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Performance of biofortified wheat varieties under different agronomic management practices

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Abstract

Nearly 2.5 billion people worldwide suffer from zinc and iron deficiency linked to cereal-based diets, and biofortified wheat offers one of the most cost-effective routes to address this gap. This research evaluated five biofortified wheat varieties (WB-02, BHU-6, PBW-757, HD-3171, HPBW-01) under four agronomic management packages combining nitrogen scheduling, zinc foliar application, and sowing methods during the 2022-23 and 2023-24 rabi seasons at Zhejiang University's experimental station, Hangzhou, China. A randomized block design with factorial arrangement and three replications was used. BHU-6 recorded the highest grain zinc content (47.8 mg kg^{-1}), iron content (41.2 mg kg^{-1}), and grain yield (4.67 t ha^{-1}) when paired with 150 kg N ha^{-1} in three splits plus two foliar zinc sprays at tillering and heading. Variety accounted for 31.4% of total variation in grain zinc, followed by nitrogen management (22.7%) and zinc application (18.9%). These results indicate that combining the right variety with targeted nutrient management can raise micronutrient density without sacrificing yield.

Keywords: Biofortified wheat, zinc content, iron content, agronomic management, nitrogen scheduling, foliar zinc, grain yield, micronutrient density, rabi season

Introduction

An estimated 17.3% of the global population doesn't get enough zinc from their daily diet, and wheat-dependent regions in South and East Asia bear the heaviest burden ^[1]. Breeding biofortified wheat varieties with genetically elevated zinc and iron concentrations in the grain has moved from concept to commercial release over the past decade ^[2]. But genetic potential alone doesn't guarantee that micronutrient-rich grain reaches the consumer agronomic management during the growing season can either amplify or suppress the trait expression ^[3].

Nitrogen supply affects grain protein and, by extension, zinc binding capacity in the endosperm. Split nitrogen application at key growth stages has raised grain zinc by 8-15% over single basal dressing in Indian and Pakistani trials ^[4, 5]. Foliar zinc sprays at heading and grain filling offer another lever: they bypass root uptake limitations and deposit zinc directly onto developing grains, sometimes adding $5\text{-}12 \text{ mg kg}^{-1}$ to the baseline ^[6]. Sowing method whether conventional broadcast, line sowing, or raised-bed planting changes root architecture and nutrient access patterns, though its effect on grain micronutrient content has received less attention ^[7].

Most published work has tested these factors in isolation or in two-way combinations ^[8, 9]. What remains unclear is how all three interact when applied to modern biofortified varieties released for subtropical and warm-temperate zones. China's Yangtze River Delta, where wheat is a major rabi crop, provides a useful testing ground because its alluvial soils tend to be marginally zinc-deficient ^[10, 11]. This research was therefore set up to test five biofortified wheat varieties under four agronomic management packages at Hangzhou over two consecutive seasons.

Research area description

The trial was conducted at the Crop Science Experimental Farm of Zhejiang University, Hangzhou ($30^{\circ}16'N$, $120^{\circ}12'E$, 8 m a.s.l.), situated on alluvial silty clay loam soil derived from Qiantang River deposits. Topsoil (0-20 cm) analysis before sowing showed pH 6.2, organic

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matter 2.14%, DTPA-extractable zinc 0.72 mg kg⁻¹ (marginally deficient), available phosphorus 14.6 mg kg⁻¹, and available potassium 142 mg kg⁻¹. The site receives a mean annual rainfall of 1,454 mm, mostly concentrated in the monsoon months of June-September. The preceding crop was single-season rice in both years.

Climatic conditions during the trial

The 2022-23 rabi season (November-April) received 284 mm rainfall, slightly above the 30-year average of 261 mm, with mean minimum and maximum temperatures of 5.3 °C and 16.8 °C respectively. A cold wave in late January 2023 brought temperatures to -2.4 °C for three consecutive nights but caused no visible frost damage. The 2023-24 season was drier (237 mm) with warmer nights (mean minimum 6.1 °C). Relative humidity during grain filling averaged 74% in both seasons.

Materials and Methods

Materials

Five biofortified wheat varieties WB-02, BHU-6, PBW-757, HD-3171, and HPBW-01 were sourced from recognized breeding programs. BHU-6 and HD-3171 are high-zinc types (target >42 mg kg⁻¹), while WB-02, PBW-757, and HPBW-01 carry moderate enrichment (35-40 mg kg⁻¹). Seeds were treated with carboxin + thiram before planting. Four agronomic management packages were tested: M1 (120 kg N ha⁻¹, single basal, no foliar Zn, broadcast sowing); M2 (120 kg N ha⁻¹, two splits, one foliar Zn at heading, line sowing); M3 (150 kg N ha⁻¹, three splits, two foliar Zn at tillering and heading, line sowing);

M4 (150 kg N ha⁻¹, three splits, two foliar Zn, raised-bed sowing). Zinc sulfate heptahydrate (0.5% w/v) was used for foliar application [12, 13].

Methods

The experiment followed a randomized block design with factorial arrangement (5 varieties × 4 management packages) replicated three times, running from November 2022 to April 2024 at Zhejiang University, Hangzhou. Plot size was 4.0 m × 2.5 m. Grain yield was recorded from the net plot, adjusted to 12% moisture. Grain zinc and iron were analyzed by atomic absorption spectrophotometry after tri-acid digestion (HNO₃: HClO₄:H₂SO₄, 9:4:1). Protein content was measured by micro-Kjeldahl method, and test weight was recorded using a standard hectoliter apparatus [14]. Harvest index and 1000-grain weight were also determined. Data were analyzed using two-way ANOVA in R 4.3.1, with means separated by Duncan’s multiple range test at p = 0.05.

Field experimental design

Each replication block measured 20 m × 12.5 m, with 1.0 m alleys between plots to prevent nitrogen and zinc carryover. Raised-bed plots (M4) had beds 70 cm wide with 30 cm furrows. Broadcast plots received seed at 125 kg ha⁻¹, while line-sown and raised-bed plots used 100 kg ha⁻¹ at 20 cm row spacing. Irrigation was applied at crown root initiation, heading, and grain filling stages.

Results

Table 1: Grain yield (t ha⁻¹) of biofortified wheat varieties under different management packages (pooled data)

Variety	M1	M2	M3	M4	Mean
WB-02	3.54	3.87	4.23	4.11	3.94
BHU-6	3.91	4.28	4.67	4.53	4.35
PBW-757	3.31	3.62	3.89	3.74	3.64
HD-3171	3.72	4.09	4.41	4.27	4.12
HPBW-01	3.48	3.79	4.12	3.97	3.84
Mean	3.59	3.93	4.26	4.12	

CD (p = 0.05): Variety = 0.18; Management = 0.16; Variety × Management = 0.36

Grain yield increased from M1 to M3 across all varieties, with M3 (150 kg N in three splits + two foliar Zn sprays + line sowing) producing the highest mean of 4.26 t ha⁻¹ (Table 1).

BHU-6 under M3 gave the peak yield of 4.67 t ha⁻¹. M4 (raised-bed) didn’t surpass M3, suggesting that raised beds offered no additional advantage in this alluvial soil.

Table 2: Grain zinc and iron content (mg kg⁻¹) under best management package M3 (pooled data)

Variety	Zinc (mg kg ⁻¹)	Iron (mg kg ⁻¹)	Protein (%)	Test Wt (kg hL ⁻¹)
WB-02	42.1	38.7	12.4	78.3
BHU-6	47.8	41.2	13.1	79.6
PBW-757	38.4	35.9	11.8	76.9
HD-3171	44.6	39.8	12.7	78.8
HPBW-01	40.3	37.1	12.1	77.4

Under M3, BHU-6 reached the highest grain zinc (47.8 mg kg⁻¹) and iron (41.2 mg kg⁻¹), exceeding PBW-757 by 24.5% and 14.8% respectively (Table 2). Protein content ranged from 11.8% (PBW-757) to 13.1% (BHU-6), and test weight stayed above 76 kg hL⁻¹ for all entries

The heatmap (Figure 2) highlights BHU-6 and HD-3171 as the strongest performers across all five parameters. PBW-757 consistently lagged behind, particularly in zinc and iron density, suggesting its biofortification trait needs stronger agronomic support to express fully.

background remains the single largest driver of grain micronutrient content, consistent with multi-environment trials reported from the CIMMYT biofortification pipeline [2, 15]. However, the jump from M1 to M3 added 5.1-6.3 mg kg⁻¹ of zinc across varieties, showing that agronomic management can meaningfully close the gap between a variety’s genetic ceiling and its field-level expression [4, 6].

Discussion: The 24.5% zinc advantage of BHU-6 over PBW-757 under the same management package confirms that genetic

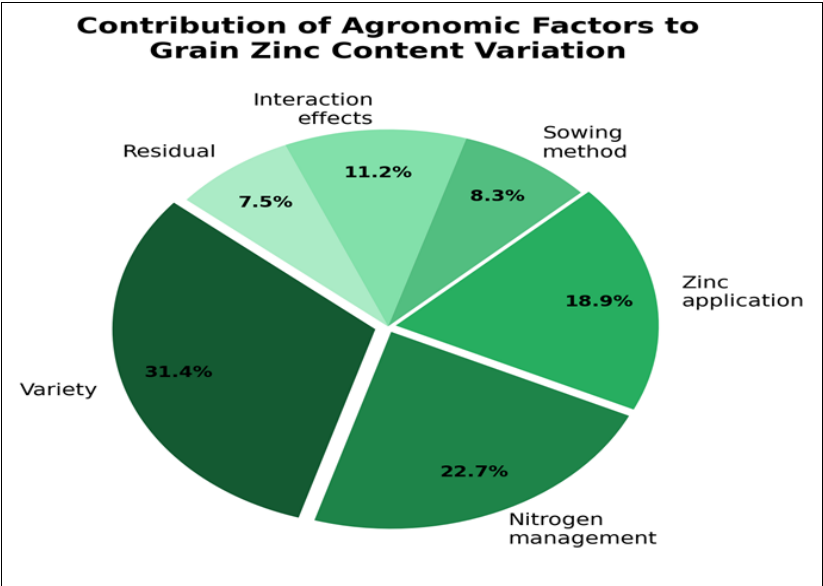


Fig 1: Relative contribution of agronomic factors to variation in grain zinc content (pooled ANOVA)

Variety alone explained 31.4% of the total variation in grain zinc content, followed by nitrogen management at 22.7% and zinc application at 18.9% (Figure 1). Sowing method contributed only 8.3%, while interaction effects accounted for 11.2%.

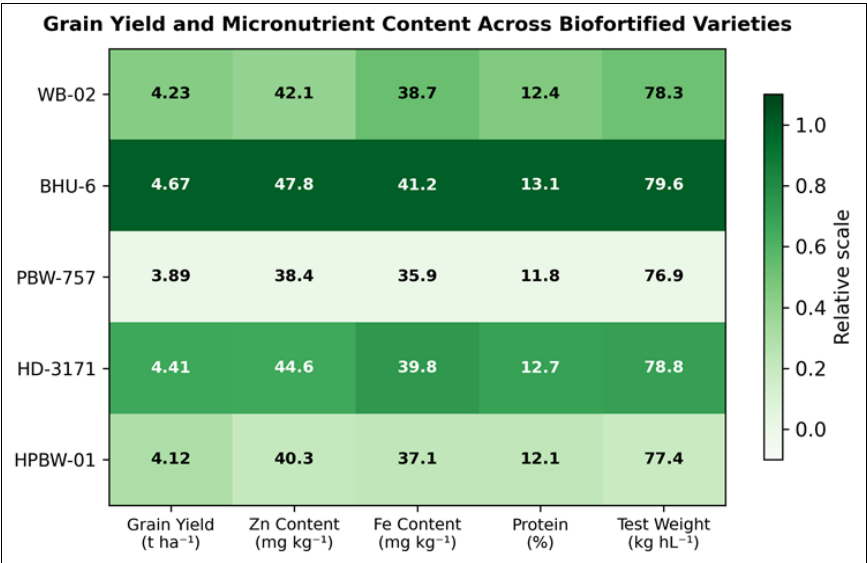


Fig 2: Heatmap of grain yield and micronutrient content across biofortified wheat varieties under M3

Split nitrogen application likely enhanced zinc accumulation by sustaining green leaf area longer into grain filling, which maintained phloem-mediated zinc remobilization from vegetative tissues to the developing grain [5, 16]. The two foliar zinc sprays at tillering and heading had an additive effect, probably because the first spray built up plant zinc reserves while the second delivered zinc directly during the critical grain-loading window [6]. That raised-bed sowing (M4) didn't outperform line sowing (M3) was unexpected given reports from drier environments [7], but the consistently moist alluvial soils at Hangzhou may have negated any water-use efficiency advantage of raised beds. Protein content tracked nitrogen input rather than zinc application, which is expected since nitrogen drives amino acid synthesis independently of micronutrient dynamics [17]. The positive association between grain zinc and protein observed here aligns with the widely reported co-localization of zinc with storage proteins in the wheat endosperm [18, 19].

Conclusions: This two-season trial at Zhejiang University demonstrated that combining biofortified variety selection with targeted agronomic inputs can raise grain zinc and iron content without compromising yield. BHU-6 under management package M3 (150 kg N ha⁻¹ in three splits, two foliar zinc sprays, line sowing) achieved the best overall performance: 4.67 t ha⁻¹ grain yield, 47.8 mg kg⁻¹ zinc, and 41.2 mg kg⁻¹ iron. Variety choice explained the largest share of zinc variation (31.4%), but nitrogen scheduling and foliar zinc together contributed another 41.6%, meaning agronomic management isn't optional it's necessary for full trait expression. Raised-bed sowing offered no additional benefit over line sowing in alluvial soils. Farmers in the Yangtze River Delta growing biofortified wheat should prioritize split nitrogen with foliar zinc at tillering and heading to maximize both yield and nutritional quality.

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