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Agronomic zinc management enhances zinc partitioning, grain retention, and protein quality in biofortified rice varieties grown on zinc-deficient soil

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Abstract

Zinc (Zn) deficiency in rice-growing soils compromises grain nutritional quality and is a major contributor to micronutrient malnutrition in rice-dependent populations. To address this problem, a pot experiment was carried out during the 2022-2023 boro season (November 2022 to May 2023) at Gazipur Agricultural University, Bangladesh, to assess how agronomic Zn management affects Zn accumulation, grain Zn enrichment, and protein content in biofortified rice grown on Zn-deficient soil. Five biofortified rice varieties and four agronomic Zn management practices were arranged factorially in a randomized complete block design with three replications. Rice variety, agronomic Zn management, and their interaction significantly affected Zn concentration in the stem, leaf, and whole grain, while differences in brown rice Zn concentration and protein content were not statistically significant. Zn accumulation in vegetative tissue rose most under basal Zn application (F1), whereas whole-grain Zn enrichment and overall grain nutritional quality peaked under the integrated nutrient management treatment (F3) combining farmyard manure (FYM), split nitrogen application, and foliar Zn spray. Among the varieties tested, BRRI dhan74 consistently showed the greatest Zn uptake, translocation, and post-milling retention. Milling lowered brown rice Zn concentration, reflecting the loss of Zn-rich bran layers; even so, Zn-enriched treatments retained numerically higher Zn levels than the control. Zn fertilization was also associated with numerically higher protein content in brown rice, though this difference was not statistically significant. Taken together, these results indicate that pairing Zn-efficient varieties with suitable agronomic practices can substantially enhance both grain Zn concentration and protein enrichment on Zn-deficient soils, an integrated biofortification strategy with the potential to help reduce Zn malnutrition in rice-based food systems.

Keywords: Agronomic biofortification, grain zinc, micronutrient enrichment, rice, zinc deficit soils

Introduction

More than half of the world's population depends on rice (*Oryza sativa* L.) as a staple food and a major source of dietary energy and protein (Sarkar *et al.* 2016, Mohidem *et al*. 2022), with the crop supplying roughly 27% of dietary energy and 20% of dietary protein across developing countries. In Bangladesh, rice dominates the cereal sector and accounts for nearly 70-80% of the population's daily caloric intake (FAO 2022). Yet despite this central role in food security, rice grain is a poor source of essential micronutrients, particularly zinc (Zn), a shortfall that contributes substantially to hidden hunger across developing countries.

Among micronutrient disorders affecting human health, Zn deficiency ranks as one of the most widespread (Prasad and Singh 2011, Krebs *et al.* 2014). Its consequences include impaired immune function, stunted growth, cognitive impairment, reduced reproductive performance, and heightened disease susceptibility, with children and pregnant women particularly at risk. Because polished rice forms the principal dietary staple across Bangladesh and much of South Asia, the naturally low Zn concentration of rice grain is regarded as a major nutritional concern in the region.

Beyond its role in human nutrition, Zn is itself an essential micronutrient for plant growth and development (Broadley *et al.* 2007), being required for enzyme activation, chlorophyll synthesis, carbohydrate and protein metabolism, membrane integrity, auxin production, and reproductive development. In rice cultivation, Zn availability is frequently constrained in submerged soils, calcareous soils, and high-pH soils, where adsorption, precipitation, and reduced mobility limit the Zn pool

accessible to roots. The resulting plant Zn deficiency manifests as poor tillering, chlorosis, delayed maturity, greater spikelet sterility, and ultimately reduced grain yield.

Against this backdrop, agronomic biofortification through soil and foliar Zn fertilization has emerged as a practical, cost-effective strategy for raising grain Zn concentration in cereals (Cakmak 2008, Velu *et al.* 2014). Zn fertilization has been shown to significantly enhance grain Zn accumulation, Zn bioavailability, and overall grain nutritional quality (Farooq *et al.* 2018, Wang *et al.* 2024), although its effectiveness depends heavily on varietal characteristics, soil properties, the timing and method of Zn application, and interactions with other nutrients.

Although several Zn-enriched rice varieties have recently been released in Bangladesh, how they respond to agronomic Zn management on Zn-deficient soils remains insufficiently explored. A further complication is that milling substantially lowers grain Zn concentration, since Zn is concentrated mainly in the outer bran and aleurone layers of the grain (Akhter *et al.* 2023). Understanding how agronomic management shapes Zn partitioning, post-milling retention, and protein enrichment is therefore essential for designing sustainable biofortification strategies.

We hypothesized that integrated agronomic Zn management would enhance Zn accumulation, grain Zn concentration, and protein retention in biofortified rice grown on Zn-deficient soils. Accordingly, this study evaluated the interactive effects of biofortified rice varieties and agronomic Zn management practices on (i) Zn concentration in stem and leaf tissues across growth stages, (ii) grain Zn enrichment and Zn retention after milling, and (iii) protein concentration in brown rice under Zn-deficient soil conditions.

Materials and methods

Experimental site and duration: The experiment was carried out during the boro season, from November 2022 to May 2023, at the Department of Agronomy, Gazipur Agricultural University, Gazipur, Bangladesh (24.09° N, 90.26° E; elevation 8.4 m above mean sea level). The site lies within a subtropical climatic zone, and temperature and humidity remained moderate throughout the experimental period (Figure 1) (BRRI, 2024).

Experimental soil: Soil used in the pot experiment was collected from Agro-Ecological Zone (AEZ)-11, the High Ganges River Floodplain, an area characterized by Zn-deficient soils, and was transported to Gazipur Agricultural University for the pot culture study. It was sandy loam in texture (56% sand, 37.28% silt, 6.72% clay). Organic matter content was 1.33%, and available Zn concentration was only 0.39 ppm (SRDI, 2023), below the 0.80 ppm critical level for rice cultivation in Bangladesh (Mayer *et al.* 2010). Soil pH (1:2.5 soil-water

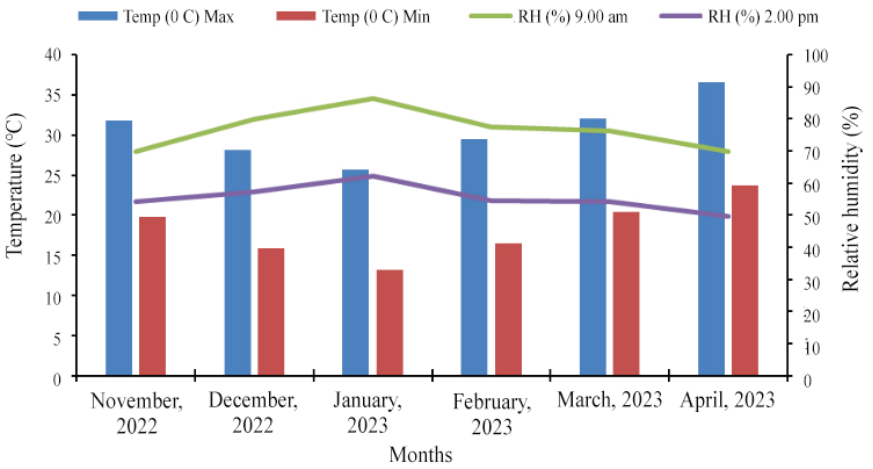


Figure 1. Monthly weather data (temperature, rainfall, humidity) during the experimental period, Gazipur, Bangladesh (BRRI, 2024).

suspension) was 7.5, and cation exchange capacity (CEC) was 16 cmol(+) kg^{-1} (SRDI, 2023); both are important determinants of Zn availability in flooded rice soils, since Zn solubility declines sharply as pH rises above neutral and clay/organic colloids with higher CEC can retain a larger exchangeable Zn pool. This inherently low Zn status made the site well suited for evaluating agronomic Zn biofortification strategies.

Experimental treatments and design: The trial was established in 60 plastic pots (24 cm diameter × 30 cm height), each filled with 12 kg of soil, and comprised two factors arranged factorially in a Randomized Complete Block Design (RCBD) with three replications.

Factor A: Rice varieties. V1 = BRRI dhan74; V2 = BRRI dhan84; V3 = BRRI dhan100; V4 = BRRI dhan102; V5 = BU dhan2.

Factor B: Agronomic Zn management practices.

F1 = Basal Zn application at 4 kg Zn ha^{-1} as ZnSO4·H2O + recommended NPKS.

F2 = Basal Zn at 2 kg Zn ha^{-1} + 1% foliar Zn spray at milking stage + recommended NPKS.

F3 = FYM at 5 t ha^{-1} + 50% PKS basal + split nitrogen application + 1% foliar Zn spray at milking stage.

F4 = Control (no Zn fertilizer) with recommended NPKS.

Seeds were sown in nursery trays on 29 November 2022, and thirty-day-old seedlings were transplanted into the pots on 29 December 2022 at two seedlings per pot. Irrigation, weeding, thinning, and plant protection measures were applied uniformly across all treatments, and plants were hand-harvested at maturity on 15 May 2023.

Pots were spaced approximately 25 cm apart within each replication block, and pot positions were re-randomized every two weeks throughout the growth period to distribute incident light and micro-environmental variation evenly and thereby minimize border/edge effects. Because two seedlings per pot were used specifically to limit intra-pot competition for the applied Zn treatments and enable a clear assessment of per-plant Zn uptake and translocation, edge effects were considered minimal in this pot-culture design; nonetheless, we acknowledge that the small plant population per pot is a limitation relative to field conditions and recommend field-scale validation of these findings.

Plant sampling and zinc analysis: Stem and leaf Zn concentration. Leaf and stem samples were collected at the tillering, heading, and maturity stages, then oven-dried, ground into fine powder, and analyzed for Zn concentration by Atomic Absorption Spectrophotometry (AAS). For digestion, a 0.5 g plant sample was treated with 5-6 mL of nitric-perchloric acid mixture (5:1) and heated at 120-200°C for 1.5-2 h until digestion was complete. The digest was then cooled, filtered, and diluted to 50 mL with distilled water before Zn concentration was determined on an Atomic Absorption Spectrophotometer (Model UNICAM 969, England).

Grain and brown rice sample preparation: Whole grain samples were sun-dried to approximately 12% moisture content and ground into fine powder for Zn analysis. Brown rice was prepared by manually dehusking the grain and grinding the samples into powder, and Zn and protein retention were assessed both before and after 10% degree of milling (DoM). Milling was performed using a McGill No. 2 laboratory rice miller for a duration of 10 minutes to achieve the targeted 10% DoM, with DoM calculated as the percentage weight loss of brown rice after milling.

Determination of grain Zn concentration: Zinc concentration in whole grain and brown rice was determined using Atomic Absorption Spectrophotometry following acid digestion procedures similar to plant tissue analysis (Yoshida and Coronel 1976).

Determination of protein content: Protein concentration in brown rice was determined by the Kjeldahl method: a 0.25 g sample was digested with concentrated sulfuric acid in the presence of a catalyst mixture (K2SO4, selenium powder, and CuSO4), and the resulting digest was distilled and titrated against 0.1 N HCl. Nitrogen concentration was calculated from the titration and multiplied by the conversion factor 5.95 to obtain the protein percentage.

Statistical analysis: Collected data were analyzed using CROPSTAT 7.2 statistical software. Two-way analysis of variance (ANOVA) was performed to determine the effects of rice variety, agronomic Zn management, and their interactions. Treatment means were separated using the Least Significant Difference (LSD) test at the 5% probability level following Gomez and Gomez (1984).

Results

Stem Zn concentration at both the tillering and heading stages was significantly influenced by agronomic Zn management (Figure 2). At tillering, values ranged from 21.33 to 53.00 ppm. Basal Zn application (F1) produced significantly greater accumulation than the other practices; the unfertilized control (F4) recorded the lowest concentration.

By the heading stage, stem Zn concentration had risen to between 21.33 and 64.76 ppm. BRRI dhan74 under basal Zn fertilization again produced the highest accumulation, while delayed Zn application and the control lagged behind. This overall rise in stem Zn from tillering to heading points to active Zn uptake and translocation as the crop entered reproductive development.

At maturity, stem Zn concentration again differed significantly among treatments (Figure 3), ranging from about 30 to 213 ppm. The integrated nutrient management treatment (F3: FYM, split nitrogen application, and foliar Zn spray) produced the greatest stem Zn accumulation, and BRRI dhan74 under this treatment continued to maintain the highest Zn concentration, consistent with superior Zn uptake and translocation efficiency in this variety.

Leaf Zn concentration was shaped significantly by the interaction between variety and agronomic management (Figure 4). At tillering, values ranged from 21.76 to 38.00 ppm, with BRRI dhan100 under integrated nutrient management (F3) reaching the highest concentration and BRRI dhan102 under the control recording the lowest.

At heading, leaf Zn concentration ranged from 21.33 to 36.00 ppm, peaking in BU dhan2 under basal Zn fertilization. Zn-treated plants consistently maintained significantly higher leaf Zn concentration than untreated plants, reflecting the improved Zn uptake achieved through agronomic management.

At maturity, leaf Zn concentration ranged from about 26 to 224 ppm (Figure 5), with the integrated nutrient management treatment (F3) again outperforming the control. BRRI dhan74 under F3 recorded the highest leaf Zn concentration at this stage, while BRRI dhan102 under the control (F4) showed the lowest accumulation.

Both rice variety and agronomic Zn management significantly shaped whole grain Zn concentration (Figure 6), which ranged from about 28 to 48 ppm overall. The integrated nutrient management treatment (F3) produced significantly higher grain Zn concentration than the control, and BRRI dhan74 and BRRI dhan84 showed the greatest grain Zn enrichment, both under this treatment.

Brown rice Zn concentration showed numerical differences among treatments both before and after milling, though

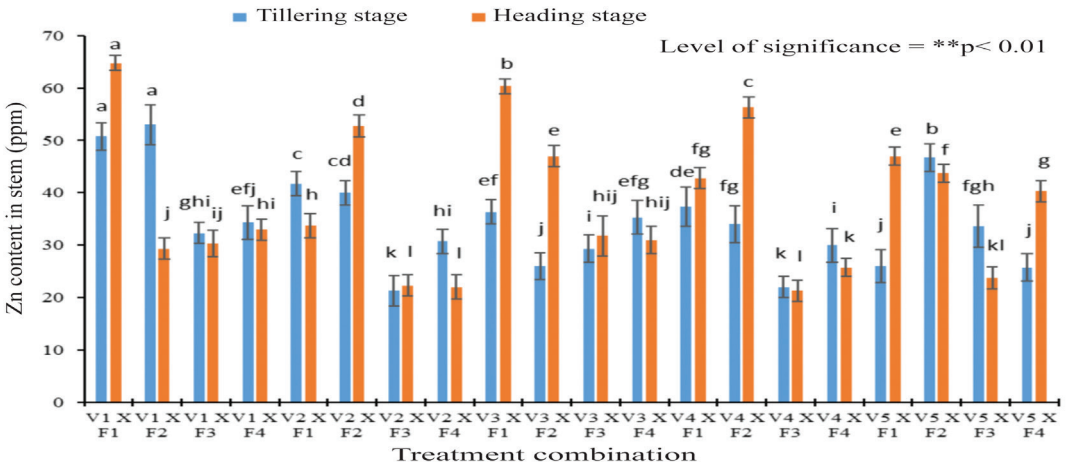


Figure 2. Stem Zn concentration of biofortified rice varieties under different agronomic Zn management practices at tillering and heading stages. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments. ** indicates significance at P ≤ 0.01.

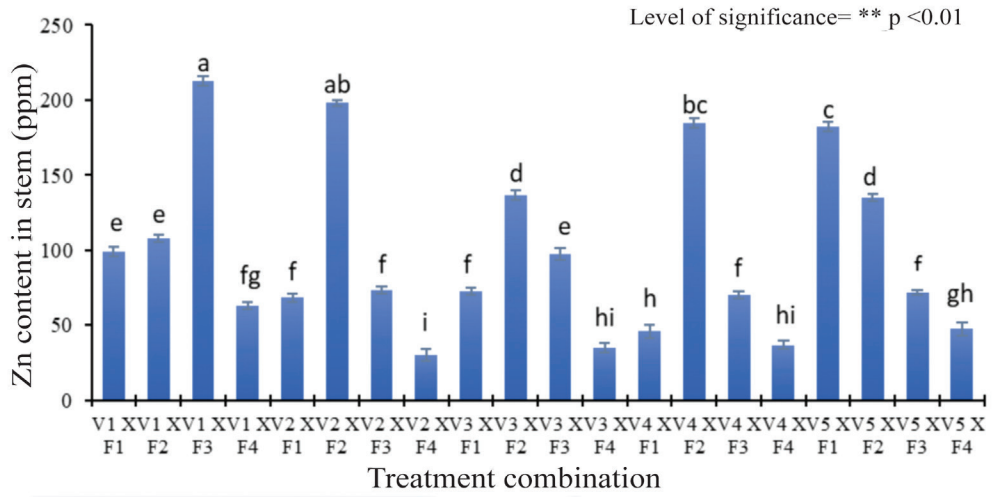


Figure 3. Stem Zn concentration of biofortified rice varieties at maturity under different agronomic Zn management practices in Zn-deficient soil. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments. ** indicates significance at P ≤ 0.01.

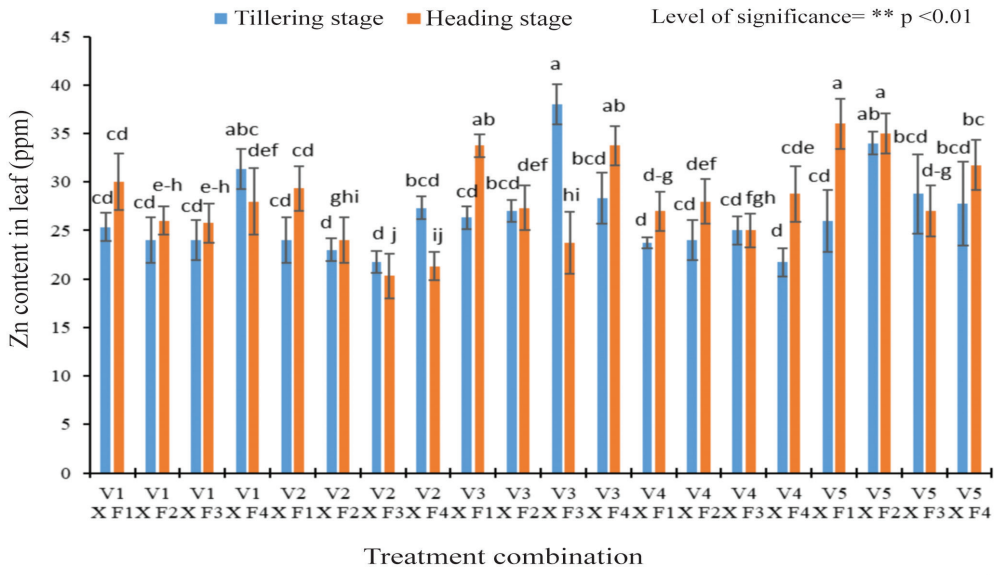


Figure 4. Leaf Zn concentration of biofortified rice varieties at tillering and heading stages under different agronomic Zn management practices in Zn-deficient soil. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments. ** indicates significance at P ≤ 0.01.

these differences were not statistically significant (Figure 7). Before milling it ranged from about 18 to 37 ppm, with BRRI dhan74 under integrated nutrient management (F3) reaching the highest numerical value; after milling, concentrations declined across all treatments, to between about 16 and 29 ppm. Zn-fertilized treatments still tended to retain more Zn numerically than the control, consistent with milling removing a considerable share of grain Zn through the loss of the Zn-rich bran and aleurone layers, although this trend did not reach statistical significance.

Protein concentration in brown rice showed numerical variation among treatments, although this variation was not statistically significant (Figure 8). Before milling, values ranged from about 9.6% to 12.8%, numerically

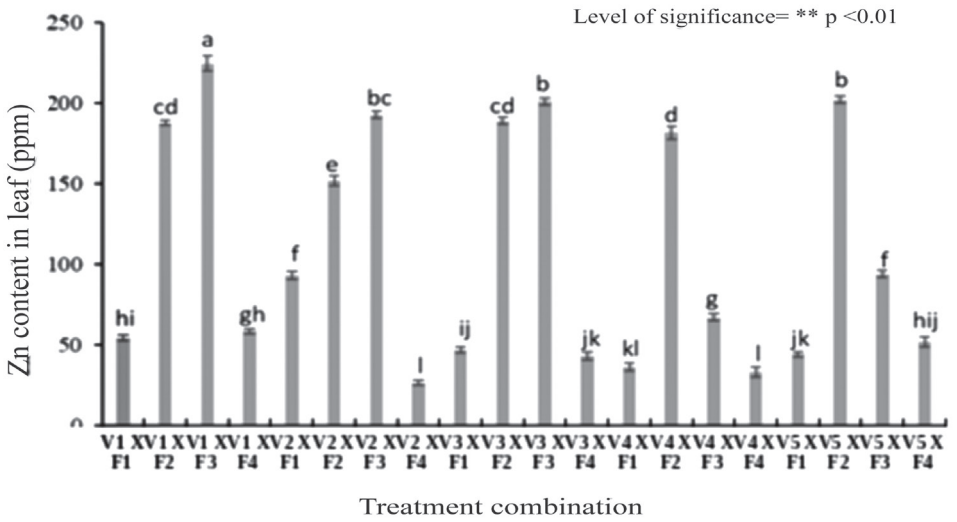


Figure 5. Leaf Zn concentration of biofortified rice varieties at maturity under different agronomic Zn management practices in Zn-deficient soil. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments. ** indicates significance at P ≤ 0.01.

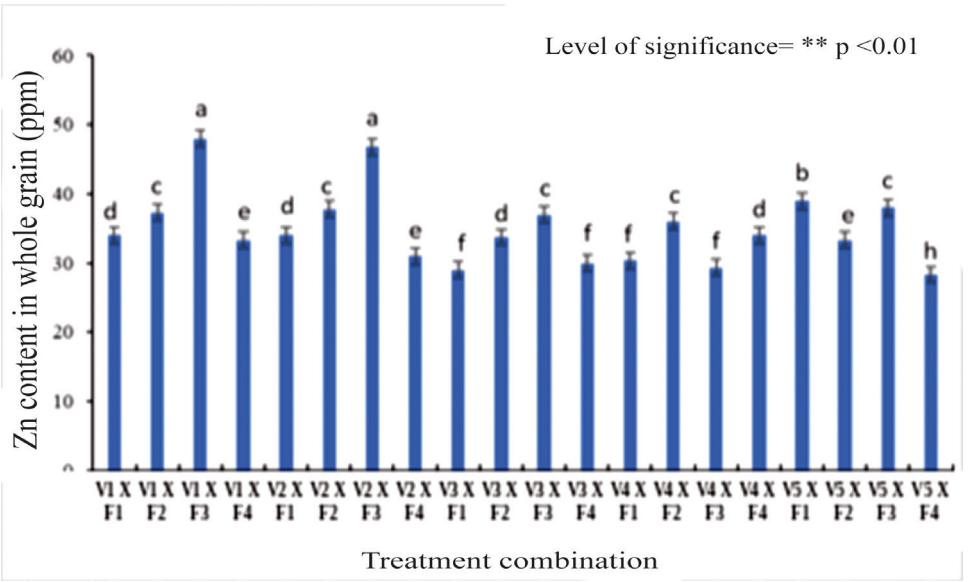


Figure 6. Whole grain Zn concentration of biofortified rice varieties under different agronomic Zn management practices in Zn-deficient soil. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments. ** indicates significance at P ≤ 0.01.

highest in BRRI dhan74 under integrated nutrient management; after milling, protein concentration declined, to between about 7.5% and 10.2%, with Zn-treated rice tending to retain somewhat higher protein levels than the untreated control, though this difference was not statistically significant. These results suggest that Zn fertilization may modestly influence protein retention, but this effect needs confirmation with a larger sample size or replicated field trials.

Discussion

The marked variation in Zn concentration across treatments underscores the important role that agronomic management and varietal characteristics play in regulating

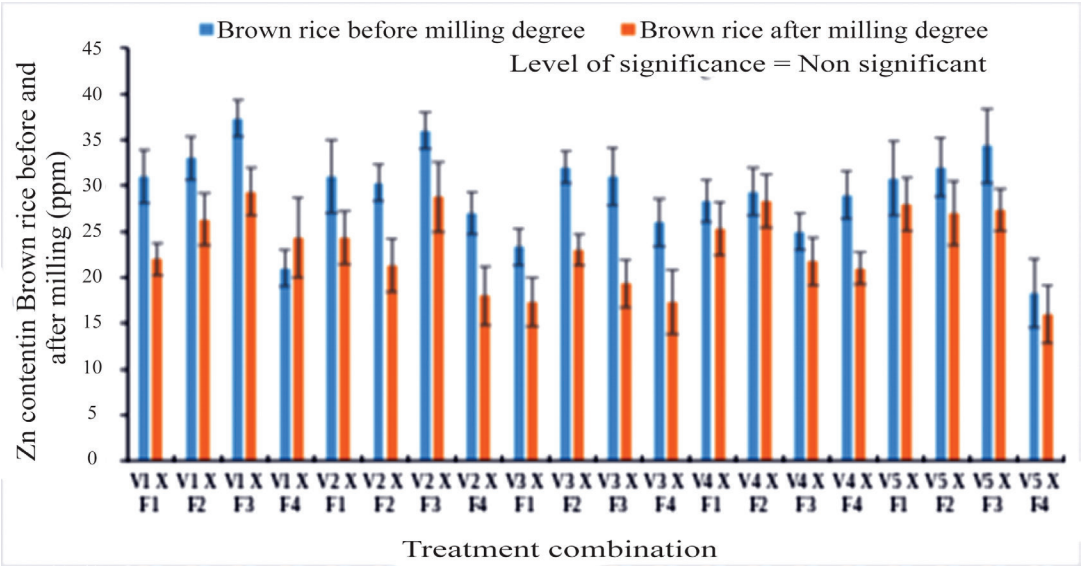


Figure 7. Brown rice Zn concentration of biofortified rice varieties before and after milling under different agronomic Zn management practices in Zn-deficient soil. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments.

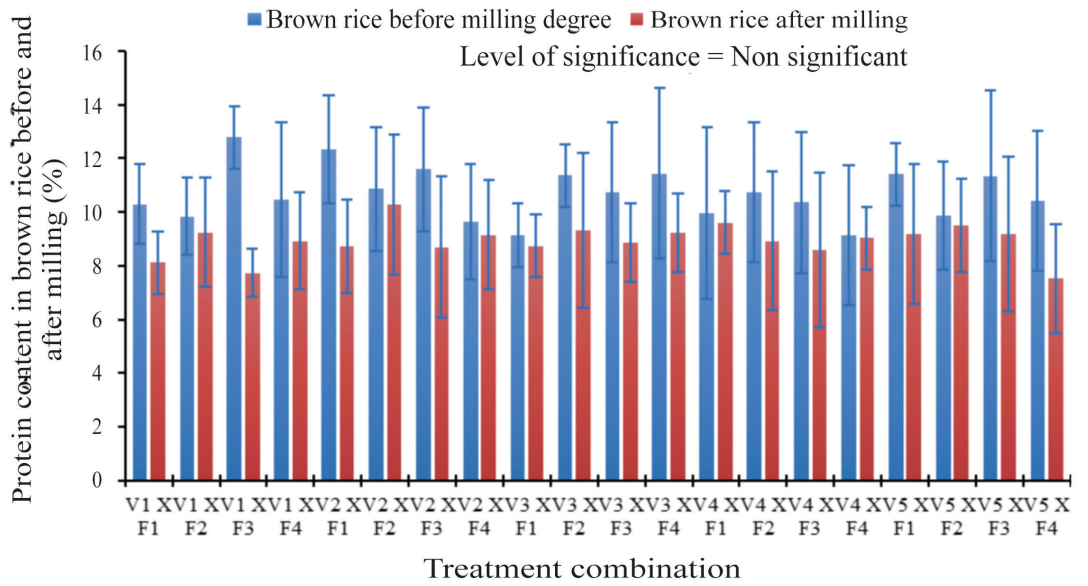


Figure 8. Protein concentration in brown rice of biofortified rice varieties before and after milling under different agronomic Zn management practices in Zn-deficient soil. V1-V5 represent rice varieties and F1-F4 represent Zn management treatments.

Zn uptake, translocation, and accumulation in rice. Basal Zn fertilization consistently raised stem and leaf Zn concentration across all growth stages. This suggests that early Zn availability promotes root uptake and drives Zn accumulation in vegetative tissue, a pattern consistent with Ghasal *et al.* (2016) and Mutambu *et al.* (2023).

That stem Zn concentration rose steadily from tillering to heading points to active Zn transport during reproductive development, in line with Impa and Johnson-Beebout (2012) and Lee *et al.* (2018). As Khalid *et al.* (2022) and Rehman *et al.* (2018) note, Zn is critical for enzyme activation, chlorophyll biosynthesis, carbohydrate

metabolism, and reproductive growth, so an adequate early-season Zn supply likely enhances metabolic activity and facilitates the subsequent remobilization of Zn toward developing grains (Boonchuay *et al.* 2013). Leaf Zn at maturity likewise contributes substantially to grain Zn loading, consistent with Shehu and Jamala (2010) and Gao *et al.* (2011).

Relative to the other varieties, BRRI dhan74 consistently accumulated and retained more Zn, echoing the genotypic variation in Zn uptake and biofortification efficiency reported by Wissuwa *et al.* (2008) and Johnson-Beebout *et al.* (2016). This variety-level difference likely reflects underlying genotypic differences in root Zn uptake efficiency, internal Zn translocation, and grain loading capacity.

Both genotype and Zn management practice significantly shaped leaf Zn concentration: fertilized plants showed higher leaf Zn, reflecting greater nutrient availability and more efficient uptake, whereas plants receiving no Zn consistently showed lower leaf Zn, consistent with restricted uptake and limited translocation.

That grain Zn concentration rose under Zn fertilization confirms the effectiveness of agronomic biofortification for improving rice nutritional quality, corroborating comparable gains reported by Yin *et al.* (2016), Tuiwong *et al.* (2022), and Kamran *et al.* (2023). While basal Zn application was most effective at the earlier vegetative stages, the integrated nutrient management treatment (F3) produced the greatest whole grain Zn enrichment, underlining the value of combining organic amendments, split nitrogen, and foliar Zn spray for maximizing grain enrichment; the higher grain Zn concentration observed under Zn-treated conditions likely stems from greater Zn accumulation in vegetative tissue followed by its remobilization during grain filling.

Because Zn is concentrated mainly in the outer bran and aleurone layers, milling substantially reduced brown rice Zn concentration, mirroring reductions reported by Hunt *et al.* (2002), Paul *et al.* (2019), and Roy *et al.* (2011). Even so, Zn-fertilized treatments retained numerically more Zn than untreated controls after milling, although this difference was not statistically significant, suggesting a trend in which agronomic biofortification may still help improve Zn intake even after grain processing.

Zn fertilization was also numerically associated with higher brown rice protein concentration, though this difference was not statistically significant, echoing a similar direction of gains in grain nutritional quality reported by Yang *et al.* (2021) and Wang *et al.* (2024). Given Zn's role in nitrogen metabolism, protein synthesis, and enzyme activation, the numerically higher protein content observed under Zn fertilization may reflect improved nitrogen assimilation and metabolic activity, although this trend would need confirmation with larger sample sizes or field-scale replication. This tentative link between Zn enrichment and protein accumulation suggests that agronomic Zn management may help improve both micronutrient content and overall nutritional quality simultaneously.

The integrated nutrient management treatment, combining FYM, split nitrogen application, and foliar Zn spray, likewise improved Zn accumulation and grain nutritional quality. As Khalid *et al.* (2022) and Salako *et al.* (2025) suggest, organic amendments can enhance Zn availability by improving soil physical and chemical properties, reducing Zn fixation, and stimulating microbial activity, while split nitrogen application may further promote Zn uptake and translocation by supporting root growth and physiological activity.

Taken together, these findings show that agronomic Zn biofortification, whether through integrated nutrient management or direct Zn fertilization, can meaningfully improve both Zn concentration and nutritional quality in biofortified rice grown on Zn-deficient soils.

Conclusion

This study shows that agronomic Zn management significantly enhances Zn accumulation and grain Zn concentration, and is numerically associated with higher protein content in biofortified rice grown under Zn-deficient soil conditions. Among the varieties tested, BRRI dhan74 stood out for its superior Zn uptake, translocation, and post-milling retention, while basal Zn fertilization and integrated nutrient management (FYM, split nitrogen application, and foliar Zn spray) substantially improved Zn enrichment and grain nutritional quality. Although milling reduced both Zn and protein concentration in brown rice, Zn-fertilized treatments retained comparatively

higher nutritional quality than the control. Together, these findings indicate that pairing Zn-efficient rice varieties with appropriate agronomic Zn management can effectively raise rice nutritional quality and help alleviate Zn malnutrition in rice-dependent populations. Field-scale, multi-location studies are now needed to confirm the effectiveness of these agronomic biofortification strategies across diverse agro-ecological conditions.

Author contributions

M. H. Ashik and M. A. Mannan conceived and designed the study. M. H. Ashik conducted the pot experiment, collected plant and grain samples, and performed the laboratory analyses. M. A. Karim and M. Ahiduzzaman contributed to data analysis and interpretation. M. Akter and M. T. Akter assisted with sample processing and statistical analysis. M. H. Ashik wrote the original draft, and all authors reviewed, edited, and approved the final manuscript.

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare no conflict of interest.

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