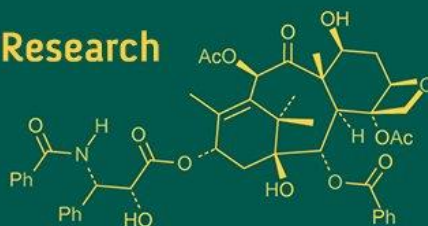


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Genetic variability, heritability and cross-environment consistency of grain zinc concentration in rice (*Oryza sativa* L.) under contrasting moisture regimes

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Abstract

Breeding rice for higher grain zinc is a durable response to dietary zinc deficiency, but efficient genetic gain depends on knowing how much of the observed variation is heritable and whether genotype ranking holds across environments. Twenty-two genotypes, including the zinc-biofortified checks Zinco rice (IET 25477), DRR Dhan 45 and DRR Dhan 48 alongside IR 64 and Samba Mahsuri, were evaluated during kharif 2020 at two field locations (Raipur, Umariya) and in a rainout shelter imposing moisture stress, in a randomised complete block design with three replications per environment. Zinc and iron in polished grain were determined by energy-dispersive X-ray fluorescence. Pooled analysis of variance detected a highly significant genotype effect for grain zinc ($F = 22.17$, $P < 0.001$) but a non-significant genotype $\times$ environment interaction ($F = 1.32$, $P = 0.121$) and a non-significant environment effect ($P = 0.299$). Grain zinc ranged from 11.48 to 21.48 ppm about a mean of 16.18 ppm. The genotypic coefficient of variation (17.42%) closely approached the phenotypic value (17.96%), broad-sense heritability reached 94.04% and genetic advance was 34.79% of the mean, indicating predominantly additive control and good prospects for direct phenotypic selection. RRHP-MI 30 (IET 25461; 21.48 ppm) and MI 127 (21.07 ppm) equalled the biofortified check and held their rank in all three environments. Grain iron averaged 3.85 ppm with a coefficient of variation of 32.05% and declined systematically across spectrometer scan sessions; iron results are reported as indicative only.

Keywords: Rice biofortification, grain zinc, heritability, genetic advance, genotype $\times$ environment interaction, EDXRF

1. Introduction

Inadequate dietary zinc remains among the most widespread micronutrient deficiencies in South and South-East Asia, and the problem is tied closely to rice-dominated diets. Milling removes the aleurone layer and embryo in which most of the grain's mineral reserve is held, leaving polished grain of popular varieties with roughly 16 ppm zinc and about 2 ppm iron. Biofortification delivers the nutrient through the seed itself and so reaches subsistence households that supplementation and industrial fortification do not. Against a baseline of 16 ppm, the HarvestPlus programme set a breeding target of 28 ppm zinc in polished rice, the concentration estimated to supply some 30 percent of the average requirement; a parallel iron target of 13 ppm has proved unattainable by conventional breeding in polished grain, where reported values remain below 8 ppm.

Converting the substantial genetic variation documented for grain zinc into genetic gain turns on two questions. The first is how much of the phenotypic variation is heritable, since mineral concentration is measured on a small sample and is exposed to considerable non-genetic noise. The second is whether genotype ranking is stable across production environments: where interaction is large, selection demands testing across many sites and seasons, whereas where it is small a leaner testing programme suffices. The evidence is not settled. Multi-environment studies of zinc-biofortified rice conducted across eight to nine locations and four seasons have reported significant interaction, while analyses of the Indian coordinated biofortification trials characterise grain zinc, iron and protein as oligogenic traits with high heritability and comparatively minor interaction effects. This study evaluated twenty-two genotypes across two field locations and a rainout shelter in order to partition the variation in grain zinc, estimate the variability parameters governing response to selection,

assess the consistency of genotype ranking, and identify entries combining high concentration with consistent expression.

2. Materials and Methods

Twenty-two rice genotypes were evaluated (Table 3). The set comprised advanced breeding lines of the Chhattisgarh programme (prefixes R-RHZ, R-RHP, CGZR), lines from collaborating institutions (MI 127, MI 156, IET 26383, two CR pedigree selections) and released checks, namely the zinc-biofortified entries Zinco rice (IET 25477), DRR Dhan 45 and DRR Dhan 48, the popular standards IR 64 and Samba Mahsuri, and CR Dhan 310 and CR Dhan 311.

The trial was conducted during kharif 2020 in three environments: the research farm at Raipur, Chhattisgarh under normal irrigated management (E1); the Umariya site under rainfed lowland conditions (E2); and a rainout shelter in which irrigation was withheld during the reproductive phase to impose moisture stress (E3). Each environment was laid out as a randomised complete block design with three replications, managed according to the recommended package of practices, with no differential zinc fertilisation between entries.

Grain was harvested at physiological maturity from each plot, sun-dried to uniform moisture, dehusked and polished. Zinc and iron in the polished grain were determined by energy-dispersive X-ray fluorescence spectrometry following the calibration approach established for rice grain and are reported in ppm on a dry-weight basis, with a reference check sample scanned at intervals to monitor instrument performance. Samples were presented to the spectrometer grouped by replication, so each replication corresponds to a distinct scanning session; this aliasing bears on the interpretation of the iron data and is addressed below.

Analysis of variance was performed separately for each environment and the homogeneity of the resulting error variances tested by Bartlett's test. Pooled analysis over environments partitioned the variation into environments, replications within environments, genotypes, genotype $\times$ environment interaction and pooled error, with the environment mean square tested against replications within environments and the genotype and interaction mean squares against the pooled error. Genotypic, interaction and error variance components were derived from the pooled analysis, phenotypic variance obtained as $\sigma^2_p = \sigma^2_g + \sigma^2_{ge/e} + \sigma^2_{e/re}$, and coefficients of variation, broad-sense heritability and genetic advance ($k = 2.06$) estimated by standard procedures. Consistency across environments was characterised by Shukla's stability variance, Wricke's ecovalence, the environmental coefficient of variation of Francis and Kannenberg, the superiority measure of Lin and Binns and the rank-sum index of Kang; the regression approach of Eberhart and Russell and AMMI analysis were also applied and are reported in the supplementary material. Correlations between zinc and iron were computed at plot level within environments and on genotype means. Analyses used Python 3 with NumPy, pandas, SciPy and statsmodels.

3. Results

Mean grain zinc was 16.73 ppm at Raipur, 15.93 ppm at Umariya and 15.86 ppm under the rainout shelter, giving a trial mean of 16.18 ppm; the corresponding iron means were 4.64, 3.67 and 3.24 ppm about a trial mean of 3.85 ppm.

Bartlett's test rejected homogeneity of error variances across environments for both zinc ($\chi^2 = 31.38$, $P < 0.001$) and iron ($\chi^2 = 17.99$, $P < 0.001$), the error mean square under the rainout shelter being appreciably the largest for zinc, and the pooled analysis is reported with this qualification.

Pooled analysis of variance (Table 1) showed a highly significant genotype effect for grain zinc, the genotype mean square exceeding the pooled error twenty-two fold, while neither the environment main effect ($P = 0.299$) nor the genotype $\times$ environment interaction ($P = 0.121$) was significant. The interaction accounted for 8.2 percent of the total sum of squares against 69.0 percent for the genotype main effect. Grain iron also showed a significant but far weaker genotype effect, with non-significant environment ($P = 0.389$) and interaction ($P = 0.528$) terms. The coefficient of variation was 11.44 percent for zinc but 32.05 percent for iron. The replications-within-environments term was highly significant for iron ($F = 20.27$, $P < 0.001$), reflecting a monotonic decline in mean iron across successive scanning sessions, from 5.75 to 2.35 ppm at Umariya and from 5.72 to 4.01 ppm at Raipur (Figure 2); rainout samples, scanned within a single session, showed no such gradient, and zinc exhibited the same pattern at about one-tenth the relative magnitude.

Variability parameters are given in Table 2. For grain zinc the genotypic variance (7.94) greatly exceeded both the interaction (0.37) and error (3.42) components, and the genotypic coefficient of variation (17.42%) closely approached the phenotypic value (17.96%), the narrow gap indicating slight environmental modification. Broad-sense heritability reached 94.04 percent with genetic advance of 34.79 percent of the mean, a combination pointing to predominantly additive gene action. Grain iron was markedly less favourable: a small genotypic variance (0.28) relative to error (1.52), a wider gap between genotypic and phenotypic coefficients of variation (13.71% against 17.38%) and moderate heritability (62.20%).

Genotype means for grain zinc spanned 11.48 to 21.48 ppm against a critical difference of 1.73 ppm (Table 3). HP-5 (RRHP-MI 30, IET 25461) recorded the highest concentration at 21.48 ppm, followed by HP-14 (MI 127) at 21.07 ppm and the biofortified check HP-12 (Zinco rice) at 20.77 ppm; differences among these three did not exceed the critical difference, so the first two are best described as equalling rather than surpassing the check, though both exceeded every non-biofortified entry by a wide margin. HP-18 (R-RHZ-IH-82, 19.72 ppm), HP-7 (CGZR-1, 19.04 ppm) and HP-16 (IET 26383, 18.53 ppm) formed a second promising group. At the lower end HP-8 (CR Dhan 310) recorded 11.48 ppm and IR 64 13.58 ppm, so the best entries carried an advantage of some 58 percent over IR 64. No entry approached the HarvestPlus target of 28 ppm, and the trial mean corresponds closely to the accepted baseline for non-biofortified polished rice.

Genotype rankings were closely preserved across the three environments, the leading entries showing near-parallel response profiles without crossover (Figure 1). HP-14 combined the second-highest mean with the lowest Shukla stability variance among the high-zinc group (0.06), the lowest environmental coefficient of variation among those entries (3.70%) and the most favourable Kang rank-sum index in the trial ($YS_i = 4.0$). HP-16 showed the lowest environmental coefficient of variation overall (0.65%) but at an intermediate concentration, while HP-5 varied somewhat

more (CV 6.02%), performing better under moisture stress (22.40 ppm) than at Umariya (20.00 ppm). The Lin and Binns superiority measure ranked HP-5 first ($P_i = 0.17$), then HP-14 (0.67) and HP-12 (1.38). Because the interaction was not significant, the environmental index underlying the Eberhart and Russell regression spanned only 0.87 ppm for zinc and the resulting regression coefficients (-1.66 to 3.69) were correspondingly unstable; they are reported in the supplementary material rather than interpreted here.

Genotype means for grain iron ranged from 2.78 to 5.11 ppm (Table 3), highest in HP-16 and HP-18, but given the scan-session gradient and a coefficient of variation of 32 percent these differences are indicative only. Iron and zinc were positively but weakly associated at plot level ($r = 0.328$, $P < 0.001$, $n = 198$), most strongly under moisture stress ($r = 0.471$, $P < 0.001$) and not significantly at Raipur ($r = 0.182$, $P = 0.145$); on genotype means the correlation was moderate ($r = 0.524$, $P = 0.012$).

4. Discussion

The central finding is a very large genotype main effect for grain zinc combined with an interaction that did not reach significance, and this has direct consequences for how a biofortification programme should allocate testing resources. Where interaction is substantial, ranking shifts between environments and reliable selection demands many sites and seasons; where it is negligible relative to the genotype effect, ranking established in a few environments can be expected to hold, and resources are better spent screening more entries than replicating fewer more widely. The result aligns with analyses of the Indian coordinated biofortification trials but contrasts with multi-environment studies at IRRI in which significant interaction was detected. The discrepancy is readily explained by scale: those studies spanned four seasons and eight to nine locations, whereas the present trial comprised a single season and three environments whose mean zinc concentrations differed by less than one part per million. The non-significant interaction should therefore be read as evidence that ranking was preserved across the particular contrasts sampled, not as a general claim. Notably, the rainout shelter depressed mean grain zinc by only 0.87 ppm relative to Raipur, so the moisture contrast achieved was narrower than the design intended.

The variability parameters reinforce this reading. A genotypic coefficient of variation of 17.42 percent against a phenotypic value of 17.96 percent leaves very little variation attributable to environment, and the resulting heritability of 94 percent is high even for grain mineral traits. High heritability with genetic advance of 35 percent of the mean is the classical signature of additive gene action and indicates that straightforward phenotypic selection in early

generations should be effective, without recourse to progeny testing, although markers would still accelerate the pyramiding of favourable alleles. Among the entries, MI 127 presents the most attractive profile for immediate use, combining the second-highest mean with the most favourable Kang index, the lowest Shukla variance among high-zinc entries and near-identical expression in all three environments. RRHP-MI 30 recorded the highest mean and ranked first on the Lin and Binns measure, and its relatively better performance under moisture stress may interest rainfed programmes, though this needs confirmation. That both matched the released check Zinco rice indicates the Chhattisgarh material carries useful alleles. Even so, no entry approached the 28 ppm target, and reaching it from a base at the non-biofortified baseline will require introgression of higher donors rather than recombination within the present set.

The iron results warrant explicit caution on three grounds. The coefficient of variation of 32 percent is well above what is acceptable for a replicated field trial. More seriously, mean iron declined systematically across successive scan sessions, and because samples were grouped by replication at the instrument, the field replication term is confounded with scan session, so the highly significant replications-within-environments mean square for iron is attributable at least partly to instrument or sample-presentation effects rather than field variation. Finally, the concentrations recorded lie close to the value expected in polished rice and near the practical resolution of the method. This is a consequence of milling rather than a defect peculiar to this study: the aleurone and embryo holding most of the grain's iron are removed during polishing, which is why conventional programmes have not approached the iron target in polished grain and why the EDXRF calibration work for rice was developed on whole grain. Selection for grain iron is better pursued on whole grain with reference to inductively coupled plasma spectrometry.

Three limitations bound these conclusions. The single season leaves the genotype $\times$ season component of interaction unestimated, and the environments proved less contrasting than intended. Bartlett's test rejected homogeneity of error variances, which weakens the formal basis of the pooled analysis; the very large ratio of genotype to error mean square for zinc makes it unlikely that this alters the qualitative conclusion, but exact probability levels should be treated as approximate. Agronomic and quality traits were not recorded alongside the mineral measurements, and since high grain zinc has been reported to associate unfavourably with grain yield and several cooking quality attributes above about 25 ppm, the entries identified here require evaluation for those traits before advancement.

Table 1: Pooled analysis of variance over three environments for grain zinc and iron (ppm).

Source	df	Zn SS	Zn MS	Zn F	Zn P	Fe SS	Fe MS	Fe F	Fe P
Environment (E)	2	31.15	15.574	1.49	0.299	68.56	34.282	1.11	0.389
Rep within E	6	62.92	10.487	3.06	0.008	185.27	30.879	20.27	<0.001
Genotype (G)	21	1594.87	75.946	22.17	<0.001	83.71	3.986	2.62	<0.001
G $\times$ E	42	190.09	4.526	1.32	0.121	62.16	1.48	0.97	0.528
Pooled error	126	431.54	3.425			191.91	1.523		
Total	197	2310.57	11.729			591.61	3.003		

Environment tested against replications within environments; genotype and interaction against pooled error.

Table 2: Variance components and genetic variability parameters.

Trait	σ^2_g	σ^2_{ge}	σ^2_e	σ^2_p	GCV (%)	PCV (%)	h^2_{bs} (%)	GA	GAM (%)	SEm $\pm$	CD 5%	CV (%)
Zn	7.94	0.37	3.42	8.44	17.42	17.96	94.04	5.63	34.79	0.62	1.73	11.44
Fe	0.28	0.00	1.52	0.45	13.71	17.38	62.20	0.86	22.27	0.41	1.15	32.05

Table 3: Grain zinc by environment, genotype means for zinc and iron, and measures of cross-environment consistency.

Code	Genotype/pedigree	Raipur	Umariya	Rainout	Zn mean	CV (%)	Shukla σ^2_i	Kang YSi	Fe mean
HP-3	R-RHZ-MB-119	13.3	12.47	12.2	12.66	4.53	-0.18	22	4.32
HP-4	IET 25470, R-56 (Protagene)	16.1	15.57	15.97	15.88	1.75	0.24	14	3.03
HP-5	RRHP-MI 30 IET 25461	22.03	20	22.4	21.48	6.02	4.79	15	4.64
HP-6	R-RHZ-IB-80	16.17	15.93	13.23	15.11	10.79	6.1	30	3.31
HP-7	CGZR-1/IET 23824	20.7	18.07	18.37	19.04	7.57	2.86	16	3.41
HP-8	CR Dhan 310 (IET 24780)	11.07	9.87	13.5	11.48	16.13	13.4	43	2.78
HP-9	R-RHZ-SM-14	14.87	13.83	12.8	13.83	7.47	1.1	24	3.21
HP-10	MI 156	15.67	13.63	13.4	14.23	8.76	1.69	24	3.22
HP-11	Samba Mahsuri	16.23	14.03	14.47	14.91	7.82	1.42	20	4.41
HP-12	Zinco rice (IET 25477)	22.83	19.83	19.63	20.77	8.63	5.41	20	3.68
HP-13	DRR Dhan 45	17	17.43	15.93	16.79	4.6	1.77	17	3.71
HP-14	MI 127	21.97	20.63	20.6	21.07	3.7	0.06	4	4.31
HP-15	IR 64	13.93	14.67	12.13	13.58	9.6	4.9	35	3.16
HP-16	IET 26383	18.63	18.4	18.57	18.53	0.65	0.34	10	5.11
HP-17	CGZR-2 (IET 23829)	16.97	15.03	17.43	16.48	7.72	4.81	24	3.94
HP-18	R-RHZ-IH-82	20.87	18.87	19.43	19.72	5.23	0.95	9	5.01
HP-19	DRR Dhan 49	14.13	14.67	14.73	14.51	2.27	1.96	24.5	4.31
HP-20	R-RH2-M1-93	15.87	16.97	16.4	16.41	3.35	3	23	3.79
HP-21	CR Dhan 311	14.47	13.47	10.5	12.81	16.1	9.68	40	3.89
HP-22	DRR Dhan 48	18.17	16.87	18.67	17.9	5.19	2.86	19	4.52
HP-23	CR 2818-1-11-1-B-1-2-B-1	13.8	12.87	15.8	14.16	10.59	9.27	36	3.03
HP-24	CR 2819-1-5-3-2B-12-1	13.4	17.33	12.8	14.51	16.97	23.14	36.5	3.9

Zn trial mean 16.18 ppm, CD (5%) 1.73 ppm; Fe trial mean 3.85 ppm, CD (5%) 1.15 ppm. Lower Shukla and Kang values denote greater consistency. Fe values are indicative only (Section 4).

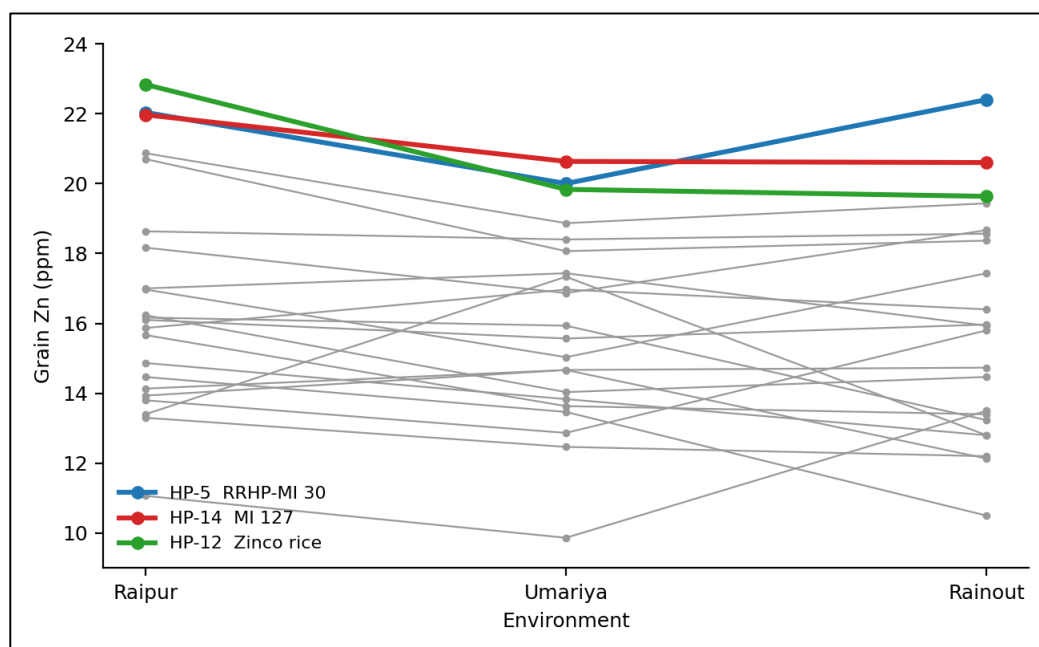


Fig 1: Response of individual genotypes for grain zinc across the three environments. Grey lines show all 22 genotypes; coloured lines highlight the three highest-ranking entries, which retain their rank in every environment. The limited re-ranking among intermediate genotypes corresponds to the non-significant genotype $\times$ environment interaction.

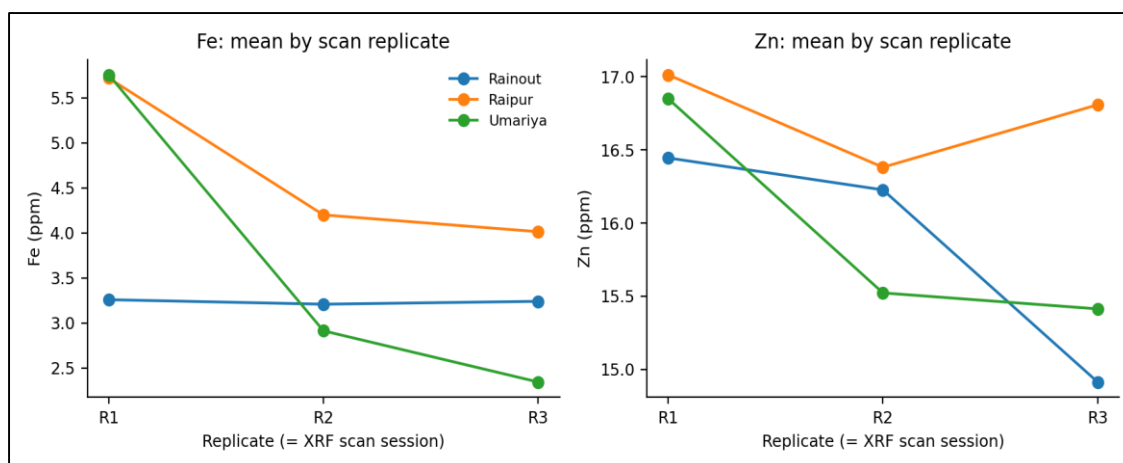


Fig 2: Mean grain iron (left) and zinc (right) by replication within each environment. Samples were presented to the spectrometer grouped by replication, so each replication is a distinct scan session. The systematic decline for iron at Raipur and Umariya, absent in the single-session rainout samples, indicates a measurement rather than a field effect.

5. Conclusion

Grain zinc in the twenty-two genotypes examined was under strong genetic control, with broad-sense heritability of 94 percent, genetic advance of 35 percent of the mean and a genotypic coefficient of variation almost equal to the phenotypic value. The genotype $\times$ environment interaction was not significant across two field locations and a rainout shelter, and genotype ranking was preserved throughout. RRHP-MI 30 (IET 25461) and MI 127 matched the released biofortified check Zinco rice and merit further evaluation, MI 127 being the more consistent. Grain iron in polished grain proved unsuitable for selection under the protocol used and should in future be determined on whole grain. Confirmation over additional seasons and more widely contrasting environments, with concurrent assessment of yield and grain quality, is the necessary next step.

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