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Genotype × soil zinc interaction constrains grain zinc loading in a biofortified common bean under farmer management

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Biofortified crops are deployed to combat micronutrient deficiencies, but their efficacy in nutrient-poor soils is poorly understood. We evaluated the zinc (Zn) loading capacity of a biofortified bean variety NUA45 across nine smallholder-managed sites in Malawi with contrasting soil Zn status. NUA45 frequently failed to achieve its target grain Zn concentration (56 mg kg^{-1}), with grain Zn strongly correlated with soil Zn availability ($R = 0.419$, $p < 0.001$). In contrast, local varieties, though not bred for enhanced Zn, often accumulated higher grain Zn concentrations, averaging 8.5 percent more than NUA45, and showed no dependence on soil Zn status ($R = -0.019$, $p = 0.939$). Across 78% of the sites had soils with DTPA-extractable Zn below the agronomic threshold of 1 mg kg^{-1}. The analysis revealed a significant genotype × soil Zn interaction ($p = 0.043$) indicating that soil factors strongly modulate the expression of biofortified traits. These findings reveal that biofortified genotypes alone cannot ensure adequate Zn accumulation where soil Zn is limiting. Integrated interventions combining soil management, Zn fertilization, and breeding for enhanced translocation efficiency are therefore required to achieve nutritional targets in legumes and other smallholder crops globally.

KEYWORDS

zinc biofortification, common bean, genotype-environment interaction, DTPA-extractable zinc, nutrient bioavailability, soil zinc availability

1 Introduction

Zinc (Zn) deficiency is one of the most critical micronutrient constraints in Malawi's agricultural systems. National-scale soil surveys have shown that nearly half of the country's cultivated soils contain DTPA-extractable Zn below the agronomic threshold of 1 mg kg^{-1} (Snapp, 1998; Lindsay and Norvell, 1978). These deficiencies arise primarily from low soil organic matter content that limits Zn complexation and retention (Almås et al., 2000), soil acidity that reduces Zn solubility and root uptake (Alloway, 2008; Cakmak, 2002) and continuous nutrient mining coupled with limited use of mineral fertilizers under smallholder conditions (Snapp et al, 2010; Omuto and Vargas, 2018). The widespread occurrence of Zn-deficient soils has direct implication on human nutrition as grain Zn concentration closely reflects soil Zn availability (Cakmak et al., 1999; Gibson, 2012). For instance, although the global prevalence of inadequate Zn intake is estimated at about 17%, more than 62% of the population in Malawi, especially in rural areas, are at risk of deficiency (Kumssa et al., 2015; Likoswe et al., 2020; NSO, 2017).

In response, the Malawian Government, in collaboration with international breeding programs, introduced three Zn-biofortified common bean varieties (NUA35, NUA45, and NUA59). These first-generation genotypes, developed by the International Center for Tropical Agriculture (CIAT), were specifically bred for elevated Zn and iron content to support the HarvestPlus target of increasing grain Zn concentration in common bean from 35 mg kg^{-1} to 56 mg kg^{-1} (Beebe et al., 2000; Blair et al., 2010; Pfeiffer and McClafferty, 2007).

Biofortified crops are varieties that have been deliberately enhanced to contain higher levels of essential micronutrients such as Zn, iron, or vitamin A in their edible parts through genetic plant breeding techniques or agronomic practices (Cakmak, 2008; White and Broadley, 2011). However, their nutritional efficacy is strongly influenced by environmental factors, particularly soil micronutrient availability. Several studies in Malawi and across sub-Saharan Africa have demonstrated a positive correlation between DTPA-extractable soil Zn and grain Zn concentrations in various staple crops (Chilimba et al., 2011; Gashu et al., 2021; Siyame et al., 2013). However, these studies have not specifically quantified the genotype-by environment (G x E) interaction for biofortied varieties under heterogenous and nutrient-depleted conditions of smallholder farms, where inputs are minimal. Consequently, a critical gap remains in understanding how such varieties respond to variable and Zn-depleted soils under typical smallholder conditions, where agronomic inputs are often minimal and management is heterogeneous.

This study, therefore, evaluates the performance of a biofortified common bean variety relative to local bean varieties across heterogeneous, Zn-deficient soils in Malawian smallholder farming systems. Unlike most evaluations conducted under structured research conditions or formal breeding trials, this farmer-managed, multi-site assessment captures the agronomic variability typical of real-world production systems. It examines how differences in soil Zn availability influence grain Zn accumulation. We hypothesize that the nutritional advantage of the biofortified variety is contingent upon adequate soil Zn availability and that under the widespread Zn deficient conditions of Malawi, it will fail to outperform the non biofortified varieties. The results could provide insight into how environmental and agronomic conditions shape the performance of biofortified crops, supporting future strategies to improve their impact in resource-limited agricultural contexts.

2 Materials and methods

2.1 Study sites

The study was conducted in five districts that play a significant role in bean production in Malawi; Central (Ntchisi and Dedza) and Northern (Chitipa, Rumphi and Mzimba). Two sites were selected in all districts except for Chitipa where only a single site was sampled. These sites represent Extension Planning Areas (EPAs), the lowest administrative units used by the Ministry of Agriculture for agricultural planning and implementation of agricultural activities in Malawi. The selected sites fall in two of the agro-ecological zones (AEZ) of Malawi, the mid- and high-altitude zones (Table 1).

Biofortified bean seeds of NUA45 variety were distributed to ten farmers in each selected site except in Bembeke, Linthipe and Kalira where eleven farmers received 1 kg seed of the biofortified variety that was planted on 0.01 ha. In addition to the biofortified variety, some farmers planted local bean varieties. The beans were cultivated as a monocrop and without the use of fertilizers or manure as is usual agricultural practice in Malawi. The farmers independently planted the seed in their fields and maintained the crop; researchers monitored the fields during the podding stage and collected soil and bean samples at harvest. Other agronomic practices for bean production (weed management, pest and disease control) were followed as stipulated in the Guide to Agricultural production and Natural Resources Management Guidelines (MoAFS, 2018).

2.2 Bean grain and soil sampling

Bean and soil sampling followed the protocol described in Gashu et al. (2020). Briefly, the center of each field was designated as the initial sampling point. Four additional sampling points were located 2 meters from the center in each of the cardinal directions (north, south, east, and west). At each point, two adjacent bean plants were harvested. All harvested plants from a field were combined to form a composite sample, from which a 200 g subsample was taken for further processing. Soil samples were collected near the base of the harvested bean plants using a Dutch soil auger (15 cm flight length, 3.5 cm diameter) at a depth of 0–20 cm. Five soil cores were taken per field and combined to create a composite sample, from which a 300 g subsample was extracted for further processing.

Out of the 93 farmers that received NUA45 seed, paired bean-soil samples were collected from 79 farmers as 14 lost their crop due

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TABLE 1 Description of study sites and number of soil and bean grain samples collected from each site.

Region	Site	District	Agroecological zone	Total samples	NUA45 Samples	Other varieties samples
Central	Bembeke	Dedza	High altitude	13	12	1
Central	Linthipe	Dedza	Mid altitude	12	11	1
Central	Kalira	Ntchisi	High altitude	12	10	2
Central	Kanjiwa	Ntchisi	Mid altitude	12	10	3
Northern	Mhuju	Rumphi	Mid altitude	6	4	2
Northern	Ntchenachena	Rumphi	High altitude	6	4	2
Northern	Khonsolo	Mzimba	High altitude	12	10	2
Northern	Manyamula	Mzimba	Mid altitude	11	9	2
Northern	Mwamkumbwa	Chitipa	High altitude	11	9	2
TOTAL	9	5	2	96	79	17

to disease pressure and mismanagement. These losses occurred randomly across sites hence minimizing the sampling bias. Paired samples of soil and ordinary common bean varieties were also collected from 17 farmers that grew monocrop common beans close to the biofortified plots in the same way as the NUA45 varieties. This facilitated a comparison of the nutrient mining efficiency of the different varieties. The limited number of samples for ordinary common bean varieties is attributed to the common practice in Malawi of growing beans in an intercrop, typically with maize, during the rainy season. It was important to exclude beans grown in this way as the national fertilizer standard for maize in Malawi, is formulated as 23N:10P:5K+6S+1.0Zn and would have supplied an additional source of Zn to any intercropped beans. Sampling was undertaken in the months of April and May 2021 when the plants had reached physiological maturity and were ready for harvesting.

2.3 Sample management and preparation

At Chitedze Agricultural Research Station (CARS), the bean grain samples were air dried and later ground in a laboratory mill (Christy and Norris Ltd, Suffolk, UK). To ensure the integrity of each sample, the mill was cleaned with a non-abrasive cloth after processing each sample. Soil samples were air dried and sieved to pass through a 2 mm mesh. A 20 g sub-sample of both ground bean and soil was shipped to the University of Nottingham, UK for further analysis.

2.4 Soil analysis

2.4.1 pH and loss on ignition

Soil sieved to a particle size of less than 2 mm was mixed with deionized water at a ratio of 5 g to 12.5 mL. The mixture was then shaken for 30 minutes on a rotary shaker before centrifugation at a speed of 3000 revolutions per second for 10 minutes. The pH of the resulting solution was determined using a combined pH meter and electrode (Mettler-Toledo AG, Toledo Group, Switzerland). Before obtaining pH readings, the electrode was calibrated using buffers at pH levels of 4.01 and 7.00. Following each measurement, the glass electrode was rinsed with Milli-Q water (18.2 MΩ cm; Merck Millipore Milli-Q, Darmstadt, Germany).

The organic matter (OM) content in soils was determined by calculating the percentage loss on soils that had been oven-dried and ignited. The procedure involved weighing 5 g of soil, which had been sieved to a particle size of less than 2 mm, in a crucible of known mass. The soil was then subjected to oven drying at 105 °C for 14–16 hours to remove water tightly bound to soil particles. Subsequently, the dried soils were placed in a furnace with a temperature of 550°C for 4 hours. Percentage OM was calculated from the change in weight of the sample before and after ignition. To correct for the overestimation of OM caused by the release of structural water from clay minerals, OM was estimated as 0.58 x LOI following the adjustment factor (Howard and Howard, 1990).

2.4.2 DTPA-available zinc concentration

To extract DTPA-available Zn (ZnDTPA), 5 g of soil was mixed with 10 mL of an extracting solution containing 0.005 M DTPA, 0.1 M triethanolamine (TEA), and 0.01 M $CaCl_2$, buffered at pH 7.3. The mixture was agitated for 2 hours using an end-over-end shaker, following the method described by Lindsay and Norvell (1978). After shaking, the samples were centrifuged and filtered through a 0.22 μm membrane.

2.5 Digestion of bean grains

The determination of Zn content in bean flour followed the procedure outlined in Mossa et al. (2020). In summary, 0.4 g of bean flour was soaked in 8 mL of 68% nitric acid (Trace Metal Analysis Grade) within a 50 mL digestion tube for 16 hours. Subsequently, the mixture was digested on a heating block (Anton Paar, Austria) for 2 hours at 105°C to facilitate release of minerals. Each cycle comprised 44 bean flour samples, 2 blanks, 2 certified reference

material (CRM; Wheat flour SRM 1567b, NIST, Gaithersburg, MD, US; Zn concentration = 11.61 mg kg^{-1}). The blanks and reference material were included to determine the accuracy of the analyses and the limit of detection (LOD) for quality control. After digestion, the samples were left to cool down. Each tube was later adjusted to a final volume of 50 mL by adding 38 mL of Milli-Q water (18.2 MΩ cm; Merck Millipore Milli-Q, Darmstadt, Germany) and stored at room temperature, awaiting Zn analysis. Immediately before analysis, the samples were further diluted with Milli-Q water in a ratio of 1:10 to achieve an acid concentration of less than 5%.

2.6 Elemental analysis

Zinc was analyzed using a single quadrupole ICP-MS (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific Inc., Waltham, MA, USA) in kinetic energy discrimination mode (KED). Samples were introduced at a flow rate of 1.2 mL min^{-1} from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific).

2.7 Statistical analysis

The data were analyzed with a linear mixed model, using the nlme and emmeans packages on the R platform (Pinheiro et al., 2021). The linear mixed model fitted by maximizing the residual log-likelihood (REML) is particularly suitable for survey data sets such as this which are imbalanced with respect to numbers of observations in the different varieties. There was a nested set of random effects which reflect the sample design: a between-district effect, a between-site within-district effect, and a between-respondent (farm) within-site effect, in addition to a residual. The fixed effects were the bean variety (biofortified, or non-biofortified local dwarf variety), and AEZ. An interaction of AEZ and variety was considered, to capture any difference in the relative performance of the varieties in different environmental conditions. The model was fitted using the lme function from nlme. The marginal residuals were examined, with summary statistics, a histogram, boxplot and QQ plot to evaluate the plausibility of an assumption of normality, and a plot of the residuals against the fitted values was examined to assess the homogeneity of the residual variation.

The next step was to examine the effect of adding soil properties as predictors of Zn concentration in bean grains. This was done by adding the results from the corresponding soil sample for each bean sample as a linear fixed effect. Because the AEZ effect was not significant in the original model, a main effect of the soil property, and an interaction with variety was tested. This was done for soil Zn concentration (log-transformed), soil organic carbon and soil pH.

3 Results

The variation of soil organic matter content and soil pH was investigated by a nested mixed effects model with a single mean as the only fixed effect (Table 2). Individual soil pH measurements ranged from moderately acidic (5.2) to slightly alkaline (7.9). but the dominant source of variation in soil pH is within-site variation, probably reflecting differences in management. Note that less than 4 percent of the variance in soil pH was attributable to differences

TABLE 2 Variation of soil pH, organic matter content and DTPA-extractable Zn from sample sites. The standard errors (SE) are from the pooled within-site variance, with variable sample sizes.

	pH		Organic matter (%)		DTPA-extractable Zn mg kg^{-1}	
Source	Variance components					
Between-AEZ	0.01		0.52		0.00	
Between-site	0.01		2.63		0.14	
Within-site	0.24		7.40		1.07	
Site	Mean	SE ±	Mean	SE ±	Mean	SE ±
Ntchenachena	5.57	0.20	6.43	1.11	0.20	0.42
Mwamkumbwa	6.40	0.15	9.33	0.82	0.78	0.31
Mhuju	5.90	0.20	4.91	1.11	0.31	0.42
Manyamula	6.05	0.15	3.57	0.82	0.43	0.31
Linthipe	5.99	0.14	7.17	0.79	0.59	0.30
Khonsolo	6.09	0.14	7.79	0.79	0.35	0.30
Kanjiwa	6.24	0.14	3.85	0.75	0.69	0.29
Kalira	6.15	0.14	6.73	0.79	1.03	0.30
Bembeke	6.02	0.14	6.89	0.75	1.75	0.29

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between sites, Ntchenachena had the smallest value (mean pH = 5.57 ± 0.20 SE), and Mwamkumbwa had the largest (mean pH = 6.4 ± 0.15 SE). Organic matter content also varied widely, with the largest variance component at within-site level (again, probably reflecting variation in management, including use of manures) but also substantial between-site variation, accounting for 25% of the variance. Measured values ranged from 1.2% to 14.9%. Mwamkumbwa had the largest mean soil OM content (9.33% ± 0.82 SE), whereas Manyamula had the smallest (3.57% ± 0.82 SE).

The variation of DTPA-extractable soil Zn concentrations was dominated by within-site variation, but 12% of the variation was seen between the sites (Table 2, Figure 1). The largest concentration was at Bembeke 1.75 mg kg^{-1} ± 0.29 SE), whereas Ntchenachena had the smallest (0.20 mg kg^{-1} ± 0.42 SE). At all but two (78%) of the sites and 78% of the individual farms, the concentration of DTPA-extractable Zn was smaller than the agronomic critical threshold of 1 mg kg^{-1} required for optimal plant growth (Alloway, 2009). Analytical accuracy was confirmed by an 87% recovery from the certified reference material (CRM), indicating acceptable methodological performance.

The between-site variance component for grain Zn concentration in NUA45 beans was substantial, accounting for 33% of the total variance (Table 3). Mean values ranged from 23.1 mg kg^{-1} at Khonsolo to 30.0 mg kg^{-1} at Kalira, with a pooled range of 18.3-36.0 mg kg^{-1} (n = 79). Notably, the mean grain Zn concentration in NUA45 (25.9 mg kg^{-1}, ± 0.42 SE) was less than half the HarvestPlus biofortification target of 56 mg kg^{-1}, indicating that the variety did not achieve its intended nutritional potential under smallholder field conditions. Analytical reliability was supported by a 102% recovery of the Certified Reference Material (CRM 1567b).

On average, local varieties accumulated 8.5% more Zn than the biofortified variety (Table 4), with mean concentrations of 28.2 mg kg^{-1} compared with 25.9 mg kg^{-1} for NUA45. The analysis of variance in Table 5 confirmed that this difference was significant (p = 0.020). Among the collected varieties, variety Nyauzembe had the largest mean grain Zn concentration (34.8 mg kg^{-1}; n = 2), while another variety, Katawetawe, had the smallest (21.0 mg kg^{-1}; n = 1) (Table 4).

Results from the second analysis of variance model showed evidence for a significant positive relationship between DTPA-extractable soil Zn concentration and Zn concentration in the bean (p = 0.0464) (Table 5). There was also evidence for an interaction of DTPA soil Zn concentration and variety (NUA45 or local; p = 0.043) suggesting that genotypes differed in their capacity to accumulate Zn under varying soil Zn availability. The evidence for this is not strong because of the small number of non NUA45 varieties, and their unbalanced distribution in the data set. However, Figure 2 suggests that the interaction is seen in the contrast between a linear relationship of Zn concentration in NUA45 beans and DTPA-Zn in the soil and no such relationship for the local varieties.

Soil pH and organic matter content were considered as possible covariates in two further models (just one of the two included along with variety and DTPA soil Zn as fixed effects), but there was no evidence for an improved fit (p = 0.34 and 0.21 respectively.) and organic matter content (p = 0.21) were not significant predictors.

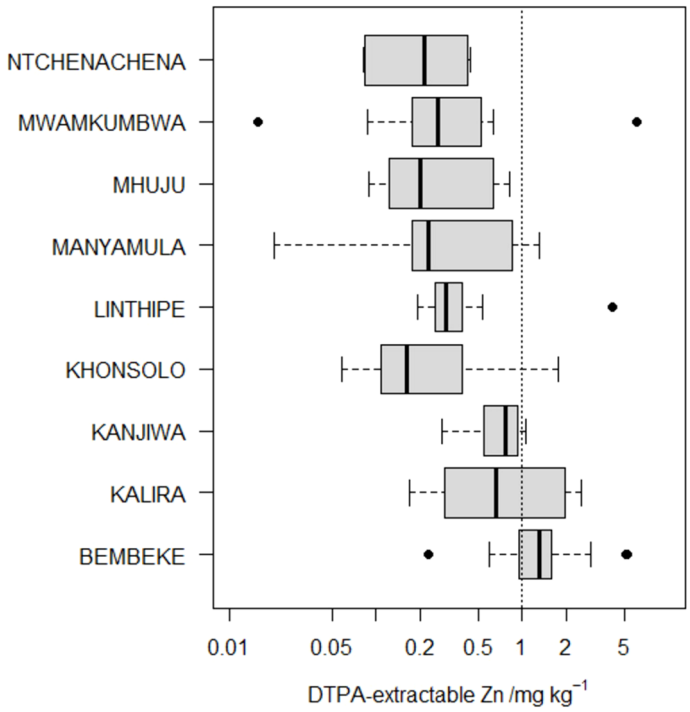

FIGURE 1
Box and whisker plots of DTPA-extractable soil Zn (mg kg^{-1}) at nine sites. Note that the concentration scale is logarithmic. The dotted vertical line represents the critical threshold of 1.0 mg kg^{-1} of Zn below which Zn deficiency is likely to limit plant growth (Alloway, 2009).

TABLE 3 Variation of grain Zn concentration (mg kg^{-1}) of NUA45 samples. The standard errors are from a pooled within-site variance, with variable sample sizes.

Variance components		
Source	Variance component	
Between-AEZ	0.57	
Between-site, within-AEZ	4.64	
Within-site	8.74	
Site means		
Site	Grain Zn concentration/mg kg^{-1}	
	Mean	Standard error
Ntchenachena	27.3	1.48
Mwamkumbwa	23.9	0.99
Mhuju	24.1	1.48
Manyamula	25.4	0.99
Linthipe	24.2	0.89
Khonsolo	23.1	0.93
Kanjiwa	25.4	0.93
Kalira	30.0	0.93
Bembeke	28.9	0.85
Mean	25.8	0.42

TABLE 4 Average grain Zn concentration and number of samples collected of NUA45 and local common bean varieties across all sampling sites.

Variety	Number of collections	Mean[+] of Zn concentration (mg kg^{-1})	Range
NUA45	79	25.8	24.1-27.9
Jandalala	2	27.8	24.6-30.9
Kamtauzgeni	2	27.2	24.6-29.8
Katawetawe	1	21.0	–
Kholophethe	2	25.7	24.7-26.7
Manthondo	1	26.6	–
Napilira	2	29.3	27.0-31.6
Nyauzembe	2	34.8	31.7-37.9
Phalombe	2	29.8	29.7-29.8
Salima	1	29.1	–
SER 124	1	29.5	–
Sesenya	1	23.3	–

[+]Where multiple samples were available, the average is shown. For single-sample varieties, the measured value is reported.

4 Discussion

This study provides the first strong field-based evidence from Malawi that Zn loading in a biofortified bean (NUA45) is conditional rather than intrinsic, with genetic enhancement failing to deliver nutritional gains in the absence of adequate micronutrient supply. Across nine smallholder-managed sites, 78% of the study sites had DTPA-extractable soil Zn below the agronomic threshold of 1 mg kg^{-1} (Alloway, 2009), creating widespread constraints to micronutrient uptake. Under these conditions, NUA45 frequently failed to achieve its targeted grain Zn concentration, highlighting a genotype × soil interaction that has not been quantified previously under smallholder field conditions.

The soil-dependence of NUA45 can be explained by well-established but often underappreciated constraints on Zn mobility and uptake in tropical soils. Elevated soil pH reduces Zn solubility through precipitation as carbonates and hydroxides (Alloway, 2008), while high phosphorus and calcium inputs exacerbate deficiency through antagonistic interactions at the rhizoplane (Fageria and Moreira, 2011). By contrast, organic matter enhances Zn bioavailability by forming soluble complexes and stimulating microbial processes such as siderophore production that mobilize micronutrients in the rhizosphere (Sharma et al., 2013). These mechanisms provide a clear explanation for why NUA45 failed to consistently reach target Zn concentrations under the predominantly Zn-deficient soils of Malawi. Similar soil-mediated bottlenecks have been observed in biofortified wheat and rice, where genetic gains in grain micronutrients were only realized with complementary fertility management (Cakmak, 2008; Joy et al., 2015).

These findings expose a critical blind spot in current biofortification pipelines. Breeding programs typically select lines under research-station or moderately fertile conditions, which do not reflect the heterogeneous and nutrient-constrained realities of smallholder systems (Bouis and Saltzman, 2017). Consequently, varieties that appear robust under controlled conditions may underperform when deployed in environments where Zn deficiency co-occurs with other stresses such as drought and low organic matter. Our results show that NUA45 exhibits high plasticity for grain Zn as it responds well in fertile soils but performs poorly in Zn-deficient ones. The high plasticity highlights the need for breeding programs to incorporate traits for stability to ensure consistent performance across variable soil fertility gradients. This could be achieved by using genomic tools to select for alleles associated with Zn uptake efficiency under deficiency (for example, from the ZIP transporter family), coupled with field validation in nutrient-poor soils. Addressing this gap requires systematic evaluation of biofortified germplasm in nutrient-poor soils, alongside soil diagnostics, to ensure that varietal recommendations are both scientifically robust and contextually relevant (White and Broadley, 2011; Zhao and McGrath, 2009; Zia et al., 2020).

While the central finding concerns the soil-dependent performance of NUA45, the contrasting stability of local landraces provides additional insight. The significant variety × soil Zn interaction observed in this study is likely driven by this fundamental difference in nutrient acquisition strategy between the responsive NUA45 and the stable non-biofortified varieties. Despite

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TABLE 5 Analysis of variance tables for a linear mixed model in which (a) the concentration of Zn in the bean is modelled in terms of Agro-ecological zone and variety (NUA45 or local variety) as fixed effects with an interaction and with Site as a random effect and (b) AEZ is dropped as a fixed effect, but a linear effect of DTP-extracted soil Zn, and its interaction with variety is included as a fixed effect and the random effect is unchanged.

(a)	Numerator df	Denominator df	Variance ratio	P-value
Variety	1	9	8.02	0.020
AEZ	1	7	1.11	0.044
AEZ•Variety	1	9	0.02	0.925
(b)	**Numerator df**	**Denominator df**	**Variance ratio**	**P-value**
Variety	1	8	5.54	0.047
DTPA-Zn	1	8	5.52	0.046
Variety•DTPA-Zn	1	8	5.80	0.043

df, degrees of freedom; AEZ, Agroecological zone.

lacking deliberate selection for micronutrient density, local varieties often accumulated equal or higher grain Zn concentrations, and their performance was largely independent of soil Zn availability. This suggests the presence of adaptive traits such as greater root proliferation, exudation of Zn-mobilizing organic acids, and enhanced symbioses with arbuscular mycorrhizae and rhizobacteria (Huang et al., 2020; Khoshgoftarmanesh et al., 2009; Ryan et al., 2001). These traits, shaped by long-term adaptation to marginal soils, represent valuable resources for breeding. Integrating them into biofortified genetic backgrounds could improve resilience and ensure nutritional gains are maintained even under deficient soils.

A further consideration is the role of seed systems. In decentralized or informal seed networks, trait dilution during multiplication or local adaptation of the biofortified line may reduce its intended nutritional effect (Huertas et al., 2022). Strengthening certified seed production, incorporating phenotypic validation, and providing farmer training on agronomic requirements could help safeguard the nutritional integrity of biofortified varieties as they move through local seed systems.

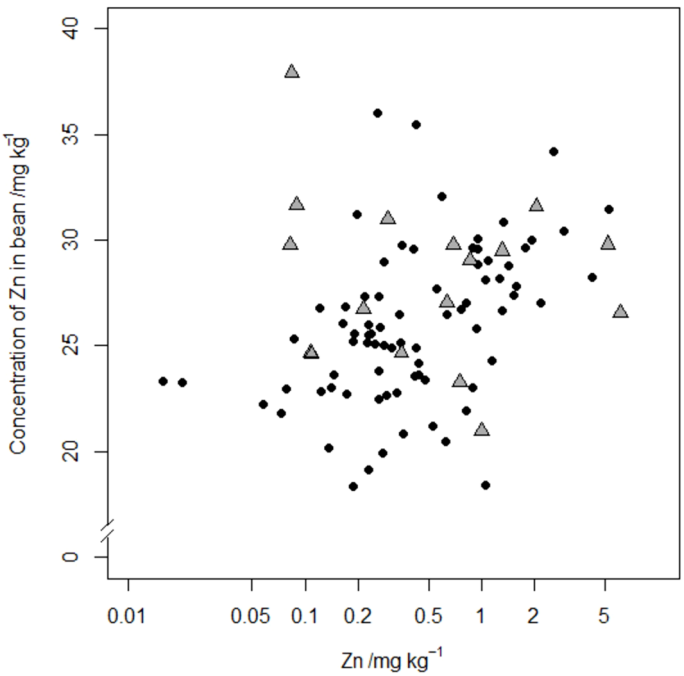

FIGURE 2
Plot of bean grain Zn concentration against the concentration of DTPA-extracted Zn in the corresponding soil sample (logarithmic scale) for NUA45 samples (n = 79, solid circles) and local bean varieties (n =17, grey triangles). The regression coefficients in the mixed model for the soil Zn effect, and 95% confidence limits, are 0.74 ± 0.64 (NUA45) and 0.26 ± 1.72 (Local varieties).

Translating these insights into practice requires integrated interventions that align genetic and agronomic innovations. Evidence from cereals and legumes shows that Zn-enriched fertilizers, foliar sprays timed at reproductive stages, seed priming, and microdosing can markedly improve grain Zn when bundled with biofortified varieties (Cakmak et al., 2010; Joy et al., 2015; Manzeke et al., 2017). However, our findings also highlight a delivery dilemma for policymakers. Realizing nutritional benefits requires either targeting biofortified varieties to soils with adequate Zn, which necessitates reliable and affordable soil testing, or investing in the development of genotypes that maintain grain Zn concentration under deficiency. Both strategies demand coordinated support, infrastructure, and time, underscoring the need for policies that link breeding programs with national soil information systems and extension networks. Both strategies demand coordinated support, infrastructure and time. This calls for policies that link breeding programs with soil information systems and extension networks to ensure that genetic gains translate into nutritional outcomes for population masses in sub-Saharan Africa.

5 Study limitations and recommendations

While this study offers novel and robust field evidence, it is bounded by its single-season scope and modest sampling of local varieties. Future work should prioritize multi-season, multi-location trials to capture genotype × environment interactions more fully and to elucidate the physiological and genetic mechanisms underpinning Zn loading in both biofortified and indigenous germplasm. This would enable breeding programs to balance nutritional targets with adaptive traits, ensuring that biofortification strategies are not only nutritionally effective but also resilient, adoptable, and scalable under real-world conditions. Efforts should also focus on developing and testing integrated strategies that strengthen Zn biofortification outcomes. These could include improving soil fertility management and optimizing Zn fertilizer application. Such combined interventions would help translate research findings into more reliable and farmer-relevant solutions for smallholder production systems.

6 Conclusion

This study demonstrates that the nutritional benefits of genetic biofortification are conditional rather than intrinsic, with the expression of high-Zn traits in NUA45 strongly constrained by soil micronutrient availability. The clear genotype × soil Zn interaction shows that biofortified beans can only realize their nutritional advantage when micronutrient supply is sufficient. Under the widespread Zn-deficient conditions observed in Malawian smallholder fields, NUA45 frequently failed to achieve its breeding target, while local varieties often matched or exceeded its performance through adaptive traits that buffer against nutrient limitations. These findings challenge the assumption of universal efficacy in biofortification and highlight the risk of overestimating its impact when evaluated only under research-station or moderately fertile conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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