Fig. 7 represented the genotypes› which-won-where pattern based on presumed yields in each environment and the demarcation of mega environments. For GYP, the genotype V13 excelled in environments E1, E2, E3, and E5, while the genotype V3 performed well in E4. In the case of GZC, genotype V1 predicted high mean values in environments E1, E2, E3, and E5, while genotype V19 excelled in environment 4.
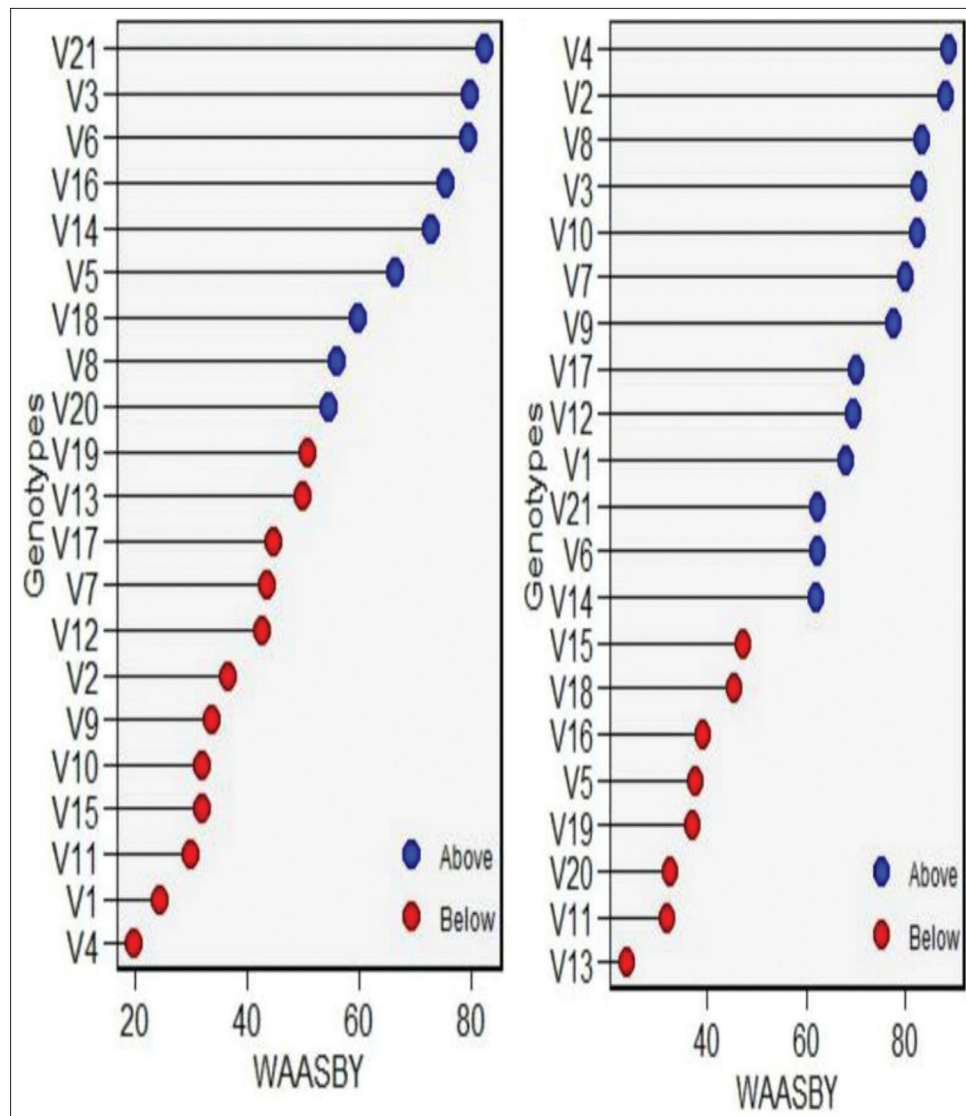

Fig 4. Estimated values of WAASBY for A- GYP and B- GZC in 21 high zinc rice genotypes.

Best linear unbiased predictor (BLUP) based genetic parameters using linear mixed effects model (LMM)

The estimated genetic parameters and variance components from LMM are presented in Table 4. A close examination of these results indicated that genotype (G), G x E variances were significant for traits based on the likelihood ratio test. The highest contribution from environmental variance was reported for GYP (39.20%), followed by GZC (10.49%). The coefficient of determination (R2GEI) of genotype (G) and environment (E) interaction, i.e., G x E, was low to moderate. For GZC, the selection accuracy (AS), genotypic correlation (rge), genotypic coefficient of variation, mean, standard error (SE), and standard deviation (SD) were reported to be quite higher than for GYP.

The Quantile-Quantile (Q–Q) plot was used to check the normality of the random effects of genotypes and interaction effects of 21 high zinc rice genotypes for GYP and GZC (Supplementary Fig. 8). The BLUP mean values of GYP and GZC of rice genotypes are shown in Supplementary tables 4 and 5 and Fig. 5. In Fig. 5, the vertical error bars represent the 95% confidence interval of prediction considering a two-tailed t test. Out of 21 genotypes, ten genotypes had an above-average predicted mean value for GYP, whereas for GZC, eleven genotypes had an above-average predicted mean value. The genotype V13 had the highest anticipated mean values for GYP, followed by V6 and V5, while the lowest reported was in V17. The maximum predicted mean values for GZC were found in genotype V1, followed by V4, V7, and V2 with a minimum in V20.

Evaluation and selection of rice genotypes using MTSI based stability method

The Eigen values, explained variance, cumulative variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis are presented in the supplementary Table 6. In the present experiment, a multi-trait stability index (MTSI) was calculated based on

grain yield per plant along with thirteen different traits mentioned in the Supplementary Table 7 and Table 5. The communality value ranged from 0.59 for days to 1st flowering date to 0.9 for the grain weight per panicle. A total of five principal components with Eigen values greater than 1 are selected, contributing about 80.13% of the total variation. The ranking of the 21 rice genotypes was done with the help of a multi-trait stability index, i.e., the MTSI index. The first three genotypes (V7, V13, and V19) were selected as multi-trait high-performing genotypes according to the MTSI score and display of 14 traits free from multi-collinearity (Supplementary Table 7 and Fig. 6). The genotype V20 was closer to the selection intensity circle and could have desirable features. All the traits are included in the five factors. Grain yield per plant is included in FA1, whereas GZC is included in FA5. Selection differential for both mean performances and WAASBY are noted as positive in GYP but negative in GZC. This indicates the efficiency of MTSI index in selecting desirable genotypes.

In the GYP, the selection differential (%)of mean performances is 19.62%, and the selection differential (%) of the WAASBY index is 50.91%. The selection gain (%) is found to be 15.35% for GYP. It is reported oppositely in the GZC: the selection differential (%) of mean performances is -14.24 %, and the selection differential (%) of the WAASBY index is -28.27 %. The selection gain (%) is found to be 12.8% for GZC (Table 5). The contribution of each component to the MTSI is categorized as either less significant or more significant. The central position is reserved for the more significant contributing factors, while the less significant ones are positioned towards the outer edges. Fig. 6 unveils the strength and weakness view of selected genotypes out of 21 rice genotypes based on the MTSI score. This figure is further supported by Supplementary Table 8. All the selected genotypes are weak contributors to all the traits present in five different factors. V6, V21 and V13 are desirable for grain yield per plant, grain zinc content, and other traits.

Table 4: Estimation of BLUP based genetic parameters and variance components from METs

Sl. No.	Parameter	GZC	GYP	Sl. No.	Parameter	GZC	GYP
1	GEN	8.43***	1.89***	12	Mean	22.16	11.62
2	GEN: ENV	3.562***	1.83***	13	SE	0.32	0.26
3	Error	3.905	2.404	14	SD	5.71	4.68
4	% GV in PV	22.66	30.88	15	CV	25.83	40.32
5	% IV in PV	9.57	29.92	16	Min	9.84 (V5 in E4)	4.54 (V17 in E5)
6	% EV in PV	10.49	39.20	17	Max	32.54 (V1 in E5)	20.36 (V13 in E3)
7	PV	37.22	6.13	18	Min ENV (Avg.)	E4 (14.27)	E5 (7.01)
8	R^2_{GEI}	0.22	0.30	19	Max ENV (Avg.)	E5 (26.41)	E3 (16.53)
9	As	0.95	0.88	20	Min GEN (Avg.)	V20 (16.61)	V17 (8.89)
10	r_{ge}	0.48	0.43	21	Max GEN (Avg.)	V1 (26.81)	V13 (14.64)
11	CVg	13.15	11.84				

As - Accuracy the selective accuracy; R^2_{ge} - the coefficient of determination of the interaction effects; r_{ge} - the genotype-environment correlation; CV_g - the genotypic coefficient of variation
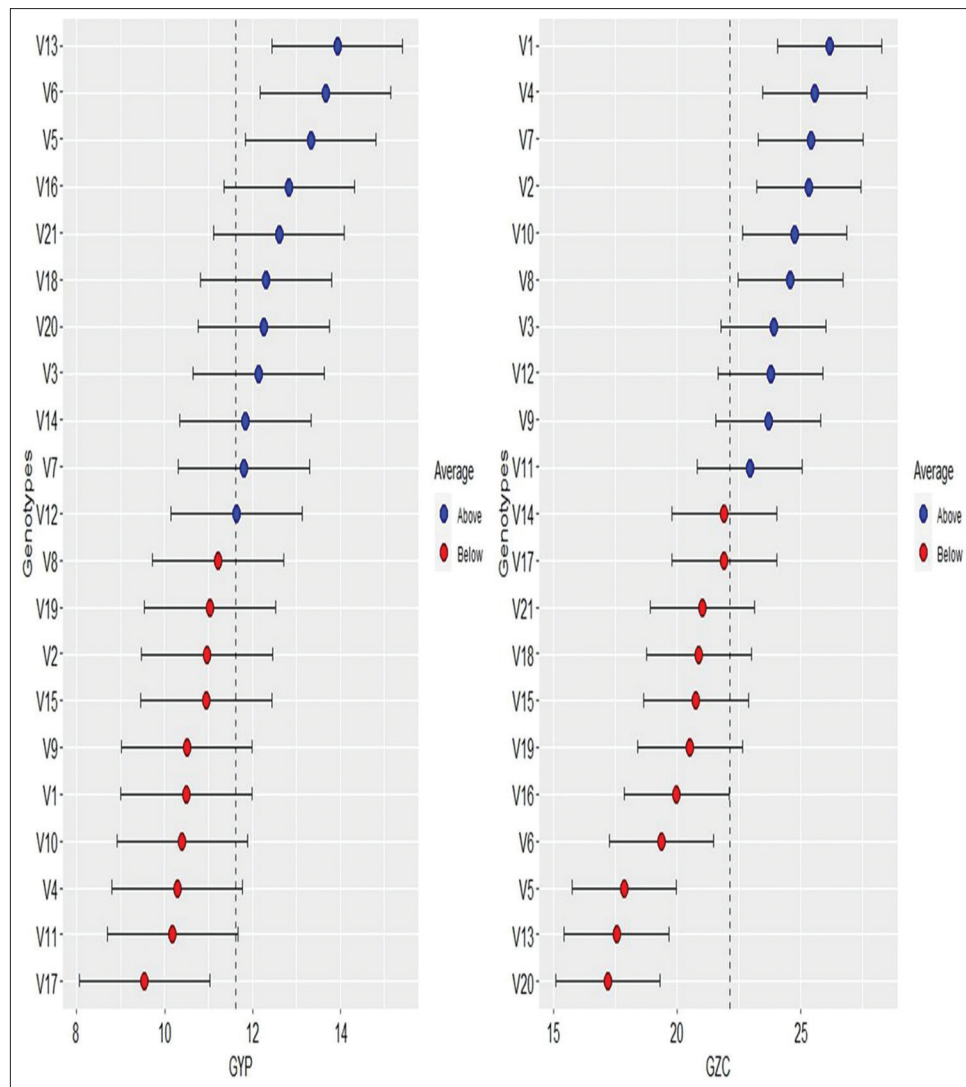
Fig 5. BLUP mean of GYP and GZC in 21 high zinc rice genotypes. Vertical error bars represent the 95% confidence interval of prediction considering a two-tailed *t* test.

Table 5: Selection differential and genetic gain of the mean and WAASBY index for 14 rice traits based on MTSI

VAR	Factor	NS				WAASBY				h2	SG	SG (%)
		Xo	Xs	SD	SD (%)	Xo	Xs	SD	SD (%)			
PH	FA 1	106.72	110.72	4.00	3.75	54.83	63.67	8.84	16.11	0.81	3.22	3.02
NSP	FA 1	109.27	145.17	35.90	32.85	46.47	63.71	17.24	37.11	0.90	32.44	29.69
NGP	FA 1	83.12	105.09	21.97	26.44	45.77	67.41	21.64	47.27	0.91	20.06	24.14
GWP	FA 1	1.51	1.95	0.44	29.13	51.18	70.20	19.02	37.17	0.87	0.38	25.29
GYP	FA 1	11.62	13.90	2.28	19.62	50.73	76.55	25.82	50.91	0.78	1.78	15.35
FstDF	FA 2	93.75	99.02	5.28	5.63	47.47	49.30	1.82	3.84	0.99	5.23	5.58
FDF	FA 2	97.96	102.69	4.73	4.83	57.34	68.15	10.81	18.86	0.97	4.58	4.68
DM	FA 2	126.81	132.58	5.77	4.55	53.82	61.37	7.55	14.02	0.99	5.69	4.49
SFP	FA 3	76.37	73.04	-3.34	-4.37	50.05	36.78	-13.26	-26.50	0.67	-2.23	-2.93
STW	FA 3	18.26	18.60	0.34	1.85	63.64	66.11	2.46	3.87	0.94	0.32	1.74
ENT	FA 4	7.87	7.31	-0.56	-7.14	60.08	59.64	-0.44	-0.73	0.86	-0.48	-6.10
GLBR	FA 4	4.00	3.96	-0.04	-1.04	62.91	68.41	5.50	8.74	0.80	-0.03	-0.83
PL	FA 5	26.01	26.63	0.61	2.36	43.06	37.70	-5.36	-12.45	0.86	0.53	2.03
GZC	FA 5	22.16	19.00	-3.16	-14.24	60.51	43.34	-17.17	-28.37	0.90	-2.84	-12.80

Where, Xo, mean for WAASBY index of the original population and Xs, mean for WAASBY index of the selected genotypes (V6, V13 and V21).
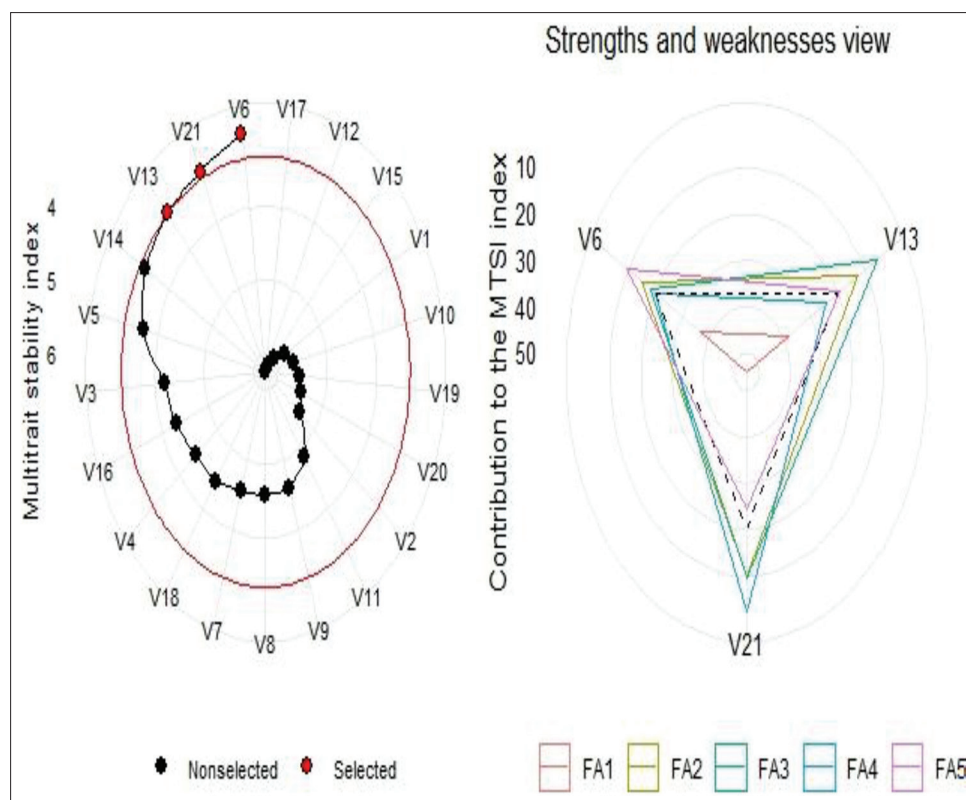
Fig 6. Genotype ranking and selection of genotypes based on the multi-trait stability index (MTSI) considering a selection intensity of 15%.

DISCUSSION

Rice cultivation is prevalent throughout different ecologies all over the world. Climate change necessitates the identification of adaptable and stable rice genotypes for cultivation by Seck et al. (2010) and Khush, 1997. Keeping this under consideration, our research was aimed at finding stable and adaptable rice genotypes for grain yield with good zinc content. In this study, AMMI was successfully employed to study the genotype and environmental interactions in rice. This technique was successfully used in rice for the evaluation of stability and adaptability for grain yield by Kumar et al. (2017), Sandhu et al. (2019), Jain et al. (2019), and Kesh et al. (2021).

Compared to earlier classical methods, the use of linear mixed models with best linear unbiased prediction (BLUP) provides an efficient way of estimating genotype-environment interactions (GEI). This approach is used to estimate different genetic parameters and is also used in calculating different multi-trait-based stability evaluation methods (Olivoto et al., 2019a; Olivoto et al., 2019b). The multi-trait stability index (MTSI) suggests using the mean performance and stability of multiple desirable agronomic characters to improve varietal recommendations. The ideal or ideotype genotype is supposed to have a maximum WAASBY score of 100 for all the considered characters.

The genotype with the lowest MTSI score is selected as it is closer to the ideotype. These multi-trait-based stability evaluation methods were successfully implemented by Debsharma et al. (2023) in rice, Sousa et al. (2019) in cowpea for immature seed production, Koundinya et al. (2021) in 25 cassava genotypes and Yue et al. (2022) in 28 maize genotypes. In this present investigation, simultaneous selection for grain yield and zinc content has been carried out through MTSI.

Mean performances and trait association studies in 21 rice genotype

The evaluation of the agronomic performance of genotypes is primary step in identifying stable genotypes across different environments. The results of the mean performances of genotypes for grain yield and GZC indicated the presence of sufficient genetic variation among the genotypes and all these variations might be attributed to the differential expression of the genetic makeup of the rice genotypes according to the environmental conditions that prevailed at test locations. Genotypes V13 for GYP and V1 for GZC are considered the highest yielding, respectively. These genotypes are suitable for future breeding practices.

Yield is a complex trait that results from the interaction of different factors that either positively or negatively correlate with yield, as well as with each other. The correlation coefficient is a valuable tool for assessing the impact of

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different traits on crop yield and their interrelationships during the selection process. In the selection process for maximizing GYP, priority is given to traits that show significant positive or negative correlations with yield. The grain yield per plant (GYP) had a significant positive correlation with the number of seeds per panicle (NSP), number of grains per panicle (NGP) and grain weight per panicle (GWP) and significant negative correlation with the GZC. Panicle length had a positive but non-significant correlation with GYP, while it had a significant positive correlation with GWP. This indicates that lager panicles can bear a greater number of grains, resulting in a high yield. This result is in harmony with Jeke et al. (2021). PH had shown a significant negative association with ENT (-0.57); this result was not supported by Sadeghi (2011), Babu et al. (2012) and Thorat et al. (2019). The most important trait, i.e., GZC, expressed a significant negative correlation with seven other traits, namely, 1st DF, 50% DF, DM, NSP, NGP, GYP, and GWP (Nagesh et al., 2013).

Evaluation and selection of rice genotypes using AMMI based stability method

Identification of high-yielding and stable genotypes by employing AMMI and BLUP simultaneously is advisable (Verma and Singh, 2021). The AMMI stability model is commonly applied to decipher GEI patterns and select stable genotypes in target environments (Gauch, 2013). AMMI is a multivariate tool and it has been found to be a powerful tool for determining genotype-environment interactions and identifying stable and adaptive genotypes (Jain et al., 2019). There was sufficient significant G × E interaction among the genotypes and the two principal components (PCs) contributed more than 70% to the total variation of GEI, suggesting the AMMI bi-plot is effective in identifying the stable genotypes. The genotypes with the lowest values of AMMI stability indices are considered the most stable. As per the PC1 vs. grain yield AMMI bi-plot, V20 and V13 were the high yielding genotypes with relatively less GEI and were considered stable varieties (Anuradha et al., 2021). Comparing different AMMI stability indices, the genotypes V11, V5 and V21 for GYP and V14, V10 for GZC are found to be stable and common. V16 and V21 for GYP and V9, V2 and V10 for GZC were identified as stable based on the AMMI model and bi-plot. The identified stable genotypes can be used in the variety development programme.

Evaluation and selection of rice genotypes using WAASB based stability method

The WAASB index is a mixed-model version of the AMMI that treats genotypes as random variables and accounts for all IPCAs while calculating genotypic stability. Olivoto et al. (2019a) proposed it for WAASB. The WAASB stability index has been used to identify stable genotypes in a variety

of studies. In the present study, based on WAASB-based stability scores, the stable genotypes identified for GYP are V3 and V6 for GZC, respectively. Based on the WAASBY score, the desirable genotypes identified for GYP are V21 and V4 for GZC, respectively. Similarly, some stable genotypes for various economic traits were discovered by various workers, including wheat grain yield (Verma and Singh, 2020), various cassava traits (Koundinya et al., 2020), barley grain yield (Ahakpaz et al., 2021), soyabean grain yield (Nataraj et al., 2021), and cassava mosaic and anthracnose disease resistance (Tize et al., 2021). To select genotypes based on mean performance and the WAASB stability score, a superiority index that allows weighing between mean performance and stability (WAASBY) was created by Olivoto et al. (2019a). Breeders can prioritise weights for yield and stability based on their breeding goals and varietal recommendations. In this study, the grain yield and WAASB score weights were 50 and 50, respectively, to come up with this superiority score.

Significant GEIs for complex variables like grain yield will prevent selection efficiency, making it difficult to generate adaptable cultivars (Rao et al., 2011). The mean vs. WAAS bi-plot can also be used to determine genotype-environment interaction. Nataraj et al. (2021) also used the WAASB stability model to understand GEI in soybean. The interactions of the environments were similar to genotypes when they flocked together on the bi-plot. The higher the WAASB score of an environment, the more it interacts with genotypes. If the environmental factors are above average, they have a favourable impact on the quality. The lower-than-average environmental mean values indicate that the environment has a negative impact. If the WAASB score is really high, then the genotypes' influence on the environment was extremely high. The environment effect, followed by GEIs, produced the most variation in this study. This is consistent with previous findings in Cassava yield trials by Koundinya et al. (2020); Bhartiya et al. (2018) in Soyabean and Olivoto et al. (2019a) in Oat, suggesting the importance of environmental factors in yield trials of the above mentioned crops.

Normally, the GGE (Genotype + Genotype x Environment) bi-plot is employed to locate mega-environments. In order to portray the same, Olivoto et al. (2019a) presented a graphic representation by plotting the character nominal means (yij) against IPCA1 values in the environment. Through these graphs (Fig. 9), genotypes were identified that perform well in each environment, which leads to genotype recommendations for specific regions or environments to exploit the use of narrow adaptations because, in the majority of cases, no single genotype consistently wins in every environment (Olivoto et al., 2019a; Koundinya et al., 2020).

Evaluation and selection of rice genotypes using BLUP based stability method

The AMMI ANOVA is based on a fixed-effects model in which genotype and environmental effects are assumed to be constant. The mixed model techniques allow for the estimation of significant parameters in quantitative genetics, such as genotypic and interaction variances, genetic correlations, and coefficients of determination, which are important from the perspective of a plant breeder. These details are crucial in a plant breeding effort, and they should be used in evaluation as well. Piepho (1994) recommends using mixed models to estimate the G x E interaction because the genotypic effects are unpredictable. In LMM, G x E was calculated using the genotype effect as a random variable. The BLUP provides more accurate forecasts of genotypes' future mean values by combining genotype and environment effects in a mixed model approach. It is more helpful to plant breeders since it is better at dealing with unbalanced or incomplete data (Smith et al., 2005).

Similar to the analysis of variance (ANOVA) of AMMI, the ANOVA of LMM revealed substantial differences among genotypes and their interactions with the environment. The percent of genotypic variation in phenotypic variance was higher than the percent of environmental variation in phenotypic variation for GZC and vice versa in the case of GYP. The environment has a considerable impact on the phenotypic expression of traits. The low to medium R2GEI of GYP and GZC revealed the presence of high residual variance in the G x E interaction component. The correlation between observed and expected values is the genotypic accuracy of selection (As) (Olivoto et al., 2019a). The high As values of GYP and GZC characters represent the trustworthiness of this model's ability to pick superior genotypes. The low to medium rge for GYP and GZC indicated complications in selecting superior stable genotypes for these traits and the need for detailed, accurate information. Based on BLUP-based parameters, the desirable genotypes identified for GYP is V13 and V1 for GZC, respectively. Similar results were found in cowpea for immature seed production by Sousa et al. (2019) and in cassava for harvest index, days to maturity by Koundinya et al. (2020) and Debsharma et al. (2023) in rice.

Evaluation and selection of rice genotypes using MTSI based stability method

The MTSI Index is a unique and simple selection approach that allows breeders and agronomists to pick plants based on their performance and stability at the same time when multiple traits data is present (Olivoto et al., 2019a). Plant breeders primary goal is to select genotypes with a high mean and stable performance; thus, the MTSI index is a useful tool for them (Olivoto et al., 2019 b). A genotype with the lowest MTSI is considered closer to the ideotype

and thus selected. This index indicated that three genotypes (V7, V13, and V19) had the highest performance for all studied traits. Genotype V14, located in close proximity to the cut point, may possess some beneficial traits and so further investigation is required for it. More genetic gains can be predicted from the selected genotypes for all the considered traits as per the MTSI index. The high heritability and genetic gain of the genotypes chosen based on the WAASBY score suggested their stability and suitability for these attributes. By selecting and including these genotypes in the varietal development and ideotype breeding strategies, the traits mentioned above might be improved. Trait strength and weakness can be assessed using factor analysis, specifically by examining the contribution of each component to the MTSI. Traits that closely resemble the ideotype have lower proportions, which can be attributed to the factor located near the outer edge of the figure. These genotypes are suitable for breeding high-yielding, high-GZC varieties. The strength and weakness plot is a useful graphical tool for evaluating and selecting genotypes based on their respective traits. Based on the MTSI score, the contribution of each component is divided into two categories: those that contribute less (displayed towards the edge) and those that contribute more (displayed in the middle). It allows for the identification of genotypes that can be improved in terms of their strengths and weaknesses. All the selected genotypes (V6, V21 and V13) are weak contributors to all the traits, suggesting these genotypes could be used in ideotype breeding. In Soyabean, Zuffo et al. (2020) also used MTSI to identify stable genotypes under drought and salinity conditions at the same time. Similar kinds of results are also reported by Olivoto et al. (2021) in Strawberry; Yue et al. (2022) in maize hybrids; Olivoto et al. (2019 b) in oat and Debsharma et al. (2023) in rice.

CONCLUSION

Zinc is essential for gene expression, protein structural integrity, cell division, insulin action enhancement, micronutrient regulation, and the body's defence system enhancement. Increasing the micronutrient content of staple crops like rice might help reduce hidden hunger, as milled rice lacks micronutrients. Plant breeding programmes need to understand GE structure and nature to identify superior genotypes. So, using agronomical and grain iron and zinc data from multiple sites makes it possible to find lines with good grain yield and stable grain zinc levels, as well as to understand how the growing environment affects the expression of micronutrients. This helps breeders reach their goal of making bio-fortified rice varieties. The genetic association study revealed significant negative correlations between grain zinc content and grain

yield per plant but positive correlations between 1000 seed weight, spikelet fertility percent, total effective tiller number, and grain zinc content. There was a significant G × E interaction and V16 and V21 for GYP and V9, V2 and V10 for GZC were identified as stable based on the AMMI model and bi-plot. V11, V5 and V21 for GYP and V14, V10 for GZC are found to be stable and common in all AMMI stability parameters. V6, V13 and V5 for GYP and V10, V8 and V2 for GZC were identified as stable based on the mean vs. WAASB bi-plot. V21 for GYP and V4 for GZC were the highest yielder and widely adaptable based on WAASBY scores. V13 for GYP and V1 for GZC were all-time winners. V13 and V1 have the highest predicted mean for GYP and GZC, respectively, based on BLUP. V6, V21 and V13 were identified as stable and selected based on the MTSI. Using BLUP-based stability methods, MTSI, and strength and weakness plots are potential and more efficient tools that make evaluating and selecting genotypes for varietal recommendations easier. These results of this investigation will also provide a pipeline for future Zn-fortified rice breeding studies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest on the manuscript's content and study undertaken.

AUTHOR CONTRIBUTIONS

Conceptualization, S.K.S. and P.P.B.; methodology, P.P.B. and S.K.S.; software, R.N.S and P.P.B; validation, S.K.S. and R.N.S.; formal analysis, S.K.S. and R.N.S.; investigation, S.K.S.; resources, S.K.S; data curation, P.P.B. and S.K.S.; writing—original draft preparation, P.P.B., P.K.M., K.S., B.J.R.; writing—review and editing, P.P.B., N.S., P.K.M., N.B.; visualization, R. N. S., S. K. S.; supervision, S.K.S. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

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SUPPLEMENTARY TABLES

Table S1: List of high zinc rice genotypes and 14 traits used for the experiment (Source: IRRI South Asia Hub, Hyderabad, India)

Entry No.	Entry Name	Genotype Code	Grain Zinc Content (Ppm)	Sl. No.	Trait Name	Abbreviation
1	IR 95044:8-B-5-22-19-GBS	V1	20.6	1	Days to 1st flowering date	Fst DF
2	IR 84847-RIL 195-1-1-1-1	V2	21.8	2	Days to 50% flowering	FDF
3	IR 99704-24-2-1	V3	14.67	3	Days to maturity	DM
4	IR 99647-109-1-1	V4	23.7	4	Total effective tiller number	ENT
5	IR 97443-11-2-1-1-1-1 -B	V5	14.45	5	Plant height (cm)	PH
6	IR 97443-11-2-1-1-1-3 -B	V6	23.47	6	Panicle length (cm)	PL
7	IR 82475-110-2-2-1-2	V7	24.73	7	Number of spikelets per panicle	NSP
8	IR 96248-16-3-3-2-B	V8	27.18	8	Number of grains/ panicles	NGP
9	R-RHZ-7	V9	26.61	9	Spikelets fertility Percentage	SFP
10	CGZR-1	V10	24.43	10	Grain weight per panicle	GWP
11	BRRI dhan 62	V11	23.33	11	Weight of 1000 Seed (gm)	STW
12	BRRI dhan 64	V12	24.97	12	Grain Length/Breadth ratio	GLBR
13	BRRI dhan 72	V13	20.7	13	Grain Zinc content (ppm)	GZC
14	DRR Dhan 45	V14	18.13	14	Grain yield/plant (gm)	GYP
15	DRR Dhan 48	V15	19.2			
16	DRR Dhan 49	V16	17.63			
17	IR 64	V17	23.57			
18	MTU1010	V18	21.70			
19	Sambamahsuri	V19	24.47			
20	Swarna	V20	18.89			
21	Local check (HUR3022)	V21	16.9			

Table S2: Mean Performance of 21 high zinc rice genotypes and mean value of traits in individual environment for GZC (ppm) and GYP (gm)

Geno-types code	GenotypeName	Mean performance of GYP						Mean performance of GZC						Mean Value of Traits in Individual Environment			
		E1	E2	E3	E4	E5	Mean	E1	E2	E3	E4	E5	Mean	Sl. No	Environment	GZC	GYP
V1	IR 95044:8-B-5-22-19-GBS	10.86	12.92	13.05	7.68	6.01	10.11	29.63	27.25	29	15.61	32.54	26.81	1	E1	24.05	12.63
V2	IR 84847-RIL 195-1-1-1-1	11.50	13.66	13.74	7.99	6.57	10.69	27.63	25.67	28.67	17.62	29.66	25.85	2	E2	22.31	14.94
V3	IR 99704-24-2-1	13.12	15.58	14.33	9.96	8.39	12.27	25.23	23.66	27.28	18.43	26.64	24.25	3	E3	23.75	16.53
V4	IR 99647-109-1-1	10.79	12.77	15.28	5.35	5.12	9.86	28.79	26.41	27.47	14.22	31.93	25.76	4	E4	14.27	7.01
V5	IR 97443-11-2-1-1-1 –B	14.90	17.62	18.01	9.10	9.31	13.79	19.61	18.12	17.47	9.84	22.43	17.49	5	E5	26.41	6.99
V6	IR 97443-11-2-1-1-3 –B	15.39	18.15	20.30	7.59	9.10	14.11	19.88	18.73	20.66	14.6	21.46	19.07				
V7	IR 82475-110-2-2-1-2	12.91	15.30	15.61	8.35	7.66	11.97	29.14	26.68	27.4	13.65	32.5	25.87				
V8	IR 96248-16-3-3-2-B	12.22	14.42	17.81	5.09	6.01	11.11	27.34	25.16	26.32	14.22	30.25	24.66				
V9	R-RHZ-7	11.25	13.33	15.49	5.88	5.63	10.31	25.92	23.95	25.35	14.45	28.54	23.64				
V10	CGZR-1	10.96	13.03	13.57	7.33	5.96	10.17	26.57	24.77	27.93	17.77	28.39	25.09				
V11	BRRIdhan 62	10.76	12.76	14.95	5.65	5.21	9.86	24.67	22.99	25.32	15.99	26.66	23.13				
V12	BRRIdhan 64	12.73	15.01	18.73	4.98	6.32	11.56	26.26	24.24	25.56	14.36	28.96	23.88				
V13	BRRIdhan 72	15.92	18.78	20.36	8.38	9.75	14.64	18.26	17.14	17.91	12.26	20.21	17.16				
V14	DRR Dhan 45	12.99	15.33	18.31	5.81	6.80	11.85	24.05	22.24	23.2	13.38	26.65	21.9				
V15	DRR Dhan 48	11.52	13.69	13.69	8.07	6.61	10.72	21.88	20.48	22.4	14.86	23.72	20.67				
V16	DRR Dhan 49	14.34	16.95	18.11	8.13	8.58	13.22	22.13	20.37	20.04	10.76	25.12	19.68				
V17	IR 64	9.63	11.46	12.84	5.96	4.54	8.89	24.22	22.4	23.38	13.45	26.82	22.05				
V18	MTU1010	13.67	16.14	18.63	6.56	7.54	12.51	22.97	21.24	21.78	12.49	25.63	20.82				
V19	Sambamahsuri	11.91	14.12	15.11	7.28	6.59	11.00	20.07	19.13	22.71	17.63	20.86	20.08				
V20	Swarna	13.85	16.29	21.02	4.47	6.91	12.51	17.32	16.37	17.55	12.87	18.94	16.61				
V21	Local check (HUR3022)	13.95	16.49	18.11	7.52	8.09	12.83	23.53	21.61	21.36	11.11	26.65	20.85				

Table S3: AMMI based stability indices and Weighted average of absolute scores for GYP and GZC in high zinc rice genotypes

GEN	GYP						GZC					
	Y1	ASV	SIPC	EV	ZA	WAASB	Y2	ASV	SIPC	EV	ZA	WAASB
V1	10.19	2.17	1.68	0.05	0.23	0.77	26.64	2.64	2.48	0.07	0.25	0.95
V10	10.07	1.70	1.70	0.06	0.20	0.68	25.06	0.80	0.88	0.01	0.08	0.30
V11	9.79	0.20	0.28	0.00	0.03	0.08	23.05	0.57	1.15	0.02	0.09	0.30
V12	11.65	1.88	2.78	0.18	0.26	0.83	23.97	0.90	1.07	0.01	0.10	0.37
V13	14.58	1.36	1.50	0.03	0.17	0.54	17.04	1.66	1.44	0.03	0.14	0.55
V14	11.90	1.31	1.20	0.03	0.14	0.47	21.89	0.53	0.54	0.00	0.05	0.19
V15	10.76	1.89	1.70	0.05	0.20	0.66	20.62	1.42	1.97	0.07	0.16	0.60
V16	13.18	0.92	1.11	0.03	0.11	0.35	19.74	1.06	1.21	0.02	0.11	0.41
V17	8.97	1.41	1.60	0.05	0.17	0.56	21.89	0.97	1.43	0.03	0.12	0.43
V18	12.50	1.40	1.75	0.05	0.19	0.60	20.75	0.84	1.74	0.05	0.13	0.45
V19	10.87	1.49	2.02	0.10	0.19	0.60	20.35	4.70	3.09	0.13	0.37	1.48
V2	10.79	1.92	1.45	0.03	0.19	0.66	25.70	1.58	2.07	0.08	0.17	0.59
V20	12.44	3.32	2.21	0.10	0.30	1.02	16.65	2.83	1.93	0.05	0.22	0.86
V21	12.88	0.47	0.48	0.00	0.05	0.18	20.91	1.49	1.49	0.03	0.15	0.56
V3	12.29	2.36	1.70	0.06	0.22	0.74	24.11	1.70	2.19	0.06	0.19	0.71
V4	9.92	1.11	1.23	0.06	0.11	0.33	25.97	2.05	2.28	0.06	0.21	0.80
V5	13.80	0.41	0.76	0.01	0.07	0.21	17.39	0.59	1.63	0.05	0.11	0.38
V6	14.23	1.38	1.32	0.03	0.15	0.51	19.06	2.32	2.06	0.05	0.21	0.81
V7	11.86	0.90	0.90	0.02	0.10	0.32	25.79	3.14	2.22	0.07	0.25	0.97
V8	11.11	1.53	1.63	0.04	0.19	0.61	24.87	1.49	1.77	0.04	0.16	0.60
V9	10.20	0.49	0.80	0.01	0.07	0.21	23.87	1.15	1.32	0.04	0.11	0.37

*Y1- yield/plant, Y2-grain zinc content, ASV- AMMI stability values, SIPC-Sums of the absolute value of the IPC scores, EV- Averages of the squared eigenvector values, ZA-Absolute value of the relative contribution of IPCs to the interaction and WAASB- Weighted Average of Absolute Scores.

Table S4: Mean grain yield per plant (gm) and zinc content (ppm) and WAASB and WAASBY scores of 21 high zinc rice genotypes

Sl. No	Code	GYP						GZC					
		Y	rResp	WAASB	rWAASB	WAASBY	rWAASBY	Y	rResp	WAASB	rWAASB	WAASBY	rWAASBY
1	V1	10.19	17	0.62	19	24.37	20	26.64	1	0.81	20	67.78	10
2	V2	10.79	14	0.52	15	36.57	15	25.70	4	0.31	4	87.88	2
3	V3	12.29	8	0.11	1	79.61	2	24.11	7	0.26	2	82.60	4
4	V4	9.92	19	0.65	20	19.98	21	25.96	2	0.32	6	88.66	1
5	V5	13.80	3	0.48	11	66.22	6	17.38	19	0.48	12	37.75	17
6	V6	14.23	2	0.35	8	79.23	3	19.06	18	0.16	1	62.08	12
7	V7	11.86	10	0.56	17	43.62	13	25.79	3	0.48	11	79.97	6
8	V8	11.11	12	0.29	6	55.96	8	24.87	6	0.32	5	83.19	3
9	V9	10.20	16	0.49	12	33.75	16	23.87	9	0.34	7	77.42	7
10	V10	10.07	18	0.50	14	31.89	17	25.06	5	0.36	9	82.33	5
11	V11	9.79	20	0.49	13	29.89	19	23.04	10	1.16	21	32.02	20
12	V12	11.65	11	0.54	16	42.63	14	23.97	8	0.51	14	69.31	9
13	V13	14.58	1	0.81	21	50.00	11	17.04	20	0.73	18	23.79	21
14	V14	11.90	9	0.15	3	72.66	5	21.89	11	0.45	10	61.99	13
15	V15	10.76	15	0.59	18	31.84	18	20.62	15	0.61	15	47.37	14
16	V16	13.18	4	0.28	5	75.33	4	19.74	17	0.69	17	39.18	16
17	V17	8.97	21	0.18	4	44.69	12	21.88	12	0.29	3	69.90	8
18	V18	12.50	6	0.41	9	59.68	7	20.75	14	0.67	16	45.40	15
19	V19	10.87	13	0.33	7	50.72	10	20.35	16	0.79	19	37.22	18
20	V20	12.44	7	0.48	10	54.40	9	16.64	21	0.51	13	32.73	19
21	V21	12.88	5	0.14	2	82.24	1	20.90	13	0.35	8	62.09	11

Table S5: Prediction of GZC and GYP of 21 high zinc rice genotypes over five environments through BLUP analysis

GEN	GZC				GYP			
	BLUPg	BLUPge	BLUPg+ge	Predicted	BLUPg	BLUPge	BLUPg+ge	Predicted
V1	4.03	0.33	4.36	26.52	-1.12	-0.22	-1.34	10.28
V2	3.18	0.26	3.45	25.60	-0.65	-0.13	-0.78	10.84
V3	1.76	0.14	1.90	24.06	0.53	0.10	0.63	12.25
V4	3.42	0.28	3.70	25.86	-1.33	-0.26	-1.59	10.03
V5	-4.29	-0.35	-4.64	17.52	1.71	0.33	2.04	13.66
V6	-2.78	-0.23	-3.01	19.15	2.05	0.40	2.44	14.06
V7	3.27	0.27	3.54	25.69	0.19	0.04	0.23	11.84
V8	2.44	0.20	2.63	24.79	-0.40	-0.08	-0.48	11.14
V9	1.54	0.13	1.67	23.83	-1.11	-0.22	-1.33	10.29
V10	2.61	0.21	2.82	24.98	-1.21	-0.23	-1.45	10.17
V11	0.80	0.07	0.86	23.02	-1.43	-0.28	-1.71	9.91
V12	1.63	0.13	1.77	23.92	0.02	0.00	0.03	11.65
V13	-4.60	-0.38	-4.98	17.18	2.31	0.45	2.76	14.38
V14	-0.24	-0.02	-0.26	21.90	0.22	0.04	0.26	11.88
V15	-1.38	-0.11	-1.50	20.66	-0.67	-0.13	-0.80	10.82
V16	-2.17	-0.18	-2.35	19.81	1.22	0.24	1.46	13.07
V17	-0.24	-0.02	-0.26	21.90	-2.07	-0.40	-2.47	9.15
V18	-1.26	-0.10	-1.37	20.79	0.69	0.13	0.83	12.45
V19	-1.63	-0.13	-1.76	20.40	-0.59	-0.11	-0.70	10.92
V20	-4.96	-0.41	-5.36	16.80	-0.59	-0.11	-0.70	10.92
V21	-1.13	-0.09	-1.22	20.94	0.99	0.19	1.18	12.80

Table S6: BLUP parameters for the trait GZC (ppm) and GYP (gm) in 21 high zinc rice genotypes

GEN	BLUP Parameters of GZC									BLUP Parameters of GYP								
	Y (GZC)	HM GV	HM GV_R	RP GV	RP GV_Y	RP GV_R	HM RP GV	HM RP GV_Y	HM RP GV_R	Y (GZC)	HM GV	HM GV_R	RP GV	RP GV_Y	RP GV_R	HM RP GV	HM RP GV_Y	HM RP GV_R
V1	26.64	24.72	1	1.19	26.31	1	1.18	26.22	1	10.19	8.95	16	0.90	10.40	16	0.88	10.19	17
V2	25.70	24.49	3	1.16	25.66	3	1.15	25.57	3	10.79	9.64	12	0.95	11.03	13	0.94	10.88	13
V3	24.11	23.32	7	1.10	24.29	7	1.09	24.13	7	12.29	11.23	6	1.09	12.66	6	1.07	12.45	6
V4	25.97	24.54	2	1.16	25.80	2	1.16	25.76	2	9.92	7.84	20	0.84	9.71	20	0.81	9.37	20
V5	17.39	15.98	21	0.78	17.25	19	0.77	17.09	20	13.80	12.43	3	1.20	13.96	3	1.20	13.89	3
V6	19.06	18.59	17	0.87	19.33	18	0.87	19.25	18	14.23	12.52	2	1.22	14.19	2	1.22	14.17	2
V7	25.79	23.67	5	1.15	25.40	4	1.14	25.26	4	11.86	10.64	7	1.04	12.03	8	1.03	11.96	8
V8	24.87	23.60	6	1.12	24.79	6	1.12	24.74	6	11.11	8.80	17	0.92	10.72	15	0.91	10.54	15
V9	23.87	22.88	8	1.08	23.92	8	1.08	23.84	9	10.20	8.48	18	0.87	10.07	18	0.86	9.98	18
V10	25.06	24.13	4	1.14	25.15	5	1.13	25.08	5	10.07	8.98	15	0.88	10.27	17	0.88	10.20	16
V11	23.05	22.11	10	1.04	23.10	10	1.04	23.07	10	9.79	8.24	19	0.84	9.71	19	0.83	9.65	19
V12	23.97	22.77	9	1.08	23.90	9	1.08	23.89	8	11.65	9.73	11	1.00	11.56	11	0.97	11.28	11
V13	17.04	16.35	19	0.77	17.17	20	0.77	17.14	19	14.58	12.75	1	1.25	14.53	1	1.24	14.46	1
V14	21.89	20.72	11	0.98	21.82	11	0.98	21.81	11	11.90	10.09	9	1.01	11.73	10	1.01	11.70	9
V15	20.62	19.89	14	0.94	20.77	14	0.93	20.72	13	10.76	9.97	10	0.96	11.15	12	0.95	11.03	12
V16	19.74	18.40	18	0.89	19.62	17	0.88	19.53	17	13.18	11.64	4	1.14	13.25	4	1.13	13.17	4
V17	21.89	20.42	12	0.98	21.72	12	0.98	21.66	12	8.97	7.81	21	0.78	9.09	21	0.78	9.02	21
V18	20.75	19.45	15	0.93	20.64	16	0.93	20.57	16	12.50	10.56	8	1.06	12.28	7	1.05	12.24	7
V19	20.35	20.27	13	0.95	21.00	13	0.93	20.57	15	10.87	9.36	14	0.94	10.92	14	0.93	10.77	14
V20	16.65	16.32	20	0.77	16.96	21	0.76	16.90	21	12.44	9.46	13	1.02	11.81	9	0.99	11.48	10
V21	20.91	19.41	16	0.94	20.73	15	0.93	20.63	14	12.88	11.37	5	1.11	12.90	5	1.11	12.89	5

Table S7: Eigen values, explained variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis

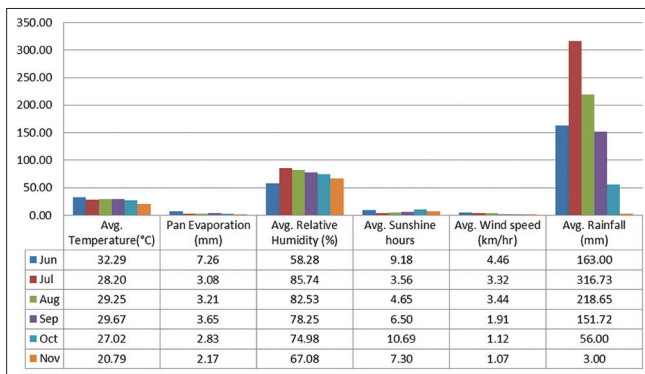| Sl. No.	Traits	FA1	FA2	FA3	FA4	Communality
1	Days to 1st flowering date	-0.2	-0.96	-0.1	0	0.98
2	Days to 50% flowering	-0.19	-0.97	-0.08	0.03	0.98
3	Days to maturity	-0.19	-0.97	-0.08	0	0.98
4	Total effective tiller number	0.52	-0.23	-0.57	-0.42	0.83
5	Plant height (cm)	-0.18	0	0.81	0.16	0.71
6	Panicle length (cm)	-0.22	-0.06	0.13	0.88	0.83
7	Number of spikelets per panicle	-0.97	-0.16	0.1	0.05	0.98
8	Number of grains/panicles	-0.97	-0.15	-0.06	0.14	0.98
9	Spikelets fertility Percentage	0.13	0.06	-0.78	0.42	0.8
10	Grain weight per panicle	-0.79	-0.01	0.5	0.34	0.99
11	Weight of 1000 Seed (gm)	0.4	0.3	0.77	0.26	0.91
12	Grain Length/Breadth ratio	0.13	-0.24	-0.33	-0.15	0.21
13	Grain Zinc content (ppm)	0.71	0.5	0.07	0.09	0.76
14	Grain yield/plant (gm)	-0.78	-0.29	0.29	0.18	0.8
15	Eigen Value	5.51	3.69	1.51	1.04	
16	Variance	39.36	26.32	10.78	7.44	
17	Cumulative Variance	39.36	65.68	76.46	83.9	

SUPPLEMENTARY FIGURES

Fig S1. Weather data of rice crop growth cycle during kharif season 2017 (June-November).
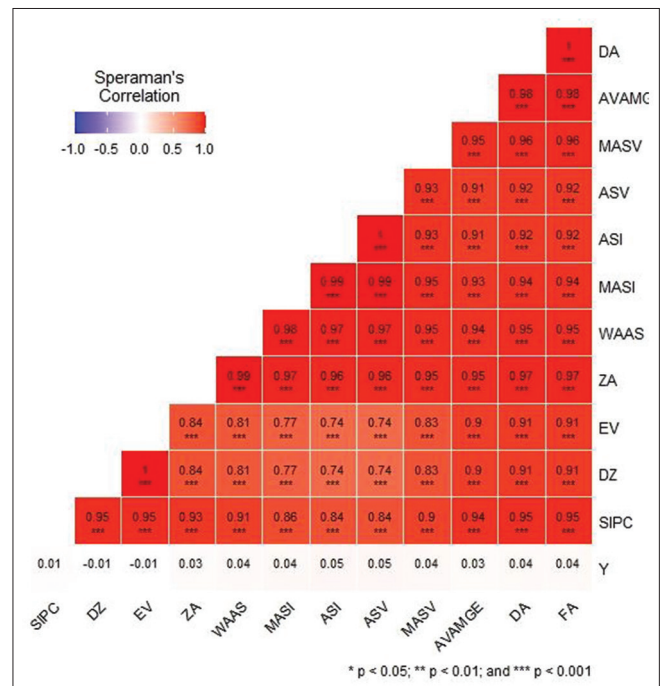

Fig S2. Correlation heat map of AMMI based stability indices.
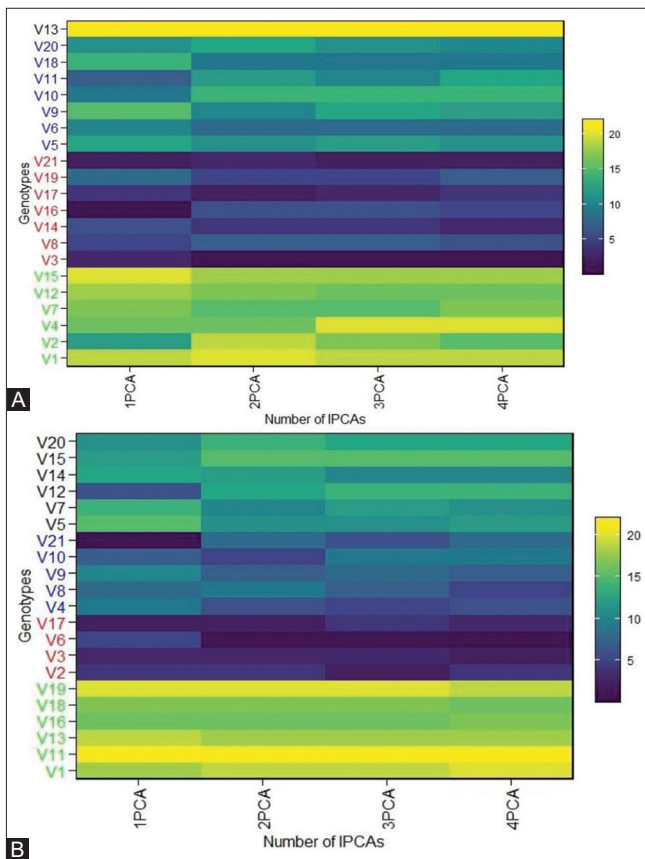
Fig S3. Heat map showing the ranks of 21 high zinc rice genotypes for (A) GYP and (B) GZC evaluated in five environments in relation to the number of interaction principal component axes (IPCA) used in WAASB genotype vs. environment interaction estimation.
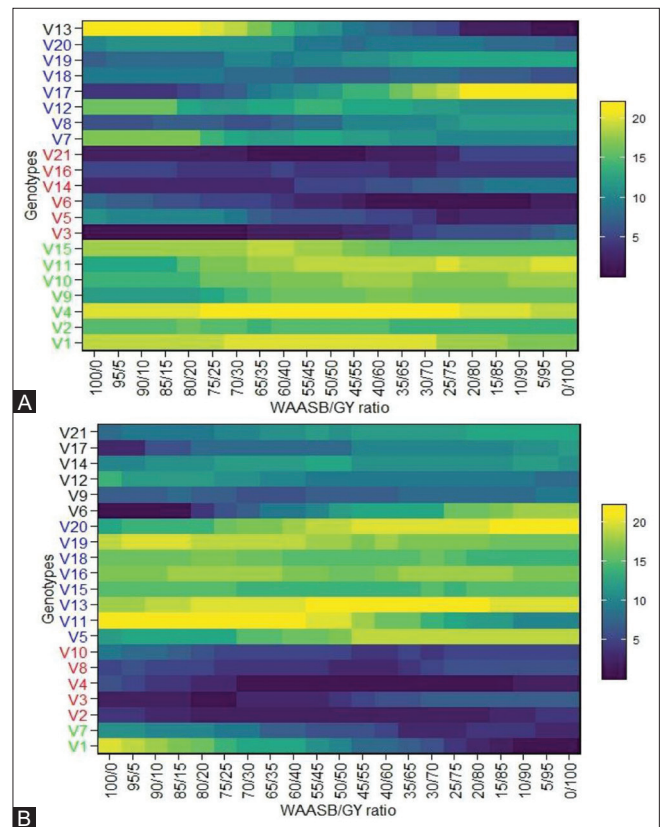
Fig S4. Ranks of 21 high zinc rice genotypes for A-GYP and B-GZC considering different weights for stability and yielding. The most-left ranks were obtained considering the stability only. The most right-ranks were obtained considering the grain yield (A) and grain zinc content (B).
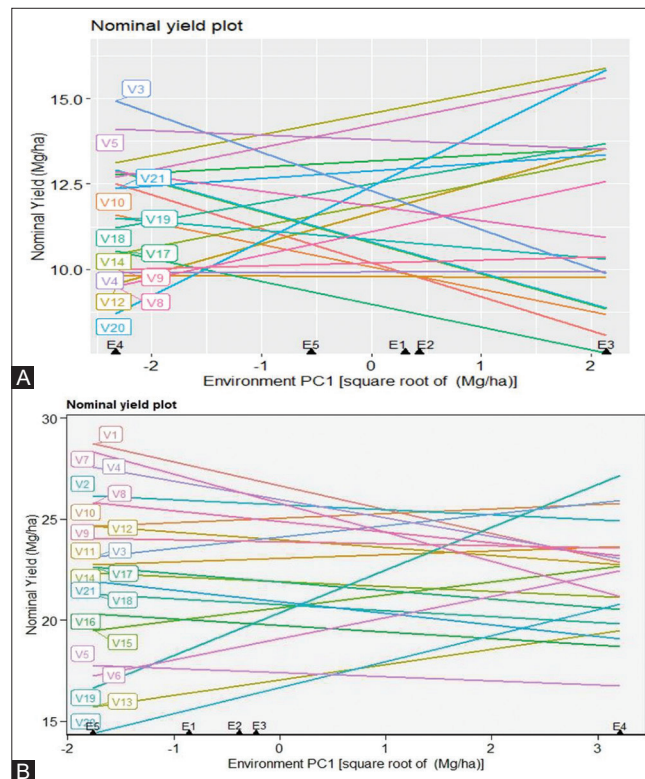
Fig S5. Which-won-where plots based on predicted yields (Nominal yield- (A) GYP, (B) GZC) in each environment for 21 high zinc rice genotypes as a function of the environment scores of the first interaction principal component axis (IPCA1).
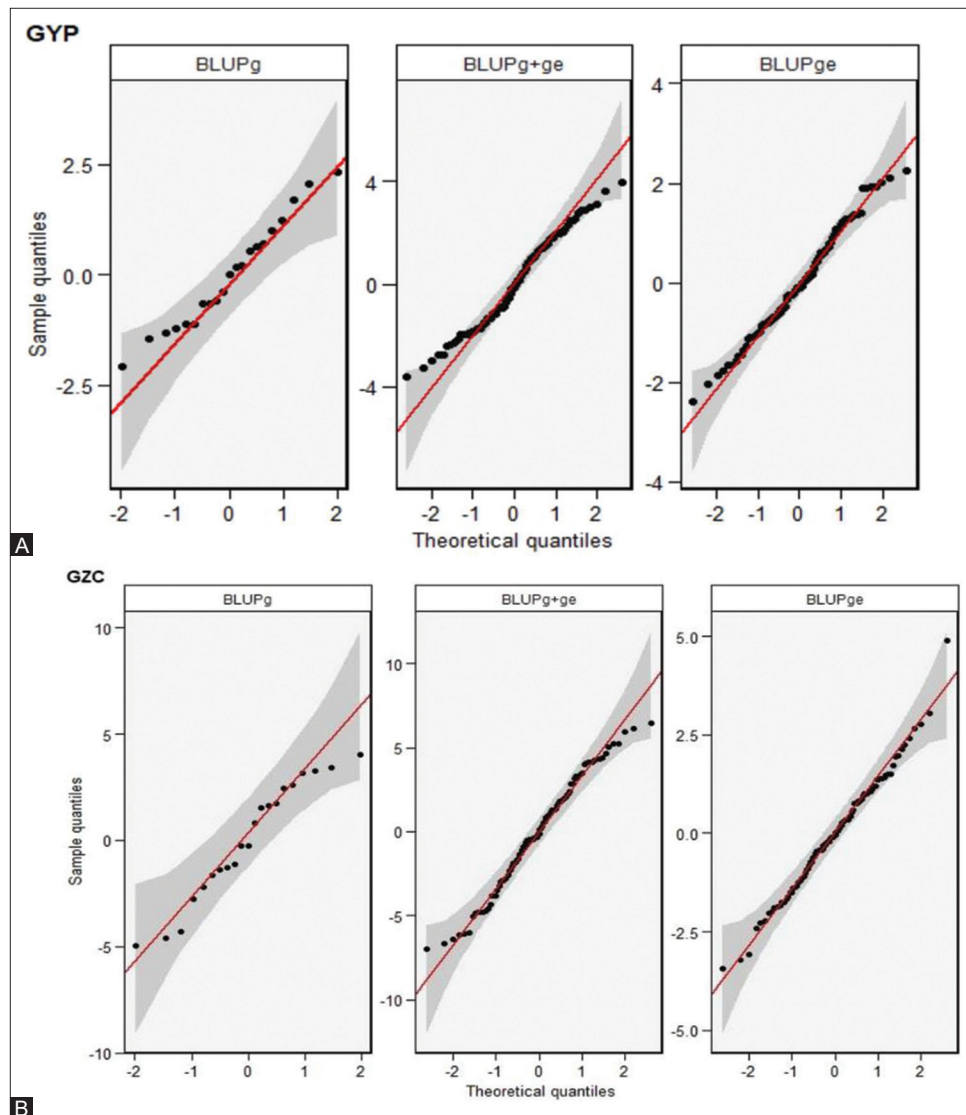
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Fig S6. Quantile-Quantile (Q – Q) plot to check normality of the random effects of genotypes and interaction effects of 21 high zinc rice genotypes for (A) GYP and (B) GZC.