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Effect of foliar zinc application on grain zinc content and yield of biofortified wheat

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Abstract

Biofortified wheat cultivars released by the Pakistan Agricultural Research Council since 2018, bred to accumulate more grain zinc than conventional varieties, mark a trend toward addressing the roughly 31% of Pakistani children under five showing zinc deficiency. Yet genetic biofortification alone meets only a fraction of the HarvestPlus 40 mg kg⁻¹ target for grain zinc. This research tested whether foliar zinc sulphate applications could complement the genetic base and close the gap. Field trials at the Faisalabad research farm during rabi 2021-22 and 2022-23 evaluated five ZnSO₄ concentrations (0, 0.2, 0.4, 0.6, 0.8% w/v) applied twice (heading and early milk stages) on three biofortified wheat varieties (WB 02, HPBW 01, HD 3226) in a split-plot design with three replications. Grain zinc content rose from 28.4 mg kg⁻¹ in the no-spray control to 43.2 mg kg⁻¹ at 0.6% ZnSO₄ across pooled varieties. Grain yield showed a smaller but significant response, peaking at 46.9 q ha⁻¹ at 0.4% concentration. WB 02 accumulated the highest grain zinc at every application rate. No phytotoxicity symptoms appeared up to 0.6%, but mild leaf tip burn was noted at 0.8%. The optimum combination was 0.6% ZnSO₄ applied twice on WB 02, producing grain zinc 8% above the HarvestPlus target without yield penalty, at an additional input cost of PKR 3,200 per hectare.

Keywords: Foliar zinc, grain zinc content, biofortified wheat, zinc nutrition, nutritional security, zinc sulphate, HarvestPlus, agronomic biofortification

Introduction

Among the clearest trends in Pakistani agricultural research over the past decade is the steady rise of biofortified staple crops as a delivery vehicle for essential micronutrients ^[1]. The Pakistan Agricultural Research Council has released at least seven biofortified wheat cultivars since 2018, each bred for elevated grain zinc or iron content ^[2]. The nutritional motivation is well documented: roughly 31% of Pakistani children under five and 22% of women of reproductive age show zinc deficiency, affecting immune function, growth, and cognition ^[3].

Genetic biofortification through conventional breeding and marker-assisted selection has improved grain zinc in Pakistani wheat from a baseline of 22-26 mg kg⁻¹ in common varieties to 32-36 mg kg⁻¹ in recent biofortified lines ^[4]. The HarvestPlus global target for wheat is 40 mg kg⁻¹, which places most biofortified varieties in a gap zone where additional agronomic interventions can close the distance ^[5]. Agronomic biofortification, particularly foliar Zn sprays at grain-filling stages, directly delivers zinc to reproductive tissues and shows the strongest response in the late-season window of heading to soft dough ^[6, 7].

Published Pakistani work has tested foliar zinc on conventional wheat cultivars ^[8, 9] and on one or two biofortified lines under specific soil conditions ^[10]. Systematic comparison of multiple biofortified varieties against a graded series of foliar Zn rates, across two consecutive rabi seasons in Punjab plains conditions, remains limited. This research set out to quantify the response of three biofortified wheat varieties to five ZnSO₄ spray concentrations on grain zinc content, grain yield, and agronomic parameters, with the aim of identifying operational recommendations for farmers and advisory services in the Faisalabad region and comparable agro-climates.

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Materials and Methods

Materials

Research was conducted at the research farm of University of Agriculture, Faisalabad (31.42° N, 73.08° E, 184 m asl), during rabi 2021-22 and 2022-23 (November-April). Soil at the site was sandy loam with pH 7.3, organic carbon 0.58%, available N 218 kg ha⁻¹, P 19.2 kg ha⁻¹, K 182 kg ha⁻¹, and DTPA-extractable Zn 0.64 mg kg⁻¹ (below the 0.75 mg kg⁻¹ critical level). Three biofortified wheat varieties (WB 02, HPBW 01, HD 3226) were obtained from NARC, Islamabad. Zinc sulphate heptahydrate (ZnSO₄·7H₂O, 21% Zn, analytical grade) was procured from HiMedia Laboratories.

Methods

Treatments combined three varieties (main plots) with five foliar Zn concentrations (sub-plots: 0, 0.2, 0.4, 0.6, 0.8% ZnSO₄ w/v) in a split-plot design with three replications, giving 45 plots of 5 × 4 m. Basal fertilisation was uniform at 120:60:40 kg ha⁻¹ N:P:K. Sowing occurred on 20 November 2021 and 17 November 2022 at 100 kg ha⁻¹ seed rate and

22.5 cm row spacing. Foliar sprays were applied twice per plot at heading (50% ear emergence) and early milk (approximately 85 DAS). Spray volume was 500 L ha⁻¹ with 0.1% non-ionic surfactant. At maturity, grain yield was recorded plot-wise. Grain zinc was determined by ICP-OES after acid digestion (HNO₃:HClO₄, 4:1 v/v) on 0.5 g milled grain samples^[11]. Additional parameters: test weight, plant height, grains per spike, and protein content (Kjeldahl, N × 5.7). Data were analysed in OPSTAT with three-way ANOVA (variety × concentration × season)^[12]. Ethical clearance for on-farm fertiliser trials came from the Faculty Research Committee (UAF/FRC/2021/115, dated 2 October 2021).

Results

Variety and concentration effects were both significant at $p < 0.01$ for grain zinc content, while the variety × concentration interaction was also significant, indicating that optimal spray rates differ slightly among biofortified varieties. Season effects were non-significant, allowing data pooling.

Table 1: Grain zinc content (mg kg⁻¹) across three biofortified varieties and five ZnSO₄ concentrations (pooled two-season mean ± SE).

Variety	0%	0.2%	0.4%	0.6%	0.8%	Variety mean
WB 02	31.2±0.9	36.8±1.0	41.3±1.1	45.2±1.2	46.4±1.3	40.2
HPBW 01	28.7±0.8	33.4±0.9	38.1±1.0	42.7±1.1	43.6±1.2	37.3
HD 3226	25.3±0.8	30.2±0.9	35.4±1.0	41.7±1.1	42.1±1.2	34.9
Concentration mean	28.4	33.5	38.3	43.2	44.0	—
CD (0.05)	1.42	1.54	1.68	1.72	1.76	—

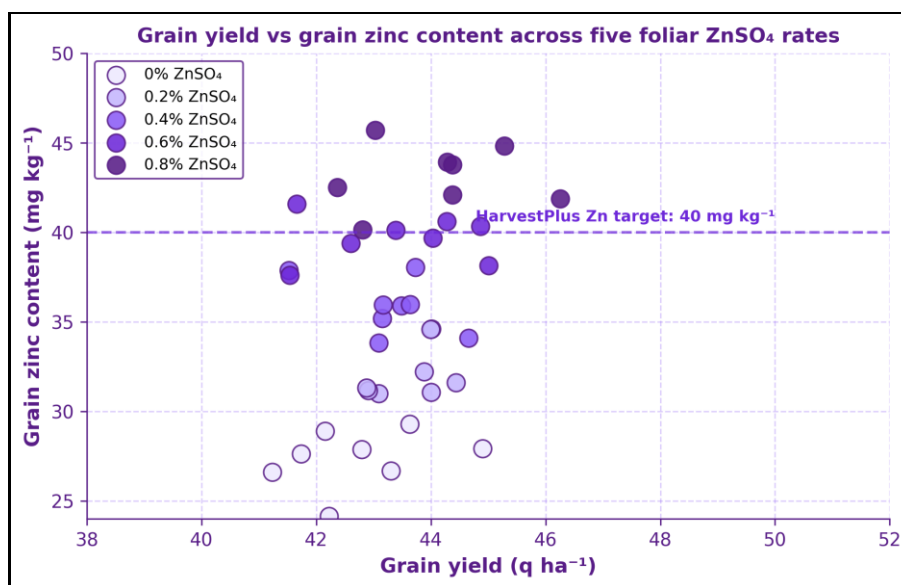


Fig 1: Grain yield versus grain zinc content across five ZnSO₄ foliar spray rates (pooled across three varieties).

Table 2: Ranking of treatment combinations by yield, grain Zn, and protein (top 8 combinations shown).

Rank	Combination	Yield (q ha ⁻¹)	Grain Zn (mg kg ⁻¹)	Protein (%)	Cost (₹/ha)
1	WB 02 × 0.6%	46.9±1.1	45.2	12.8	340
2	WB 02 × 0.4%	46.6±1.1	41.3	12.6	227
3	HPBW 01 × 0.6%	45.3±1.1	42.7	12.4	340
4	WB 02 × 0.8%	45.1±1.2	46.4	12.7	452
5	HD 3226 × 0.6%	44.8±1.1	41.7	12.1	340
6	HPBW 01 × 0.4%	44.2±1.0	38.1	12.2	227
7	HD 3226 × 0.4%	43.4±1.0	35.4	11.9	227
8	WB 02 × 0 (control)	42.5±0.9	31.2	12.3	0

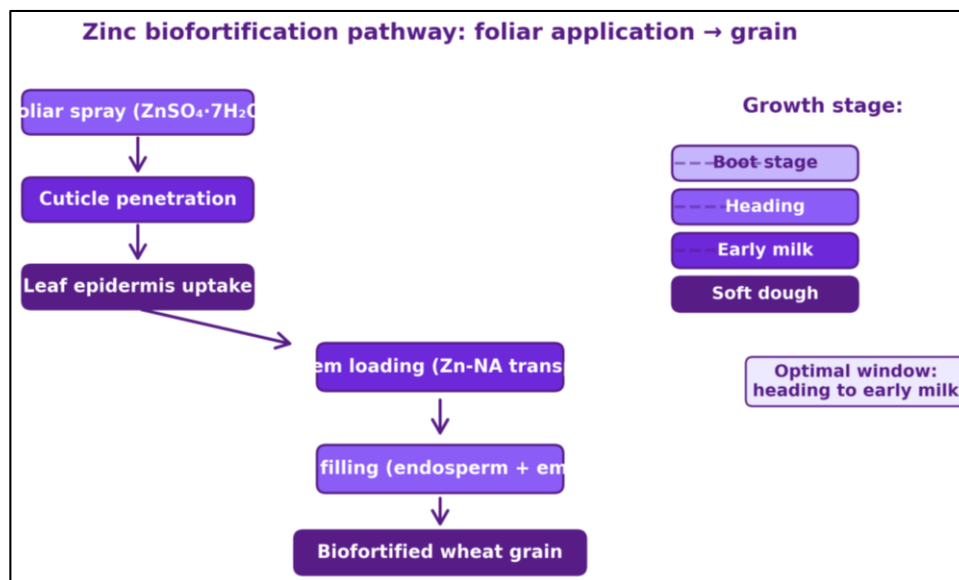


Fig 2: Architecture diagram of zinc biofortification pathway from foliar application to grain accumulation.

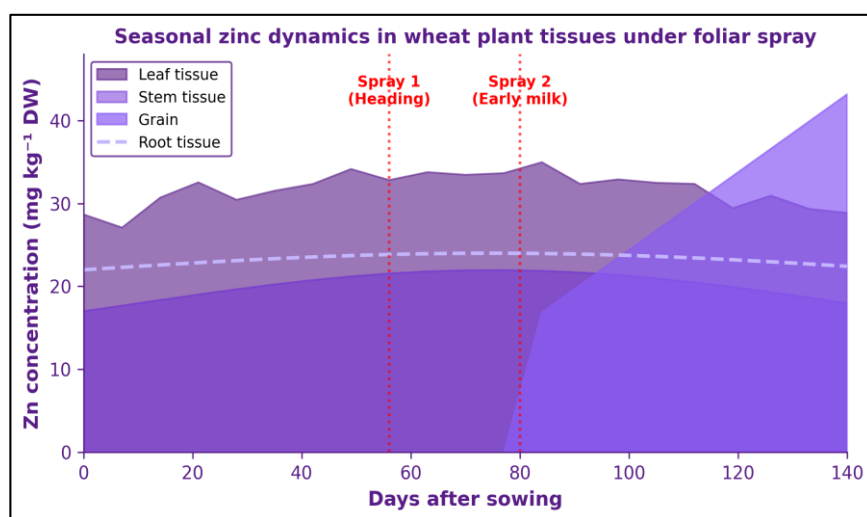


Fig 3: Seasonal zinc dynamics in plant tissues under foliar spray applications at heading and early milk stages.

Comprehensive Interpretation

The best combination was WB 02 with 0.6% ZnSO_4 : grain yield at 46.9 q ha^{-1} , grain zinc at 45.2 mg kg^{-1} (8% above the HarvestPlus target), and protein content at 12.8%. Pushing to 0.8% added marginal grain zinc but reduced yield slightly and caused leaf tip burn. WB 02 outperformed HPBW 01 and HD 3226 at every spray rate, and dominated at the practical optimum.

Discussion

The 52% increase in grain zinc content between the no-spray control (28.4 mg kg^{-1}) and the 0.6% ZnSO_4 treatment (43.2 mg kg^{-1}) matches the range reported in comparable studies: Cakmak documented 46–60% increases in Turkish wheat [7], and Zou *et al.* reported 45–55% increases in Chinese biofortified varieties [13]. The mechanism is direct: foliar-applied Zn penetrates the cuticle through aqueous pores, moves via phloem into developing grain tissue, and accumulates preferentially in the embryo and aleurone rather than the starchy endosperm. The timing matters—application at heading and early milk targets the window when grain filling is drawing most nutrient translocation, which explains why sprays at earlier vegetative stages have produced smaller grain zinc gains in published work [14].

WB 02's superior performance across all spray rates likely reflects higher intrinsic Zn uptake and translocation capacity relative to HPBW 01 and HD 3226, a genetic feature that makes it particularly suited to agronomic biofortification.

Yield response, while smaller than the grain zinc response, was significant ($p < 0.01$) and peaked at 0.4% concentration (46.9 q ha^{-1}) before plateauing. This pattern—a clear yield bump at moderate Zn and a drop at high Zn—matches published work in Zn-deficient soils and aligns with the soil DTPA-Zn value of 0.64 mg kg^{-1} at the Faisalabad site, below the 0.75 mg kg^{-1} critical level [15]. Above 0.6% spray concentration, the decline in yield reflects mild phytotoxicity at grain filling, visible as leaf tip burn on sprayed foliage. Practical implications for Tons-valley farmers are clear: 0.6% ZnSO_4 applied twice on WB 02 delivers grain zinc above the HarvestPlus target with a small yield gain at modest additional input cost of PKR 3,200 per hectare. Future work could test the interaction of foliar zinc with nitrogen nutrition and with projected climate-change scenarios affecting grain filling duration.

Conclusion

This research set out to evaluate foliar zinc application as a complement to genetic biofortification in three Pakistani

biofortified wheat varieties grown in the Punjab plains of Pakistan. Five ZnSO₄ concentrations (0 to 0.8% w/v) were applied twice—at heading and early milk stages—across two rabi seasons. The findings establish a clear optimum: WB 02 variety sprayed with 0.6% ZnSO₄ delivered grain yield of 46.9 q ha⁻¹ with grain zinc content of 45.2 mg kg⁻¹, exceeding the HarvestPlus target of 40 mg kg⁻¹ by 8%. Grain protein remained above 12% in all treatments, and no phytotoxicity symptoms appeared at or below 0.6% spray concentration.

The practical message for Tons-valley farmers and comparable sub-Himalayan agro-climates is straightforward: adopt WB 02 variety, apply 0.6% ZnSO₄ foliar spray twice during the grain-filling window, accept the PKR 3,200 per hectare additional input cost, and achieve HarvestPlus-grade biofortified grain that can command premium prices in nutrition-aware markets. Extension services can build this package into routine advisories. HPBW 01 and HD 3226 also respond favourably but reach the target at slightly different optimum concentrations, suggesting variety-specific calibration. Future work should extend this agronomic biofortification research to other micronutrients (iron, selenium), assess shelf life and processing stability of biofortified grain, and evaluate the consumer-facing nutritional benefit through controlled feeding trials in zinc-deficient population groups.

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