



Karuppunel: A promising donor for high zinc content in rice (*Oryza sativa*) grain

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ABSTRACT

Complex inheritance of high iron (Fe) and zinc (Zn) traits in polished grains, coupled with large genotype (G) × environment (E) interaction components are major challenges for rice biofortification programmes. Understanding such G × E interactions through multi-location trials and quantifying their magnitude using appropriate statistical models is the major pre-requisite in identification of stable donors for micronutrient traits for development of nutrient rich varieties. In the present study, we evaluated a set of 28 rice genotypes for Fe, Zn and key agronomical traits during wet season 2017 in 5 diverse environments in India. Combined analysis of variance revealed significant main effects due to genotypic, environmental and G × E interaction effects. Grain Fe showed maximum contribution from the effect of the genotype while in case of Zn, it was influenced significantly by environmental effect. Mega-environments were identified for stable evaluation of genotypes for Zn and Fe content, based on GGE biplot analysis. Yield stability index identified the genotype, G14 (Karuppunel) to be superior for grain Zn content (41.1 ppm) with high mean performance and high stability across the environments followed by the genotypes G4 (Taraori Basmati), G18, G25 (Tilakasturi and IC36704) and G2 (Edavankudipokkali). The findings from the study have significant implications for the development of high grain Zn containing rice varieties, so that the hidden hunger can be addressed in the right perspective.

Keywords: Biofortification, Donor for high zinc, Environments, G × E interactions, Rice genotypes

Malnutrition and Micronutrient deficiency is affecting most of the low and middle-income countries, where rice is the major staple food crop. Biofortification through genetic enhancement is a promising and proven strategy for increasing micronutrient content of staple food crops (Bouis *et al.* 2019). Rice being major staple food, providing >50% of daily calories to more than half of the world's population is a key target for biofortification. Zn is a critical element for growth and development in human beings, particularly for children and women. Using conventional breeding, by utilizing donors with high grain Zn and without compromising yield, 6 rice varieties (DRRDhan 45, DRRDhan 48, DRRDhan 49, CRDhan 311, Zinco rice and Surabhi) have been released in India with grain Zn content ranging from 20–24 ppm in polished rice. With the

threshold target levels of grain Zn content increased to 28 ppm by HarvestPlus, identification of stable donor genotypes with higher grain Zn would hasten the development of the Zn biofortified rice varieties. From stability and G × E analysis, high Zn rice varieties (Inabangan-Asilo *et al.* 2019), stable promising Zn donors (Madhu *et al.* 2020) and stable genotypes for Zn (Naik *et al.* 2020) were identified. High Zn landrace 'Karuppunel' identified in the study is being used in biofortification breeding programmes to develop high Zn biofortified rice varieties.

Under biofortification projects, various research groups in India have identified promising donors for Fe and Zn after evaluation of several thousands of germplasm. Thus, by pooling these promising donors identified by 4 research groups of India, the present study was carried out with the following objectives, (i) Evaluation of set of landraces and breeding lines along with a control (check) varieties for grain Zn and Fe content in polished rice, single plant yield, days to 50% flowering, plant height and panicle length across five diverse environments, (ii) Analyze the G × E interactions for 6 key agronomic traits and grain micronutrient content and (iii) identify promising and stable donors for grain Zn/Fe content across environments as well as in specific environments.

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MATERIALS AND METHODS

Experimental materials and design: The experimental material included 28 rice genotypes (G1-G28) comprising landraces and breeding lines. The experimental trials were conducted during wet season 2017 at 5 locations in India, viz. experimental farms ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, Telangana (E1); ICAR-Indian Agricultural Research Institute (IARI), New Delhi (E2); ICAR-National Rice Research Institute (NRRRI), Cuttack, Odisha (E3); Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh (E4) and Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu (E5) as test environments. The 3 released rice varieties BPT5204, Sneha and Uphar were used as checks. Randomized Complete Block Design (RCBD) with 3 replications was adopted as the experimental design to evaluate the genotypes in the 5 test environments (E1-E5). The five locations are situated at 4 different agro-ecological zones of India with diverse soil and climatic conditions as detailed in Supplementary Table 1.

Phenotyping: Data on the agronomical traits, viz. days to 50% flowering (DFF), plant height (PH), panicle length (PL) and single plant yield (SPY) were recorded from the middle 5 plants following the standard protocols. The paddy harvested from the 5 test locations was sent to ICAR-IIRR where the samples were dehusked (Jingjian Huayuan International Trade Co., Ltd, sponsored by Harvest Plus) and polished in a specially designed K-710 Non-Ferrous Rice Polisher (Krishi International India Ltd., India). The SPY from 3 plants were pooled and divided into 3 parts and analyzed as 3 replicates. The polished rice (5 g) was subjected to energy dispersive X-ray fluorescent spectrophotometer (ED-XRF) (OXFORD Instruments X-Supreme 8000, sponsored by Harvest Plus) at ICAR-IIRR.

Statistical analyses: The descriptive statistics, viz. mean, range, coefficient of variation were analyzed using Microsoft Excel and the statistical package STAR 2.0.1 (IRRI 2014 a, b). Combined analysis of variance (ANOVA) to assess the magnitude of genotypic, environment and

genotype × environment effects was done by Additive Main effect and Multiplicative Interactions (AMMI) model by considering genotypes as fixed effects and environments as random effects. AMMI model was executed using the R package *agricolae* (De Mendiburu and Simon 2015).

RESULTS AND DISCUSSION

Genotypic variation: Wide phenotypic variation was observed for the studied traits in the 28 genotypes. Genotypic means ranged from 82 (G20:ThukiGurmatiya) to 109 days (G11:Chittimuthyalu) for DFF, 85.06 (G6:BPT5204) to 162.83 cm (G21:ShyamJeera) for PH, 19 (G6:BPT 5204) to 39.8 cm (G21:ShyamJeera) for PL, 6.4 (G16:Jeeradhan) to 22.8 g (G8:ARC10075) for SPY, 1.6 (G26:ARB6001) to 4.3 ppm for Fe (G14:Karuppunel), 13.9 (G10:Sneha) to 41.0 ppm (G14:Karuppunel) for Zn. Wide variation was also observed among the locations for DFF, PH, PL and Zn while for the traits PL and Fe comparatively similar means were recorded across the locations (Table 1). G11 and G6 showed late maturity, while G20 and G9 were found to be early maturing. G21 and G22 showed maximum height with longer panicles, while G6 recorded minimum height with smallest panicles. Maximum SPY was recorded in G8 and G10, while G16, G25 and G15 were found to be poor yielders. For the micronutrient traits, G14 (Karuppunel) recorded exceptionally higher mean values for Zn (41.05 ppm), while G10 and G26 showed minimum values for Fe and Zn in polished rice (Supplementary Table 2). “Karuppunel” identified as best donor for high Zn (>28 ppm), was found to be early flowering (97 days) with 136 cm PH, 22 cm PL and SPY of 12 g.

In the present study, Fe showed limited variability in the range of 1.6–4.3 ppm and none of the 28 accessions recorded Fe concentration of ≥ 12 ppm as per the standards set by the Harvest Plus. The results obtained supports earlier reports on limited variability for Fe concentration in polished rice (Bashir *et al.* 2013) and indicates that there is only a limited scope of improvement for Fe from the existing variability

Table 1 Descriptive statistics of micronutrient and agronomic traits in 5 test environments

Environment	Fe		Zn		DFF		PH		PL		SPY	
	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
E1	2.7 \pm 0.2	1.1 – 5.6	20.8 \pm 1.5	10.7 – 48.2	86.8 \pm 1.6	72 - 94	141.0 \pm 5.0	87.4 – 176.3	26.2 \pm 0.7	19.6 – 33.6	12.4 \pm 1.1	3.3 – 27.4
E2	2.64 \pm 0.2	1.2 - 4.8	19.0 \pm 0.9	13 – 36.9	122.9 \pm 2.4	97.7 – 151.7	118.8 \pm 5.0	54.7 – 167.3	24.7 \pm 0.5	15.5 – 31.9	25.11 \pm 1.78	10.3 – 42.3
E3	3.5 \pm 0.2	1.5 – 6.0	30.9 \pm 1.2	18.8 – 41.4	96.6 \pm 2.9	63.3 – 120.7	136.7 \pm 6.0	69.2 – 176.5	25.6 \pm 0.9	17.8 – 34.5	11.6 \pm 1.2	1.9 – 30.3
E4	2.6 \pm 0.2	1.2 – 5.6	20.0 \pm 1.5	8.4 – 49.7	91.8 \pm 2.3	71.3 – 114.3	145.1 \pm 4.4	91.5 – 183.6	26.7 \pm 0.5	21.1 – 33.2	10.1 \pm 0.7	4 – 17.3
E5	2.4 \pm 0.2	0.97 – 4.5	13.8 \pm 0.9	8.1 – 29.1	103.4 \pm 0.9	99 – 119.3	110.1 \pm 3.4	79 – 139.8	24.1 \pm 0.5	19.8 – 28.5	10.8 \pm 0.9	5.9 – 27.3

Fe, iron in polished rice; Zn, zinc in polished rice; DFF, days to 50% flowering; PH, plant height; PL, panicle length and SPY, single plant yield.

Environment details are given in Materials and Methods.

in the primary gene pool. In contrast to Fe, wide variability was observed for Zn indicating the scope of its improvement through appropriate breeding strategies. Seven of the 28 genotypes recorded Zn concentration of >24 ppm in at least two of the test locations. G14 (Karuppunel) appeared to be the best genotype for Zn accumulation in endosperm as it recorded >28 ppm of Zn in polished rice in all the 5 test locations and was identified as promising donor for the development of biofortified varieties with high grain Zn.

Correlation: Grain Zn and Fe content showed significant ($P<0.05$) positive correlation at E2, E3, E5, while no correlation was observed between these two traits at E1 and E4. A slight negative correlation between Zn and SPY was observed at E1 and E4. Positive correlation was observed between PH and PL at all locations. Significant positive correlation between Fe and Zn was observed in earlier reports (Anuradha *et al.* 2012). Earlier studies also reported significant negative correlation between Zn and yield (Dixit *et al.* 2019), to absence of correlation (Gangashetty *et al.* 2013). The absence of correlation between the Zn and yield observed in this study could be owing to the relatively lesser yields of landraces. High positive correlation was observed for Fe among environments E1, E2 and E5, followed by E4 and E5. Positive correlation was observed between E1 and E4; E2 and E5 for Zn; between E1 and E3 for PH, PL and SPY; between E3 and E4 for PH and PL.

Combined AMMI-ANOVA analysis: Combined AMMI-ANOVA analysis was performed to partition the observed total phenotypic variation into genotype (G), environment (E) and $G \times E$ interaction components, for 5 environments, showed highly significant ($P<0.0001$) contribution of main effects due to genotypes and environments and the interaction effect due to $G \times E$ interaction for the five traits studied (Table 2). Zn has highest contribution from environment effect (40.3%) followed by genotype (34.8%) and $G \times E$ interaction (19.1%). Contrarily Fe has major contribution from genotype (42.1%) followed by $G \times E$ interaction (31.8%) and environment effect (10.9%). Among

the agronomic traits, PH (55.02%) and PL (55.04%) had major portion of total variation contributed by the genotype effect, whereas for DFF and SPY the maximum proportion of variation was explained by environment effect (DFF: 54.1%, SPY: 40.66%) followed by $G \times E$ interaction (DFF: 27.9%, SPY:31.4%) and genotype (DFF: 17.5%, SPY:19.8%). The first four principal components of $G \times E$ interaction were highly significant for Fe, Zn and DFF and for PH, PL and SPY, the first three principal components were significant. IPC1 and IPC2 together explained maximum proportion of interaction variance ranging from 69.5 (DFF) to 91.7% (PH).

Stability analysis based on AMMI biplots: AMMI 1 biplots presenting the main effects plotted against the IPCA1 scores were used to identify the best performing genotypes in the respective environments (Fig 1). The AMMI 2 biplots showing IPCA1 scores were plotted against IPCA2 scores are given in Supplementary Fig 1.

For Zn, AMMI 2 biplot showed 83.2% goodness of fit and E3 was found to be the promising environment. E5 was found to be poor for expression of grain Zn content. G15 and G16 were stable across the environments. For Fe, the AMMI 2 biplot showed a goodness of fit of 72% for Fe and E3 was favourable for the expression of most of the genotypes. G16 and G11 were stable across the environments. For DFF, AMMI2 biplot explained 69.5% of the total variation G9 and G13 showed stable performance across the environments. For PH, E2 and E5 were favourable environments and for PL E4, E1 and E3 were the top performers. For SPY, AMMI 2 biplot showed 89.2% of fitness and G2 and G3 were stable across the environments.

The study showed significance of $G \times E$ interaction effects for the 6 traits in confirmation with the earlier reports (Rerkasem *et al.* 2015). Grain Fe, PH and PL showed maximum contribution from genotype, while grain Zn and SPY depicted greater contribution from the environment effect. These results highlighted that grain Zn and SPY are more sensitive to environmental factors as compared to Fe, PH and PL substantiating the present study. Significant

Table 2 Combined AMMI analysis of variance for the agronomic and mineral micronutrient traits

Source of variation	df	DFF		PH		PL		SPY		Fe		Zn	
		MS	VE (%)	MS	VE (%)	MS	VE (%)	MS	VE (%)	MS	VE (%)	MS	VE (%)
ENV	4	16657.3***	54.1	19263.8***	21.6	94.8***	6.6	3303.4***	40.7	16.5***	10.9	3297.3***	40.3
REP(ENV)	10	14.2	0.1	29.0	0.0	0.5	0	32.9***	0.2	0.6**	0	3.9***	0.01
GEN	27	797.3***	17.5	7263.0***	55.0	117.5***	55.0	238.8***	19.8	9.4***	42.1	421.8***	34.8
ENV:GEN	108	318.9***	27.9	661.6***	20.1	13.4***	25.1	94.6***	31.4	1.8***	31.8	57.7***	19.1
PC1	30	473.1**	41.2	1653.19***	69.4	30.6***	63.4	230.4***	67.6	2.7***	42.5	97.1***	46.8
PC2	28	347.7**	28.3	568.5***	22.3	11.4***	22.1	78.9***	21.6	2.0***	29.5	81.0***	36.4
PC3	26	322.5**	24.3	219.7***	8	7.7***	13.8	39.8***	10.1	1.3***	18.3	26.4***	11
PC4	24	88.9**	6.2	9.3	0.3	0.4	0.7	2.5	0.6	0.8***	9.8	15.0**	5.8
Residuals	270	1.9		42.5		2.8		8.5		0.3		6.8	
Total	419	294.2		850.5		13.8		77.6		1.4		78.0	

Fe, iron; Zn, zinc; DFF, days to 50% flowering; PH, plant height; PL, panicle length and SPY, single plant yield.

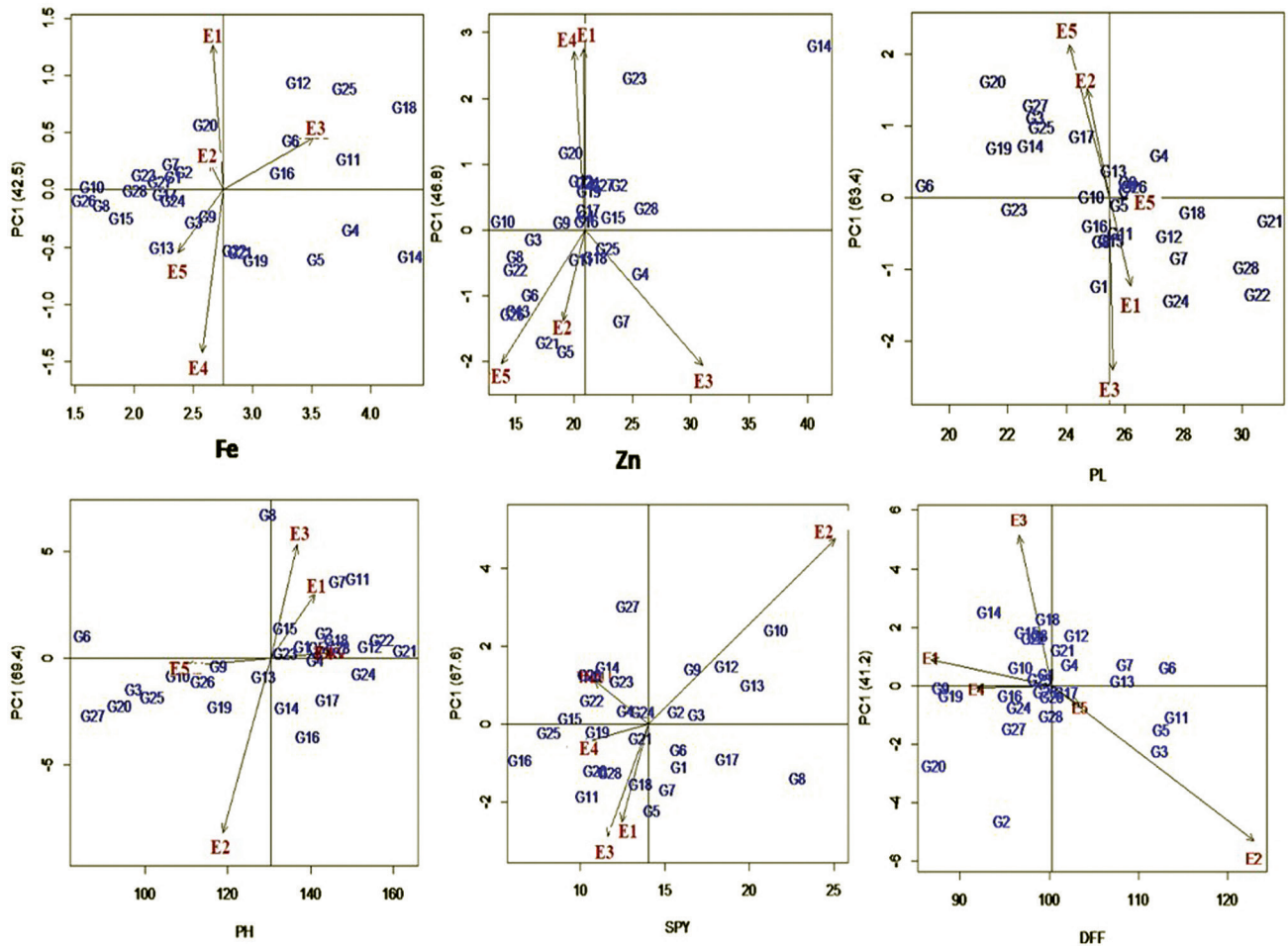


Fig 1 AMMI 1 biplots for the traits Fe (iron), Zn (zinc), PL (panicle length), PH (plant height), SPY (single plant yield) and DFF (days to 50% flowering).

contribution of environment to the total variation for grain Zn and yield were previously reported (Bashir *et al.* 2014). Thus, the identification of stable donors for high grain Zn across the environments is an encouraging outcome of this study.

Ranking environments and identification of mega-environments through GGE biplot analysis: Two distinct environment groups were identified for Zn (g1: E3, g2: E1, E2, E4 and E5) and Fe (g1: E1, E2 and E3, g2: E4 and E5). Three mega-environments were identified for PH (g1: E3, g2: E1, E4 and E5, g3: E2), two mega-environments each were identified for DFF (g1: E2, E4 and E5, g2: E1 and E3), PL (g1: E2 and E5, g2: E1, E3 and E4), and SPY (g1: E1, E3 and E4, g2: E2 and E5). The most discriminating environments were E4, E3 and E1 for Zn; E1, E2 and E4 for Fe; E1 and E3 for SPY; E3 and E2 for PH and DFF, and E3 and E1 for PL. E3 was the least discriminating environments for Fe, E4 for SPY, and E5 for Zn, DFF, PH and PL (Supplementary Fig 2).

The environment E4 was found to be the representative location for all the traits except for Fe for which E2 was the representative environment. In our study, environments E3 and E4 were found to be ideal test environments for

selecting genotypes with general adaptation for Zn, while E3 for selecting specific adapters for the agronomical traits.

In our study, soil Fe content ranged from 12.3 (E2) to 34.2 ppm (E1), soil Zn content ranged from 1.2 (E5) to 3.5 ppm (E3). E3 with high soil Zn content was identified as promising environment for the best expression of Zn content with highest location mean of 30.99 ppm and Karuppunel stood as best genotype for high Fe and Zn across environments.

Evaluation of the genotypes based on GGE biplots: Identification of mega-environments and superior genotypes for respective mega-environments is the major application of GGE biplot analysis. The principal component axes of GGE biplot explained 76.42 and 85.56% of the G + GE variation for Fe and Zn respectively. For the agronomical traits, GGE biplots explained a variation of 73.84 to 94.24% (DFF: 73.84%, SPY: 84.83%, PL: 88.79%, PH: 94.24%). For Zn, G14 stood as winner in mega-environment 2 represented by E1, E2, E4 and E5, while G15 showed superior performance in mega-environment 1 represented by E3. E3 was sitting right on the dividing line between G14 and G15, both the genotypes are expected to show similar Zn concentration in E3. Further, G14 followed by G23 and G28 was in close

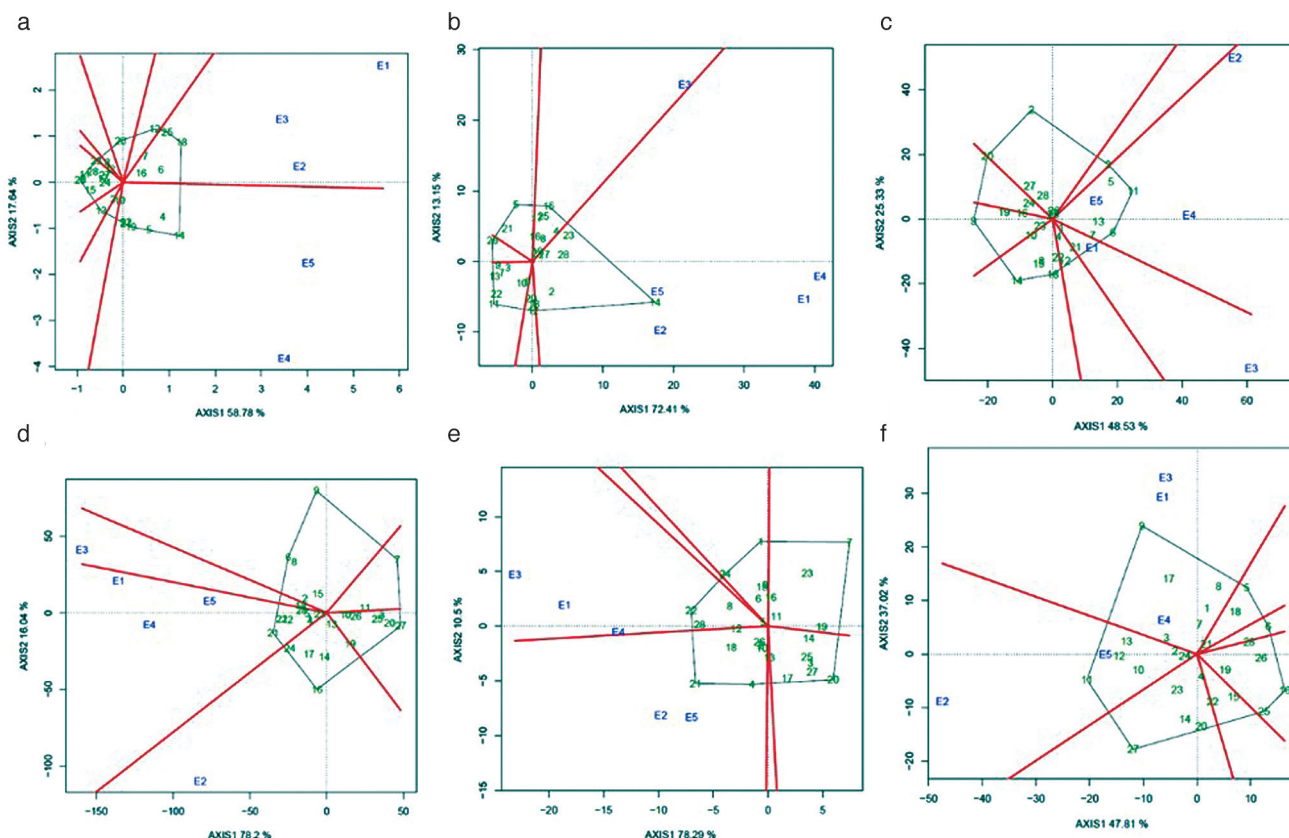


Fig 2 Which-won-where view of GGE biplot for the traits Fe (a), Zn (b), DFF (c), PH (d), PL (e) and SPY (f).

proximity to the ideal genotype and hence considered ideal for Zn accumulation in endosperm. For Fe, G18 was the winner in mega-environment 1 while G14 was superior in mega-environment 2. G6, G4, G18, G16 and G14 were ideal genotypes having high mean as well as high stability

(Fig 2). For SPY, G9 stood as winners for SPY in the mega-environment 1 and G11 was winner at mega-environment 2. Several studies earlier adopted GGE biplot analysis to identify mega-environments and recommend genotypes for specific adaptation (Amiri *et al.* 2015). Our findings

Table 3 *Ysi* ranking and significance of the stability variance of the genotypes for Fe, Zn and SPY based on combined analysis

Genotypes	Fe	Zn	Fe + Zn	SPY	Fe + Zn + SPY	Genotypes	Fe	Zn	Fe + Zn	SPY	Fe + Zn + SPY
G1	+10	+8	18	+15	33	G17	7	+10	17	+20	37
G2	+12	+17	29	+20	49	G18	+22	+12	34	6	40
G3	+13	4	17	+17	34	G19	+12	+11	23	6	29
G4	+21	+21	42	+11	53	G20	6	2	8	-5	3
G5	+18	0	18	+10	28	G21	+11	-3	8	+13	21
G6	+20	+8	28	-7	21	G22	+10	-7	3	2	5
G7	+19	-5	14	+18	32	G23	-4	+20	16	2	18
G8	6	+19	25	+11	36	G24	5	+13	18	7	25
G9	-8	0	-8	+23	15	G25	+19	+15	34	-9	25
G10	7	5	12	+16	28	G26	-2	-9	-11	-3	-14
G11	-5	-10	-15	+22	7	G27	5	+13	18	4	22
G12	+16	3	19	+19	38	G28	-6	+22	16	0	16
G13	-2	-6	-8	+21	13	Trait mean	2.7	20.92		14	
G14	+23	+23	46	-1	45	<i>Ysi</i> mean	9	7.464		8.142	
G15	1	+16	17	0	17	LSD	0.3	1.575		1.755	
G16	+18	7	25	-10	15						

Fe, iron; Zn, zinc; SPY, single plant yield.

on the representativeness of discriminating power of the environments tested have useful implications in designing future varietal testing trials focused on biofortification.

Stability analysis of the genotypes based on Ysi: The stability of the 28 genotypes was assessed based on *Ysi* for the traits Zn, Fe and SPY. Of the 28 genotypes, 15 were stable with positive *Ysi* ranking for both Zn and Fe, while 14 were stable for SPY (Table 3). Three genotypes were superior for all the 3 traits and 11 genotypes were superior for 2 of the 3 traits. For both Fe and Zn, G14 was superior followed by G4, G18, G25, G2 and G6.

In conclusion, the landrace ‘Karuppnel’ identified in the present study could be a game changer for high grain Zn and can be efficiently used as donor in varietal improvement programmes to develop micronutrient healthier rice varieties with high yield potential.

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