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High-throughput XRF phenotyping of rice recombinant inbred lines identifies zinc-dense transgressive segregants for biofortification breeding

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Abstract

Zinc and iron deficiency remain widespread in populations dependent on rice as a staple, and breeding cultivars with higher grain micronutrient density is a durable, delivery-free intervention. This study screened 249 F7 recombinant inbred lines (RILs), advanced by single seed descent from four biparental crosses, together with the parents Chandrahasini and RKVY-211, for zinc and iron concentration in polished grain by energy-dispersive X-ray fluorescence (XRF) using a rice-specific calibration established against ICP-OES, following evaluation in a randomised complete block design with three replications in the wet season of 2025 at Indira Gandhi Krishi Vishwavidyalaya, Raipur. Zinc in polished grain averaged 21.08 ± 4.23 ppm and was normally distributed (Shapiro-Wilk $W = 0.995$, $p = 0.591$), with a coefficient of variation of 20.1% and a range of 10.7 to 33.8 ppm. The RKVY-211 $\times$ Chandrahasini population ($n = 230$) averaged 21.73 ppm zinc against 15.49 ppm for MTU1010 $\times$ CGR-77, a difference corresponding to a large effect size (Hedges' $g = 1.60$; Kruskal-Wallis $H = 36.25$, $p < 0.001$). The zinc range of the RKVY-211 $\times$ Chandrahasini population extended well beyond both parents, with 41.3% of lines exceeding the high-parent value and the best lines reaching 33.8, 32.9 and 32.5 ppm — evidence of transgressive segregation arising from complementary alleles dispersed between the parents. Thirteen lines (5.2%) met the HarvestPlus target of 28 ppm zinc for polished rice and 65 lines (25.9%) met the 24 ppm threshold applied in Indian biofortification trials. Fourteen iron entry means above 7.2 ppm were excluded as suspected extraneous contamination, and iron over the screened dataset averaged 3.44 ± 1.27 ppm with no significant differences among crosses ($H = 5.43$, $p = 0.143$). Grain iron and zinc were positively but only moderately correlated ($r = 0.365$, $p < 0.001$), indicating that simultaneous improvement is feasible but that index selection on both traits is required. Twenty-three lines fell in the upper quartile for both micronutrients and are nominated for replicated multi-environment evaluation as candidate varieties or as donors in the biofortification breeding programme.

Keywords: *Oryza sativa*, biofortification, grain zinc, grain iron, recombinant inbred lines, transgressive segregation, X-ray fluorescence

1. Introduction

Micronutrient malnutrition affects a large share of the world's population, and zinc and iron deficiencies are the most consequential of these for human health, contributing to impaired immune function, stunting, cognitive deficits and anaemia (Elegbeleye *et al.* 2025) [5]. The Global Burden of Disease assessment for 2021 quantifies the scale of the iron-deficiency burden in particular (Huang *et al.* 2025) [8]. The burden falls heaviest on populations whose diets are dominated by a single cereal staple. In much of South and Southeast Asia that staple is rice, which is intrinsically poor in both micronutrients, particularly after milling removes the aleurone and embryo where much of the mineral fraction is concentrated.

Biofortification — raising the micronutrient density of the grain through breeding rather than through supplementation or post-harvest fortification — is attractive because the improvement is embedded in the seed (Bouis *et al.* 2011) [2]. Once a biofortified cultivar is adopted, no recurring delivery infrastructure, consumer behaviour change or additional cost is required, and the benefit reaches subsistence households that commercial fortification channels do not. HarvestPlus and national programmes have accordingly set breeding targets for grain zinc in rice, and several zinc-dense varieties have been released in India (Sanjeeva Rao *et al.* 2020; Senguttuvel *et al.* 2023) [12, 14], although sustaining and scaling such programmes presents continuing challenges (Bouis *et al.* 2024) [3].

Success in such a programme depends on the availability of parental material carrying favourable alleles and on the identification of segregants that combine those alleles. Recombinant inbred line populations are especially valuable at this stage. Because RILs are near-homozygous and genetically fixed, a line identified as superior can be recovered from seed, re-evaluated, multiplied and used directly either as a candidate variety or as a donor in further crossing — an advantage that early segregating generations do not offer. Structured populations of this kind are the principal link between genetic analysis and applied breeding (Scott *et al.* 2020)^[13], and rice RIL populations have been used to resolve the genetic basis of grain iron and zinc concentration (Anuradha *et al.* 2012)^[1] and, in the breeding programme at this centre, of nitrogen use efficiency under direct-seeded conditions (Verulkar *et al.* 2018)^[21].

The present study screened RIL populations derived from four biparental crosses maintained in the rice breeding programme at Indira Gandhi Krishi Vishwavidyalaya, Raipur, for grain zinc and iron concentration. The objectives were to characterise the extent and distribution of variation for both micronutrients, to test for transgressive segregation relative to the parents, to quantify the association between the two traits as it bears on selection strategy, and to identify individual lines meriting advancement to replicated multi-environment testing as candidate varieties or as donors.

2. Materials and Methods

2.1 Plant material

Two hundred and forty-nine recombinant inbred lines (RILs) at the F7 generation, advanced by single seed descent, were evaluated together with the parents Chandrahasini and RKVY-211. The populations comprised RKVY-211 × Chandrahasini (230 lines), MTU1010 × CGR-77 (13 lines), Swarna × M55 (4 lines) and Pusa Basmati × M55 (2 lines). All four crosses are maintained in the institutional breeding programme for the selection of lines suitable for release as varieties or for use as donors in micronutrient improvement.

At F7 the expected residual heterozygosity is below 1%, so each line is effectively homozygous and true-breeding. Single seed descent also minimises the inadvertent selection that pedigree advance can impose. Any line identified as superior is therefore genetically fixed and can be recovered from seed, multiplied and re-evaluated without further inbreeding.

Progeny numbering in the RKVY-211 × Chandrahasini population runs to 237, of which 230 lines were available for assay; seven lines (39, 41, 60, 88, 96, 103 and 184) were not evaluated.

2.2 Experimental site and season

The material was grown during the wet season of 2025 at the Research cum Instructional Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. The farm lies at approximately 21°16' N and 81°36' E at an elevation of about 299 m above mean sea level, in a region classified as dry sub-humid, receiving most of its rainfall from the south-west monsoon.

The trial was conducted on a Vertisol of the Arang II series, a deep heavy clay soil known locally as Kanhar. Composite soil samples from the trial field gave a pH (1:2.5) of 7.73, an electrical conductivity of 0.22 dS m⁻¹ and organic carbon of 0.64%. Available nitrogen, phosphorus and potassium were 211.9, 16.12 and 433 kg ha⁻¹ respectively — medium in nitrogen and phosphorus, and high in potassium.

2.2.1 Crop management

Seedlings were raised on well-prepared nursery beds and given light, frequent irrigation until transplanting. The main field was prepared by two criss-cross ploughings followed by harrowing and levelling, and bunds were raised to limit seepage.

Fertiliser was applied at 100:60:40 kg N:P:K per hectare as urea, single super phosphate and muriate of potash. Phosphorus and potassium were applied basally at transplanting and nitrogen in three splits. No zinc-containing fertiliser or foliar spray was applied at any stage.

Twenty-three-day-old seedlings were transplanted singly per hill at 15 × 15 cm spacing. Standing water of 5 ± 2 cm was maintained after establishment, weeds were removed by hand at fortnightly intervals, and plant protection was applied as required.

The trial was laid out in a randomised complete block design with three replications, each line occupying one plot per replication. Grain was sampled from each plot separately, and all values reported here are entry means over the three replications.

2.3 Grain sampling and micronutrient estimation

Grain samples were harvested at physiological maturity, sun-dried to a uniform moisture content and cleaned before analysis.

Harvested grain was dehulled and polished on a Zaccaria PAZ-1/DTA laboratory testing rice mill (Indústria Machina Zaccaria S/A, Limeira, SP, Brazil), which husks by rubber rolls and whitens by friction using an abrasive ring and rubber brake, with a 100 g paddy sample capacity. Samples were dried to a moisture content of 12–14% before milling, since grain moisture affects milling performance (Tharanya *et al.* 2025)^[17], and each sample was milled and polished for 1–2 min. Milling settings were held constant across all samples so that the degree of polish was uniform and independent of operator. All values reported here are therefore for polished (milled) rice — the form in which rice is predominantly consumed.

Grain zinc and iron were determined by energy-dispersive X-ray fluorescence spectrometry on an Oxford Instruments X-Supreme 8000 at ICRISAT, Patancheru, Telangana, India. A rice-specific calibration was applied, established against reference values determined by inductively coupled plasma optical emission spectrometry (ICP-OES) following the XRF method of Paltridge *et al.* (2012)^[10] and validated for high-throughput screening on this instrument (Govindaraj *et al.* 2016)^[6].

All concentrations are reported in parts per million (ppm; equivalent to mg kg⁻¹) on a dry-weight basis. Sampling and handling followed protocols designed to minimise extraneous mineral contamination (Stangoulis and Sison 2008)^[15].

2.4 Data screening for extraneous iron

The iron data were strongly right-skewed (skewness 3.50) and heavy-tailed (excess kurtosis 21.28) despite a tightly clustered core: the interquartile range was only 1.90 ppm while the maximum reached 22.2 ppm, about six times the median. A normal quantile-quantile plot followed the reference line up to approximately 7 ppm and then broke sharply upward — the pattern expected when a well-behaved distribution is contaminated by aberrant values rather than one that is intrinsically log-normal.

Because XRF measures total iron present on and within the sample without distinguishing physiologically accumulated grain iron from residues of soil, dust or contact with threshing, dehulling and polishing equipment, and because the aberrant values were strictly unidirectional with no corresponding cluster of implausibly low readings, these observations were attributed to extraneous iron contamination. Overestimation of grain iron

arising from dust contamination is a recognised limitation of XRF screening: aluminium density above 5 mg kg⁻¹ has been established as a marker of such contamination in cereal grain (Pfeiffer and McClafferty 2007)^[11], but XRF does not permit aluminium or titanium to be measured as an index element, so contamination cannot be flagged directly and must instead be identified from the distribution of the iron values themselves (Govindaraj *et al.* 2016)^[6].

Iron readings exceeding the Tukey upper fence of 7.2 ppm (Q3 + 1.5 × IQR) were therefore excluded prior to analysis. Fourteen of 251 observations (5.6%) were removed on this criterion, leaving 237 for the iron analyses. Excluding these observations reduced the coefficient of variation for iron from 57.7% to 37.0% and skewness from 3.50 to 0.63. Descriptive statistics for the full unscreened dataset are provided as supplementary material. No observations were excluded for zinc.

2.5 Statistical analysis

Descriptive statistics comprised the mean, standard deviation, standard error, coefficient of variation, quartiles, range, skewness and excess kurtosis. Normality was assessed by the Shapiro-Wilk, Kolmogorov-Smirnov, Anderson-Darling and Jarque-Bera tests. Differences among crosses were tested by the Kruskal-Wallis test across all four populations and by Welch's t-test between the two adequately sampled populations, with Hedges' g reported as the effect size. Association between the two micronutrients was quantified by Pearson, Spearman and Kendall coefficients.

Selection differentials were computed at 5%, 10% and 20% selection intensity and expressed both as a percentage of the population mean and in phenotypic standard deviation units. Genotypic and phenotypic coefficients of variation, heritability and expected genetic advance were *not* estimated. These parameters require partitioning of phenotypic variance into genotypic and error components, which is obtained from the analysis of variance on plot-level data; the analyses reported here were performed on entry means, from which the error variance cannot be recovered.

All analyses were performed in Python 3.12.3, using pandas 3.0.2 and NumPy 2.4.4 for data handling, SciPy 1.17.1 for the descriptive, distributional and inferential statistics, and Matplotlib 3.10.8 for the figures (Harris *et al.* 2020; Virtanen *et al.* 2020)^[7, 22].

3. Results

3.1 Variation for grain zinc concentration

Zinc concentration in polished grain averaged 21.08 ± 4.23 ppm across all 251 lines, ranging from 10.7 to 33.8 ppm — a 3.2-fold spread (Table 1). The distribution was symmetric and closely normal (skewness 0.15, excess kurtosis 0.11; Shapiro-Wilk W = 0.995, p = 0.591), and the mean and median were effectively identical at 21.08 and 20.80 ppm (Figure 1). The coefficient of variation of 20.1% indicates substantial working variation for selection.

Table 1: Descriptive statistics for grain zinc and iron concentration (ppm) in rice RILs, wet season 2025, IGKV Raipur. Iron statistics are computed on the screened dataset (n = 237) as defined in Section 2.4

Statistic	Zinc (ppm)	Iron (ppm)
Number of lines	251	237
Mean	21.08	3.44
Standard deviation	4.23	1.27
Standard error	0.275	0.083
Coefficient of variation (%)	20.1	37.0
Minimum	10.7	1.0
First quartile	18.40	2.40
Median	20.80	3.30
Third quartile	23.80	4.10
Maximum	33.8	7.2
Range	23.1	6.2
Skewness	0.15	0.63
Excess kurtosis	0.11	0.25
Shapiro-Wilk W	0.995	0.967
Shapiro-Wilk p	0.591	< 0.001

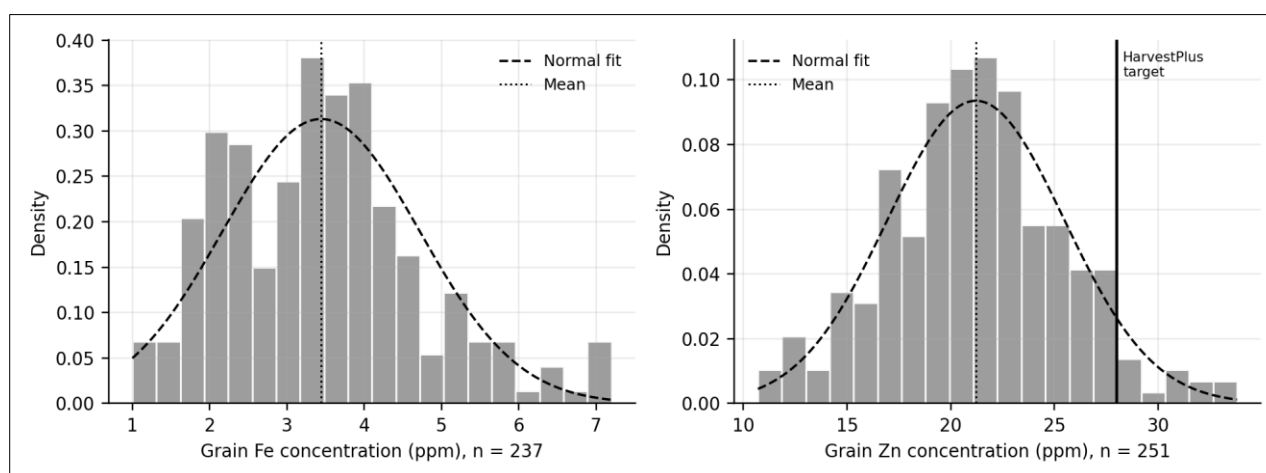


Fig 1: Frequency distribution of grain iron (left) and zinc (right) concentration in rice RILs after screening for extraneous iron. Dashed curve, fitted normal density; dotted vertical line, mean.

Because the grain was analysed in polished form, the values compare directly with published biofortification targets, which are defined for polished rice. Thirteen lines (5.2%) met or exceeded the HarvestPlus target of 28 ppm, and 65 (25.9%) met the 24 ppm threshold applied in AICRIP biofortification trials. All 13 target-meeting lines came from the RKVY-211 × Chandrahasini population. Classifying lines by concentration showed that 65 lines (25.9%) and 12 lines (4.8%) fell below 14 ppm, comparable to the least favourable commercial varieties.

3.2 Variation for grain iron concentration

After screening, grain iron averaged 3.44 ± 1.27 ppm with a range of 1.0 to 7.2 ppm (Table 1). The distribution was mildly right-skewed (skewness 0.63), unimodal and continuous. The coefficient of variation of 37.0% exceeded that for zinc, but the absolute range was narrow and the values low — consistent with

published values for polished rice, in which popular varieties carry roughly 2 ppm iron. No line approached the HarvestPlus iron target of 13 ppm.

3.3 Association between grain zinc and iron

Grain zinc and iron were positively and significantly correlated but the association was moderate (Pearson $r = 0.365$, $p < 0.001$; Spearman $\rho = 0.375$, $p < 0.001$; $n = 237$; Figure 2). The close agreement between the parametric and rank-based coefficients after screening indicates that the estimate is not driven by extreme values; on the unscreened data the Pearson coefficient was depressed to 0.267 while the rank-based estimates were essentially unchanged. Classifying lines above and below the median of each trait gave a highly significant association ($\chi^2 = 23.70$, $p < 0.001$), yet 34.3% of lines still fell into discordant classes — high in one micronutrient and low in the other.

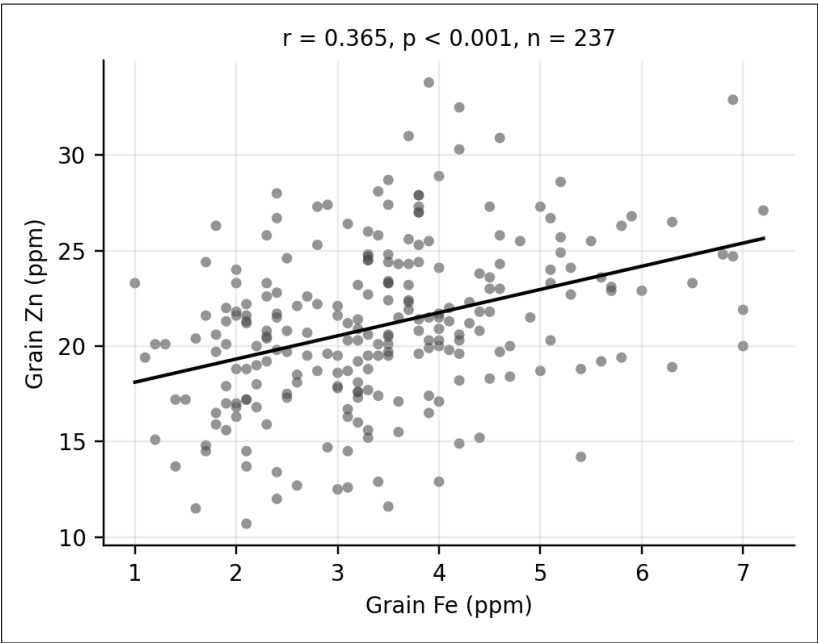


Fig 2: Relationship between grain iron and zinc concentration in rice RILs after screening for extraneous iron (n = 237). Solid line, least-squares regression.

3.4 Differences among crosses

The four RIL populations differed markedly for grain zinc (Kruskal-Wallis $H = 36.25$, $p < 0.001$; Table 2, Figure 3). The RKVY-211 × Chandrahasini population averaged 21.73 ppm against 15.49 ppm for MTU1010 × CGR-77, a difference of more than 6 ppm confirmed by Welch's t-test ($t = 6.58$, $p < 0.001$) with a very large effect size (Hedges' $g = 1.60$). Swarna × M55 and Pusa Basmati × M55 recorded the lowest means at 13.10 and 13.75 ppm respectively, although with only four and two lines

these values are descriptive and no inference is drawn from them. For grain iron the differences among crosses were not significant after screening ($H = 5.43$, $p = 0.143$), and the comparison between the two adequately sampled populations was likewise non-significant ($t = 1.65$, $p = 0.115$). The apparent superiority of RKVY-211 × Chandrahasini for iron on the unscreened data was therefore an artefact of contamination rather than a genetic difference.

Table 2: Grain zinc and iron concentration by cross. Zinc statistics use all lines; iron statistics use the screened dataset (Section 2.4), so the two n values differ where readings were excluded.

Population	n Zn	n Fe	Zn mean ± SD	Zn range	Fe mean ± SD
RKVY-211 × Chandrahasini	230	216	21.73 ± 3.92	13.4 - 33.8	3.49 ± 1.31
MTU1010 × CGR-77	13	13	15.49 ± 3.29	12.0 - 22.6	3.19 ± 0.57
Swarna × M55	4	4	13.10 ± 3.05	10.7 - 17.5	2.20 ± 0.45
Pusa Basmati × M55	2	2	13.75 ± 3.04	11.6 - 15.9	2.90 ± 0.85
Chandrahasini (parent)	1	1	22.20	—	2.80
RKVY-211 (parent)	1	1	21.50	—	2.40

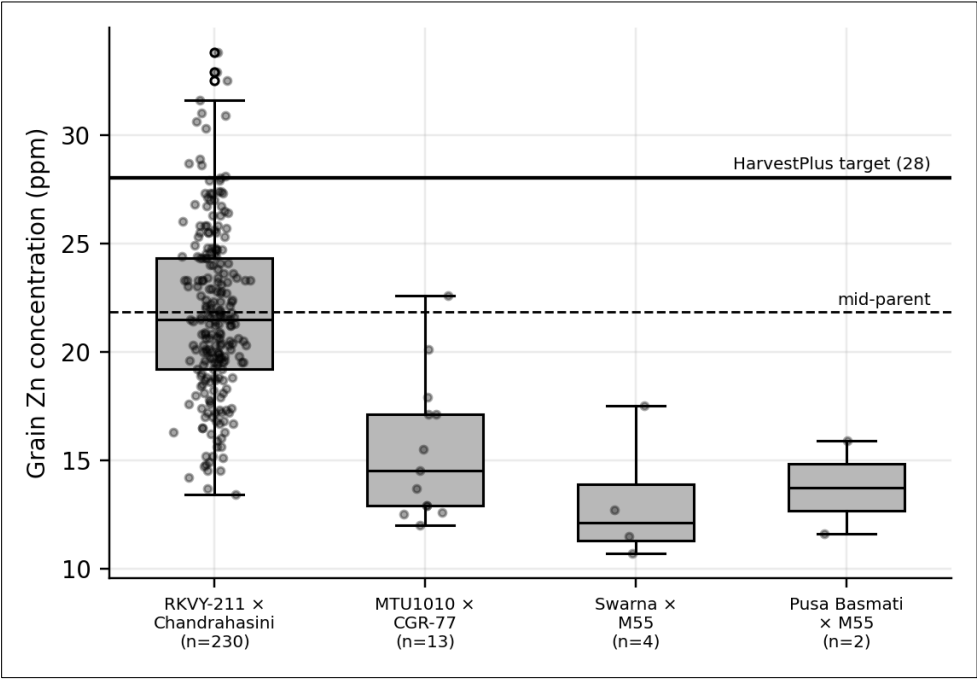


Fig 3: Distribution of grain zinc concentration by cross after screening. Individual lines are overlaid as points; the dashed horizontal line marks the mid-parent value of RKVY-211 × Chandrahasini (21.85 ppm).

3.5 Transgressive segregation and superior lines

Chandrahasini and RKVY-211 recorded 22.20 and 21.50 ppm zinc respectively, giving a mid-parent value of 21.85 ppm and a high-parent value of 22.20 ppm. Within the RKVY-211 × Chandrahasini population, 95 lines (41.3%) exceeded the high-parent value and the population mean did not differ significantly from the mid-parent value ($t = -0.45$, $p = 0.654$). The population range of 13.4 to 33.8 ppm extended well beyond the parental range in both directions, and the highest lines reached 33.8, 32.9 and 32.5 ppm — approximately 50% above the high parent. This bidirectional transgression indicates that favourable alleles are dispersed between the two parents and that recombinants combining them exceed either parent, the classic signature of complementary gene action. Because dominance and most

epistatic effects are largely dissipated by the F7 generation, the variation expressed here is predominantly additive and therefore directly usable in selection. Selection differentials in this population were substantial. Selecting the highest 5% for zinc (12 lines, threshold 28.05 ppm) yielded a differential of 8.92 ppm, equivalent to 41.1% of the population mean or 2.28 phenotypic standard deviations; at 10% intensity the differential was 7.30 ppm (1.86 SD) and at 20% intensity 5.66 ppm (1.44 SD). Twenty-three lines fell in the upper quartile for both zinc and iron simultaneously and constitute the priority set for advancement (Table 3). These lines combine high zinc density with iron in the upper part of the available range and, being near-homozygous, can be recovered from seed for immediate re-evaluation.

Table 3: Top ten rice RILs identified for combined grain zinc and iron concentration. All are from the RKVY-211 × Chandrahasini population.

Rank	Line	Zn (ppm)	Fe (ppm)
1	RKVY-211 × Chandrahasini-172	32.9	6.9
2	RKVY-211 × Chandrahasini-161	32.5	4.2
3	RKVY-211 × Chandrahasini-207	30.9	4.6
4	RKVY-211 × Chandrahasini-196	30.3	4.2
5	RKVY-211 × Chandrahasini-98	28.6	5.2
6	RKVY-211 × Chandrahasini-89	27.3	5.0
7	RKVY-211 × Chandrahasini-163	27.3	4.5
8	RKVY-211 × Chandrahasini-202	27.1	7.2
9	RKVY-211 × Chandrahasini-208	26.8	5.9
10	RKVY-211 × Chandrahasini-222	26.7	5.1

Three further lines merit note for zinc alone, recording the highest concentrations in the study while carrying iron nearer the population mean: RKVY-211 × Chandrahasini-143 (33.8 ppm Zn), -206 (31.0) and -176 (28.9).

4. Discussion

4.1 Target-meeting zinc exists within adapted material

Lines capable of meeting international biofortification targets already exist within the crosses this programme maintains. Thirteen reached the HarvestPlus target of 28 ppm and 65 the 24 ppm AICRIP threshold, against a commercial baseline of 12 to 16 ppm in polished grain. The significance lies in the genetic background rather than the concentrations. Zinc-dense rice is not scarce: national screening

has reported roughly 7 to 53 mg kg⁻¹ in brown rice, and several hundred accessions and mapping lines already exceed 28 mg kg⁻¹ in polished grain (Sanjeeva Rao *et al.* 2020) ^[12], while surveys of traditional Indian genotypes have documented comparable variation in white rice (Maganti *et al.* 2020) ^[9]. The recurring difficulty is that such donors are usually landraces or unadapted introductions whose agronomic background must be rebuilt over several cycles. The lines reported here arose in crosses already made for regional adaptation, so trait and background are

combined at the outset — which shortens the route to deployment more than any increment in concentration would.

The distribution supports selection. A 3.2-fold spread, symmetric and closely normal with a coefficient of variation of 20.1%, indicates a quantitative character under many loci of small effect rather than one or two major genes — architecture that responds predictably to recurrent phenotypic selection. The trial soil was also slightly alkaline (pH 7.73), which suppresses zinc uptake, so these concentrations are conservative rather than flattering.

4.2 Chandrahasini is the donor, and recombination has improved on it

The cross contrast locates the programme's zinc alleles. RKVY-211 × Chandrahasini averaged 21.73 ppm against 15.49 ppm for MTU1010 × CGR-77 — over 6 ppm, with a Hedges' *g* of 1.60 — and every target-meeting line came from it. Chandrahasini is confirmed as the effective donor among the parents examined, and Chandrahasini-derived material should carry the primary weight in future crossing.

More useful is what the population did relative to its own parents. Forty-one per cent of lines exceeded the high parent and the best reached 33.8 ppm, roughly 50% above it, while the population mean sat almost exactly on the mid-parent value. Bidirectional transgression on this scale establishes that favourable alleles are dispersed between the parents and that recombination has assembled superior combinations — the signature of predominantly additive gene action, and the most favourable situation available to a breeder, since additive effects respond to selection and transmit intact.

The F7 generation makes this an asset rather than an observation. At effective homozygosity, dominance and most epistasis have dissipated, so the advantage of the superior lines is fixed rather than transient, and single seed descent means the population was advanced with minimal inadvertent selection. Each top line is therefore both a candidate variety and an improved donor, beginning from a higher zinc baseline than Chandrahasini itself. The differentials are substantial: selecting the top 5% yields 8.92 ppm, or 2.28 phenotypic standard deviations; at 10% intensity, 7.30 ppm.

4.3 Iron is constrained by physiology and processing

The iron results are sobering. Grain iron averaged 3.44 ppm with no differences among crosses, and no line approached the 13 ppm target — a concentration reached in polished grain only through transgenic intervention (Trijatmiko *et al.* 2016) [18]. The obstacles are structural. Genetic variation for grain iron in cultivated rice is narrow, and iron concentrates in the aleurone and embryo, precisely the fractions milling removes, whereas zinc partitions more evenly into the endosperm and survives milling in usable quantity. That asymmetry explains why biofortification has progressed far further on zinc than on iron in rice, and it applies here.

A measurement issue compounds the biology. Fourteen entry means above 7.2 ppm showed the pattern of extraneous contamination — a tight core with an abrupt, unidirectional upper tail — and their removal cut the coefficient of variation from 57.7% to 37.0% and skewness from 3.50 to 0.63. Critically, the nominally significant cross difference for iron disappeared after screening: had those readings been retained, this study would have reported a genetic difference that does not exist. XRF cannot measure aluminium or titanium as index elements, so such contamination must be inferred from the iron distribution itself; friction whitening, which brings grain into repeated contact with metal and abrasive surfaces, is an additional source in milled rice.

Comparable behaviour has been reported for iron in earlier XRF screening of rice from this programme, where readings drifted systematically across spectrometer scan sessions and iron was consequently reported as indicative only (Upadhyay and Shukla 2026) [19]. Iron is therefore not a trait this material can currently be selected on.

4.4 Selection strategy: an index, not a proxy

The correlation between the micronutrients ($r = 0.365$) is favourable in sign but too weak to exploit. There is no antagonism, so selecting for zinc will not depress iron; but shared control accounts for only about 13% of common variance, and 34.3% of lines were high in one micronutrient and low in the other. Selecting on zinc alone would misclassify roughly a third of the material with respect to iron. Where both are targeted an index is necessary, and the twenty-three dual upper-quartile lines are its output here; where resources are constrained, selecting on zinc and monitoring iron is more efficient.

One dimension is missing. Yield and agronomic performance were not assessed, and a zinc-dense line that yields poorly has no route to adoption. Micronutrient-yield associations in cereals are generally weak rather than strongly negative, so a favourable combination should be attainable — but it must be demonstrated. Yield, maturity and grain quality should be recorded alongside micronutrient density in the evaluation that follows.

4.5 Nutritional significance

The 28 ppm target is not arbitrary. It derives from a daily rice intake of about 422 g, zinc absorption near 20% and retention near 90% after processing, set to supply close to 30% of the estimated average requirement (Swamy *et al.* 2016) [16]. A line meeting it therefore delivers a quantified fraction of daily need. On that basis the material identified here is nutritionally meaningful: against a 12 to 16 ppm baseline, the nominated lines represent approximately a doubling of dietary zinc from the same quantity of rice, requiring no change in consumption habits, no processing infrastructure and no recurring cost once a variety is adopted. That is the case for biofortification over supplementation, and the reason this screening justifies replicated testing.

4.6 Interpretation and next steps

The design defines what the results support. Evaluation was replicated within a single season at one location, so the entry means reported here are averaged over three replications and are correspondingly more reliable than single observations, but they remain specific to one environment and genotype × environment interaction could not be assessed. Because the analyses were performed on entry means rather than plot-level data, genotypic and error variances could not be separated and heritability and genetic advance were therefore not estimated. Earlier work on 22 genotypes from this programme, evaluated at two locations and under imposed moisture stress, found high broad-sense heritability for grain zinc and a non-significant genotype × environment interaction, with the leading entries holding their rank in all three environments (Upadhyay and Shukla 2026) [19]; those parameters remain to be established for the present material. Multi-environment testing is nevertheless required here, not because genotype ranking is expected to shift, but because each nominated line must demonstrate that it holds its zinc concentration under the range of conditions a released variety would encounter. The lines nominated here are candidates that met the target in one environment, not validated genotypes. These constraints bear on interpretation, not on the value of the screening, whose purpose is to reduce a large population to a set

worth the cost of replicated testing. That purpose is served: 249 lines narrowed to 13 meeting the international target and 23 combining upper-quartile values for both micronutrients. Because the material is fixed at F7, every nominated line is recoverable from seed. The next step is replicated multi-location evaluation of that set, recording yield and agronomic traits alongside micronutrient density, with ICP-OES confirmation at the final selection stage.

5. Conclusion

Screening of 249 F7 rice RILs from four biparental crosses revealed substantial and normally distributed variation for grain zinc concentration, ranging from 10.7 to 33.8 ppm with a mean of 21.08 ppm in polished grain — well above the 12 to 16 ppm typical of popular varieties. Thirteen lines met the HarvestPlus target of 28 ppm and 65 met the 24 ppm national threshold. The RKVY-211 × Chandrahasini population was clearly superior for zinc and exhibited marked transgressive segregation, with 41.3% of lines exceeding the high parent and the best lines approximately 50% above it. Grain iron was low and, once readings attributable to extraneous contamination were excluded, showed no significant variation among crosses. The two micronutrients were positively but moderately correlated, indicating that index selection is required where both are targeted. The practical outcome is a shortlist worth the cost of replicated testing. Thirteen lines meeting the international zinc target and twenty-three combining upper-quartile values for both micronutrients — headed by RKVY-211 × Chandrahasini-143, -172, -161 and -207 — are recommended for replicated multi-location evaluation with ICP-OES confirmation, after which the best performers may be advanced as candidate varieties or deployed as donors carrying a higher zinc baseline than either original parent.

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