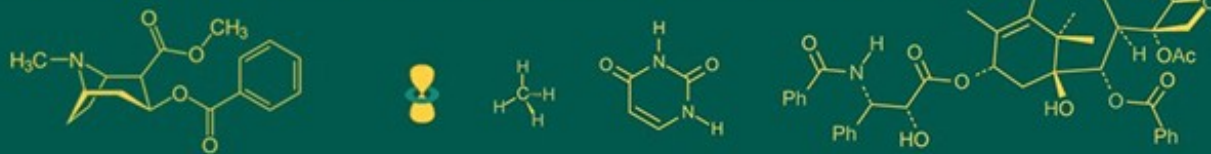


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating: (2026): 5.29
 Indexing: Embase
 IJABR 2026; SP-10(10): 211-223
www.biochemjournal.com
 Received: 12-08-2026
 Accepted: 17-09-2026
 Published: 02-10-2026

Atul Kumar Shukla
 Department of Genetics and
 Plant Breeding, B.N.P.G.
 College, Rath, Hamirpur, Uttar
 Pradesh, India

Shubham Rajpoot
 Department of Genetics and
 Plant Breeding, B.N.P.G.
 College, Rath, Hamirpur, Uttar
 Pradesh, India

Gyanendra Shrivastav
 Department of Genetics and
 Plant Breeding, B.N.P.G.
 College, Rath, Hamirpur, Uttar
 Pradesh, India

Genetic diversity and evaluation of aerobic rice hybrids for grain zinc content in rice (*Oryza Sativa* L.)

Atul Kumar Shukla¹, Shubham Rajpoot², Gyanendra Shrivastav³

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i10Sc.10280>

ABSTRACT

Grain zinc (Zn) deficiency is widespread among rice-dependent populations of South Asia, while shrinking irrigation supplies are moving rice towards non-flooded culture. Variability, divergence and combining ability for grain Zn and yield traits were examined under aerobic conditions to identify parents and hybrids uniting productivity with Zn density. Twenty-four genotypes (16 restorers, five elite lines, three checks) and 12 hybrids from a 3×4 line $\times$ tester design were evaluated in randomised complete block designs with three replications at B.N.P.G. College, Rath, Hamirpur, Uttar Pradesh, during two successive kharif seasons. Genotypes differed significantly ($p \leq 0.01$) for all ten traits. Grain Zn ranged from 18.06 to 30.37 mg kg⁻¹, and R13-Aerobic13, R8-Aerobic08 and R11-Aerobic11 exceeded 28 mg kg⁻¹. Broad sense heritability was high for every trait (70.65 to 92.11%), and high genetic advance as per cent of mean for grain yield per plant (40.04%) and grain Zn (25.91%) indicated largely fixable variation. Grain Zn correlated positively with grain yield ($r = 0.906$) through indirect effects of 1000 grain weight and spikelet fertility. Mahalanobis D^2 grouped the panel into four Tocher clusters, grain yield accounting for 61.23% of divergence. Among hybrids, CMS-B $\times$ R13-Aerobic13 recorded the highest grain Zn (31.24 mg kg⁻¹) with significant positive SCA, and CMS-A $\times$ R13-Aerobic13 the highest yield (33.22 g plant⁻¹) with 27.93 mg kg⁻¹ Zn. Non-additive variance predominated among hybrids, yet R13-Aerobic13 was the best general combiner for both traits and is the preferred restorer for Zn-dense aerobic hybrids.

Keywords: Biofortification, D^2 analysis, Combining ability, Heritability, Heterosis, Water-saving cultivation

Introduction

Rice supplies the bulk of daily energy for more than half of the world's population, and in South and Southeast Asia it is eaten two or three times a day by households that have little access to animal-source foods (Muthayya *et al.*, 2014) [1]. A diet built so heavily on one cereal carries a hidden cost. Milled rice is poor in bioavailable Zn, and populations relying on it rarely meet their requirement for the element. Estimates based on national food supplies place roughly one person in six at risk of inadequate Zn intake worldwide, with the highest prevalence in South Asia (Wessells *et al.*, 2012) [2]. Deficiency weakens immune function, contributes to childhood stunting and raises mortality from diarrhoea and pneumonia among young children (Black *et al.*, 2013) [3]. Rising atmospheric CO₂ is expected to depress the Zn concentration of C₃ cereals further, which would enlarge the population at risk precisely in the rice-eating regions of India and its neighbours (Myers *et al.*, 2015) [4].

Supplementation and fortification reach urban consumers reasonably well but seldom reach the rural poor who grow and eat their own grain. Breeding staple crops for higher micronutrient density avoids this delivery problem because the nutrient travels inside the seed that farmers already save and sow (Welch *et al.*, 2004) [5]. HarvestPlus fixed a target increment of 12 mg kg⁻¹ over a baseline of 16 mg kg⁻¹ in polished rice, giving a breeding target of 28 mg kg⁻¹ (Pfeiffer *et al.*, 2007) [6]. Programmes pursuing that target have since released Zn-biofortified rice varieties across Asia and demonstrated that nutritionally relevant gains can be achieved without yield sacrifice (Bouis *et al.*, 2017) [7]. In India, several Zn-enriched rice varieties have been notified through the national testing system, although most are inbred cultivars bred for irrigated, transplanted culture (Rao *et al.*, 2020) [8].

That culture is itself under pressure. Puddled transplanted rice consumes two to three times more water per unit of grain than other cereals, much of it lost through seepage, percolation and evaporation from standing water (Tuong *et al.*, 2003) [9]. Aerobic rice, in which high-yielding, input-responsive varieties are dry seeded and grown in unpuddled, non-saturated soil with supplementary irrigation, can cut irrigation water substantially while keeping yields at acceptable levels (Bouman *et al.*, 2005) [10]. Direct seeding also lowers labour demand and shortens the crop cycle, which explains its rapid spread in the Indo-Gangetic Plains (Farooq *et al.*, 2011; Kumar *et al.*, 2011) [11, 12]. The shift is not neutral for plant nutrition. The loss of standing water

Corresponding Author:

Atul Kumar Shukla
 Department of Genetics and
 Plant Breeding,
 B.N.P.G. College, Rath,
 Hamirpur, Uttar Pradesh,
 India

changes soil redox status, pH and the solubility of micronutrients, and rice roots growing in aerobic soil face a physical and chemical environment very different from that of a flooded paddy (Kato *et al.*, 2014) [13]. For Zn in particular, flooding favours the formation of sparingly soluble sulphides, carbonates and hydroxides in calcareous and sodic soils, so the aerobic condition may alter both the amount of Zn a genotype can acquire and the ranking of genotypes for grain Zn (Impa *et al.*, 2012) [14].

Hybrid rice offers a yield advantage commonly placed at 15 to 20% over the best inbred cultivars grown under similar management (Virmani, 1994) [15]. The three-line system, which combines a cytoplasmic male sterile (CMS) line, its maintainer and a fertility restorer, underpins most commercial hybrids in China and India (Cheng *et al.*, 2007) [16]. If hybrid vigour can be joined with high grain Zn, a single cultivar could address both productivity and nutrition. Whether grain Zn in hybrids is governed by additive effects, which parents transmit predictably, or by dominance and interaction effects, which must be sought in specific combinations, decides how such a programme should be organised (Velu *et al.*, 2014) [17].

Parent selection in any hybrid or recombination programme gains from knowing how genotypes differ across many traits simultaneously. Mahalanobis D^2 statistics condense multivariate differences into a single distance and, together with Tocher clustering, group genotypes so that parents can be drawn from clusters that are both divergent and agronomically superior (Mahalanobis, 1936) [18]. Divergence is useful only up to a point, since very distant parents may combine poorly, and the most productive crosses often come from parents of moderate divergence and high mean performance (Arunachalam, 1981) [19]. Germplasm surveys have shown wide variation for grain Zn in rice, and several genomic regions controlling it have been mapped (Swamy *et al.*, 2016) [20]. Nearly all of this evidence comes from inbred material evaluated under flooded culture. Information on the variability of restorer and elite lines for grain Zn under aerobic management, on the divergence among them and on how Zn density is inherited in their hybrids remains scarce, and without it breeders cannot choose restorers for Zn-dense aerobic hybrids on a sound basis.

The present investigation was designed with four objectives. The first was to estimate genetic variability, heritability and genetic advance for grain yield, its components and grain Zn in a panel of restorers, elite lines and checks grown under aerobic conditions. The second was to measure the association of grain Zn with yield traits and to partition the correlations with grain yield into direct and indirect effects. The third was to quantify genetic divergence with Mahalanobis D^2 and group the panel into clusters for parent selection. The fourth was to evaluate 12 CMS-based hybrids for mean performance, heterosis and combining ability for grain yield and grain Zn under the same management.

Materials and Methods

Experimental site and weather

The investigation was conducted at the B.N.P.G. College, Rath, Hamirpur, Uttar Pradesh. The diversity panel was evaluated during a kharif season and the hybrids during the following kharif season under aerobic conditions, following the recommended agronomic package of practices.

Genetic material

The diversity panel comprised 24 genotypes, namely 16 restorer lines (R1-Aerobic01 to R16-Aerobic16), five elite breeding lines (EL-01 to EL-05) and three checks. The restorers were drawn from a larger set of 56 restorer lines maintained at lies at 17°19' N latitude and 78°23' E longitude, at an altitude of 542 m above mean sea level, in the Southern Zone of Telangana State, and receives an average annual rainfall of about 750 mm, which included promising backcross inbred

lines (BC₂F₆) derived from KMR-3R/Nagina 22 together with parental genetic lines. The checks were KMR-3R (Check-1), the restorer of the hybrid KRH2 developed at ZARS, Mandya, University of Agricultural Sciences, Bengaluru; Nagina 22 (Check-2), a heat tolerant line maintained at B.N.P.G. College, Rath, Hamirpur, Uttar Pradesh; and ZINCO-RICE (Check-3), a Zn-biofortified variety released by Indira Gandhi Krishi Vishwavidyalaya, Raipur.

On the basis of their per se performance for grain Zn and agronomic traits in the diversity panel, four restorers were chosen as testers, namely R2-Aerobic02 (higher Zn with acceptTable-yield), R4-Aerobic04 (high Zn with good spikelet fertility), R8-Aerobic08 (high Zn with good yield potential) and R13-Aerobic13 (high Zn with profuse productive tillering). These were crossed with three CMS lines in a line × tester mating design (Kempthorne, 1957) [21] to produce 12 F₁ hybrids. The lines were APMS6A (CMS-A) from the Agricultural Research Station, Maruteru, CRMS32A (CMS-B) from ICAR-National Rice Research Institute, Cuttack, and IR80555A (CMS-C) from the International Rice Research Institute, Philippines.

Hybrid seed production

Hybrid seed was produced following standard three-line procedures (Virmani, 1994) [15]. Because the parents differed in duration, sowing dates were staggered to synchronise flowering; the CMS line was sown once, and each restorer was sown three times at three-day intervals. Restorer plants were transplanted in two rows at 15 × 15 cm, and the CMS line was planted in eight rows at 30 × 15 cm between the restorer rows. Off-type plants were rogued out before flowering to maintain genetic purity. At booting, about half of the flag leaf of the CMS plants was clipped with a sharp sickle to ease pollen dispersal. Supplementary pollination was done by shaking the restorer plants with a rope or bamboo pole three to four times a day during peak anthesis for 6 to 10 days. At maturity, the seed and pollen parents were harvested and threshed separately, and hybrid seed from the CMS rows was dried, processed, packed and labelled by cross combination.

Experimental design and aerobic crop management

The diversity panel and the hybrids were evaluated independently, each in a randomised complete block design (RCBD) with three replications. The field was prepared by disc ploughing, cultivator and rotavator operations, followed by harrowing and levelling, to obtain a fine, unpuddled seedbed for dry direct seeding. Seed was sown manually at a spacing of 20 × 15 cm. The crop was maintained under aerobic conditions and was never continuously flooded. Supplementary irrigation was applied whenever hairline cracks appeared on the soil surface, or when leaf tip rolling in the uppermost leaves indicated impending water stress, so that the root zone stayed moist but unsaturated throughout the season. Fertilisers were applied at 100 kg N, 40 kg P and 60 kg muriate of potash ha⁻¹ together with 10 kg ZnSO₄ ha⁻¹. Nitrogen was applied in three splits, and the whole of the phosphorus and muriate of potash was applied basally to dry soil before the last ploughing. Weed control and plant protection followed the recommended package of practices for aerobic rice at B.N.P.G. College, Rath, Hamirpur, Uttar Pradesh, with prophylactic measures to keep the crop healthy.

Observations recorded

Observations were recorded on five randomly tagged competitive plants per plot per replication for plant height (cm), panicle length (cm), total number of tillers per plant, number of productive tillers per plant, total grains per panicle, spikelet fertility (%), 1000 grain weight (g) and grain yield per plant (g). Days to 50% flowering was recorded on a whole plot basis as the number of days from sowing to the date on which half of the plants in a plot had exerted panicles. Spikelet fertility was calculated as the proportion of filled grains to total spikelets on the main panicle, expressed as a percentage. Grain yield per plant was recorded after threshing and sun drying the harvested grain to a standard moisture content.

Grain sampling and zinc estimation

At maturity, grain from the tagged plants of each plot was bulked, cleaned of empty spikelets and dried. Samples were prepared for Zn estimation by a non-contaminating milling procedure that avoided contact with metallic surfaces, and grain Zn concentration (mg kg^{-1}) was determined with appropriate calibration against grain reference standards of known Zn concentration. Each replication was analysed separately so that the Zn data could enter the same analysis of variance as the agronomic traits.

Statistical analysis

Replication-wise plot means were subjected to analysis of variance for an RCBD following Panse *et al.* (1985) [22], and genotype means were compared with the critical difference at $p = 0.05$ (Gomez *et al.*, 1984) [23]. The standard error of the mean was computed as the square root of the error mean square divided by the number of replications. Genotypic and phenotypic variances were derived from the expected mean squares, and the genotypic (GCV) and phenotypic (PCV) coefficients of variation were estimated after Burton *et al.* (1953) [24] and classified as low (below 10%), moderate (10 to 20%) or high (above 20%) following Sivasubramanian *et al.* (1973) [25]. Broad sense heritability and expected genetic advance at 5% selection intensity ($k = 2.06$), expressed also as per cent of the mean, were estimated by the method of Johnson *et al.* (1955) [26]. Heritability was classed as low, moderate or high following Robinson *et al.* (1949) [27], and genetic advance as per cent of mean was classed as low (below 10%), moderate (10 to 20%) or high (above 20%).

Phenotypic correlation coefficients among the ten traits were computed on genotype means following Al-Jibouri *et al.* (1958) [28] and tested against tabulated r at 22 degrees of freedom. The correlations of nine traits with grain yield per plant were partitioned into direct and indirect effects by path coefficient analysis (Dewey *et al.*, 1959) [29], with grain yield per plant as the dependent variable.

Genetic divergence was measured with Mahalanobis (1936) [18] D^2 statistic. The pooled error variance and covariance matrix was obtained from the RCBD analysis of variance and covariance, and the genotype means were transformed into uncorrelated variables through triangular (Cholesky) decomposition of the error matrix, so that D^2 between any two genotypes equalled the sum of squared differences of the transformed means (Rao, 1952) [30]. Genotypes were grouped by Tocher's method, taking the largest of the minimum D^2 values of individual genotypes as the threshold for cluster formation (Singh *et al.*, 1977) [31]. Average intra- and inter-cluster distances were computed from the pairwise D^2 values, and the relative contribution of each trait to divergence was estimated from the number of times it ranked first across all 276 pairwise comparisons (Singh, 1981) [32]. Principal component analysis was carried out on the correlation matrix of genotype means to verify the dimensional structure of the variation.

Hybrid data were analysed first as an RCBD with 12 crosses, and the cross sum of squares was then partitioned into lines, testers and line $\times$ tester components (Kempthorne, 1957) [21]. Lines and testers were tested against the line $\times$ tester mean square, and the line $\times$ tester mean square against the error. General combining ability (GCA) effects of lines and testers, specific combining ability (SCA) effects of crosses, their standard errors and the variances due to GCA (σ^2_{gca}) and SCA (σ^2_{sca}) were estimated after Singh *et al.* (1977) [31]. The ratio $\sigma^2_{\text{gca}}/\sigma^2_{\text{sca}}$ was used to judge the relative importance of additive and non-additive gene action (Baker, 1978) [33]. Heterosis was calculated as the per cent deviation of the F_1 mean from the mid-parent value (relative heterosis, MPH) and from the parent with the higher mean (heterobeltiosis, BPH). All analyses were carried out with standard biometrical procedures as described in the references cited.

Results

Results and Discussion are presented as separate sections so that the experimental evidence can be read independently of its interpretation.

Analysis of variance and mean performance

Genotypes in the diversity panel differed significantly ($p \leq 0.01$) for all ten traits (Table-1). The genotype mean square was 18.9 to 36.0 times the error mean square for days to 50% flowering, grain yield per plant, grain Zn and productive tillers per plant, which shows that genotypic differences dominated the observed variation for these traits. Replication effects were small and non-significant, and the coefficients of variation were low, ranging from 1.74% for days to 50% flowering to 8.14% for total tillers per plant, which attests to the precision of the aerobic field trial. Among hybrids, crosses differed significantly for eight traits; plant height ($p = 0.066$) and panicle length did not vary significantly. The line $\times$ tester interaction was significant for total tillers, total grains per panicle, spikelet fertility, grain yield per plant and grain Zn, whereas lines did not differ significantly for any trait when tested against the interaction.

Mean performance is summarised in Table-2. Grain Zn in the panel ranged from 18.06 mg kg^{-1} in R12-Aerobic12 to 30.37 mg kg^{-1} in R13-Aerobic13 around a general mean of 24.24 mg kg^{-1} (Figure 1). Taking the biofortified check ZINCO-RICE (Check-3, 25.79 mg kg^{-1}) as the standard and 1.73 mg kg^{-1} as the critical difference, four entries were significantly superior, namely R13-Aerobic13, R8-Aerobic08 (28.66 mg kg^{-1}), R11-Aerobic11 (28.17 mg kg^{-1}) and EL-03 (27.78 mg kg^{-1}). The first three exceeded the HarvestPlus target of 28 mg kg^{-1} . Grain yield per plant varied more than twofold, from 16.22 g in R12-Aerobic12 to 33.00 g in R13-Aerobic13, and R13-Aerobic13, EL-03 (31.68 g), R4-Aerobic04 (31.66 g) and R9-Aerobic09 (31.60 g) outyielded Check-3 (28.43 g) significantly. The same pattern ran through the yield components. R13-Aerobic13 had the most grains per panicle (243.13), the highest spikelet fertility (91.63%) and the boldest grain (24.39 g per 1000 grains), whereas R12-Aerobic12, R7-Aerobic07 and Check-2 sat at the opposite extreme for almost every trait. Flowering ranged from 86.00 days in R4-Aerobic04 to 99.57 days in R14-Aerobic14, and most high-yielding, Zn-dense entries flowered in fewer than 90 days.

Hybrid grain Zn ranged from 24.43 mg kg^{-1} in CMS-C $\times$ R2-Aerobic02 to 31.24 mg kg^{-1} in CMS-B $\times$ R13-Aerobic13, the latter being significantly higher than every other cross ($CD = 1.95 \text{ mg kg}^{-1}$). Five further hybrids, CMS-A $\times$ R13-Aerobic13, CMS-A $\times$ R8-Aerobic08, CMS-C $\times$ R4-Aerobic04, CMS-C $\times$ R13-Aerobic13 and CMS-C $\times$ R8-Aerobic08, clustered between 27.60 and 27.93 mg kg^{-1} , within reach of the target. CMS-A $\times$ R13-Aerobic13 was the highest yielding hybrid (33.22 g plant $^{-1}$), followed by CMS-B $\times$ R4-Aerobic04 (32.44 g), and it combined early flowering (87.67 days) with the highest spikelet fertility (91.63%) and grain weight (24.91 g) among hybrids. Crosses involving R2-Aerobic02 had the lowest grain Zn irrespective of the CMS line, and CMS-B $\times$ R8-Aerobic08 was both the latest (95.60 days) and the lowest yielding hybrid (24.21 g).

Genetic variability, heritability and genetic advance

Estimates of genetic parameters are given in Table-3. PCV exceeded GCV for every trait, but only by small margins (0.33 to 2.33 percentage points), so the environment contributed little to the expression of these traits under the uniform aerobic regime. Grain yield per plant alone showed high GCV (20.25%) and PCV (21.10%). Moderate GCV was recorded for productive tillers per plant (16.17%), total grains per panicle (13.82%), grain Zn (13.23%) and total tillers per plant (13.06%), while the remaining traits showed low values. Heritability in the broad sense was high throughout, from 70.65% for panicle length to 92.11% for grain yield per plant, with grain Zn close behind at 90.31%. Genetic advance as per cent of mean was high for grain yield per plant (40.04%), productive tillers (30.83%), total grains per panicle (25.97%), grain Zn (25.91%) and total tillers (22.83%), moderate for plant height, 1000 grain weight and spikelet fertility, and low for days to 50% flowering (8.30%) and panicle

length (9.31%). Expected gain from selecting the top 5% of the panel for grain Zn was 6.28 mg kg⁻¹.

Correlation and path analysis

Grain yield per plant was significantly and positively correlated ($p \leq 0.01$) with every trait except days to 50% flowering, with which it was negatively correlated ($r = -0.825$) (Table-4). The strongest associations of yield were with spikelet fertility ($r = 0.936$), productive tillers ($r = 0.935$), total tillers ($r = 0.934$) and total grains per panicle ($r = 0.929$). Grain Zn followed the same pattern. It correlated positively with spikelet fertility ($r = 0.909$), grain yield per plant ($r = 0.906$), 1000 grain weight ($r = 0.886$) and panicle length ($r = 0.865$), and negatively with days to 50% flowering ($r = -0.729$). Plant height showed the weakest associations of all traits, and its correlation with flowering ($r = -0.454$) was significant only at $p \leq 0.05$.

Path analysis (Table-5) explained 97.2% of the variation in grain yield per plant, leaving a residual effect of 0.167. The largest positive direct effects came from 1000 grain weight (0.443) and spikelet fertility (0.357), followed by plant height (0.191) and total tillers (0.158). Days to 50% flowering exerted a negative direct effect (-0.175), and panicle length had a sizeable negative direct effect (-0.391) despite its strong positive correlation with yield, the correlation being carried by positive indirect effects via grain weight and spikelet fertility. The direct effect of grain Zn on yield was negligible (-0.020). Its correlation with yield was built almost entirely from indirect contributions through 1000 grain weight (0.393) and spikelet fertility (0.325).

Genetic divergence and clustering

Pairwise D^2 values ranged from 8.75 (EL-05 and Check-3) to 704.10 (R12-Aerobic12 and R13-Aerobic13), with a mean of 147.01. Using a threshold of 39.87, Tocher's method grouped the 24 genotypes into four clusters (Table-6; Figure 2). Cluster I was the largest, holding 12 genotypes that included all four restorers used as testers, the other Zn-dense entries R11-Aerobic11 and EL-03, and the best check, Check-3. Cluster II held eight genotypes, Cluster III three, and R5-Aerobic05 formed a solitary Cluster IV. Cluster means separated the groups sharply. Cluster I combined the highest grain yield (29.16 g), grain Zn (26.86 mg kg⁻¹), spikelet fertility (88.56%) and grain number (220.21) with the earliest flowering (88.93 days). Cluster II was its mirror image, being late (96.22 days), short, low yielding (19.16 g) and poor in Zn (21.07 mg kg⁻¹). Cluster III was intermediate for all traits, and the solitary R5-Aerobic05 combined late flowering and tall stature with the lowest cluster mean for grain Zn (19.39 mg kg⁻¹).

Intra-cluster D^2 was highest in Cluster I (37.27) and lowest in Cluster III (25.61) (Table-7). The greatest inter-cluster distance separated Clusters I and II (292.22), followed by Clusters I and IV (208.19), while Clusters III and IV were closest (72.84). Grain yield per plant ranked first in 169 of the 276 comparisons and accounted for 61.23% of total divergence. Days to 50% flowering and grain Zn each contributed 9.42%, plant height 8.33% and spikelet fertility 4.71%, while total tillers per plant made no contribution. Principal component analysis confirmed the dominance of a single axis of variation. The first component explained 84.34% of total variance, with nearly equal loadings (0.32 to 0.34) for all yield components and grain Zn and an opposite loading for flowering (-0.29). The second component (6.62%) was defined mainly by plant height (0.84) and flowering (0.38).

Combining ability and heterosis

Testers contributed most of the variation among crosses for grain Zn (65.0%) and days to flowering (74.5%), whereas the line $\times$ tester interaction contributed the largest share for grain yield per plant (51.5%) and spikelet fertility (55.8%). Lines contributed less than 14% for every trait except panicle length. The variance due to SCA exceeded that due to GCA for grain Zn (σ^2_{sca} 1.443 against σ^2_{gca} 0.160) and far more so for grain yield per plant (7.217 against 0.064), giving $\sigma^2_{gca}/\sigma^2_{sca}$ ratios well below unity (Table-1). Only 1000 grain weight showed a ratio above one (1.18).

GCA effects are presented in Table-8. Among testers, R13-Aerobic13 was the best general combiner, with significant positive effects for grain Zn (1.920), grain yield per plant (1.520), grains per panicle, spikelet fertility and 1000 grain weight, together with a significant negative (desirable) effect for days to flowering. R4-Aerobic04 was the best combiner for grain yield (1.958) and also contributed earliness and shorter plants, but its GCA for Zn was close to zero. R2-Aerobic02 was a poor combiner for nearly every trait, including grain Zn (-1.940) and yield (-2.668), and R8-Aerobic08 transmitted lateness and height. None of the GCA effects of the three CMS lines reached significance for grain Zn or grain yield. CMS-A showed positive tendencies for yield, spikelet fertility and grains per panicle, and CMS-B a small positive tendency for Zn.

SCA effects and heterosis are presented in Table-9. CMS-B $\times$ R13-Aerobic13 had the only significant positive SCA effect for grain Zn (1.931), and it also showed the highest relative heterosis (17.07%) and a positive heterobeltiosis (2.87%) for the trait. Heterobeltiosis for grain Zn was modest overall, the highest values occurring in CMS-A $\times$ R2-Aerobic02 (4.88%) and CMS-B $\times$ R2-Aerobic02 (4.49%), both of which involved a low Zn tester and hence a low better parent. For grain yield per plant, CMS-C $\times$ R2-Aerobic02 recorded a significant positive SCA effect (2.583) and the highest heterobeltiosis (12.54%), followed closely by CMS-A $\times$ R8-Aerobic08 (12.24%). Relative heterosis for yield exceeded 17% in five crosses. CMS-B $\times$ R8-Aerobic08 carried significant negative SCA effects for both grain yield (-3.207) and grain Zn (-1.617), which accounts for its poor performance.

Discussion

Extent of variability and its genetic basis

The significant genotypic differences for all traits, obtained with coefficients of variation below 9%, show that the panel carried genuine variability for grain Zn and yield that the aerobic regime allowed to express. A near 1.7-fold range in grain Zn, from about 18 to 30 mg kg⁻¹, lies within the spread reported for cultivated germplasm, although germplasm-wide screens that include traditional and aromatic landraces have recorded considerably wider ranges (Swamy *et al.*, 2016) [20]. The narrower spread here is expected. The panel consisted of breeding lines that had already passed through selection for plant type and yield, a process that tends to trim the extremes of mineral concentration. Mapping populations derived from contrasting inbred parents have similarly shown transgressive but bounded variation for grain Zn (Anuradha *et al.*, 2012; Garcia-Oliveira *et al.*, 2009) [34, 35]. The small gap between PCV and GCV, and heritability above 70% for all traits, indicate that the phenotype was a reliable guide to the genotype in this environment. Heritability in the broad sense includes all genetic variance, so on its own it cannot separate additive from non-additive effects (Falconer *et al.*, 1996) [36]. Its value for selection becomes clearer when read together with genetic advance. High heritability combined with high genetic advance as per cent of mean, as found for grain yield per plant, grain Zn, productive tillers, total tillers and grains per panicle, is generally taken to reflect additive gene action, since additive variance is what produces a response to selection among homozygous lines (Johnson *et al.*, 1955) [26]. Because the restorers and elite lines are advanced inbreds, the genetic variance among them consists largely of additive and additive $\times$ additive components, and simple pedigree or recurrent selection within the panel should raise grain Zn. The expected gain of more than 6 mg kg⁻¹ in one cycle is large relative to the 12 mg kg⁻¹ increment that HarvestPlus seeks. Days to flowering and panicle length, which combined high heritability with low genetic advance, are more likely influenced by non-additive effects, and selection for them would yield modest returns.

The high heritability of grain Zn deserves comment, since several studies across locations have found grain Zn strongly influenced by environment and by genotype $\times$ environment interaction (Norton *et al.*, 2010) [37]. The two findings are compatible. Heritability esti

mated in a single environment includes genotype $\times$ environment variance within the genotypic component and so tends to overstate the heritability relevant across sites and seasons (Falconer *et al.*, 1996) [36]. Soil Zn availability also governs whether genotypic differences are expressed at all. Genotypic differences in grain Zn become larger on soils where Zn supply is adequate and shrink where it is limiting (Wissuwa *et al.*, 2008) [38]. The present estimates describe the potential of the panel in this soil and season, and multi-environment testing is needed before they are applied to selection across the target region.

Why grain zinc and yield moved together

The strong positive correlation between grain Zn and grain yield ($r = 0.906$) runs against the widely reported dilution effect, in which higher starch accumulation lowers the concentration of minerals in the grain. Long-term records from wheat show that the introduction of high-yielding semi-dwarf cultivars coincided with a decline in grain Zn and Fe concentration (Fan *et al.*, 2008) [39]. Rice appears less prone to this penalty. In wheat and barley, much of the grain Zn is remobilised from senescing leaves, whereas in rice the bulk of grain Zn is taken up by roots during grain filling and moves directly to the developing grain, with remobilisation from leaves contributing a smaller and more variable share (Sperotto, 2013; Stomph *et al.*, 2009) [40, 41]. A genotype that sustains active root uptake and transpiration late into grain filling can raise both yield and grain Zn together, provided soil Zn supply continues. Under aerobic culture this link may be tighter than under flooding. Rice roots in aerobic soil explore a larger soil volume and depend more on root length, fine root proliferation and the maintenance of root activity under intermittent drying (Kato *et al.*, 2014) [13]. The same root vigour that sustains yield under aerobic conditions would enhance Zn acquisition, so Zn density and productivity may share a common physiological basis in this system.

Zn chemistry under non-flooded conditions reinforces this interpretation. Flooding raises the pH of acid soils, lowers that of alkaline soils and encourages precipitation of Zn with sulphide, carbonate and iron or manganese hydroxides, while bicarbonate accumulation in flooded calcareous soils impairs root function (Impa *et al.*, 2012) [14]. Aerobic soils avoid some of these constraints but introduce others, such as lower diffusion of Zn in drier soil. Pot and field studies comparing flooded and aerobic culture have found that Zn uptake is not uniformly higher under aerobic conditions and that the outcome depends on soil type and genotype (Gao *et al.*, 2006) [42]. In the present panel, the genotypes that coped best with aerobic management were also those that captured most Zn, a pattern consistent with root-driven acquisition rather than with dilution.

The path analysis clarifies the nature of the association. Grain Zn had almost no direct effect on grain yield, and its correlation with yield passed through 1000 grain weight and spikelet fertility. Well-filled, heavy grains in fertile panicles reflect uninterrupted assimilate and water supply during grain filling, which is also the period when root-derived Zn is loaded into the grain. Loading involves a sequence of transport steps. Zn is taken up by transporters of the ZIP family such as *OsZIP4*, which is expressed in roots and in the phloem (Ishimaru *et al.*, 2005) [43], while node-localised *OsZIP3* directs Zn preferentially to developing tissues (Sasaki *et al.*, 2015) [44]. Chelation by nicotianamine, whose synthesis depends on the *OsNAS* genes, and the activity of YSL transporters that move metal complexes in the phloem both influence how much Zn reaches the endosperm (Palmgren *et al.*, 2008) [45]. Overexpression of *OsNAS* genes has raised endosperm Zn in transgenic rice, which shows that the phloem supply rather than grain sink capacity often limits accumulation (Johnson *et al.*, 2011) [46]. Genotypes with higher spikelet fertility and grain weight offer more active sinks at the time this transport system operates, which explains why Zn density tracked grain filling traits. Some of the large negative direct effect of panicle length on yield probably reflects multicollinearity among the strongly intercorrelated components rather than a true physiological penalty, and it should not be read as a reason to select for short panicles.

The practical implication is encouraging. In this material, selection for yield under aerobic management would not lower grain Zn and would tend to raise it, and simultaneous selection for both traits should face little genetic resistance. The negative association of flowering with both yield and Zn also indicates that earlier genotypes performed better, most likely because they completed grain filling before the late-season decline in soil moisture and temperature that aerobic crops commonly face. A useful caution is that correlations across genotype means capture the association among these particular lines. A panel that included lines with very different yield potential, or a Zn-deficient soil, could reveal the dilution effect that the present data did not show (Swamy *et al.*, 2016) [20].

Genetic divergence and parent selection

Grouping 24 genotypes into four clusters, with half of them in one cluster, shows that the panel was less diverse than its size suggests. This is expected in material of this kind, since many of the restorers trace to a common KMR-3R/Nagina 22 backcross background and all had been selected for a similar plant type. The dominance of grain yield per plant in the contribution to divergence, and the concentration of 84% of variance on the first principal component, indicate that the genotypes differed mainly along one axis of overall vigour and productivity. Yield components and grain Zn moved together along this axis, while plant height and flowering contributed independent, smaller dimensions. Grain Zn contributed 9.42% of divergence in its own right, equal to flowering, which means the transformed Zn values still discriminated among genotypes after accounting for their correlation with preceding traits.

The most important observation for the hybrid programme is that all four restorers used as testers belonged to Cluster I. They were chosen on *per se* Zn and yield, which favoured genotypes from the productive cluster, but this choice restricted the genetic distance among testers. The D^2 values among them ranged from 24.63 (R4-Aerobic04 and R13-Aerobic13) to 110.55 (R2-Aerobic02 and R13-Aerobic13), all well below the inter-cluster distances of the panel. Parents of moderate divergence and high mean performance often give the best crosses, because very distant parents may lack complementary favourable alleles or show disrupted coadaptation (Arunachalam, 1981) [19]. Cluster II was the most distant from Cluster I, but its members were poor *per se* for every trait, and crossing them with Cluster I genotypes would add divergence mainly at the cost of productivity. For improving the restorer pool itself through recombination, crosses among high performing members of Cluster I with the greatest mutual distance are more promising. R8-Aerobic08 $\times$ R13-Aerobic13 ($D^2 = 75.46$) and R11-Aerobic11 $\times$ R13-Aerobic13 ($D^2 = 43.81$) join parents that are all above the target for grain Zn and differ appreciably in flowering, plant height and grain number, which should allow transgressive recombinants to be recovered. R11-Aerobic11, which ranked third for grain Zn and fifth for yield but was not used as a tester, deserves inclusion in the next test-cross cycle, as does EL-03 once its fertility restoring ability has been confirmed.

Gene action in hybrids and the choice of parents

The hybrid analysis gives a different picture of gene action from that drawn from the inbred panel. Among crosses, σ^2_{sca} exceeded σ^2_{gca} for grain Zn and far exceeded it for grain yield, which indicates that non-additive effects dominated the variation among these 12 hybrids (Baker, 1978) [33]. The two results are not contradictory. Variance among homozygous lines is largely additive, whereas variance among F_1 hybrids adds dominance and its interactions, which are expressed only in the heterozygous state (Falconer *et al.*, 1996) [36]. Two features of the design also depressed σ^2_{gca} . With only three lines and four testers, GCA variance rests on very few degrees of freedom, and the testers were drawn from a single cluster, which narrowed the range of GCA effects among them. Work on wheat has more often found predominantly additive control of grain Zn (Velu *et al.*, 2014) [17], and a larger mating design with testers from several clusters would be expected to reveal more additive variance.

Even within this limited set, the testers explained about two thirds of the variation among crosses for grain Zn, and GCA of the restorer clearly governed the Zn level of its hybrids. R13-Aerobic13, with the highest per se Zn, produced three of the six Zn-richest hybrids, while every cross with R2-Aerobic02 was low in Zn. This agreement between per se performance and GCA for Zn makes the restorer's own grain Zn a practical first screen. The grain harvested from an F₁ plant is F₂ seed, and its mineral content depends largely on the uptake and remobilisation capacity of the F₁ mother plant (Stomph *et al.*, 2009) [41]. Restorer alleles that improve root uptake or phloem loading would be expressed in the hybrid plant irrespective of the grain's own genotype, which explains the strong paternal influence. The CMS lines contributed little variation for any trait, and none had significant GCA for Zn or yield. This is expected from female lines selected mainly for s Table-sterility and outcrossing traits, and it points to the maintainer lines as a neglected target for Zn improvement.

Heterosis for grain Zn was mostly positive over the mid-parent but small over the better parent, a pattern also typical of grain mineral concentration in wheat, where dominance effects for Zn are usually small (Velu *et al.*, 2014) [17]. Several crosses showed positive heterobeltiosis only because their better parent was the weakest tester, R2-Aerobic02, so heterobeltiosis on its own would mislead parent choice here. CMS-B × R13-Aerobic13 stands apart. Its grain Zn exceeded the HarvestPlus target, it exceeded its high Zn restorer parent and it carried the only significant positive SCA for the trait, which points to favourable complementation between the two parents. Its yield was good but not outstanding, and CMS-B carried a negative, though non-significant, GCA for yield. CMS-A × R13-Aerobic13 offers the better balance, with the highest yield, the best spikelet fertility and grain weight among hybrids, early flowering and grain Zn close to the target. CMS-A × R8-Aerobic08 and CMS-C × R4-Aerobic04 combine yields above 30 g plant⁻¹ with Zn near 27.8 mg kg⁻¹ and form a useful second tier.

Relating grain zinc to the HarvestPlus target

The HarvestPlus target of 28 mg kg⁻¹ refers to polished rice (Pfeiffer *et al.*, 2007) [6]. Four entries reached it in the present study, namely R13-Aerobic13, R8-Aerobic08 and R11-Aerobic11 in the panel and CMS-B × R13-Aerobic13 among hybrids, and six others came within 0.5 mg kg⁻¹. All three panel entries also exceeded, by a significant margin, the released biofortified variety ZINCO-RICE grown alongside them, which gives an internal benchmark independent of the grain fraction analysed. How close these values are to nutritional usefulness depends on the grain fraction analysed. Zn is more concentrated in the bran and embryo than in the endosperm, so milling can remove a substantial part of the Zn present in brown rice, although rice loses proportionally less Zn on polishing than it does Fe (Impa *et al.*, 2012) [14]. The values reported here should be confirmed on fully polished grain before the entries above 28 mg kg⁻¹ are regarded as meeting the target. The estimation method matters as well. ED-XRF offers rapid, inexpensive screening but requires careful calibration against reference methods to give values comparable with ICP-OES (Paltridge *et al.*, 2012) [47].

Agronomic management offers a further margin. The basal application of 10 kg zinc sulphate ha⁻¹ in the present trials would have kept

soil Zn supply from limiting uptake, a condition under which genotypic differences in grain Zn are most fully expressed (Wissuwa *et al.*, 2008) [38]. Soil or foliar Zn application during grain filling can raise grain Zn in cereals substantially and complements genetic improvement, particularly on Zn-deficient soils (Cakmak *et al.*, 2018) [48]. Combining a Zn-efficient restorer such as R13-Aerobic13 with timely foliar Zn in aerobic fields may allow hybrids to exceed the target reliably. Bioavailability must also be considered, since phytate in rice binds Zn and reduces absorption. Breeding targets were set assuming typical phytate levels, and genotypes that raise Zn without raising phytate will deliver more benefit (Bouis *et al.*, 2017) [7].

Limitations

Each experiment was conducted in a single kharif season at one location, so genotype × environment interaction could not be separated from genotypic variance, and the heritability and ranking reported here need confirmation across sites with contrasting soil Zn status. The panel and the hybrids were evaluated in successive kharif seasons, so differences between the two experiments, such as the higher mean grain Zn of the hybrids, are confounded with season, and the hybrids could not be compared statistically with the checks. Parental values for heterosis estimation were not taken from the same trial as the hybrids, and heterosis for panicle length was not computed because the per se panicle length values available for the CMS lines were not comparable with those of the other entries. Grain Fe and phytate were not measured, grain Zn was not compared across degrees of milling, and root traits were inferred rather than recorded. The combining ability estimates rest on three lines and four related testers and describe these parents rather than the wider germplasm.

Conclusion

The aerobic evaluation revealed substantial, highly heritable variation for grain Zn among restorers and elite lines, with a spread of more than 12 mg kg⁻¹ and three restorers exceeding the HarvestPlus target. Grain Zn and grain yield increased together, and the association arose through grain filling traits rather than through any direct effect, so selection for productivity under aerobic management need not sacrifice Zn density in this material. The panel fell into four clusters along a single dominant axis of vigour, and the restorers chosen as testers all came from the most productive cluster, which limited divergence and heterosis among the resulting hybrids. Within the hybrids, non-additive effects dominated, yet the restorer's own Zn level and GCA largely set the Zn level of its crosses. R13-Aerobic13 emerged as the best restorer for both yield and grain Zn. CMS-B × R13-Aerobic13 is the most Zn-dense hybrid, and CMS-A × R13-Aerobic13 offers the best combination of high yield and near-target Zn, with CMS-A × R8-Aerobic08 and CMS-C × R4-Aerobic04 as promising alternatives. These hybrids merit multi-location evaluation across soils of differing Zn status with analysis of genotype × environment interaction, measurement of polished-grain Zn, phytate and bioavailability, and a wider line × tester design that includes R11-Aerobic11, EL-03 and restorers from other clusters. Marker-assisted transfer of grain Zn loci into R13-Aerobic13 and its maintainer counterparts would accelerate the development of Zn-biofortified aerobic rice hybrids.

Source of variation	df	DFF	PH (cm)	PL (cm)	TNT	NPT	TGPP	SF (%)	GW (g)	GYPP (g)	Zn (mg kg ⁻¹)
(a) Diversity panel											
Replications	2	0.029	13.051	0.260	0.202	0.018	14.143	1.503	0.343	1.916	0.987
Genotypes	23	50.531**	214.809**	5.957**	6.062**	5.883**	2370.091**	72.852**	8.627**	77.659**	31.985**
Error	46	2.561	18.152	0.725	0.695	0.312	149.957	4.952	0.817	2.156	1.105
(b) Hybrids (line × tester)											
Replications	2	3.525	1.585	0.519	0.921	0.087	116.751	10.623	0.982	5.948	1.425
Crosses	11	14.631**	35.548	0.982	1.702**	1.162*	622.522**	18.871*	3.734*	26.995**	9.379**
Lines (CMS)	2	5.597	7.270	1.495	0.334	0.797	225.686	14.523	1.105	9.328	1.078
Testers (restorers)	3	39.940*	109.150**	1.470	3.596	3.137**	1135.637	20.896	10.087*	41.749	22.353
Line × tester	6	4.988	8.173	0.567	1.211*	0.296	498.242**	19.308*	1.434	25.507**	5.659**
Error	22	2.342	16.864	0.903	0.350	0.474	131.459	6.189	1.181	3.856	1.331
σ ² _{gca}		0.416	1.181	0.018	0.021	0.037	5.361	-0.019	0.099	0.064	0.160
σ ² _{sca}		0.882	-2.897	-0.112	0.287	-0.059	122.261	4.373	0.084	7.217	1.443
σ ² _{gca} /σ ² _{sca}		0.472	NE	NE	0.074	NE	0.044	-0.004	1.177	0.009	0.111
Contribution of lines (%)		6.96	3.72	27.68	3.57	12.47	6.59	13.99	5.38	6.28	2.09
Contribution of testers (%)		74.45	83.74	40.81	57.61	73.63	49.75	30.20	73.67	42.18	65.00
Contribution of line × tester (%)		18.59	12.54	31.51	38.81	13.90	43.66	55.81	20.95	51.54	32.91

Genotype / cross	DFF	PH (cm)	PL (cm)	TNT	NPT	TGPP	SF (%)	GW (g)	GYPP (g)	Zn (mg kg ⁻¹)
(a) Diversity panel										
R1-Aerobic01	91.500	90.167	24.000	10.367	8.467	194.367	83.700	20.320	21.070	23.637
R2-Aerobic02	86.567	100.933	24.800	10.933	9.233	195.867	86.333	21.220	26.430	24.300
R3-Aerobic03	94.133	85.300	23.900	8.767	6.933	171.833	81.200	20.383	20.360	20.977
R4-Aerobic04	86.000	94.700	25.567	12.467	9.867	219.267	87.733	23.237	31.657	27.067
R5-Aerobic05	96.267	101.633	23.867	9.067	7.767	194.067	78.667	21.060	22.293	19.387
R6-Aerobic06	88.633	94.567	26.900	12.033	10.033	237.733	90.200	21.550	26.617	25.023
R7-Aerobic07	98.833	84.767	22.233	8.567	6.200	156.100	77.367	18.903	18.503	19.280
R8-Aerobic08	93.200	107.500	26.900	10.333	9.300	211.233	87.567	23.430	27.487	28.657
R9-Aerobic09	88.000	92.233	25.300	12.433	10.933	225.300	90.233	22.360	31.597	25.920
R10-Aerobic10	95.967	96.667	24.033	9.200	7.400	162.267	82.667	20.190	20.777	23.693
R11-Aerobic11	89.100	114.500	25.367	11.767	9.000	230.367	87.667	21.510	30.113	28.170
R12-Aerobic12	94.867	89.967	22.567	7.800	5.933	149.133	76.833	17.313	16.217	18.063
R13-Aerobic13	87.133	100.233	26.267	12.533	10.400	243.133	91.633	24.387	32.997	30.370
R14-Aerobic14	99.567	89.800	22.667	9.133	7.233	178.967	78.433	19.657	19.780	21.030
R15-Aerobic15	89.067	105.867	25.533	11.067	10.100	215.433	87.967	22.090	29.020	26.810
R16-Aerobic16	96.800	97.233	24.533	10.367	8.267	195.767	85.567	21.407	24.373	24.430
EL-01	91.933	99.033	24.467	10.600	8.633	207.700	85.800	21.700	26.033	26.407
EL-02	93.267	88.300	23.433	8.800	7.367	184.567	82.367	19.853	22.180	23.747
EL-03	87.933	112.467	26.567	11.600	9.667	226.933	90.000	23.487	31.683	27.780
EL-04	97.933	96.433	23.433	8.833	6.600	163.667	78.567	19.563	18.727	21.430
EL-05	91.100	106.633	25.200	10.533	8.900	206.567	87.700	20.943	27.817	26.043
Check-1	90.433	97.600	24.067	9.667	7.933	175.000	82.267	20.563	23.640	23.513
Check-2	95.200	82.233	22.167	8.367	6.900	155.067	75.300	18.273	16.757	20.347
Check-3	88.500	99.267	25.633	10.600	9.167	222.967	89.900	22.030	28.433	25.787
General mean	92.164	97.001	24.558	10.243	8.426	196.804	84.403	21.060	24.773	24.244
SE(m)±	0.924	2.460	0.492	0.481	0.322	7.070	1.285	0.522	0.848	0.607
CD (<i>p</i> = 0.05)	2.630	7.002	1.399	1.370	0.918	20.126	3.657	1.486	2.413	1.728
CV (%)	1.736	4.392	3.466	8.137	6.627	6.222	2.636	4.292	5.928	4.335
(b) Hybrids										
CMS-A × R2-Aerobic02	89.333	99.300	27.400	11.700	9.733	202.367	89.700	21.280	24.413	25.487
CMS-A × R4-Aerobic04	89.367	96.100	28.300	12.733	9.900	208.733	87.800	23.237	30.147	26.243

(continued on next page)

Genotype / cross	DFF	PH (cm)	PL (cm)	TNT	NPT	TGPP	SF (%)	GW (g)	GYPP (g)	Zn (mg kg ⁻¹)
CMS-A × R8-Aerobic08	91.633	103.000	27.167	10.367	9.433	226.433	86.267	22.337	30.850	27.820
CMS-A × R13-Aerobic13	87.667	96.333	27.700	12.600	10.167	217.100	91.633	24.910	33.223	27.933
CMS-B × R2-Aerobic02	89.367	98.933	28.133	11.233	8.967	195.100	87.400	21.490	25.550	25.390
CMS-B × R4-Aerobic04	87.967	94.700	27.967	12.067	9.833	219.967	87.033	23.687	32.443	27.123
CMS-B × R8-Aerobic08	95.600	106.033	28.000	10.933	8.267	207.200	85.400	23.333	24.207	25.803
CMS-B × R13-Aerobic13	89.567	101.167	28.533	11.833	10.167	229.600	86.767	23.323	30.693	31.240
CMS-C × R2-Aerobic02	88.367	98.633	27.833	10.767	8.900	202.267	82.433	21.743	29.743	24.433
CMS-C × R4-Aerobic04	88.600	96.233	29.400	12.500	9.767	203.233	89.500	22.347	30.993	27.727
CMS-C × R8-Aerobic08	91.733	103.367	27.800	12.000	8.933	185.567	88.133	21.767	30.223	27.600
CMS-C × R13-Aerobic13	88.867	100.633	28.233	11.533	10.200	232.233	90.833	23.840	28.353	27.717
General mean	89.839	99.536	28.039	11.689	9.522	210.817	87.742	22.774	29.237	27.043
SE(m)±	0.883	2.371	0.549	0.341	0.397	6.620	1.436	0.627	1.134	0.666
CD (<i>p</i> = 0.05)	2.591	6.954	1.609	1.001	1.166	19.415	4.213	1.840	3.325	1.953
CV (%)	1.703	4.126	3.389	5.058	7.230	5.439	2.835	4.772	6.717	4.266

Trait	Range	Mean	GCV (%)	PCV (%)	h ² bs (%)	GA	GAM (%)
DFF	86.00 to 99.57	92.16	4.34	4.67	86.19	7.65	8.30
PH (cm)	82.23 to 114.50	97.00	8.35	9.43	78.31	14.76	15.22
PL (cm)	22.17 to 26.90	24.56	5.38	6.40	70.65	2.29	9.31
TNT	7.80 to 12.53	10.24	13.06	15.39	72.03	2.34	22.83
NPT	5.93 to 10.93	8.43	16.17	17.48	85.62	2.60	30.83
TGPP	149.13 to 243.13	196.80	13.82	15.16	83.15	51.10	25.97
SF (%)	75.30 to 91.63	84.40	5.64	6.22	82.05	8.88	10.52
GW (g)	17.31 to 24.39	21.06	7.66	8.78	76.11	2.90	13.77
GYPP (g)	16.22 to 33.00	24.77	20.25	21.10	92.11	9.92	40.04
Zn (mg kg ⁻¹)	18.06 to 30.37	24.24	13.23	13.93	90.31	6.28	25.91

Trait	DFF	PH	PL	TNT	NPT	TGPP	SF	GW	GYPP	Zn
DFF	1.000									
PH	-0.454*	1.000								
PL	-0.741**	0.699**	1.000							
TNT	-0.825**	0.536**	0.841**	1.000						
NPT	-0.834**	0.552**	0.878**	0.952**	1.000					
TGPP	-0.766**	0.622**	0.894**	0.926**	0.928**	1.000				
SF	-0.817**	0.615**	0.930**	0.915**	0.930**	0.925**	1.000			
GW	-0.682**	0.634**	0.894**	0.855**	0.877**	0.879**	0.875**	1.000		
GYPP	-0.825**	0.677**	0.877**	0.934**	0.935**	0.929**	0.936**	0.923**	1.000	
Zn	-0.729**	0.680**	0.865**	0.853**	0.853**	0.855**	0.909**	0.886**	0.906**	1.000

(continued on next page)

Trait	DFE	PH	PL	TNT	NPT	TGPP	SF	GW	Zn	r with GYPP
DFE	-0.175	-0.087	0.290	-0.130	-0.053	-0.090	-0.292	-0.302	0.015	-0.825**
PH	0.080	0.191	-0.273	0.085	0.035	0.073	0.220	0.281	-0.014	0.677**
PL	0.130	0.134	-0.391	0.133	0.056	0.105	0.332	0.396	-0.018	0.877**
TNT	0.145	0.102	-0.329	0.158	0.061	0.109	0.327	0.379	-0.017	0.934**
NPT	0.146	0.105	-0.343	0.150	0.064	0.109	0.332	0.389	-0.017	0.935**
TGPP	0.134	0.119	-0.350	0.146	0.059	0.118	0.330	0.390	-0.017	0.929**
SF	0.143	0.117	-0.364	0.144	0.060	0.109	0.357	0.388	-0.018	0.936**
GW	0.120	0.121	-0.350	0.135	0.056	0.103	0.312	0.443	-0.018	0.923**
Zn	0.128	0.130	-0.338	0.135	0.055	0.101	0.325	0.393	-0.020	0.906**

Cluster	No.	Genotypes	DFE	PH (cm)	PL (cm)	TNT	NPT	TGPP	SF (%)	GW (g)	GYPP (g)	Zn (mg kg ⁻¹)
I	12	EL-05, Check-3, R15-Aerobic15, R11-Aerobic11, EL-03, R8-Aerobic08, R6-Aerobic06, R4-Aerobic04, EL-01, R2-Aerobic02, R9-Aerobic09, R13-Aerobic13	88.93	102.33	25.71	11.41	9.60	220.21	88.56	22.33	29.16	26.86
II	8	R14-Aerobic14, EL-04, R7-Aerobic07, R3-Aerobic03, R10-Aerobic10, Check-2, EL-02, R12-Aerobic12	96.22	89.18	23.05	8.68	6.82	165.20	79.09	19.27	19.16	21.07
III	3	R1-Aerobic01, Check-1, R16-Aerobic16	92.91	95.00	24.20	10.13	8.22	188.38	83.84	20.76	23.03	23.86
IV	1	R5-Aerobic05	96.27	101.63	23.87	9.07	7.77	194.07	78.67	21.06	22.29	19.39

Cluster	I	II	III	IV
I	37.27 (6.11)	292.22 (17.09)	103.86 (10.19)	208.19 (14.43)
II		35.90 (5.99)	78.14 (8.84)	80.91 (9.00)
III			25.61 (5.06)	72.84 (8.53)
IV				0.00 (0.00)

Trait	Times ranked first	Contribution (%)
GYPP	169	61.23
DFE	26	9.42
Zn	26	9.42
PH	23	8.33
SF	13	4.71
NPT	8	2.90
GW	6	2.17
TGPP	4	1.45
PL	1	0.36
TNT	0	0.00

Table 8. General combining ability (GCA) effects of CMS lines and restorers for yield, yield attributes and grain Zn content

Parent	DFE	PH (cm)	PL (cm)	TNT	NPT	TGPP	SF (%)	GW (g)	GYPP (g)	Zn (mg kg ⁻¹)
Lines (CMS)										
CMS-A	-0.339	-0.853	-0.397	0.161	0.286	2.842	1.108	0.166	0.422	-0.172
CMS-B	0.786	0.672	0.119	-0.172	-0.214	2.150	-1.092	0.184	-1.013	0.346
CMS-C	-0.447	0.181	0.278	0.011	-0.072	-4.992	-0.017	-0.350	0.592	-0.174
SE (gi) lines ±	0.442	1.185	0.274	0.171	0.199	3.310	0.718	0.314	0.567	0.333
Testers (restorers)										

(continued on next page)

Parent	DFF	PH (cm)	PL (cm)	TNT	NPT	TGPP	SF (%)	GW (g)	GYPP (g)	Zn (mg kg ⁻¹)
R2-Aerobic02	-0.817	-0.581	-0.250	-0.456*	-0.322	-10.906**	-1.231	-1.270**	-2.668**	-1.940**
R4-Aerobic04	-1.194*	-3.858*	0.517	0.744**	0.311	-0.172	0.369	0.316	1.958**	-0.012
R8-Aerobic08	3.150**	4.597**	-0.383	-0.589**	-0.644*	-4.417	-1.142	-0.296	-0.810	0.031
R13-Aerobic13	-1.139*	-0.158	0.117	0.300	0.656**	15.494**	2.003*	1.250**	1.520*	1.920**
SE (g) testers ±	0.510	1.369	0.317	0.197	0.229	3.822	0.829	0.362	0.655	0.385

Note. Abbreviations as in Table-1. Negative GCA effects are desirable for DFF and PH. * and ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively (t test with 22 error df).

Table 9. Specific combining ability (SCA) effects and heterosis (%) over mid-parent (MPH) and better parent (BPH) for selected traits in CMS-based aerobic rice hybrids

Cross	SCA DFF	SCA SF	SCA GW	SCA TGPP	SCA GYPP	SCA Zn	MPH DFF	MPH GYPP	BPH GYPP	MPH Zn	BPH Zn	MPH GW	BPH GW	MPH SF	BPH SF
CMS-A × R2-Aerobic02	0.650	2.081	-0.391	-0.386	-2.577*	0.556	0.056	-2.210	-7.630	8.685	4.883	2.013	0.283	4.708	3.900
CMS-A × R4-Aerobic04	1.061	-1.419	-0.020	-4.753	-1.469	-0.616	0.412	9.313	-4.770	5.678	-3.042	6.257	0.000	1.660	0.076
CMS-A × R8-Aerobic08	-1.017	-1.442	-0.309	17.192*	2.002	0.918	-1.044	21.012	12.236	8.552	-2.920	1.692	-4.666	-0.019	-1.485
CMS-A × R13-Aerobic13	-0.694	0.781	0.719	-12.053	2.045	-0.858	-2.121	17.612	0.687	5.469	-8.023	10.991	2.146	3.755	0.000
CMS-B × R2-Aerobic02	-0.442	1.981	-0.198	-6.961	-0.006	-0.059	-1.015	5.513	-3.330	7.357	4.486	4.778	1.272	2.622	1.236
CMS-B × R4-Aerobic04	-1.464	0.014	0.413	7.172	2.262	-0.254	-2.259	20.929	2.485	8.349	0.209	10.077	1.937	1.359	-0.798
CMS-B × R8-Aerobic08	1.825	-0.108	0.671	-1.350	-3.207**	-1.617*	2.137	-2.169	-11.933	-0.097	-9.957	7.950	-0.413	-0.447	-2.474
CMS-B × R13-Aerobic13	0.081	-1.886	-0.885	1.139	0.950	1.931**	-1.104	11.619	-6.981	17.070	2.865	5.567	-4.360	-1.196	-5.311
CMS-C × R2-Aerobic02	-0.208	-4.061**	0.589	7.347	2.583*	-0.496	-1.578	17.959	12.536	4.864	0.549	4.737	2.466	-4.054	-4.517
CMS-C × R4-Aerobic04	0.403	1.406	-0.393	-2.419	-0.793	0.869	-1.006	11.373	-2.095	12.330	2.438	2.657	-3.830	3.329	2.014
CMS-C × R8-Aerobic08	-0.808	1.550	-0.362	-15.842*	1.205	0.699	-1.468	17.403	9.956	8.327	-3.687	-0.450	-7.099	1.849	0.647
CMS-C × R13-Aerobic13	0.614	1.106	0.166	10.914	-2.995*	-1.073	-1.332	-0.509	-14.072	5.247	-8.737	6.698	-2.242	2.559	-0.873
SE (sij) ±	0.883	1.436	0.627	6.620	1.134	0.666									

Note. Abbreviations as in Table-1. SCA significance by t test with 22 error df; * and ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. Better parent was taken as the parent with the higher mean. Heterosis for panicle length is not presented because per se panicle length values of the CMS lines were not comparable with those of the other entries. Heterosis for other traits is available on request.

Figures

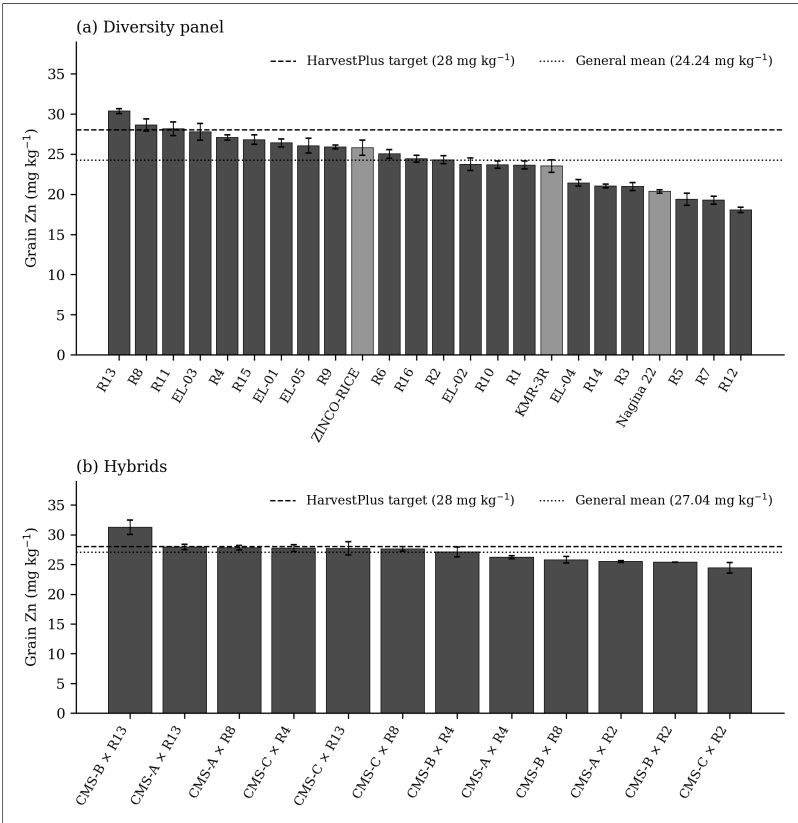


Figure 1. Grain Zn content (mg kg⁻¹) of (a) 24 restorers, elite lines and checks and (b) 12 CMS-based hybrids evaluated under aerobic conditions. Bars are means of three replications arranged in descending order, with checks shown in light grey; vertical bars indicate standard error of the mean ($n = 3$). The dashed horizontal line marks the HarvestPlus breeding target for rice (28 mg kg⁻¹) and the dotted line the general mean of each experiment (24.24 and 27.04 mg kg⁻¹, respectively).

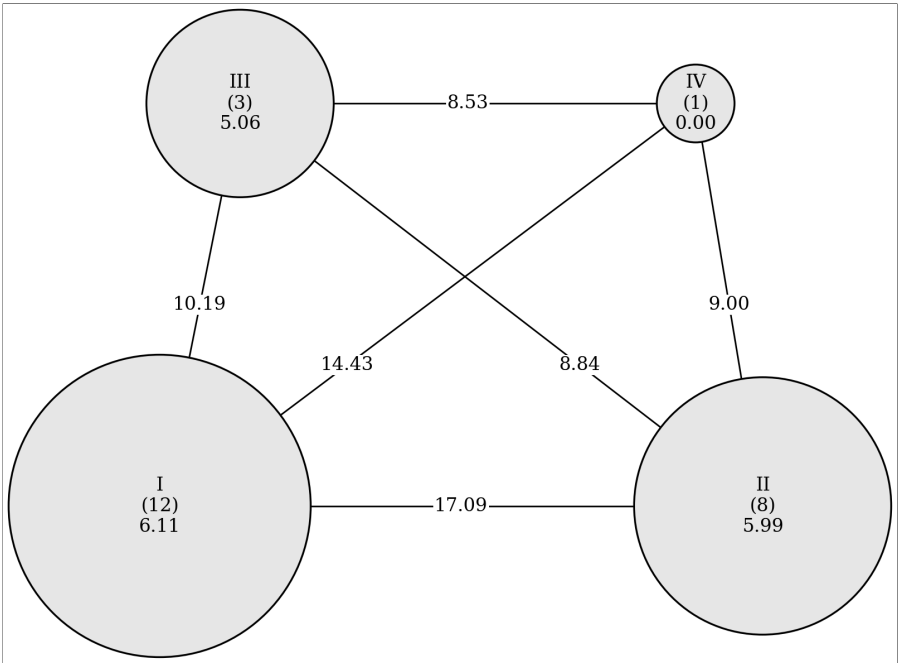


Figure 2. Diagrammatic representation of the four Tocher clusters of 24 genotypes based on Mahalanobis D² computed from ten traits. Circles represent clusters, with the number of genotypes in parentheses and the average intra-cluster distance ($D = \sqrt{D^2}$) written inside; lines connecting circles give average inter-cluster D values (I and II 17.09, I and III 10.19, I and IV 14.43, II and III 8.84, II and IV 9.00, III and IV 8.53). Circle size reflects cluster size and is not drawn to scale.

References

1. Muthayya S, Sugimoto JD, Montgomery S, Maberly GF. An overview of global rice production, supply, trade, and consumption. *Annals of the New York Academy of Sciences*. 2014;1324(1):7–14.

2. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS ONE*. 2012;7(11):e50568.

3. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham-McGregor S, Katz J, Martorell R, Uauy R, Maternal and Child Nutrition Study Group. Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet*. 2013;382(9890):427–451.
4. Myers SS, Wessells KR, Kloog I, Zanolletti A, Schwartz J. Effect of increased concentrations of atmospheric carbon dioxide on the global threat of zinc deficiency: A modelling study. *The Lancet Global Health*. 2015;3(10):e639–e645.
5. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*. 2004;55(396):353–364.
6. Pfeiffer WH, McClafferty B. HarvestPlus: Breeding crops for better nutrition. *Crop Science*. 2007;47(S3):S88–S105.
7. Bouis HE, Saltzman A. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security*. 2017;12:49–58.
8. Rao DS, Neeraja CN, Madhu Babu P, Nirmala B, Suman K, Rao LVS, Surekha K, Raghu P, Longvah T, Surendra P, Kumar R, Babu VR, Voleti SR. Zinc biofortified rice varieties: Challenges, possibilities, and progress in India. *Frontiers in Nutrition*. 2020;7:26.
9. Tuong TP, Bouman BAM. Rice production in water-scarce environments. In Kijne JW, Barker R, Molden D, editors. *Water productivity in agriculture: Limits and opportunities for improvement*. CABI Publishing; 2003:53–67.
10. Bouman BAM, Peng S, Castañeda AR, Visperas RM. Yield and water use of irrigated tropical aerobic rice systems. *Agricultural Water Management*. 2005;74(2):87–105.
11. Farooq M, Siddique KHM, Rehman H, Aziz T, Lee DJ, Wahid A. Rice direct seeding: Experiences, challenges and opportunities. *Soil and Tillage Research*. 2011;111(2):87–98.
12. Kumar V, Ladha JK. Direct seeding of rice: Recent developments and future research needs. *Advances in Agronomy*. 2011;111:297–413.
13. Kato Y, Katsura K. Rice adaptation to aerobic soils: Physiological considerations and implications for agronomy. *Plant Production Science*. 2014;17(1):1–12.
14. Impa SM, Johnson-Beebout SE. Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. *Plant and Soil*. 2012;361:3–41.
15. Virmani SS. Heterosis and hybrid rice breeding. Springer-Verlag. 1994.
16. Cheng SH, Zhuang JY, Fan YY, Du JH, Cao LY. Progress in research and development on hybrid rice: A super-domesticated in China. *Annals of Botany*. 2007;100(5):959–966.
17. Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RP. Biofortification strategies to increase grain zinc and iron concentrations in wheat. *Journal of Cereal Science*. 2014;59(3):365–372.
18. Mahalanobis PC. On the generalised distance in statistics. *Proceedings of the National Institute of Sciences of India*. 1936;2:49–55.
19. Arunachalam V. Genetic distance in plant breeding. *Indian Journal of Genetics and Plant Breeding*. 1981;41(2):226–236.
20. Swamy BPM, Rahman MA, Inabangan-Asilo MA, Amparado A, Manito C, Chadha-Mohanty P, Reinke R, Slamet-Loedin IH. Advances in breeding for high grain zinc in rice. *Rice*. 2016;9:49.
21. Kempthorne O. An introduction to genetic statistics. John Wiley and Sons; 1957.
22. Panse VG, Sukhatme PV. Statistical methods for agricultural workers (4th ed.). Indian Council of Agricultural Research. 1985.
23. Gomez KA, Gomez AA. Statistical procedures for agricultural research (2nd ed.). John Wiley and Sons; 1984.
24. Burton GW, DeVane EH. Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal*. 1953;45(10):478–481.
25. Sivasubramanian S, Madhavamenon P. Genotypic and phenotypic variability in rice. *Madras Agricultural Journal*. 1973;60:1093–1096.
26. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans. *Agronomy Journal*. 1955;47(7):314–318.
27. Robinson HF, Comstock RE, Harvey PH. Estimates of heritability and the degree of dominance in corn. *Agronomy Journal*. 1949;41(8):353–359.
28. Al-Jibouri HA, Miller PA, Robinson HF. Genotypic and environmental variances and covariances in an upland cotton cross of interspecific origin. *Agronomy Journal*. 1958;50(10):633–636.
29. Dewey DR, Lu KH. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. *Agronomy Journal*. 1959;51(9):515–518.
30. Rao CR. Advanced statistical methods in biometric research. John Wiley and Sons. 1952.
31. Singh RK, Chaudhary BD. Biometrical methods in quantitative genetic analysis. Kalyani Publishers. 1977.
32. Singh D. The relative importance of characters affecting genetic divergence. *Indian Journal of Genetics and Plant Breeding*. 1981;41(2):237–245.
33. Baker RJ. Issues in diallel analysis. *Crop Science*. 1978;18(4):533–536.
34. Anuradha K, Agarwal S, Rao YV, Rao KV, Viraktamath BC, Sarla N. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. *Gene*. 2012;508(2):233–240.
35. Garcia-Oliveira AL, Tan L, Fu Y, Sun C. Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology*. 2009;51(1):84–92.
36. Falconer DS, Mackay TFC. Introduction to quantitative genetics (4th ed.). Longman; 1996.
37. Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, Price AH. Genetic mapping of the rice ionome in leaves and grain: Identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant and Soil*. 2010;329:139–153.
38. Wissuwa M, Ismail AM, Graham RD. Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. *Plant and Soil*. 2008;306:37–48.
39. Fan MS, Zhao FJ, Fairweather-Tait SJ, Poulton PR, Dunham SJ, McGrath SP. Evidence of decreasing mineral density in wheat grain over the last 160 years. *Journal of Trace Elements in Medicine and Biology*. 2008;22(4):315–324.
40. Sperotto RA. Zn/Fe remobilization from vegetative tissues to rice seeds: Should I stay or should I go? Ask Zn/Fe supply! *Frontiers in Plant Science*. 2013;4:464.
41. Stomph TJ, Jiang W, Struik PC. Zinc biofortification of cereals: Rice differs from wheat and barley. *Trends in Plant Science*. 2009;14(3):123–124.
42. Gao X, Zou C, Fan X, Zhang F, Hoffland E. From flooded to aerobic conditions in rice cultivation: Consequences for zinc uptake. *Plant and Soil*. 2006;280:41–47.
43. Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. OsZIP4, a novel zinc-regulated zinc transporter in rice. *Journal of Experimental Botany*. 2005;56(422):3207–3214.
44. Sasaki A, Yamaji N, Mitani-Ueno N, Kashino M, Ma JF. A node-localized transporter OsZIP3 is responsible for the preferential distribution of Zn to developing tissues in rice. *The Plant Journal*. 2015;84(2):374–384.

45. Palmgren MG, Clemens S, Williams LE, Krämer U, Borg S, Schjørring JK, Sanders D. Zinc biofortification of cereals: Problems and solutions. *Trends in Plant Science*. 2008;13(9):464–473.
46. Johnson AAT, Kyriacou B, Callahan DL, Carruthers L, Stangoulis J, Lombi E, Tester M. Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS ONE*. 2011;6(9):e24476.
47. Paltridge NG, Palmer LJ, Milham PJ, Guild GE, Stangoulis JCR. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant and Soil*. 2012;361:251–260.
48. Cakmak I, Kutman UB. Agronomic biofortification of cereals with zinc: A review. *European Journal of Soil Science*. 2018;69(1):172–180.
49. Table-1. Analysis of variance (mean squares) for grain yield, yield attributes and grain Zn content in the diversity panel (24 genotypes) and the line $\times$ tester hybrids (3 CMS lines $\times$ 4 restorers) evaluated under aerobic conditions.
50. Note. df, degrees of freedom; DFF, days to 50% flowering; PH, plant height; PL, panicle length; TNT, total tillers per plant; NPT, productive tillers per plant; TGPP, total grains per panicle; SF, spikelet fertility; GW, 1000 grain weight; GYPP, grain yield per plant; Zn, grain zinc concentration. In (b), lines and testers were tested against the line $\times$ tester mean square and the remaining sources against the error mean square. Negative variance estimates are reported as computed and were taken as zero for interpretation; NE, ratio not estimable because σ^2_{sca} was negative. * and ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.
51. Table-2. Mean performance of restorers, elite lines, checks and CMS-based hybrids for yield, yield attributes and grain Zn content under aerobic conditions.
52. Note. Abbreviations as in Table-1. Values are means of three replications. SE(m), standard error of the mean; CD, critical difference; CV, coefficient of variation. CD for the diversity panel is based on 46 error df and for hybrids on 22 error df. Check-1, KMR-3R; Check-2, Nagina 22; Check-3, ZINCO-RICE. The diversity panel and hybrids were evaluated in successive kharif seasons.
53. Table-3. Estimates of genetic variability parameters for yield, yield attributes and grain Zn content in 24 genotypes evaluated under aerobic conditions.
54. Note. Abbreviations as in Table-1. GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; h^2_{bs} , broad sense heritability; GA, genetic advance at 5% selection intensity ($k = 2.06$) in trait units; GAM, genetic advance as per cent of mean. GCV and PCV classes, low $< 10\%$, moderate 10 to 20%, high $> 20\%$; h^2_{bs} high $> 60\%$; GAM high $> 20\%$.
55. Table-4. Phenotypic correlation coefficients among yield, yield attributes and grain Zn content computed on genotype means of 24 genotypes.
56. Note. Abbreviations as in Table-1. Critical values of r at 22 df are 0.404 ($p = 0.05$) and 0.515 ($p = 0.01$). * and ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.
57. Table-5. Direct (bold diagonal) and indirect effects of yield attributes and grain Zn content on grain yield per plant.
58. Note. Abbreviations as in Table-1. Residual effect = 0.167; coefficient of determination (R^2) = 0.972. Indirect effects are read along rows. ** significant at $p \leq 0.01$.
59. Table-6. Composition of Tocher clusters based on Mahalanobis D2 and cluster means for yield, yield attributes and grain Zn content.
60. Note. Abbreviations as in Table-1. Clustering threshold (largest of the minimum D2 values of individual genotypes) = 39.87.
61. Table-7. Average intra-cluster (diagonal) and inter-cluster (above diagonal) D2 values, with D values in parentheses, and relative contribution of traits to genetic divergence Contribution of traits to divergence.
62. Note. Abbreviations as in Table-1. Contribution was estimated from the number of times each trait ranked first among 276 pairwise comparisons following Singh (1981). Cluster IV is solitary, so its intra-cluster distance is zero.

How to cite this article: Shukla AK, Rajpoot S, Shrivastav G. Genetic diversity and evaluation of aerobic rice hybrids for grain zinc content in rice (*Oryza Sativa* L.). *International Journal of Advanced Biochemistry Research*. 2026;SP-10(10):211-223. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i10Sc.10280>