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Pulse Biofortification for Nutritional Security: A Critical Narrative Review of Biological Pathways, Bioavailability and Delivery

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Micronutrient deficiencies persist where affordable diets provide insufficient quantities of bioavailable minerals and vitamins. Pulses are strategically important targets for biofortification because they combine protein, dietary fibre and micronutrients with high consumption in many low- and middle-income settings. Their value, however, is constrained by a recurrent disconnect between increased seed nutrient concentration and demonstrated nutritional benefit. This critical narrative review evaluates pathways by which pulse biofortification can contribute to nutritional security, with emphasis on common bean, lentil, chickpea, field pea, mungbean and cowpea. Literature published from 2000 to 19 June 2026 was prioritised, with earlier seminal evidence retained when necessary. Genetic, genomic, agronomic, processing, bioavailability, human efficacy and delivery evidence was integrated rather than assessed as isolated technical domains. The strongest evidence supports heritable variation in iron and zinc concentration across several pulse species, substantial genotype-by-environment effects, and agronomic responsiveness of zinc, iron and selenium under appropriate soil or foliar management. Yet total seed concentration is an incomplete endpoint because phytate, polyphenols, mineral speciation, cooking losses and food-matrix effects alter the amount ultimately available for absorption. Human evidence is markedly uneven. Iron-biofortified common bean has progressed furthest from breeding through controlled feeding trials, with improvement in iron status and associated functional outcomes in Rwandan women, while comparable efficacy evidence for lentil, chickpea, mungbean, cowpea and pea remains limited. Delivery studies further show that seed access, agronomic performance, sensory acceptance and market diffusion determine whether nutritional traits reach habitual diets. The review therefore proposes a chain-of-evidence perspective in which nutritional security requires simultaneous success in crop performance, nutrient density, retention, bioavailability, consumption and population reach. Future programmes should breed for bioavailable nutrient delivery rather than concentration alone, validate stability across target environments, embed processing and human absorption endpoints earlier in selection, and evaluate adoption and equity at scale.

Keywords: Biofortification; pulses; iron; zinc; selenium; bioavailability; genotype × environment interaction; nutritional security.

1. Introduction

Nutritional security requires more than an adequate supply of dietary energy. It depends on reliable access to foods that deliver sufficient protein, vitamins and mineral elements in forms that can be absorbed and used physiologically. Biofortification addresses one component of this problem by increasing the concentration or bioavailability of target nutrients in edible crop tissues through plant breeding, biotechnology, agronomic management or combinations of these approaches. Unlike industrial fortification, the nutrient trait is generated within the crop production system and can recur with each harvest when seed and management pathways are sustained (Saltzman *et al.*, 2013; White & Broadley, 2009). The approach is therefore attractive for populations that consume substantial quantities of locally produced staples and have irregular access to commercially fortified foods or supplements. Its relevance should nevertheless be calibrated carefully: biofortification can improve the nutrient quality of habitual foods, but it cannot compensate for all determinants of malnutrition, including low total food intake, infection, poor sanitation, inequitable intra-household allocation or lack of dietary diversity (Bouis & Saltzman, 2017; Meenakshi *et al.*, 2010).

Pulses occupy a distinctive position within this strategy. In this review, pulses refer to dry edible seeds of grain legumes, with principal attention to common bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), mungbean (*Vigna radiata* (L.) R. Wilczek) and cowpea (*Vigna unguiculata* (L.) Walp.). These crops are already nutritionally dense relative to many cereal staples, providing protein, dietary fibre, iron (Fe), zinc (Zn), folate and other micronutrients. Their contribution can therefore be increased either by raising an already meaningful nutrient concentration or by improving the fraction that remains available after storage, processing and digestion. The same seed matrix that makes pulses valuable, however, complicates biofortification. Phytate and polyphenolic compounds can reduce non-haem Fe and Zn absorption, seed coat characteristics influence cooking and leaching, and mineral accumulation responds to genotype, soil chemistry and climate. Pulse biofortification is consequently not a simple exercise in maximising milligrams of nutrient per kilogram of dry seed (Jha & Warkentin, 2020; Kumar & Pandey, 2020). Recent multi-location lentil evidence has further shown that iron and zinc concentrations vary alongside phytic acid and protein traits, supporting simultaneous evaluation of nutrient density and factors relevant to mineral utilisation (Kumar *et al.*, 2024).

The technical literature has expanded along several partially disconnected lines. Genetic studies have identified substantial variation and quantitative loci for seed Fe and Zn in common bean and lentil, while agronomic experiments have demonstrated enrichment after soil or foliar application of Zn, Fe or selenium (Se) in lentil, pea and mungbean. Food-science studies have shown that cooking and compositional differences alter the bioavailable fraction. Human absorption and efficacy trials have provided a more demanding test of whether these improvements matter nutritionally. Importantly, the results are not uniformly supportive. Early stable-isotope studies with common bean showed that higher seed Fe did not necessarily produce greater absorbed Fe when inhibitory compounds remained high, whereas subsequent controlled feeding of iron-biofortified beans improved Fe status in women with low baseline stores (Haas *et al.*, 2016; Petry *et al.*, 2012). This apparent contradiction is instructive rather than problematic: it exposes the difference between nutrient concentration, fractional absorption, total absorbed dose and longer-term physiological response. Recent chickpea work has likewise demonstrated that mineral enrichment should be evaluated beyond total concentration, because selenium and zinc bioaccessibility changed following biofortification treatments (Espriu-Corella *et al.*, 2025). Similarly, biofortified cowpea cultivars have shown differences in in vitro and in vivo iron bioavailability associated with lower levels of mineral-complexing compounds, reinforcing the importance of downstream bioavailability assessment (Medeiros *et al.*, 2024).

Recent reviews have summarised pulse biofortification broadly, genetic biofortification in lentil, and Fe/Zn improvement in common bean and chickpea (Huertas *et al.*, 2023; Jha *et al.*, 2024; Jha & Warkentin, 2020; Kumar *et al.*, 2016; Kumar & Pandey,

2020). These syntheses establish the richness of germplasm and the rapid development of molecular tools, but crop- or technique-centred organisation can obscure the translational chain from breeding target to nutritional outcome. For nutritional security, a variety with a high laboratory mineral concentration is only a proximal success. It must retain the trait under target environments, maintain yield and seed quality, survive customary processing, deliver bioavailable nutrient in the meal, be eaten in sufficient quantities and reach nutritionally vulnerable consumers through functioning seed and food markets. Evidence becomes progressively thinner as one moves along this chain, particularly outside common bean. A 2025 synthesis of eastern African common bean breeding further emphasises the need to combine higher iron and zinc concentration with agronomic adaptation, bioavailability and cooking quality when evaluating biofortification progress (Kimani, 2025).

1.1 Scope, Research Gap and Objectives

This review examines pulse biofortification as an integrated pathway to nutritional security rather than as a catalogue of high-nutrient genotypes or fertiliser responses. The primary scope encompasses common bean, lentil, chickpea, field pea, mungbean and cowpea, with emphasis on Fe, Zn and Se and selective consideration of folate where pulse-specific evidence informs breeding potential. Soybean and groundnut are excluded because their dominant production and nutritional roles are those of oilseed legumes rather than pulses. The central gap addressed is the limited integration of crop genetics, environment, agronomy, processing, bioavailability, human efficacy and delivery within a single critical framework. The objectives are to evaluate the strength and limitations of the major biofortification pathways, determine where nutrient concentration is a valid proxy and where it is not, compare the maturity of evidence among pulse crops and nutrients, identify the bottlenecks that separate biological enrichment from nutritional impact, and define research priorities capable of moving pulse biofortification from promising traits to dependable population benefit.

2. Methods for Literature Selection

A critical narrative design was used because the review question spans heterogeneous evidence that cannot be reduced meaningfully to a single intervention, comparator or effect measure. The literature includes germplasm surveys, quantitative-genetic analyses, genotype-by-environment trials, fertiliser experiments, food-chemistry studies, in vitro bioavailability models, stable-isotope absorption studies, randomised feeding trials and adoption research. A systematic review or meta-analysis would require a narrower question and more uniform endpoints, whereas the present purpose is to examine causal and translational links across disciplines. Narrative synthesis nevertheless requires transparent searching, explicit selection and critical appraisal. The conduct and reporting principles were informed by methodological guidance emphasising clear justification, search transparency, appropriate referencing and critical scientific reasoning in narrative reviews (Baethge *et al.*, 2019; Green *et al.*, 2006).

2.1 Review Design and Rationale

The unit of synthesis was the evidence pathway rather than the individual study. Studies were interpreted according to what they could validly establish. For example, a multi-environment germplasm trial can support conclusions about genetic variability and environmental stability but cannot establish human efficacy; a stable-isotope meal study can estimate absorption from a specific meal but not long-term adoption; and an adoption study can illuminate delivery without demonstrating biological efficacy. This distinction was used to prevent evidence from an upstream stage being treated as proof of a downstream outcome.

2.2 Information Sources

Literature was searched in PubMed, Europe PMC, AGRIS, OpenAlex, OpenAIRE, the Directory of Open Access Journals and Semantic Scholar. Searches of authoritative institutional resources and backward and forward citation checking were used to verify programme context, identify linked efficacy or adoption studies and detect corrections or errata. Bibliographic details and Digital Object Identifiers (DOIs) of journal articles retained in the review were checked against authoritative records, DOI landing pages or indexed bibliographic records. Sources whose identity, peer-review status or relevance to the cited claim could not be verified were not retained.

2.3 Search Strategy and Search Period

The main search period was January 2000 to 19 June 2026, reflecting the period in which modern biofortification programmes, crop genomics and nutrition-linked breeding expanded substantially. Earlier studies were included when they were seminal to mechanisms or remained necessary for interpreting later work. Search concepts combined pulse terms (pulse, grain legume, bean, lentil, chickpea, pea, mungbean and cowpea) with biofortification terms and target nutrients (iron, zinc, selenium, folate), and then with pathway terms such as breeding, genomic selection, quantitative trait locus, genome-wide association, genotype by environment, agronomic biofortification, foliar fertilisation, phytate, polyphenol, cooking, retention, bioavailability, absorption, efficacy, randomised trial, adoption, seed system and consumer acceptance. Representative combinations included "(pulse OR bean OR lentil OR chickpea OR pea OR mungbean OR cowpea) AND (biofortification OR micronutrient) AND (iron OR zinc OR selenium OR folate)" and "(biofortified bean) AND (absorption OR iron status OR cognition OR adoption)". Searches were iteratively refined when a crop-nutrient pathway required more specific retrieval.

2.4 Eligibility Criteria

Peer-reviewed original studies and high-quality reviews were eligible when they addressed a pulse crop and contributed directly to genetic variation, nutrient accumulation, agronomic enrichment, processing and retention, bioavailability, human nutritional

response, adoption or delivery. Methodological papers were included for review conduct, and authoritative institutional material was used only where programme or public-health context required it. English-language publications predominated because they could be evaluated reliably within the available sources. Studies of cereal-only biofortification, industrial fortification without a crop biofortification component, feed-only outcomes and oilseed legumes were excluded from the central synthesis. Conference abstracts, unverifiable records, promotional material and articles lacking sufficient bibliographic information were excluded. Studies published after the final search date were not included.

2.5 Screening, Duplicate Handling and Study Selection

Titles and abstracts were first assessed for direct relevance to the review pathway, followed by full-text or detailed record assessment where necessary. Duplicate records were reconciled using title, author, year and DOI. Selection was purposive rather than exhaustive: priority was given to studies with strong design, multi-environment or replicated evidence, direct measurement of nutritional endpoints, conceptual importance or clear relevance to a disputed point. Conflicting and negative studies were retained where they materially altered interpretation. Because the process was a critical narrative review with iterative citation searching rather than a prospectively registered systematic review, no PRISMA-style record counts were generated or inferred.

2.6 Critical Appraisal and Evidence Synthesis

Evidence was appraised for study design, sample or germplasm representativeness, environmental replication, analytical validity, controls, treatment comparability, measurement of anti-nutritional factors, relevance of cooking or meal preparation, appropriateness of absorption or biomarker endpoints, statistical adequacy, external validity and consistency with independent studies. Greater weight was given to evidence that moved beyond total nutrient concentration to retention, bioavailability or human outcomes. Thematic synthesis was organised around six linked questions: whether useful variation exists; whether it can be inherited or induced reliably; whether the trait is stable in target environments; whether the nutrient survives preparation and is bioavailable; whether repeated consumption changes nutritional status or function; and whether the variety can be adopted and consumed at population scale.

3. Why Pulses Are Distinct Biofortification Targets

Pulse biofortification begins from a favourable nutritional baseline. Common bean, lentil, chickpea, pea, mungbean and cowpea contribute protein and several micronutrients to diets in which animal-source foods may be limited. This creates two strategic advantages. First, the marginal increase required to reach a nutritionally meaningful target may be smaller than in a crop with very low native nutrient concentration. Second, pulses are typically eaten as a food component rather than as a purified ingredient, so improvements can be delivered through established culinary practices without creating a new consumption occasion. The same characteristics also create constraints: pulses contain compounds that regulate seed phosphorus and defence but can reduce mineral absorption, and their longer cooking requirements expose nutrients to leaching and matrix changes. The value of a biofortified pulse must therefore be defined by nutrient delivered per habitual serving rather than concentration in raw seed alone (Jha & Warkentin, 2020; Kumar & Pandey, 2020).

The dominant breeding targets have been Fe and Zn because both are nutritionally important and show exploitable seed variation. Common bean is the clearest example. QTL studies demonstrated heritable variation for seed Fe and Zn, but also revealed a polygenic architecture and sensitivity to genetic background (Blair *et al.*, 2009, 2010). Lentil germplasm likewise shows broad variation, with association mapping and multi-environment trials confirming that both genotype and environment contribute to observed concentration (Bhattacharya *et al.*, 2022; Kumar *et al.*, 2018; Singh *et al.*, 2017). Cowpea studies show transgressive segregation and useful variation for several minerals, while newer multi-environment work also measures phytic acid and potential mineral bioavailability rather than mineral concentration in isolation (Ntswane *et al.*, 2024; Fernandes Santos & Boiteux, 2015). These findings make genetic improvement plausible, but they also undermine the notion of a single, universally high-mineral genotype.

A further distinction is required between nutrient concentration and nutrient content per seed or per unit land area. Concentration can rise because nutrient uptake increases, but it can also change when seed size or carbohydrate deposition changes. Conversely, a high-yielding genotype may show lower concentration through dilution while still producing more total mineral per hectare. For nutritional security, neither metric is sufficient alone. Farmers require yield, consumers eat servings rather than hectares, and public-health benefit depends on the nutrient concentration of the portion actually consumed. Breeding trials should therefore report yield and seed size alongside mineral concentration, and where feasible estimate nutrient yield per hectare. This reduces the risk that apparent biofortification is merely a consequence of smaller seeds or lower dry-matter accumulation. It also helps identify genotypes that combine agronomic productivity with nutritional density, which are more likely to be adopted and to deliver meaningful nutrient at population scale (White & Broadley, 2009).

Selenium presents a different biological problem. Plants do not require Se in the same way that they require Fe or Zn, and seed Se concentration is strongly influenced by soil Se availability and chemical form. Lentil grown in Se-rich environments can accumulate substantial Se, much of it as selenomethionine, but the magnitude is highly location-dependent (Thavarajah *et al.*, 2008). Subsequent screening showed genetic variation and potential for broader selection, yet the environmental dependence means that Se biofortification often resembles a location-specific agronomic strategy more than a purely stable genetic trait (Thavarajah *et al.*, 2017). A programme that extrapolates high-Se performance from naturally enriched soils to deficient regions risks overestimating transferability.

Folate illustrates another opportunity and caution. Comparative analysis of common bean, lentil, chickpea and pea found considerable differences among crops, cultivars and locations, demonstrating both nutritional value and breeding variation (Jha *et al.*, 2015). Yet folate concentration is chemically more labile than total mineral concentration, and routine breeding pipelines rarely integrate folate retention through storage and cooking with human biomarkers. Consequently, folate biofortification in pulses is biologically plausible but remains less mature as a nutrition-delivery pathway than Fe biofortification in common bean.

The implications are summarised in Table 1. The table emphasises that a "biofortification target" is not a single trait. It is a crop–nutrient–food-system combination whose feasibility depends on heritable variation, environmental stability, anti-nutrient context, processing and the scale of habitual intake. Common bean Fe has the strongest translational evidence, whereas several other crop–nutrient combinations remain supported mainly by compositional or agronomic studies.

Table 1. Pulse crops as biofortification targets: opportunity, constraints and maturity of evidence

Crop and target	Biological opportunity	Principal constraint	Evidence maturity	Supporting references
Common bean – Fe/Zn	Wide genetic variation; QTL and multi-environment evidence; high habitual consumption in several target populations.	Phytate/polyphenols, cooking losses, and imperfect relation between total Fe and absorbed Fe.	Advanced for Fe: breeding, absorption, efficacy and delivery evidence available.	Blair *et al.* (2009, 2010); Haas *et al.* (2016); Katuuramu *et al.* (2021)
Lentil – Fe/Zn	Broad germplasm variation, marker associations and repeated G×E evidence.	Trait stability varies among environments; direct human efficacy evidence is sparse.	Intermediate: strong crop evidence, limited human endpoints.	Bhattacharya *et al.* (2022); Kumar *et al.* (2018); Singh *et al.* (2017)
Lentil – Se	Efficient seed accumulation and substantial variation among genotypes.	Strong dependence on soil Se supply and site; narrow margin between inadequate and excessive intake requires careful targeting.	Intermediate for agronomy/composition; limited population efficacy evidence.	Thavarajah *et al.* (2008, 2017)
Chickpea – multiple minerals	Large diversity panels and expanding genomic resources support multi-mineral selection.	Evidence is concentrated on composition/genomics rather than absorption or efficacy.	Emerging to intermediate.	Jha *et al.* (2024); Salaria *et al.* (2025)
Field pea – Zn	Foliar and combined Zn application can increase seed Zn; cooking effects have been quantified.	Agronomic response is soil- and treatment-dependent; human outcome data are lacking.	Intermediate for agronomic pathway.	Pandey *et al.* (2013); Poblaciones & Rengel (2016)
Mungbean – Fe/Zn	Responsive to targeted mineral application with potential yield and nutrient co-benefits.	Evidence is dominated by single-location agronomic trials and concentration endpoints.	Emerging.	Dhaliwal *et al.* (2023); Farooq *et al.* (2025)
Cowpea – Fe/Zn	Segregating populations and diverse accessions show mineral variation.	Bioavailability and G×E have been studied less extensively than in common bean.	Emerging to intermediate.	Ntswane *et al.* (2024); Fernandes Santos & Boiteux (2015)

Note. Fe = iron; Zn = zinc; Se = selenium; QTL = quantitative trait locus; G×E = genotype-by-environment interaction. Evidence maturity is a critical judgement based on the depth of the chain from crop trait to human or delivery outcomes, not a formal score

4. Biological Pathways for Raising Nutrient Density

Three broad pathways can raise nutrient density in pulse seed: genetic improvement, molecularly enabled manipulation of nutrient acquisition and partitioning, and agronomic enhancement of nutrient supply or uptake. In practice these pathways overlap. A breeder may select a genotype with superior Zn accumulation but still depend on adequate soil Zn; a foliar treatment may produce a larger response in genotypes with efficient remobilisation; and gene editing may eventually alter anti-nutrient or transporter pathways rather than total mineral loading alone. The relevant comparison is therefore not which pathway is intrinsically superior, but which combination produces a stable, agronomically competitive and nutritionally useful phenotype in the target environment.

4.1 Conventional Breeding, Genetic Diversity and Genotype-by-Environment Effects

Conventional breeding remains the most mature route for pulse biofortification because it can package nutrient traits with yield, disease resistance, maturity, seed type and culinary quality. Common bean provides strong evidence that seed Fe and Zn concentrations are heritable but quantitatively controlled. QTL detected in recombinant populations show that major and minor loci can contribute to accumulation, yet their effects are not necessarily constant across genetic backgrounds or environments (Blair *et al.*, 2009, 2010). This architecture favours recurrent selection and multi-location testing over dependence on a single marker. It also means that breeding targets should be expressed as stable nutrient delivery at acceptable yield rather than the maximum concentration observed in a screening nursery.

Lentil studies reinforce this point. Association mapping across Indian and Mediterranean material identified marker–trait associations for Fe and Zn, demonstrating that useful alleles exist in breeding germplasm (Singh *et al.*, 2017). Multi-year evaluations subsequently found significant environmental and genotype-by-environment effects for mineral concentration, while broader multilocation work identified genotypes with comparatively stable performance (Bhattacharya *et al.*, 2022; Kumar *et al.*, 2018). The methodological strength of these studies lies in replicated environments and direct measurement of grain minerals. Their limitation for nutritional inference is equally clear: they do not establish how much of the additional Fe or Zn survives cooking or is absorbed. Breeding programmes that terminate selection at seed concentration may therefore optimise an incomplete phenotype.

Genotype-by-environment analysis should also be aligned with the geography of intended delivery. Stability across a broad experimental network is useful, but a genotype need not be universally stable if a specific high-burden region can be served reliably. Mega-environment approaches may therefore be appropriate: identify production zones with coherent soil and climate constraints, select nutrient-dense material within those zones, and maintain a portfolio rather than insisting on one globally superior variety. This is especially relevant for pulses grown under rainfed conditions, where drought, heat and soil fertility can alter seed filling and mineral remobilisation. Evidence from lentil and common bean indicates that environment can change both absolute concentration and genotype ranking, so nutritional trait release standards should include representative stress environments rather than only favourable research stations (Bhattacharya *et al.*, 2022; Katuuramu *et al.*, 2021).

Cowpea and chickpea expand the genetic opportunity but also illustrate different stages of evidence maturity. Cowpea crosses show transgressive segregation for several minerals, indicating that recombination can produce progeny beyond parental values (Fernandes Santos & Boiteux, 2015). Multi-environment cowpea screening that includes phytic acid improves nutritional interpretability because a high-Fe line with proportionally high phytate may not be superior in practice (Ntswane *et al.*, 2024). In chickpea, current reviews describe extensive genomic resources, and a recent diversity-panel study detected marker associations for several mineral traits, including Zn (Jha *et al.*, 2024; Salaria *et al.*, 2025). These results justify continued genomic selection but should not be presented as proof of improved human nutrition.

The central breeding problem is therefore multi-objective. Nutrient density competes for selection attention with yield, yield stability, disease resistance, seed size, colour, cooking time and market class. A nutritionally superior line that yields less, cooks slowly or falls outside consumer-preferred seed types can fail before reaching the diet. Conversely, a moderate nutrient gain embedded in a high-performing popular variety may deliver more population nutrient because adoption and consumption are larger. Biofortification breeding should consequently optimise expected bioavailable nutrient delivered per unit area and per habitual serving, not concentration alone.

4.2 Molecular and Genomics-Assisted Breeding, Transgenics and Gene Editing

Molecular approaches can shorten the route from phenotypic variation to selection decisions. In lentil, marker associations for Fe and Zn and expanding genomic resources provide candidate loci for marker-assisted breeding, while common bean QTL studies have mapped regions controlling mineral concentration (Blair *et al.*, 2010; Singh *et al.*, 2017). In chickpea, the growing availability of dense marker data and diversity panels supports genome-wide association and genomic prediction for multinutrient traits (Jha *et al.*, 2024; Salaria *et al.*, 2025). Their practical value depends on prediction across environments and breeding populations. Marker effects discovered in one population should not be assumed to transfer across gene pools, especially when mineral traits show environmental sensitivity.

Transgenic and gene-editing approaches could, in principle, intervene more directly in transporters, chelators, seed-storage processes or anti-nutrient synthesis. Recent reviews of common bean and chickpea describe these possibilities, including manipulation of pathways related to Fe/Zn homeostasis and reduction of phytate (Huertas *et al.*, 2023; Jha *et al.*, 2024). The evidence base in pulses, however, is still primarily mechanistic and pre-commercial. There is little direct human efficacy evidence for gene-edited biofortified pulse varieties. Regulatory requirements, transformation efficiency, off-target or pleiotropic effects, seed-system compatibility and consumer acceptance remain relevant constraints. Gene editing is thus best treated as an enabling breeding tool rather than as an already validated nutritional intervention.

A particularly important opportunity is to change the target from total mineral concentration to bioavailability-related traits. The common bean evidence shows why. Genetic reduction of seed phytate increased Fe absorption in women, demonstrating that modifying the inhibitor environment can be nutritionally consequential (Petry *et al.*, 2013). Yet a later comparison of low-phytate and biofortified beans reported gastrointestinal symptoms associated with the low-phytate beans, showing that a single biochemical improvement can introduce other food-quality costs (Petry *et al.*, 2016). Future molecular breeding should therefore seek balanced changes in mineral loading, inhibitor profile, seed physiology and consumer-relevant quality rather than extreme modification of one pathway.

4.3 Agronomic and Rhizosphere Pathways

Agronomic biofortification raises nutrient density by increasing plant-available nutrient, improving uptake or enhancing translocation to edible seed. It can be deployed more rapidly than breeding and can complement genetic gains, but its effectiveness is intrinsically tied to soil chemistry, application method, timing, crop genotype and nutrient mobility. White and Broadley (2009) emphasised this interaction between phytoavailability and plant uptake; pulse experiments provide crop-specific examples.

Field pea responds strongly to foliar Zn under Zn-limited conditions. Controlled work showed that foliar Zn could improve seed Zn and other yield or quality attributes, while a later study comparing soil and foliar application found that combined strategies could

raise grain Zn and that cooking reduced the concentration achieved in raw seed (Pandey *et al.*, 2013; Poblaciones & Rengel, 2016). These studies are useful because they link agronomic enrichment to food processing, but the use of phytate:Zn ratios or in vitro proxies still falls short of human absorption. The agronomic dose that maximises grain Zn is not necessarily the dose that maximises cost-effectiveness, farmer adoption or absorbed Zn.

Lentil experiments similarly demonstrate responsiveness to Fe and Zn fertilisation, and Se work shows especially strong dependence on environmental availability (Dhaliwal *et al.*, 2021; Thavarajah *et al.*, 2008). The Se case is a warning against transferring recommendations without soil diagnosis. Where soil Se is naturally high, genetic differences in accumulation can be exploited; where it is low, fertiliser or other agronomic interventions may be required. Because Se has a narrower safe intake range than Fe or Zn, agronomic enrichment also demands closer quality control and geographic targeting.

Mungbean studies published in 2023 and 2025 reported increases in seed Fe and Zn and changes in productivity after different mineral application methods, with foliar or combined treatments often performing favourably (Dhaliwal *et al.*, 2023; Farooq *et al.*, 2025). These are promising field signals, yet external validity is limited when conclusions rely on one location, season or cultivar. Soil pH, baseline micronutrient status, rainfall and genotype can change response markedly. The next step should therefore be coordinated multi-environment agronomic trials that include nutrient-use efficiency, residual soil effects, economic return, post-cooking retention and bioavailability rather than repeated demonstrations that fertiliser can raise raw-seed concentration.

Table 2 compares the principal biological pathways. The critical distinction is temporal and evidential: breeding offers renewable nutrient traits through seed but requires longer development; agronomy is faster but recurring and environment-specific; molecular manipulation may target difficult traits but has greater technical and regulatory uncertainty; and integrated strategies may be most effective when they are tested against the same nutritional endpoint.

Table 2. Comparative pathways for pulse biofortification

Pathway	Primary mechanism	Strengths	Key limitations	Most defensible use	Supporting references
Conventional breeding	Selection and recombination for higher seed nutrient concentration and associated quality traits.	Seed-embedded, renewable, compatible with existing variety release systems; can combine agronomic traits.	Polygenic control, G×E, possible dilution or trade-offs, long breeding cycles.	Foundation for durable Fe/Zn improvement where heritable variation is established.	Blair *et al.* (2009, 2010); Kumar *et al.* (2018)
Marker/genomic-assisted breeding	Use of QTL, associated markers or genome-wide prediction to accelerate selection.	Can improve selection efficiency and combine multiple loci.	Marker effects may be population- and environment-specific; nutrient phenotype still requires validation.	Acceleration of multi-environment breeding, not replacement for phenotyping.	Singh *et al.* (2017); Salaria *et al.* (2025)
Gene editing/transgenic approaches	Targeted alteration of uptake, transport, storage or anti-nutrient pathways.	Potential to modify traits with limited natural variation and address bioavailability directly.	Technical, regulatory and acceptance constraints; sparse pulse-specific human efficacy evidence.	Research and trait development where conventional variation is insufficient.	Huertas *et al.* (2023); Jha *et al.* (2024)
Agronomic biofortification	Soil, foliar, seed or combined nutrient application increases plant supply and remobilisation.	Rapid, flexible and potentially synergistic with breeding.	Recurring input cost; strong dependence on soil, climate, timing and genotype; risk of over-application.	Zn, Fe or Se enrichment where baseline deficiency and crop response are demonstrated.	Dhaliwal *et al.* (2021, 2023); Poblaciones & Rengel (2016)
Integrated genetic–agronomic strategy	Combines nutrient-efficient genotypes with targeted nutrient management.	Can exploit genotype responsiveness and improve stability across production constraints.	More complex recommendations and seed/input delivery; requires coordinated testing.	High-priority pathway for target environments with known soil constraints.	White & Broadley (2009); Jha & Warkentin (2020)

Note. Fe = iron; Zn = zinc; Se = selenium; G×E = genotype-by-environment interaction; QTL = quantitative trait locus

5. From Nutrient Concentration to Bioavailable Nutrient

The principal conceptual weakness in much pulse biofortification research is the use of total seed mineral concentration as the terminal outcome. Total concentration is necessary for a concentration-based breeding target, but it is neither sufficient nor always directionally equivalent to absorbed nutrient. The translational sequence includes harvest concentration, storage stability, preparation losses, chemical speciation, interaction with inhibitors or enhancers, meal composition, fractional absorption and the physiological regulation of absorption. Any of these stages can attenuate or reverse an apparent advantage.

5.1 Phytate, Polyphenols and Mineral Speciation

Phytate is central because it is both a normal seed phosphorus store and a strong chelator of divalent minerals. Pulse seeds also contain polyphenols whose effects vary with chemical structure and seed coat. Early human studies in common bean exposed the practical consequences. Petry *et al.* (2012) compared beans differing in Fe and polyphenol characteristics and found that higher bean Fe did not automatically increase the amount absorbed. The study's importance lies less in its pessimistic conclusion than in its demonstration that concentration can be offset by lower fractional absorption. When phytate was genetically reduced, Fe absorption increased in young women, providing direct causal evidence that the inhibitor environment matters (Petry *et al.*, 2013).

Subsequent work complicated the picture. In Rwandan women with low Fe status, phytic acid concentration influenced Fe bioavailability from biofortified beans, supporting phytate as a relevant selection trait (Petry *et al.*, 2014). Yet lowering phytate cannot be treated as universally beneficial. Low-phytate beans produced absorption comparable to biofortified beans in a later study but were associated with adverse gastrointestinal symptoms (Petry *et al.*, 2016). Phytate also has roles in seed viability and may have health effects outside mineral absorption. A nutritionally rational breeding strategy should therefore seek an acceptable inhibitor–mineral balance rather than eliminating phytate indiscriminately.

The relationship between polyphenols and Fe is similarly context-dependent. Different polyphenol profiles can inhibit or sometimes exert weaker effects, and the food matrix matters. Katuuramu *et al.* (2021) found that common bean genotypes differed not only in Fe and Zn concentration but also in an in vitro measure of Fe bioavailability across environments. This is valuable because it permits selection for a composite nutritional phenotype. In contrast, relying solely on total polyphenol or total phytate concentration can miss differences in chemical form, localisation and interactions with other seed constituents.

The inhibitor problem is also nutrient-specific. Phytate can constrain Zn as well as Fe, but the relative importance of phytate, polyphenols and physiological regulation differs between minerals. A genotype selected as "low anti-nutrient" for Fe should not automatically be assumed to optimise Zn, Se or folate delivery. Selenium is governed more strongly by chemical species and environmental supply, whereas folate losses reflect chemical instability and processing rather than chelation. This argues against a generic nutritional-quality index in which all micronutrients are treated as interchangeable. Each target nutrient requires a mechanistically appropriate set of secondary phenotypes and validation assays, even when the same pulse variety is intended for multinutrient improvement (Jha *et al.*, 2015; Thavarajah *et al.*, 2008).

5.2 Cooking, Dehulling and Food-Matrix Effects

Pulses are almost always eaten after hydration and heating, so nutritional evaluation of raw seed is incomplete. Cooking can improve digestibility and reduce some anti-nutritional compounds, but it can also leach soluble minerals into discarded water. Huertas *et al.* (2022) evaluated common bean genotypes and household cooking methods using an in vitro digestion model. The study showed substantial losses of Fe and Zn during preparation and low recovery of Fe in the bioavailable fraction, with genotype-dependent chemical differences. The exact magnitude should not be generalised to all recipes, but the direction is clear: the cooking protocol is part of the biofortification phenotype.

Field pea evidence leads to the same conclusion. Agronomic Zn application increased raw grain Zn, but freezing and cooking reduced grain Zn and changed the phytate:Zn ratio (Poblaciones & Rengel, 2016). Biofortification targets set on uncooked dry seed can therefore overstate nutrient delivered if processors or households discard mineral-rich cooking liquor. Conversely, processes such as soaking, fermentation, germination or dehulling may reduce inhibitors in some pulse foods while also removing mineral-rich seed fractions. The net effect is recipe-specific and should be measured directly.

The food matrix also extends beyond the pulse itself. Composite meals containing enhancers or inhibitors can alter Fe absorption. Results from a purified bean puree or single test meal may therefore differ from habitual diets containing cereals, vegetables, animal-source foods, tea or other polyphenol sources. This partly explains why acute absorption studies and longer-term feeding trials can appear discordant: the former isolate absorption under controlled meal conditions, whereas the latter integrate repeated intake, physiological adaptation and whole-diet context.

5.3 Measurement Limitations and Better Nutritional Phenotypes

Analytical hierarchy matters. Inductively coupled plasma methods accurately quantify total minerals but do not establish chemical accessibility. Phytate:mineral molar ratios are inexpensive screening proxies but cannot reproduce human digestion, homeostatic regulation or the effects of specific polyphenols. In vitro digestion and Caco-2 cell models improve mechanistic relevance and are valuable for high-throughput selection, but they still require validation against human absorption. Stable-isotope studies offer direct measurement of fractional absorption from defined meals, while long-term randomised feeding trials determine whether repeated consumption alters biomarkers and function. Each step is more expensive and less scalable than the previous one.

The practical response should not be to apply the most expensive method to every breeding line. A tiered pipeline is more defensible: screen large populations for mineral concentration and basic anti-nutrient profiles; evaluate promising lines for cooked retention and validated in vitro bioavailability; test a small number of advanced candidates using human absorption; and reserve longer-term efficacy trials for varieties that are agronomically competitive and ready for delivery. Such staging concentrates resources on candidates that have survived earlier translational filters.

Table 3 makes explicit why evidence at one analytical level should not be promoted to the next. It also shows that the strongest claims about nutritional security require repeated-consumption outcomes and real-world reach, not only a statistically significant rise in raw-seed concentration.

Table 3. Interpreting nutritional endpoints in pulse biofortification research

Endpoint	What it establishes	What it cannot establish	Major confounders or modifiers	Supporting references
Total seed mineral concentration	Whether the harvested seed contains more of the target mineral.	Retention, absorption, physiological benefit or adoption.	Moisture basis, contamination control, yield dilution, soil nutrient status.	Blair *et al.* (2009); Singh *et al.* (2017)
Anti-nutrient/mineral ratios	Approximate potential for mineral chelation relative to substrate.	Actual fractional absorption in a person or meal.	Chemical form, polyphenol profile, meal composition, processing.	Ntswane *et al.* (2024); Petry *et al.* (2014)
Cooked retention	Amount remaining after a defined household or laboratory preparation.	Absorption or long-term efficacy.	Soaking, water ratio, decanting, pressure cooking, seed coat and hardness.	Huertas *et al.* (2022); Poblaciones & Rengel (2016)
In vitro bioavailability	Relative accessibility/uptake under a model digestive system.	Human homeostatic regulation or long-term biomarker change.	Model calibration, cell line response, matrix chemistry.	Katuuramu *et al.* (2021); Huertas *et al.* (2022)
Stable-isotope absorption	Fractional and total absorption from a controlled meal.	Long-term population impact, acceptability or delivery.	Baseline iron status, meal matrix, acute study design.	Petry *et al.* (2012, 2013, 2014, 2016)
Randomised feeding efficacy	Whether sustained consumption changes biomarkers and selected functional outcomes.	Effectiveness under routine markets and heterogeneous adherence.	Baseline status, dose, adherence, inflammation, co-diet and trial context.	Haas *et al.* (2016); Murray-Kolb *et al.* (2017); Luna *et al.* (2020)

Note. A stronger downstream endpoint does not invalidate upstream screening; it defines the level of inference that is justified

6. Human Nutritional Efficacy: Where the Evidence Is Strong and Where It Stops

Human evidence is the decisive separator between biologically plausible biofortification and demonstrated nutritional efficacy. Among pulses, common bean Fe provides the clearest chain. This evidence did not emerge as a uniformly positive sequence. Acute absorption studies first revealed constraints, followed by breeding and feeding studies that showed benefit under specific conditions. That trajectory is scientifically valuable because it demonstrates how a programme can respond to a mechanistic limitation rather than dismiss it.

6.1 Acute Absorption Evidence and the Bioavailability Problem

Stable-isotope studies in young women and Rwandan women established that common bean Fe is sensitive to the inhibitor matrix. Petry *et al.* (2012) found that high-Fe beans did not deliver a greater absorbed Fe amount under the tested meals, challenging the assumption that concentration alone would be efficacious. Petry *et al.* (2013) then showed that genetically lowering phytate increased Fe absorption, and Petry *et al.* (2014) linked phytic acid concentration to Fe bioavailability from biofortified beans in women with low Fe status. Together, these studies support two conclusions. First, common bean is not intrinsically unsuitable as a Fe vehicle; rather, the nutrient matrix determines how much of the extra Fe can be used. Second, bioavailability must be considered early enough that breeding does not select high-Fe lines with proportionally poor absorption.

Petry *et al.* (2016) added an important safety and acceptability dimension by finding gastrointestinal symptoms with low-phytate beans. This result limits a simplistic strategy of maximising absorption through drastic phytate reduction. A food must be tolerable as well as bioavailable. It also indicates that functional seed traits, digestive responses and consumer acceptance should be evaluated alongside biochemical targets when a breeding intervention alters major storage compounds.

6.2 Controlled Feeding Evidence for Iron Status and Function

The pivotal efficacy evidence came from a randomised controlled feeding trial in Rwandan women with low Fe stores. Haas *et al.* (2016) reported that consuming iron-biofortified beans for 128 days improved indicators of Fe status compared with conventional beans. The trial's strength was repeated controlled consumption and biomarker assessment in a target population. Its setting also limits direct generalisation: participants were young women in Rwanda, bean intake was high and feeding was controlled. Nevertheless, it established that a biofortified pulse can produce a physiological benefit despite the absorption constraints identified in earlier studies.

Follow-up analyses extended the outcome chain beyond biomarkers. Murray-Kolb *et al.* (2017) reported positive effects on selected cognitive performance measures in young Rwandan women after an 18-week feeding trial. Wenger *et al.* (2019) related changes in Fe status to changes in brain activity and behaviour, and Luna *et al.* (2020) linked improved Fe status during the feeding intervention to greater physical work efficiency. These studies are important because nutritional security is ultimately concerned with health and function, not only serum biomarkers. They also require cautious interpretation. The functional analyses derive from

a linked intervention context, so they do not constitute independent replications across populations, ages and dietary patterns. Cognitive tasks and work-efficiency measures are sensitive to design, learning, socioeconomic context and baseline deficiency.

Glahn *et al.* (2017) evaluated Fe bioavailability of first-generation iron-biofortified beans released in Rwanda using laboratory models, providing a bridge between breeding material and human feeding evidence. Such triangulation is valuable: controlled in vitro or animal-informed screening can help explain why varieties differ, while human trials establish whether those differences translate into physiological response. The field would benefit from applying a similar sequence to other pulses rather than moving directly from high seed concentration to claims of nutritional impact.

6.3 The Evidence Gap beyond Common Bean

The contrast with other pulses is stark. Lentil has strong data on Fe, Zn and Se concentration, genetic variability, G×E and agronomic enrichment, but comparatively little randomised human evidence linking biofortified lentil to micronutrient biomarkers. Chickpea has rich genomic and compositional research but minimal end-to-end efficacy evidence. Field pea and mungbean agronomic studies show that seed nutrient concentration can be raised, but they generally stop before human absorption or status. Cowpea research increasingly incorporates phytic acid and potential bioavailability, yet controlled human trials remain limited. This unevenness means that "pulse biofortification" should not be treated as one evidence category. Conclusions demonstrated for iron-biofortified common bean cannot be automatically transferred to Zn-biofortified pea, Se-enriched lentil or Fe-enriched mungbean.

A plausible reason is the cost and logistical complexity of human feeding studies. Breeding and agronomic trials can test dozens or hundreds of genotypes or treatments, whereas controlled feeding requires a stable food supply, ethical oversight, biomarker expertise and sustained participant adherence. This resource asymmetry creates a publication landscape rich in proximal endpoints. The solution is not to demand efficacy trials for every genotype, but to establish decision gates that move only the most agronomically and nutritionally credible candidates downstream.

Table 4 summarises the common bean human evidence and explicitly separates absorption, biomarker and functional endpoints. The collective signal is favourable for Fe-biofortified beans in the studied Rwandan population, but the chain remains concentrated geographically and demographically. Replication in children, pregnant women and other high-bean-consuming populations would strengthen external validity.

Table 4. Representative human and translational evidence for iron-biofortified common bean

Evidence stage	Population/model	Main inference	Critical limitation	Supporting references
Acute absorption	Women consuming controlled bean meals	Higher total seed Fe did not necessarily increase absorbed Fe; inhibitors can offset concentration gains.	Meal-specific, short-term studies do not predict long-term status by themselves.	Petry *et al.* (2012)
Phytate manipulation	Young women in controlled absorption studies	Lower seed phytate increased Fe absorption, showing direct importance of inhibitor reduction.	Extreme low-phytate modification may alter seed/food properties.	Petry *et al.* (2013)
Biofortified bean absorption	Rwandan women with low Fe status	Phytic acid materially influenced Fe bioavailability from biofortified beans.	Acute absorption remains sensitive to meal composition and baseline status.	Petry *et al.* (2014)
Low-phytate comparison	Rwandan women with low Fe status	Low-phytate and biofortified beans could provide comparable absorption, but gastrointestinal symptoms were reported with low-phytate beans.	Tolerability constrains purely biochemical optimisation.	Petry *et al.* (2016)
Controlled feeding efficacy	Young Rwandan women with low Fe stores	Repeated consumption of iron-biofortified beans improved Fe status over 128 days.	Controlled setting and high habitual bean intake limit generalisation to all populations.	Haas *et al.* (2016)
Cognition and neural outcomes	Young Rwandan female university students	Improved Fe exposure/status was associated with gains in selected cognitive and neural measures.	Linked trial context; functional tests require independent replication.	Murray-Kolb *et al.* (2017); Wenger *et al.* (2019)
Physical work efficiency	Iron-depleted Rwandan women	Improved Fe status during the intervention was related to increased work efficiency.	Secondary/linked analysis and specific population context.	Luna *et al.* (2020)

Note. Fe = iron. This table is representative rather than exhaustive and distinguishes what each study design can validly infer

7. Delivery, Adoption and Policy Pathways to Nutritional Security

Biological efficacy is necessary but insufficient for nutritional security. A nutrient-dense pulse delivers no benefit if farmers cannot obtain seed, if the variety is agronomically inferior, if consumers reject its colour or cooking properties, or if market channels do not

maintain varietal identity. Biofortification programmes therefore operate as seed-system and food-market interventions as much as breeding interventions. The strongest pulse-specific evidence again comes from iron-biofortified beans in Rwanda.

Consumer research with four iron-biofortified bean varieties identified distinct preference segments and showed that information about nutritional benefits and socioeconomic characteristics influenced acceptance (Murekezi *et al.*, 2017). This matters because biofortified traits are often invisible. Unlike orange-fleshed crops, higher Fe in a bean may not provide a sensory cue that helps consumers identify the product. Nutrition messaging can support demand, but it cannot compensate for poor taste, long cooking time or an unfavourable market class. Breeding programmes should therefore treat sensory and culinary traits as co-primary delivery traits rather than as post-release marketing problems.

Adoption evidence from Rwanda shows that delivery design affects both speed and persistence of uptake. Vaiknoras *et al.* (2019) found that proximity to formal seed delivery, informal dissemination through social networks and extension access influenced adoption dynamics. This demonstrates the value of plural pathways: formal distribution can seed an innovation, while farmer-to-farmer exchange expands reach. The finding also cautions against measuring programme success only by initial seed dissemination. Nutritional impact depends on continued cultivation, replacement of seed when necessary, and recurrent consumption over multiple seasons.

The agronomic performance of the variety can reinforce the nutritional objective rather than compete with it. Vaiknoras and Larochelle (2021) evaluated adoption of a widely grown iron-biofortified bean variety in Rwanda and reported gains in bean productivity and changes in household consumption, purchasing and sales. The study used observational impact methods rather than random allocation, so residual selection bias is possible despite econometric adjustment. Even so, it illustrates a crucial principle: adoption is easier to sustain when the biofortified variety also solves a farmer problem such as yield or marketability. A nutrient trait that requires farmers to accept a yield penalty will face a more difficult scaling pathway.

Economic modelling has long suggested that biofortification can be cost-effective when breeding costs are spread across large populations and farmers can recycle seed, but results depend strongly on coverage, retention of the trait, consumption levels and the health burden addressed (Meenakshi *et al.*, 2010). Synthesis across developing-country consumers likewise shows that acceptance is not determined by nutrition information alone; sensory quality, price, familiarity and market context remain important (Birol *et al.*, 2015). Pulse programmes should therefore evaluate cost per unit of *absorbed* nutrient or health gain under realistic adoption, rather than cost per unit of seed mineral increase.

Breeding-based and agronomic biofortification also create different delivery obligations. A genetically biofortified variety can reproduce the trait through seed, but only if varietal identity and seed quality are maintained. Agronomic biofortification requires recurrent access to the correct fertiliser formulation, application knowledge and cash or credit, and it may be ineffective where soil or water conditions limit uptake. These recurring requirements can shift costs from a breeding programme to farmers and extension systems. Integrated strategies should therefore be assessed for who pays, who controls the input, whether recommendations are robust under smallholder management, and whether nutrient enrichment persists when input use declines. A technically superior combination may be less equitable than a moderate seed-embedded gain if the former depends on inputs inaccessible to poorer households.

Equity is another underdeveloped dimension. Biofortified pulses may be especially useful for rural households that produce and consume their own food, but the poorest farmers may have the least access to certified seed, fertiliser or extension. Women often play major roles in pulse production, processing and meal preparation, while women of reproductive age are also a priority group for Fe nutrition. Delivery strategies that overlook gendered control of seed, land, cash and food allocation can weaken the link between production and individual intake. The Rwandan adoption evidence suggests that the identity and characteristics of household decision makers influence persistence, supporting more explicit gender analysis in future programmes (Vaiknoras *et al.*, 2019).

Policy positioning should remain complementary. Biofortified pulses can raise the nutrient quality of a food already embedded in local diets, but they should not displace supplementation where clinically indicated, mandatory or voluntary food fortification where coverage is high, infection control, or broader efforts to diversify diets. Biofortification has its greatest comparative advantage where recurrent consumption and seed multiplication can sustain exposure with relatively low marginal delivery cost. Its weakest setting is one in which the target pulse is eaten infrequently, seed replacement is poor, or the target nutrient is constrained mainly by absorption inhibitors that breeding has not addressed.

8. Integrative Critical Synthesis: A Chain-of-Evidence Model for Pulse Biofortification

The reviewed evidence supports a chain-of-evidence model in which pulse biofortification succeeds only when six links remain intact: useful biological variation or agronomic responsiveness; stable crop performance in target environments; retention of the nutrient after storage and preparation; adequate bioavailability in the relevant meal; sufficient repeated consumption to change physiological status; and delivery at scale to people at nutritional risk. The weakest link sets the ceiling on impact. A programme cannot compensate for zero adoption with excellent absorption, nor can high adoption rescue a nutrient trait that disappears during cooking.

This model explains why common bean Fe is unusually informative. Genetic and breeding evidence established that seed Fe can be increased. Human absorption studies then exposed phytate and related matrix constraints. Breeding and selection proceeded alongside nutrition research, and a controlled feeding trial demonstrated improvement in Fe status, followed by analyses of

cognition, neural function and work efficiency (Haas *et al.*, 2016; Murray-Kolb *et al.*, 2017; Petry *et al.*, 2012; Wenger *et al.*, 2019). Adoption studies subsequently examined how seed distribution and agronomic performance affected uptake in Rwanda (Vaiknoras *et al.*, 2019; Vaiknoras & Larochelle, 2021). Few other pulse–nutrient combinations have evidence across so many links.

The model also prevents overinterpretation of impressive upstream results. A high Se lentil grown on Se-rich soil may be nutritionally valuable locally but not reproducible on low-Se soils. A mungbean treatment that raises grain Fe and Zn may be agronomically promising but still requires retention and absorption evidence. A chickpea genomic locus associated with Zn is a useful breeding resource, not a health intervention. An Fe-rich bean with unfavourable phytate or cooking properties can fail despite meeting a raw-seed target. By specifying the link actually tested, the field can communicate progress without conflating biological potential with established population benefit.

Confidence is highest when independent methods converge. For example, common bean evidence combines QTL studies, multi-environment analyses, food chemistry, stable isotopes, controlled feeding and adoption research. Confidence is lower where evidence is repeatedly generated by similar agronomic trials in one region without validation in different environments or downstream nutritional testing. Future reviews and programmes should therefore report the *distribution* of evidence across the chain, not only the number of studies.

Causal claims should be calibrated to the strongest link tested. A genotype association can identify a locus linked to seed mineral concentration but does not prove that manipulating that locus will improve human status. A fertiliser experiment can establish treatment response but not effectiveness under farmer practice. An absorption study can show greater uptake from a meal but not sustained clinical benefit. A randomised feeding trial can establish efficacy under controlled exposure but not adoption or cost-effectiveness. This hierarchy is not intended to privilege human trials over crop science; it specifies how different disciplines contribute non-substitutable evidence. Nutritional security emerges only when those contributions are connected, and the chain can be strengthened incrementally without pretending that every study answers the final policy question.

Table 5 converts the chain into a research and decision framework. It highlights the main failure modes and the evidence required to move a candidate forward. The framework is intentionally demanding because nutritional security is a population-level outcome; a concentration increase is one contribution to that outcome, not its synonym.

Table 5. Chain-of-evidence framework for moving pulse biofortification towards nutritional security

Chain link	Minimum evidence before progression	Typical failure mode	Priority corrective action	Supporting references
1. Biological potential	Replicated genetic variation or agronomic response for target nutrient.	Single-environment outlier or contaminated analytical result.	Replicate across germplasm, seasons and validated analytical methods.	Singh *et al.* (2017); Dhaliwal *et al.* (2023)
2. Agronomic stability	Nutrient trait retained with acceptable yield and quality across target environments.	G×E reverses ranking; nutrient gain accompanies yield penalty.	Multi-environment selection and joint nutritional–agronomic indices.	Bhattacharya *et al.* (2022); Katuuramu *et al.* (2021)
3. Processing retention	Nutrient advantage remains after locally relevant storage and cooking.	Leaching or dehulling removes much of the gain.	Set breeding targets on cooked edible portion; standardise representative recipes.	Huertas *et al.* (2022); Poblaciones & Rengel (2016)
4. Bioavailability	Validated in vitro or human evidence that the additional nutrient is accessible/absorbed.	Phytate or polyphenols offset higher total concentration.	Co-select inhibitor profile and test advanced lines with absorption methods.	Petry *et al.* (2012, 2013, 2014)
5. Human efficacy	Repeated consumption improves nutrient status and, where feasible, functional outcomes.	Acute absorption gain does not translate to biomarker response.	Conduct adequately powered feeding trials in deficient target populations.	Haas *et al.* (2016); Murray-Kolb *et al.* (2017)
6. Delivery and reach	Seed access, adoption, repeated cultivation, consumer acceptance and sustained intake.	Variety is nutritionally effective but not grown, bought or eaten.	Integrate seed systems, market traits, nutrition communication and equity evaluation.	Murekezi *et al.* (2017); Vaiknoras *et al.* (2019); Vaiknoras & Larochelle (2021)

Note. G×E = genotype-by-environment interaction. The framework is sequential for decision making, but feedback is expected; failures at later links may require redesign of earlier breeding targets

9. Future Directions

The immediate priority is to redefine breeding targets around bioavailable nutrient delivery. For Fe and Zn, advanced lines should be selected using a combination of total concentration, anti-nutrient profile, cooked retention and a validated bioavailability assay. The common bean evidence demonstrates that this is not a theoretical refinement: higher Fe can be offset by lower fractional absorption, while phytate reduction can improve absorption but create other food-quality problems (Petry *et al.*, 2012, 2013, 2016). Multi-trait indices should therefore penalise poor yield, long cooking time and adverse inhibitor profiles rather than rank lines on

concentration alone. This approach is feasible immediately because large breeding populations can still be screened cheaply at the first stage, with more expensive assays reserved for advanced material.

A second priority is multi-environment nutritional phenotyping. G×E is repeatedly observed for Fe and Zn in lentil and common bean, yet nutritional traits are often tested in fewer environments than yield. Future programmes should evaluate mineral concentration, phytate or relevant polyphenol traits, and at least one bioavailability proxy across representative soil types and production zones. Designs should separate genotype, environment and management effects and include soil micronutrient measurements. For agronomic biofortification, factorial genotype-by-fertiliser trials are more informative than testing one variety because they can identify nutrient-efficient genotypes and avoid recommendations that work only for a particular genetic background (Bhattacharya *et al.*, 2022; Katuuramu *et al.*, 2021).

Third, processing should become a formal selection environment. Breeders generally phenotype seed before household preparation, whereas consumers eat boiled, pressure-cooked, dehulled, fermented or milled products. A standard panel of locally representative preparation methods should be developed for major pulse food systems, and biofortification targets should be expressed on the cooked edible portion. This is especially important when cooking water is discarded or dehulling removes mineral-rich fractions. Food scientists and breeders should agree on rapid assays that predict retention reliably enough for breeding decisions, with periodic confirmation using full chemical analyses (Huertas *et al.*, 2022; Poblaciones & Rengel, 2016).

Fourth, human studies should broaden beyond iron-biofortified common bean. The next candidates should be chosen where upstream evidence is strongest, habitual consumption is high and deficiency burden is relevant. Iron- or zinc-biofortified lentil is an obvious candidate because genetic variation and G×E have been well characterised. Zinc-biofortified field pea and Fe/Zn-enriched mungbean require stronger processing and absorption evidence before large efficacy trials. Selenium-enriched lentil warrants geographically targeted work that incorporates baseline Se status and safe intake because soil-driven variation can be substantial (Thavarajah *et al.*, 2008, 2017). Trials should pre-specify biomarkers appropriate to the nutrient and include functional outcomes only when mechanistically justified and adequately powered.

Fifth, molecular breeding should target bottlenecks revealed by nutrition studies rather than pursue genomic novelty for its own sake. Genomic prediction can help stabilise Fe and Zn accumulation across environments; gene editing may be useful where a specific transporter, chelator or anti-nutrient pathway limits progress. But edited lines should be assessed for seed viability, germination, plant performance, cooking quality, gastrointestinal tolerance and bioavailability. Pulse-specific evidence does not yet support treating gene editing as a mature delivery route, so proof-of-concept lines should move through the same chain of agronomic and human validation as conventionally bred varieties (Huertas *et al.*, 2023; Jha *et al.*, 2024).

Sixth, adoption studies need to be designed earlier, not after varietal release. Participatory breeding and consumer testing can identify preferred seed colour, size, texture and cooking time before expensive nutrition trials. Seed-system experiments should compare small-pack sales, community multiplication, commercial channels and informal exchange, while tracking not only first adoption but disadoption and repeated use. The Rwandan bean experience shows that formal distribution and social networks can interact to accelerate diffusion (Vaiknoras *et al.*, 2019). Similar work is needed in South Asia and other pulse-intensive regions where market structures, seed replacement rates and gender roles differ.

Finally, impact evaluation should connect agriculture and nutrition quantitatively. Programmes should estimate nutrient delivered per hectare, nutrient retained per cooked serving, expected absorbed nutrient per consumer and coverage among deficient groups. Economic analyses should incorporate these stages rather than valuing a variety solely by yield or raw nutrient gain. Longer-term work should compare biofortification with, and in combination with, fortification, supplementation and diet-diversification strategies. The relevant policy question is rarely whether biofortification "works" in isolation; it is where a biofortified pulse provides the greatest additional benefit within a portfolio of nutrition interventions.

10. Conclusions

Pulse biofortification has credible potential to strengthen nutritional security, but the evidence supports a narrower and more conditional conclusion than the phrase can imply. Heritable variation for seed Fe and Zn is established in several pulse species, and agronomic management can increase Zn, Fe or Se under responsive environments. The strongest translational case is iron-biofortified common bean, for which research has progressed from genetic improvement through absorption studies to controlled feeding, functional outcomes and adoption analysis. This evidence demonstrates that a biofortified pulse can improve nutritional status when the crop, food matrix, consumption pattern and delivery system align.

The same evidence also identifies the principal limitation of the field: higher nutrient concentration is not synonymous with higher nutritional value. Genotype-by-environment effects can destabilise mineral traits; cooking can remove part of the gain; phytate and polyphenols can reduce absorption; and consumer or farmer rejection can eliminate exposure altogether. Other pulse crops, particularly lentil, chickpea, field pea, mungbean and cowpea, possess strong biological or agronomic potential but generally lack the same depth of human efficacy and effectiveness evidence.

A defensible pathway forward is therefore to treat biofortification as a chain of linked performance criteria. Breeding and agronomy should optimise stable nutrient density together with yield and food quality; processing and bioavailability should be tested before human efficacy is assumed; and seed-system, market and equity considerations should be embedded before scale-up. Under this framework, pulses can make a meaningful contribution to nutritional security as part of diversified food and public-health strategies, while avoiding the overstatement that any high-mineral seed is inherently a nutrition intervention.

11. Limitations

This critical narrative review is intrinsically vulnerable to selection and interpretive bias despite use of a transparent search strategy, explicit eligibility criteria and structured evidence appraisal. It was designed to integrate heterogeneous biological, agronomic, food-science, nutrition and adoption evidence rather than to estimate a pooled effect, and therefore no quantitative meta-analysis was attempted. The accessible literature is uneven across crops and outcomes: common bean iron is represented by linked absorption, efficacy and delivery studies, whereas several other pulse–nutrient combinations are supported mainly by compositional or agronomic research. This imbalance limits cross-crop comparisons and may make the evidence base appear more mature for common bean than for pulses generally. English-language and openly accessible records predominated, and some relevant subscription-only full texts or regional literature may therefore have been missed. Heterogeneity in genotypes, soils, fertiliser regimes, cooking methods, bioavailability assays and human populations further limits direct comparison. Finally, the field is developing rapidly, so genomic and agronomic advances emerging after the final search date are not represented.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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