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Iron Content of Market-Identified and ISABU-Recommended Biofortified Common Bean (*Phaseolus vulgaris*) Varieties, before and after Cultivation in Ruyigi Province, Burundi: A Comparative Agronomic Evaluation

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Abstract

Background: Iron deficiency is a leading cause of childhood anemia worldwide and in Burundi. Biofortifying staple crops such as common beans (*Phaseolus vulgaris*) can increase daily iron intake. Butezi District, which has one of the highest rates of chronic malnutrition in Ruyigi Province, where childhood anemia affects 71%, illustrates this issue. Iron levels in a bean variety at harvest depend on genetic traits, farming practices, and soil conditions, highlighting the need to understand how soil-related factors influence iron content. **Objective:** This research aimed to evaluate the iron content of four locally identified biofortified bean varieties available in the market and compare them with four official ISABU varieties suited to the cultivation and climate of the Butezi health district. We compared them before and after cultivation under consistent field conditions within the study area. **Methods:** We collected and analyzed soil samples before planting. At ISABU, laboratory tests on seeds from eight varieties determined initial iron levels. We grew four of these varieties (the ISABU-recommended group) at four experimental hills, with one plot per variety at each hill, and reevaluated them after harvest. We selected the four market-identified varieties through a prior community survey in which households named the iron-biofortified varieties they recognized, followed by visits

to the markets most commonly used to purchase these varieties for laboratory analysis. Because we could not independently verify biofortified status for these seed lots, we describe them throughout as market-identified rather than confirmed biofortified varieties. We then compared iron content within each variety before and after cultivation, and between the two groups. **Results:** The four ISABU-recommended varieties in the study area had an average iron content of 115.0 mg/kg, more than twice the 53.1 mg/kg found in the four market-identified biofortified varieties. After one cultivation cycle across four soil types in Butezi, iron content decreased in 15 of 16 variety-by-soil combinations, with a mean decrease of 51.3% across these combinations (ranging from −83.7% to +57.1%); computed directly from the overall group means, the decrease was 57.9% (from 115.0 to 48.4 mg/kg). The final average was 48.4 mg/kg, below the initial levels of the market-identified varieties. Iron retention was highest in clayey soil (average: 60.3 mg/kg), while stony soil showed the lowest (37.0 mg/kg), despite having the highest total iron and phosphorus contents. Only the Kaneza variety showed an increase in iron content, specifically in clayey soil (+57.1%). Changes in zinc, calcium, and protein levels after cultivation were minimal. **Conclusion:** The anticipated genetic iron benefits of ISABU biofortified beans were inconsistent under current farming conditions in Butezi. Instead, soil type, particularly the contrast between clayey soils and phosphorus-rich stony soils, was more strongly associated with the extent to which these benefits were expressed at harvest than variety choice. Because each hill represented a single soil type, this association cannot be statistically separated from other hill-specific conditions. These results suggest that promoting iron-biofortified beans in Ruyigi should be informed by further, better-replicated soil-specific trials to confirm whether genetic traits can be realized more consistently. The observed benefit on clayey soil in Kaneza is based on a single measurement and should be considered preliminary (pilot) data, requiring further validation through additional trials.

Keywords

Biofortification, Iron Content, Common Bean, *Phaseolus vulgaris*, Soil Iron, Burundi, Child Nutrition

1. Introduction

Iron deficiency remains one of the most common micronutrient deficiencies worldwide and is the leading cause of anemia among young children. Although it has declined globally over the past thirty years, children under five in low- and middle-income countries, particularly in sub-Saharan Africa, continue to bear the heaviest burden [1] [2]. In children, iron deficiency can hinder cognitive development, slow physical growth, and lead to long-term human capital losses [3] [4]. Therefore, international guidelines stress the importance of interventions targeting young children [5] [6]. Often linked to other micronutrient deficiencies, it is

part of the broader issue of hidden hunger, which affects nearly two billion people globally. This deficiency commonly coexists with stunting and increasingly with overweight within the same populations and households [7] [8]. Zinc deficiency, closely related to iron deficiency, further complicates this challenge, particularly in countries like Burundi, with limited progress expected in the next decade [9] [10]. Repeated national surveys show high rates of anemia and stunting among children in Burundi [11]-[13], underscoring the urgent need for nutritional interventions to address these issues.

Food-based approaches are important, sustainable alternatives to supplementation and fortification, particularly in rural areas where access to processed foods and health care is limited. Biofortification increases the micronutrient content of staple food crops primarily through two approaches: agronomic biofortification, which applies mineral fertilizers during cultivation to enhance nutrient uptake, and genetic biofortification, which uses conventional plant breeding techniques to improve micronutrient content.

This latter strategy has been widely used for staple crops such as legumes and cereals distributed through international programs such as HarvestPlus, yielding richer and more cost-effective gains in dietary iron, zinc, and vitamin A in many countries.

Nevertheless, the genetic potential observed under controlled breeding conditions does not always translate into comparable nutritional benefits when crops are grown in typical farm soils [14].

The gap between genetic potential and actual crop performance is widening as climate variability reduces the micronutrient content of staple food crops, a trend influenced by both pedological factors and plant-specific physiological processes. This highlights the need for strategies to verify nutrient content under real-world cultivation conditions, rather than relying solely on breeding data [15].

Common bean (*Phaseolus vulgaris*) is the most widely cultivated and consumed legume worldwide. It plays an essential role in the diet and provides significant nutritional benefits in the Great Lakes region, where per capita consumption is high [14] [16] [17]. The region features a rich diversity of local varieties and breeding lines, with considerable variation in seed iron and zinc content, making it a prime target for biofortification efforts.

These initiatives have evolved into genetic screening programs in West Africa and Latin America [18] [19]. Conventional breeding has produced iron-rich bean varieties now available from the national research institution, ISABU, in Burundi [20] [21].

Bean seed iron content is influenced by genetics as well as agronomic and nutritional factors, underscoring the need for local testing. Variety selection can increase seed iron content by 60% - 80% compared with unselected varieties [14]. However, iron content varies considerably by genotype, soil quality, and location, and interactions among these factors can produce variation comparable to genetic differences [22] [23].

Soil characteristics such as pH, organic matter, and iron availability at planting determine how much genetic iron potential is expressed in the final seed [24] [25].

A variety selected for high iron content under controlled research conditions may perform differently across soil types. In addition, biofortified seeds sold in informal local markets, often several generations removed from certified breeding stock, may not retain the same iron content as the currently recommended ISABU varieties.

Thus, a chain runs from soil to nutrition, in which agricultural practices beyond the simple choice of cultivated varieties influence how a crop's genetic potential translates into tangible benefits for consumers [26]. The nutritional outcome depends not only on iron content but also on antinutritional factors such as phytic acid and polyphenols, which are naturally present in the seed coat and cotyledons. These substances can markedly reduce iron absorption, even when iron content is accurately measured [16] [27] [28].

Furthermore, agronomic and compositional characteristics, such as cooking time and drought tolerance [29] [30], influence a variety's adoption and use within households. Thus, the actual nutritional benefit of biofortification depends on a variety's genetic iron potential, growing conditions, and postharvest bioavailability.

This finding aligns with broader evidence from subsistence farming households in rural Burundi, where child undernutrition is more closely linked to limited dietary diversity and low nonfarm income than to agricultural production alone [31] [32]. This underscores the importance of assessing whether available and consumed biofortified beans can improve household iron intake, given local soil and ecological conditions.

This research had two main objectives. First, to compare the initial iron content of four locally available iron-biofortified bean varieties identified by the study population with four biofortified varieties on the official ISABU list that are adapted to the agroecological conditions of the study area. Second, to assess whether iron content remains stable, increases, or decreases after a full cultivation cycle on local soils, taking into account the soil iron status before planting.

2. Methodology

2.1. Study Design and Setting

We conducted fieldwork in Butezi Health District, mapping soil types across the hills of Butezi Commune. We randomly selected four hills (Mubira, Sorero, Bwagiriza, and Rubaragaza) that spanned the four soil types, reflecting the diversity of local farming conditions. Each hill represented a single soil type: loamy, stony, sandy, or clayey.

2.2. Bean Variety Selection

Eight bean varieties were selected and divided into two groups of four, each linked to iron-biofortified beans but originating from different sources. The first group, Sinamakamwe, Mukungugu, Kinure, and Mukutsapantalon, comprises the varie-

ties most frequently cited by study participants during community data collection. These well-known, locally available varieties served as reference points, representing the biofortified beans that households most often report as available in markets. This first group was identified through a two-step process: a prior community survey in which households in the study area were asked to name the iron-biofortified bean varieties they recognized and most commonly purchased, followed by a field visit to the markets most frequently used by these households to source and purchase the corresponding seed lots for laboratory analysis. Because this identification relied on farmer and vendor recognition rather than genetic testing or certification records, we could not independently confirm the biofortified status of these four varieties; we therefore refer to them throughout this article as market-identified varieties rather than confirmed biofortified varieties.

We selected the second group, Mugwiza, Rufutamadeni, Musore, and Kaneza, from ISABU's official list of iron-biofortified varieties recommended for the study area. We obtained seeds from ISABU, then analyzed, planted, harvested, and re-analyzed them to track iron content throughout the cultivation cycle.

2.3. Soil Sampling and Analysis

Before planting, we collected soil samples from four experimental hills: Mubira, Sorero, Bwagiriza, and Rubaragaza. ISABU analyzed these samples to determine total iron and key physicochemical properties, including total nitrogen, available phosphorus, and exchangeable calcium and potassium. The analysis characterized the four soil types present across the hills: loamy, stony, sandy, and clayey.

At each hill, one composite topsoil sample was submitted for analysis. Analyses were performed at the ISABU Laboratoire d'Analyse des Sols et Produits Agro-Alimentaires (LASPA), which is accredited to ISO 17025:2017 for a defined scope of tests, using the following validated methods: total nitrogen by a modified Kjeldahl procedure; available phosphorus by the Olsen method (extraction with NaHCO3, UV-spectrophotometric determination, ISO 11263); exchangeable calcium and potassium by ammonium acetate extraction with flame atomic absorption spectrometry (AAS, NF X 31-108); and total iron by acid (aqua regia) digestion followed by flame AAS (NF EN 16174/XP CEN/TS 16188).

2.4. Initial Seed Analysis

Before sowing, we analyzed seed samples from the eight varieties in the laboratory to determine iron, zinc, calcium, and crude protein content, establishing a baseline for comparison with postharvest data. For each variety, we analyzed a single composite seed sample at the same ISABU/LASPA laboratory. We determined total iron and zinc by acid digestion followed by flame AAS, under the laboratory's ISO 17025:2017 accreditation for total mineral content in agro-food matrices; we estimated crude protein from total nitrogen (modified Kjeldahl method) using the conventional nitrogen-to-protein conversion factor for legumes (N × 6.25). The ± values accompanying iron and zinc results (a constant ±6.22 mg/kg for iron and

±0.77 mg/kg for zinc across all samples) correspond to the standard measurement uncertainty of the accredited analytical method, as reported by the laboratory, rather than to biological or analytical replicates: each variety, and each variety-by-soil combination at harvest, was represented by a single composite sample, with no replicate analyses performed.

2.5. Field Trial and Planting

The four ISABU-recommended varieties were cultivated at each of the four experimental hills (Mubira, Sorero, Bwagiriza, and Rubaragaza) under uniform agronomic practices (land preparation, planting density, and management calendar) applied consistently across sites. At each hill, one plot was established per variety, yielding sixteen variety-by-soil experimental units in total (four varieties × four hills). There was no plot replication within a hill and no randomization of plot position within sites, consistent with the pilot, single-cultivation-cycle nature of this study. Each hill therefore represents both a distinct soil type and a distinct set of site-specific conditions (management history, microclimate, topography).

2.6. Postharvest Sampling and Analysis

At harvest, we collected seed samples from the four iron-biofortified varieties separately from each of the four experimental hills. We sent these samples to the ISABU laboratory for analysis of iron, zinc, calcium, and crude protein content. To ensure consistency, we applied the same analytical methods used in the initial analysis to the postharvest analysis. This postharvest evaluation focused primarily on the ISABU-recommended group to assess whether its genetic iron advantage persists under real-world cultivation conditions. As in the initial seed analysis, each variety-by-soil combination at harvest was represented by a single composite sample, which was analyzed once using the methods described in Section 2.4.

2.7. Data Analysis

Iron, zinc, calcium, and protein concentrations were assessed for each biofortified variety, comparing levels before planting (initial seed) and after harvest across the four soil types. Descriptive statistics, including means, percentage changes from baseline, and ranges, were calculated for each variety-by-soil combination. We examined these results alongside the physicochemical properties of each soil to assess differences in iron retention among soils. Given the limited number of soil types and the absence of biological replication, the study is essentially descriptive, and we did not perform inferential statistical tests. Two complementary metrics of change are reported and used consistently throughout this article: 1) the mean of the sixteen individual variety-by-soil percentage changes from baseline (a combination-level average, giving equal weight to each combination), and 2) the percentage change computed directly from the overall group means before and after cultivation (a pooled-mean average). These two metrics differ in value because they weight the underlying data differently; both are reported wherever a percent-

age change is discussed to avoid ambiguity.

2.8. Ethical Considerations

This research was conducted after approval from the national ethics committee (CNE/14/2023 and CNE/30/2024).

3. Results

3.1. Soil Types and Physicochemical Characteristics

The four hills studied in Butezi commune were classified into four main soil types, with their distribution detailed in Table 1. The hills selected for the cultivation trial, all located in Butezi, each represent one of these soil types, enabling comparison of the four biofortified varieties across different pedological conditions.

Table 1. Distribution of soil types across the studied hills of Butezi commune, former Ruyigi province.

Commune	Hill	Soil type
Butezi	Mubira	Loamy
	Sorero	Stony
	Bwagiriza	Sandy
	Rubaragaza	Clayey

Physicochemical analysis of the four soil types (Table 2) shows differences in total iron and available phosphorus. Stony soil had the highest levels of total iron (209 mg/kg), available phosphorus (44.2 mg/kg), and exchangeable calcium and potassium. In contrast, clayey soil had the lowest total iron (37.9 mg/kg), although it had similar nitrogen content and higher calcium content than sandy and loamy soils.

Table 2. Physicochemical characteristics of the four soil types identified across the experimental hills.

Soil type	Nitrogen (%)	Available P (mg/kg)	Exchangeable Ca (cmol/kg)	Exchangeable K (cmol/kg)	Total Fe (mg/kg)
Loamy	0.120	<0.04	1.87	0.468	52.5
Clayey	0.133	7.85	3.61	0.675	37.9
Sandy	0.127	0.434	1.44	0.242	60.1
Stony	0.244	44.2	5.27	0.973	209

3.2. Initial Nutritional Content (Before Planting) of the Eight Bean Varieties

At baseline, the four ISABU-recommended varieties had a mean iron content

more than twice that of the four commonly available market-identified varieties (115.0 mg/kg, ranging from 74.5 to 190 mg/kg, versus 53.1 mg/kg, ranging from 50.9 to 55.9 mg/kg), reflecting targeted genetic improvement. This iron advantage was specific, as the mean initial zinc content of market-identified varieties (27.3 mg/kg) was slightly higher than that of ISABU-recommended varieties (21.4 mg/kg). Similarly, market-identified varieties had higher initial calcium and crude protein content (327.2 mg/kg and 19.5%) than ISABU-recommended varieties (196.8 mg/kg and 17.4%). Table 3 presents complete initial data for the eight varieties.

Table 3. Initial (pre-planting) iron, zinc, calcium, and crude protein content of the four ISABU-recommended biofortified varieties and the four market-identified varieties.

Variety	Group	Fe (mg/kg)	Zn (mg/kg)	Ca (mg/kg)	Protein (%)
Mugwiza	ISABU recommended	190 ± 6.22	23.5 ± 0.77	282	15.2
Rufutamadeni	ISABU recommended	74.5 ± 6.22	16.5 ± 0.77	166	14.5
Musore	ISABU recommended	106 ± 6.22	26.1 ± 0.77	172	20.0
Kaneza	ISABU recommended	89.6 ± 6.22	19.4 ± 0.77	167	19.9
Sinamakamwe	Market identified	55.9 ± 6.22	24.9 ± 0.77	248	18.2
Mukungugu	Market identified	53.1 ± 6.22	28.4 ± 0.77	240	23.1
Kinure	Market identified	52.5 ± 6.22	24.1 ± 0.77	412	17.6
Mukutsapantalon	Market identified	50.9 ± 6.22	31.9 ± 0.77	409	19.0

3.3. Postharvest Nutritional Content of Biofortified Varieties by Soil Type

After a single cultivation cycle, iron content declined in 15 of the 16 variety-by-soil combinations. The mean decrease across these sixteen combinations was 51.3% (ranging from −83.7% to +57.1%); computed directly from the overall group means, the group means fell from 115.0 mg/kg to 48.4 mg/kg, a decrease of 57.9%. This reduction brought the postharvest iron content of the ISABU-recommended group below the initial mean of 53.1 mg/kg for market-identified varieties.

Mugwiza, which had the highest initial iron content, showed the sharpest decline across all four soils, decreasing by an average of 81.2%, from 190 mg/kg to 35.6 mg/kg. Kaneza was the only variety to increase iron content, and only on one soil, gaining 57.1% on clayey soil (from 89.6 to 140.8 mg/kg), although it lost iron on the other three soils.

Stony soil had the highest total iron and phosphorus content among the four types, whereas clayey soil had the lowest. Zinc and crude protein content showed minimal variation after harvest, with mean increases of 4.7% and 1.3%, respectively. Calcium content increased by an average of 26.4%. Table 4 and Table 5 indicate the content of iron, calcium, zinc and proteins after harvest.

Table 4. Postharvest iron content of the four ISABU-recommended biofortified varieties for each of the four experimental soil types, and percentage change from baseline.

Variety	Hill	Soil type	Fe before (mg/kg)	Fe after (mg/kg)	ΔFe (%)
Mugwiza	Bwagiriza	Sandy	190	32.9	−82.7
Mugwiza	Rubaragaza	Clayey	190	30.9	−83.7
Mugwiza	Sorero	Stony	190	39.9	−79.0
Mugwiza	Mubira	Loamy	190	38.8	−79.6
Rufutamadeni	Bwagiriza	Sandy	74.5	32.8	−56.0
Rufutamadeni	Rubaragaza	Clayey	74.5	30.3	−59.3
Rufutamadeni	Sorero	Stony	74.5	40.1	−46.2
Rufutamadeni	Mubira	Loamy	74.5	30.5	−59.1
Musore	Bwagiriza	Sandy	106	35.4	−66.6
Musore	Rubaragaza	Clayey	106	39.1	−63.1
Musore	Sorero	Stony	106	33.3	−68.6
Musore	Mubira	Loamy	106	77.2	−27.2
Kaneza	Rubaragaza	Clayey	89.6	140.8	+57.1
Kaneza	Sorero	Stony	89.6	34.9	−61.0
Kaneza	Mubira	Loamy	89.6	53.0	−40.8
Kaneza	Bwagiriza	Sandy	89.6	84.4	−5.8

Table 5. Postharvest zinc, calcium, and crude protein contents of the four ISABU-recommended biofortified varieties across the four experimental hills.

Variety	Hill	Zn after (mg/kg)	Ca after (mg/kg)	Protein after (%)
Mugwiza	Bwagiriza	18.8	285	14.7
Mugwiza	Rubaragaza	23.2	205	20.8
Mugwiza	Sorero	20.2	246	21.3
Mugwiza	Mubira	19.1	270	13.9
Rufutamadeni	Bwagiriza	23.2	310	16.4
Rufutamadeni	Rubaragaza	19.0	260	15.9
Rufutamadeni	Sorero	21.4	253	19.1
Rufutamadeni	Mubira	22.1	210	16.2
Musore	Bwagiriza	25.8	231	19.9

Continued

Musore	Rubaragaza	20.2	185	16.4
Musore	Sorero	20.2	271	15.4
Musore	Mubira	22.4	206	17.6
Kaneza	Rubaragaza	22.3	234	16.2
Kaneza	Sorero	23.9	216	22.2
Kaneza	Mubira	21.3	270	13.9
Kaneza	Bwagiriza	23.6	156	16.0

Overall, these results suggest that the initial genetic iron advantage of ISABU biofortified varieties was not consistently maintained after one cultivation cycle under current conditions in Butezi. This change varied by soil type and variety.

4. Discussion

This study aimed to assess variation in the iron content of biofortified beans identified as suitable for local cultivation by measuring seed iron content before planting and after harvest, and by analyzing soil samples from each cultivation site. This approach allows the genetic potential of each variety to be distinguished from its actual agronomic performance in Butezi commune, Ruyigi province.

The initial comparison shows that ISABU-recommended varieties had a mean iron content before planting that was more than twice that of popular market-identified varieties. This suggests that biofortified seeds sold in local markets, several generations removed from certified breeding sources, may have lower iron content than the original levels.

This trend aligns with known gaps in seed quality and varietal replacement in Burundi's bean seed systems [33].

In fifteen of the sixteen variety-by-soil combinations tested, iron content declined. The mean decrease was 51.3% when averaged across the sixteen combinations and 57.9% when computed from the overall group means. This decline was sufficient to reduce the mean postharvest iron content of the ISABU-recommended group (48.4 mg/kg) below the initial mean of 53.1 mg/kg for market-identified varieties.

Mugwiza, which initially had the highest iron content, showed the sharpest decline, losing more than 75% of its initial advantage across all tested soils. Kaneza increased its iron content primarily in clayey soil but lost iron in the other soil types. This suggests that pedological and agricultural conditions may limit iron accumulation during growth, independent of genetic potential [24] [25]. However, because soil type in this pilot trial is confounded with hill-specific management and microclimate, this association cannot be fully separated from other site-level factors.

These findings, in which the postharvest iron content of ISABU-recommended

varieties approaches or falls below that of market-identified varieties, align with other East African studies showing that the iron content of biofortified and non-biofortified varieties tends to converge under local conditions [34]. This suggests that the iron content of biofortified seeds may decline in markets as seed stock becomes increasingly removed from certified breeding cycles.

Soil-level analysis shows that postharvest iron content was lowest in stony soil, even though stony soil had the highest overall iron and phosphorus content among the four samples. Clayey soil had the highest iron concentrations despite its lower total iron content. This indicates that total soil iron alone does not determine the iron available to the plant. A pattern consistent with published evidence is that high soil phosphorus can reduce iron availability to the plant by forming poorly soluble iron phosphates [27]; however, this mechanism was not directly tested in the present study, since soil pH, organic matter, and extractable iron were not measured. It should therefore be regarded as a plausible explanation rather than a demonstrated cause. Soils are known to differ not only in iron content but also in physicochemical properties that affect iron retention [25].

Consequently, the choice of cultivated varieties should account for these soil-specific characteristics; targeted soil management strategies, such as addressing phosphorus availability, would need to be tested directly in future trials before recommendation.

Iron loss varied by variety regardless of soil type, ranging from 55.1% in Rufuta-madeni to 81.2% in Mugwiza. The notable increase observed in Kaneza on clayey soil suggests that genotype-by-environment interactions, already documented for bean seed iron and zinc, can be substantial enough to markedly alter expected outcomes [22] [23]. Given the single-hill-per-soil-type design of this pilot study, confirm this observation in trials with multiple hills per soil type before generalizing.

Current understanding of how soil components affect iron retention and bioavailability can explain these soil-related differences. Nitrogen availability is a key driver of protein accumulation in common bean seeds, often independently of iron content [25]. This aligns with the higher crude protein content observed in more fertile soils [35].

In contrast, phosphorus interacts differently with iron: high soil phosphorus levels are reported to promote the formation of poorly soluble iron phosphates in the root zone, thereby reducing the amount of iron available for uptake [27]. This mechanism offers one plausible explanation, among others, for the reduced iron retention observed in phosphorus-rich stony soil [27]; however, it remains a hypothesis rather than a demonstrated cause in this study, since we did not measure soil pH, organic matter, or extractable iron.

Calcium and potassium interact with iron and zinc differently depending on soil type. They compete with zinc and, to a lesser extent, with iron for root uptake [36]. Potassium increases zinc uptake, whereas calcium can decrease it [36].

In addition, calcium influences iron absorption in humans: dietary calcium

generally inhibits intestinal absorption of both heme and nonheme iron, likely by disrupting iron transport across the intestinal mucosa [37]. Consequently, a variety's calcium content may reduce iron availability when both nutrients are consumed in the same meal [37].

Zinc in bean seeds affects bioavailability more than retention. Iron and zinc are thought to compete for similar or overlapping intestinal absorption pathways, including binding sites in the mucus layer that influence how efficiently trace elements reach enterocytes [38]. Consequently, a bean variety rich in both iron and zinc may not deliver proportional nutritional benefits for either mineral.

The importance of crude protein for iron absorption depends on its source and amount. While some animal proteins promote nonheme iron absorption, legume proteins, such as those in common beans, often contain phytic acid, the main antinutrient that limits iron bioavailability in beans, rather than the protein itself [16] [28].

These element-specific roles suggest that soil management aimed at strengthening iron biofortification in beans in Ruyigi province may need to consider phosphorus and calcium, among other factors. Furthermore, increasing seed iron content alone does not guarantee increased iron absorption unless zinc, phytic acid, and overall dietary factors are also managed. These soil-nutrient interactions are proposed explanatory mechanisms from the broader literature; the present study did not measure the additional soil parameters (pH, organic matter, extractable iron) needed to test them directly and therefore cannot establish which mechanism, if any, accounts for the differences observed here.

Under optimal conditions, a combination of genetic potential, favorable agronomic characteristics, and market access can benefit farmers [7] [39]-[41].

The main advantage of iron-biofortified beans is their higher seed iron content, which can improve iron status and, if iron is preserved through harvest and cooking, may enhance cognitive function. This approach is also highly cost-effective compared with other public health strategies [7] [42]-[45]. However, benefits do not depend solely on selecting the right variety. Our results show that, without adequate soil management, genetic iron advantages can be substantially reduced before consumption.

Varieties must be adapted to local soils, where their characteristics are more likely to be preserved [7] [39] [41]. Multistakeholder partnerships improve access to quality seed and accelerate the adoption of improved varieties [33].

Nutrition-sensitive agricultural strategies, such as biofortification, are most effective when paired with comprehensive policies that strengthen market access, dietary diversity, and health systems [46].

4.1. Strengths of the Study

The paired before-after design, applied to the same varieties across four distinct soil types, allowed us to distinguish each variety's genetic potential from its actual agronomic performance, a distinction that single-time-point comparisons cannot

establish. In addition, analyzing four nutrients (iron, zinc, calcium, and crude protein), rather than iron alone, revealed that nutritional erosion was specific to iron and did not indicate a broader decline in seed nutritional quality.

The study highlighted a link between soil fertility and seed protein content, a finding not apparent from iron content data alone. It examined both a market-identified group and an ISABU-recommended group, focusing on the seed varieties households typically encounter rather than assuming that certified breeding material is the same as locally available seed.

4.2. Limitations of the Study

Because this study was conducted over a single cultivation cycle, with a limited number of plots per soil type and no biological replication within soils, the findings' applicability to other seasons, soil types, and climates in Ruyigi province is limited. Because a single hill represented each soil type, soil-type effects cannot be statistically separated from other hill-specific factors, including management history, microclimate, and topography; findings described in this article as soil-associated should therefore be interpreted as hill-level associations, pending confirmation across multiple hills for each soil type. The isolated increase in iron content observed in Kaneza on clayey soil should be considered preliminary rather than conclusive.

Variations in microclimate, water availability, and plot management, even within the same site, may have affected iron accumulation independently of soil type. Laboratory data reflect total seed iron content rather than its bioavailable form. Postharvest analysis was limited to the ISABU-recommended group, precluding direct comparison with market-identified varieties. The biofortified status of the four market-identified varieties could likewise not be independently verified. Further trials across seasons and sites, with biological replication, randomized plot allocation, and paired bioavailability assessments, are needed to confirm whether the observed trends, particularly the potential advantage of clayey soil, hold.

4.3. Future Research Directions

A more detailed physicochemical analysis of the soil-plant system is needed, including measurements of soil pH and organic matter content, both of which influence the availability of iron and phosphorus.

Conducting before-and-after-harvest comparisons over at least two additional cultivation seasons, including more hills per soil type across the district, with randomized plot allocation and biological replication for each soil type, would enable a better assessment of the consistency of the clayey-soil association and of Kaneza's specific results, and would allow soil-type effects to be statistically distinguished from hill-specific conditions.

Combining agronomic iron measurements with bioavailability testing, such as in vitro Caco-2 digestion models or human absorption studies, could help determine the proportion of iron actually absorbed, accounting for antinutritional

compounds and mineral interactions.

Genetic analyses of well-known market varieties using genome-wide association methods can determine whether their reduced initial iron levels are due to genuine varietal traits, genetic drift during informal seed multiplication, or prior exposure to iron-deficient soils. Such analyses could also officially verify the biofortified status of seed lots identified in the market.

In addition, a complementary study examining economic and behavioral factors, such as farmers' fertilizer use, seed sourcing, and openness to soil-adapted varieties, would help translate these agronomic findings into practical, evidence-based recommendations for households in Ruyigi province.

5. Conclusions

This study compared four ISABU-recommended iron-biofortified bean varieties with four popular market-identified varieties, before and after one cultivation cycle, across four soil types in Butezi. The initial ISABU advantage in iron, more than twice that of the market-identified varieties, decreased by an average of 51.3% across the sixteen variety-by-soil combinations after cultivation (57.9% when computed from the overall group means), falling below market levels.

Soil type was more strongly associated with this pattern than variety identity: clayey soils showed the best retention and the only net gain (Kaneza), while stony soils, despite high total iron and phosphorus content, showed the lowest retention. This pattern is consistent with a possible role of phosphorus in limiting iron availability, though this was not directly tested in the present study and each soil type was represented by only one hill.

No variety proved universally optimal: Kaneza showed the most consistent iron retention, with a net gain on clayey soil; Mugwiza showed the sharpest decline; and Musore and Rufutamadeni showed intermediate, soil-dependent profiles.

These patterns indicate that soil type, rather than variety choice alone, is a factor in whether the genetic iron advantage of ISABU-recommended varieties is expressed at harvest. On clayey soil, such as at Rubaragaza, Kaneza showed the best iron retention in this pilot trial and warrants further, better-replicated testing; on phosphorus-rich stony soil, such as at Sorero, none of the four ISABU varieties retained a majority of their initial iron content. Because this study did not test soil amendments or fertilizer regimens, we do not make specific fertilizer recommendations here; rather, we recommend that future, adequately replicated and randomized trials evaluate whether targeted soil management (for example, addressing phosphorus availability on stony soils) can improve iron retention. A soil-differentiated approach to variety promotion, together with strengthening the formal seed system, should guide further investigation of iron-biofortified beans in Butezi, complemented by additional multiseason, replicated trials.

Author Contributions

Belyse, M. conceived and designed the study, participated in field data collection,

performed the statistical analysis, interpreted the results, and drafted the manuscript. Mbazi, S., Juma, K., Sayoki, M., Sévérin, S., Caleb S. and Jonathan, N. supervised the study throughout its conduct, including the quality of data collection and analysis, and critically reviewed and revised the manuscript for important intellectual and scientific content. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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