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Biofortification Techniques for Nutritional Improvement of Vegetable Crops: A Critical Narrative Review of Agronomic, Breeding and Genome-Based Approaches

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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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Review Article

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Abstract

Background: Inadequate intakes of iodine, iron, zinc, selenium, folate and several vitamins remain widespread, and biofortification, the enrichment of edible plant parts with nutrients during crop growth, has become an established complement to supplementation and industrial fortification. Most biofortification research and policy has been built around cereals and pulses, whereas vegetables differ in portion size, water content, harvested organ, production system and consumption practice.
Purpose and Scope: This critical narrative review appraises agronomic, breeding and biotechnological techniques used to biofortify vegetables, including potato and sweetpotato, and asks which techniques produce nutritionally meaningful, bioavailable, safe and deployable gains.
Approach: Peer-reviewed literature published from January 2000 to July 2026 was identified through multidisciplinary and topic-specific scholarly sources, supplemented by citation searching and authoritative institutional documents, and was appraised for relevance, design, reporting basis, environmental replication and the level of nutritional evidence reached.

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Principal Findings: Agronomic enrichment with selenium and iodine is reliable in controlled and field conditions, but it operates within narrow safety margins and can alter other quality traits, while iron enrichment of vegetables through fertilisation is largely ineffective. Zinc responds to foliar and soil application in potato, although excessive doses reduce yield and may raise phytate. Conventional breeding has produced the strongest human evidence, notably for orange-fleshed sweetpotato and iron-dense potato, yet absorption studies show that higher concentration does not guarantee proportionally higher absorbed nutrient. Metabolic engineering and genome editing have generated striking gains in carotenoids, folate, anthocyanins, ascorbate, provitamin D3 and γ-aminobutyric acid in tomato, potato and lettuce, but few such lines have been tested for bioavailability, stability or agronomic performance across environments. Evidence for microbial and nanoscale enrichment remains largely confined to short-term controlled studies.
Unresolved Questions: Major gaps concern portion-based reporting, post-harvest retention, human bioavailability, multi-nutrient stacking and cost-effectiveness outside staple systems.
Implications: Technique choice should be matched to the nutrient, crop organ and delivery context rather than to technological novelty.
Conclusion: Vegetable biofortification is technically feasible for several nutrients, but its public-health value remains demonstrated for only a small subset of crop–nutrient combinations.

Keywords: Agronomic biofortification; genome editing; hidden hunger; micronutrient bioavailability; orange-fleshed sweetpotato; selenium and iodine enrichment; metabolic engineering; horticultural crops.

1. Introduction

Micronutrient inadequacy remains one of the most widespread nutrition problems worldwide. A modelling analysis based on dietary intake distributions for 185 countries estimated that, before fortification and supplementation are considered, more than five billion people do not consume enough iodine, vitamin E and calcium, and more than four billion do not consume enough iron, riboflavin, folate and vitamin C (Passarelli *et al.*, 2024). The mineral deficiencies among these problems are shaped both by diets dominated by starchy staples and by the composition of the soils on which food is grown (de Valença *et al.*, 2017; White & Broadley, 2009). Biofortification, understood here as increasing the concentration or bioavailability of nutrients in the edible parts of crops during plant growth rather than during food processing, emerged as a complementary food-based strategy that draws on agronomic practice, plant breeding and biotechnology (Bouis & Saltzman, 2017; Garg *et al.*, 2018; White & Broadley, 2009).

Recent assessments further emphasise that the long-term nutritional contribution of biofortification depends not only on increasing nutrient density but also on embedding nutrient-rich crops within food systems and delivery pathways that support sustained access and consumption (Bouis *et al.*, 2024).

The intellectual history of biofortification has been dominated by staple crops. Early formulations argued that breeding micronutrient density into cereals, legumes and roots eaten daily in large quantities would reach resource-poor households at low recurrent cost (Welch & Graham, 2004), and the HarvestPlus programme subsequently developed and released biofortified staple varieties with documented efficacy for several nutrients (Bouis & Saltzman, 2017). Vegetables occupy an ambiguous place in this narrative. Orange-fleshed sweetpotato [*Ipomoea batatas* (L.) Lam.], hereafter OFSP, is widely regarded as one of the most successful biofortified crops (Low *et al.*, 2017), and potato (*Solanum tuberosum* L.) has become a target for iron biofortification (Burgos *et al.*, 2023). Most vegetables, however, are consumed in modest portions, contain a high proportion of water and are harvested as leaves, fruits, inflorescences or storage organs whose physiology differs from that of cereal grains (Buturi *et al.*, 2021; White & Broadley, 2009). These features change which techniques are feasible, how gains should be expressed and whether higher concentrations translate into nutritionally meaningful intakes.

Vegetables also present distinctive opportunities. Many are grown in intensive or protected systems in which nutrient supply can be regulated closely, including soilless cultivation, where the composition of the nutrient solution can be adjusted to enrich or deplete specific elements (Renna *et al.*, 2022). Several botanical families accumulate particular elements efficiently. Brassicaceae and *Allium* species, for instance, convert absorbed selenium into organic compounds that are retained in edible tissues (Puccinelli *et al.*, 2017; Wiesner-Reinhold *et al.*, 2017). The short generation times and well-characterised genomes of tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) have made them early testbeds for metabolic engineering and genome editing directed at nutritional traits (J. Li *et al.*, 2022; Nonaka *et al.*, 2017; H. Zhang *et al.*, 2018). A genome-edited tomato enriched in γ-aminobutyric acid (GABA) was among the first genome-edited foods offered to consumers (Ezura, 2022), and an anthocyanin-enriched transgenic tomato has completed regulatory review in the United States (Animal and Plant Health Inspection Service [APHIS], 2022). A systematic review of agronomic improvement in horticultural crops also found that research coverage remains uneven across nutrient classes, with mineral biofortification receiving greater attention than vitamin enrichment (Kathi *et al.*, 2024). Recent synthesis of potato nutritional biofortification likewise documents the complementary use of agronomic and genetic approaches to improve nutritional composition, supporting the continued importance of potato within biofortification research (Li *et al.*, 2025).

The literature has nonetheless developed in a fragmented manner. Reviews of vegetable biofortification have usually addressed single element groups, especially selenium and iodine (Golubkina *et al.*, 2021; Puccinelli *et al.*, 2017; Schiavon *et al.*, 2020), agronomic mineral enrichment (Buturi *et al.*, 2021), soilless production (Renna *et al.*, 2022) or genome editing across crops in general (Kumar *et al.*, 2022). Broader syntheses remain centred on staples and on the HarvestPlus portfolio (Bouis & Saltzman, 2017; Garg *et al.*, 2018; Ofori *et al.*, 2022). A recent scoping review of biofortification in high-income countries identified 11 vegetable studies among 46 eligible articles and found particularly sparse evidence on safety, sensory properties and modelling

(Gulyas *et al.*, 2025). Recurring concerns across these reviews include the scarcity of bioavailability and human studies, poorly standardised agronomic protocols and uncertain transfer of laboratory gains to field conditions (Buturi *et al.*, 2021; de Valença *et al.*, 2017; Kah *et al.*, 2018).

Three developments justify a renewed critical synthesis. Genome editing has produced vegetable lines with nutritional traits that are qualitatively new, such as accumulation of the vitamin D3 precursor 7-dehydrocholesterol in tomato fruit (J. Li *et al.*, 2022), and the regulatory treatment of such products has changed substantially in several jurisdictions (Council of the European Union, 2026; Menz *et al.*, 2020). Stable-isotope absorption studies with biofortified potato and sweetpotato have exposed a gap between nutrient concentration and absorbed nutrient that agronomic and breeding trials cannot detect (Burgos *et al.*, 2023; Jongstra *et al.*, 2020). New enrichment platforms, including microgreens, microbial inoculants and nanoscale fertilisers, are also being promoted faster than their advantage over conventional inputs is being established (Di Gioia *et al.*, 2019; Kah *et al.*, 2018). A review that appraises these techniques side by side, using consistent criteria of nutritional relevance, evidence maturity and implementation feasibility, is therefore warranted. Recent synthesis of apocarotenoid regulation in horticultural crops further illustrates the expanding range of metabolic targets being investigated for simultaneous nutritional and quality improvement (Liu *et al.*, 2026).

1.1 Scope, Research Gap and Objectives

This review examines techniques used to increase the nutritional value of vegetable crops during cultivation. Vegetables are interpreted in the horticultural sense to include fruit vegetables, leafy vegetables, brassicas, alliums, root vegetables, microgreens and the tuber crops potato and sweetpotato. The techniques considered are agronomic enrichment through soil, foliar and soilless nutrient supply, microbial and nanoscale inputs used for enrichment, conventional and marker-assisted breeding, transgenic metabolic engineering and genome editing. The principal nutrients are the minerals selenium, iodine, zinc, iron, calcium and magnesium and the vitamins or provitamins most often targeted in vegetables, namely provitamin A carotenoids, folate, ascorbate and provitamin D3. Anthocyanins, lycopene and GABA are discussed where they illustrate the capabilities and limitations of particular techniques, with the caveat that they are not essential nutrients and that their health effects are less firmly established. Industrial food fortification, supplementation, edible fungi, cereals and pulses harvested as dry grain are excluded, except where staple-crop evidence provides a necessary comparator.

The specific gap addressed is the absence of an integrated appraisal that evaluates the full range of techniques for vegetables against the same questions: whether reported gains are expressed on a basis that is meaningful for a realistic portion, whether gains persist after storage and preparation and are absorbed, how far the evidence has progressed from controlled experiments towards human outcomes, and what trade-offs, safety margins and delivery constraints accompany each technique. The objectives are to define the conceptual and physiological features that distinguish vegetables as biofortification targets, to appraise agronomic and genetic techniques critically, to evaluate the evidence connecting nutrient concentration with nutritional outcomes, to examine cross-cutting risks and implementation constraints, and to derive a prioritised research agenda. The intended contribution is a technique-by-context framework that helps researchers, breeders and policymakers judge which combinations of crop, nutrient and technique justify investment, rather than a descriptive catalogue of enrichment studies.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative design was adopted because the evidence relevant to vegetable biofortification spans pot and hydroponic experiments, multi-environment field trials, characterisation of transgenic and genome-edited events, in vitro digestion studies, stable-isotope absorption studies, community-based efficacy trials and policy analyses. These designs use incompatible outcome metrics and address different questions, so a pooled effect estimate would be neither meaningful nor defensible. A systematic review restricted to one crop–nutrient–technique combination would have yielded greater procedural reproducibility but could not have supported the cross-technique comparison that motivates this article. Narrative synthesis does not remove the obligation to be transparent about how evidence was located, selected and weighed. The conduct and reporting of the review were therefore guided by the six quality domains of the Scale for the Assessment of Narrative Review Articles, which cover justification of importance, explicit aims, description of the literature search, referencing, scientific reasoning and presentation of relevant endpoint data (Baethge *et al.*, 2019), and by the view that narrative reviews are most valuable when they interpret heterogeneous evidence critically rather than simply aggregate it (Greenhalgh *et al.*, 2018).

2.2 Information Sources

Searches were conducted in PubMed, Europe PMC, Crossref, Semantic Scholar, Google Scholar and the Directory of Open Access Journals. CGSpace, the open-access repository of the CGIAR research centres, was searched as a topic-specific source because much of the breeding, delivery and nutrition research on biofortified sweetpotato and potato originates from CGIAR programmes and associated national partners. J-STAGE was searched as a regional source for Japanese scholarly literature, because Japan produced the first commercial release of a genome-edited vegetable with a nutritional trait and has developed a distinct regulatory approach to such products. Authoritative institutional sources were consulted for regulatory and nutritional reference values, specifically documents of the European Food Safety Authority, the Animal and Plant Health Inspection Service of the United States Department of Agriculture and the Council of the European Union. Database searching was supplemented by backward citation searching of recent reviews, forward citation searching of influential primary studies, related-article functions and targeted searches

for corrections and retractions; these checks identified retracted articles within the vegetable biofortification literature, which were excluded.

2.3 Search Strategy and Search Period

The search strategy combined four concept blocks. The first captured biofortification and related terms (biofortification, biofortified, agronomic enrichment, micronutrient enrichment, nutritional enhancement). The second captured vegetable crops through generic and crop-specific terms, including vegetable*, horticultural crop*, tomato, *Solanum lycopersicum*, potato, *Solanum tuberosum*, sweetpotato, sweet potato, *Ipomoea batatas*, lettuce, *Lactuca sativa*, spinach, *Brassica*, broccoli, cauliflower, carrot, *Daucus carota*, onion, *Allium* and microgreen*. The third captured techniques (fertili*, foliar, fertigation, hydroponic*, soilless, nanoparticle*, nanofertili*, plant growth-promoting, mycorrhiza*, breeding, marker-assisted, genomic selection, transgenic, metabolic engineering, genome editing, CRISPR). The fourth captured nutrients and outcomes (selenium, iodine, zinc, iron, calcium, magnesium, provitamin A, β-carotene, carotenoid*, folate, ascorbate, vitamin C, vitamin D, anthocyanin*, bioavailability, bioaccessibility, retention, cooking, absorption, efficacy, acceptance, adoption, safety, regulation). A representative string was: (biofortif *OR "agronomic enrichment" OR "nutritional enhancement") AND (vegetable* OR tomato OR potato OR sweetpotato OR "sweet potato" OR lettuce OR spinach OR Brassica OR broccoli OR cauliflower OR carrot OR onion OR microgreen*) AND (fertili* OR foliar OR hydroponic *OR soilless OR nano* OR mycorrhiz *OR breeding OR "marker-assisted" OR transgenic OR "metabolic engineering" OR "genome editing" OR CRISPR) AND (selenium OR iodine OR zinc OR iron OR carotenoid* OR folate OR "vitamin C" OR "vitamin D" OR anthocyanin *OR bioavailab* OR absorption OR retention). Field tags, truncation and Boolean syntax were adapted to each source, and simplified keyword combinations were used where a platform did not support nested Boolean logic.

The search period extended from 1 January 2000 to 13 July 2026. The starting year corresponds to the first engineering of a provitamin A biosynthetic pathway into a staple food tissue (Ye *et al.*, 2000) and to the subsequent consolidation of biofortification as an organised research and policy agenda, reflected in the HarvestPlus programme from 2003 onwards (Bouis & Saltzman, 2017). Foundational publications preceding 2000 and conceptual papers were admitted when they were needed to explain mechanisms or historical developments.

2.4 Eligibility Criteria

Eligible sources were peer-reviewed original studies, systematic and narrative reviews, meta-analyses and authoritative institutional documents that addressed the enrichment of vegetable crops with nutrients or health-related compounds through agronomic, microbial, nanoscale, breeding, transgenic or genome-editing techniques, or that reported the retention, bioaccessibility, bioavailability, human efficacy, safety, acceptance, regulation or cost of biofortified vegetables. Studies on staple cereals, pulses and cassava were admitted only when they supplied a necessary methodological or conceptual comparator. Experimental studies were required to describe the crop, the technique, the chemical form and dose where relevant, and an appropriate untreated or non-biofortified comparator. Human studies were required to report a defined population, intervention and biomarker or intake outcome. Publications were limited to English. Conference abstracts, trade literature, promotional materials, theses, patents offered as evidence of effectiveness and sources whose bibliographic details could not be confirmed were excluded. Preprints were not used because peer-reviewed evidence was available for all themes.

2.5 Screening, Duplicate Handling and Study Selection

Titles and abstracts were screened for relevance to the scope, after which full texts or, where full texts were inaccessible, complete abstracts and publisher records were examined to confirm that each source supported the specific claims for which it was considered. Duplicate records retrieved from different sources were identified by matching digital object identifiers and, where these were absent, by comparing titles, authors, years and journal details, and a single verified record was retained. A source was not cited when its bibliographic identity could not be confirmed from an authoritative registry or publisher record, or when the accessible material was insufficient to verify the finding attributed to it. Selection prioritised studies that used field or multi-environment designs, reported chemical forms and doses, expressed results on a fresh-weight or portion basis, examined bioavailability or human outcomes, or represented conflicting findings. Because selection was purposive and interpretive, no record counts or flow diagram are reported.

2.6 Critical Appraisal and Evidence Synthesis

Each included study was appraised for design appropriateness, adequacy of controls, replication across seasons, sites or genotypes, transparency of the chemical form and dose applied, analytical method, and the basis on which concentrations were reported. Particular attention was paid to whether results were expressed per unit dry weight or fresh weight, because the high water content of most vegetables makes dry-weight values a poor guide to nutrient delivery per portion. Evidence was classified according to the furthest stage it reached along a translational sequence running from controlled-environment concentration data, through field performance and post-harvest retention, to bioaccessibility, absorption in humans and efficacy or effectiveness in populations. Formal risk-of-bias scores were not assigned because no single recognised instrument applies across the designs involved. Themes were developed iteratively around techniques, nutrients and cross-cutting constraints, and disagreements among studies were interpreted with reference to crop species and harvested organ, element speciation, dose and timing, growing environment, genotype, analytical approach and study duration. Conclusions were graded informally as well supported, conditionally supported or preliminary according to the consistency and translational depth of the evidence.

3. Vegetables as Distinct Biofortification Targets

3.1 Beyond the Staple-Crop Paradigm

Biofortification differs from conventional fortification in that enrichment occurs within the living plant, either because the crop is supplied with a nutrient it can absorb and translocate or because its genetic capacity to synthesise, accumulate or retain a nutrient has been altered (Garg *et al.*, 2018; White & Broadley, 2009). Three technique families are conventionally recognised, namely agronomic enrichment, conventional breeding and transgenic or genome-based modification, and they differ fundamentally in how benefits are delivered (Garg *et al.*, 2018). Agronomic enrichment requires inputs to be applied every season and therefore yields recurrent costs, whereas a biofortified variety, once released and adopted, carries its trait without further inputs (de Valença *et al.*, 2017). The staple-crop rationale for favouring genetic approaches rests on two assumptions, namely that the target crop is eaten daily in large quantities by deficient populations and that improved seed can move through established seed systems (Bouis & Saltzman, 2017; Welch & Graham, 2004).

Both assumptions hold only partially for vegetables. Potato and sweetpotato are staples in parts of the Andes and eastern and southern Africa, and it is no coincidence that these two crops account for most of the human evidence on biofortified vegetables (Burgos *et al.*, 2023; Jongstra *et al.*, 2020; Low *et al.*, 2017). For most other vegetables, intake per person is lower, more seasonal and more variable, and many are propagated through hybrid seed markets or grown in commercial protected systems whose consumers are not necessarily those at greatest nutritional risk. This asymmetry does not render vegetable biofortification unimportant, but it implies that the appropriate yardstick is the contribution of a realistic portion to a defined nutrient gap in a specified population rather than the magnitude of the concentration increase. The time required to breed and release a biofortified variety, estimated at eight to ten years for a single nutrient in staple programmes, also weighs differently in vegetable markets characterised by rapid varietal turnover and private breeding (Van Der Straeten *et al.*, 2020).

3.2 Portion Size, Water Content and the Reporting Basis

The most pervasive methodological weakness in the vegetable biofortification literature is the reliance on concentrations and fold changes that do not convey nutritional relevance. Because the edible tissues of most vegetables consist largely of water, a concentration expressed per unit dry weight can exceed the corresponding fresh-weight value several-fold and therefore gives a misleading impression of the amount delivered in a portion. Studies that report both bases illustrate the point. Engineered potato tubers accumulating up to 47 μg β-carotene per gram dry weight, a 3600-fold increase over the control, were estimated to supply approximately half of the adult recommended daily allowance of vitamin A in a 250 g fresh portion (Diretto *et al.*, 2007). Similarly, provitamin D3 levels in genome-edited tomato were reported on a dry-weight basis, and the nutritional claim rested on a conversion showing that one fruit, after ultraviolet-B treatment, could contain vitamin D3 equivalent to two medium-sized eggs (J. Li *et al.*, 2022). These are informative translations, but they are the exception rather than the rule.

Fold changes create a related distortion. Agronomic studies of iodine and selenium have reported increases of up to 249-fold and 700-fold respectively (Buturi *et al.*, 2021), yet large relative changes are easily achieved when baseline concentrations are close to analytical detection limits, and they reveal nothing about whether the enriched product falls between the recommended intake and the tolerable upper intake level. A field study with carrot (*Daucus carota* L.) showed that soil application of selenium alone yielded roots of which 100 g supplied 500–650% of the recommended daily allowance for selenium (Smoleń *et al.*, 2016), whereas an iodine-enriched vegetable serving used in a human study was formulated to supply 45 μg iodine, about 30% of the adult recommended allowance (Tonacchera *et al.*, 2013). The contrast shows that the objective of agronomic enrichment is calibration to a target, not maximisation.

The reporting basis also matters for interpreting apparent historical declines in vegetable nutrient composition. An analysis of food composition data for 43 garden crops reported declines in several nutrients between 1950 and 1999 and attributed them plausibly to cultivar change (Davis *et al.*, 2004). A later critical review argued that comparisons of composition data published decades apart are unreliable because sampling, analytical methods, cultivars and origins differ, while also accepting that high-yielding cultivars can show lower mineral concentrations when carbohydrate accumulation outpaces mineral uptake, a dilution effect (Marles, 2017). The two positions converge on a point that matters for biofortification: gains in yield and gains in nutrient density are not automatically aligned, and nutrient targets must be monitored explicitly in breeding programmes.

3.3 Physiological Constraints on Uptake, Translocation and Accumulation

The feasibility of each technique depends on how the target nutrient moves from the growth medium or the site of synthesis to the harvested organ. Selenium is chemically analogous to sulfur, and selenate is taken up through sulfate transporters, whereas selenite enters mainly through phosphate transporters. Both are assimilated into selenocysteine and selenomethionine, and toxicity arises largely when these amino acids are incorporated non-specifically into proteins or when oxidative stress is induced (Gupta & Gupta, 2017; White, 2016). The sulfur pathway explains why sulfur-rich Brassicaceae and *Allium* crops accumulate selenium efficiently and why selenium supply can interfere with sulfur-containing secondary metabolites (Puccinelli *et al.*, 2017; Wiesner-Reinhold *et al.*, 2017).

Iodine is not recognised as essential for terrestrial plants, but it is absorbed by roots and leaves and can promote growth at low concentrations while inhibiting it at higher ones. Its redistribution through the phloem is limited, so it tends to accumulate in transpiring leaves rather than in fruits and seeds (Medrano-Macías *et al.*, 2016). This physiology favours leafy vegetables, although it is not an absolute barrier. Repeated root application of potassium iodide produced tomato fruits containing 1.5–10 mg iodine per

kilogram fresh weight without a significant reduction in yield at non-phytotoxic concentrations (Kiferle *et al.*, 2013). The apparent discrepancy between low phloem mobility and substantial fruit accumulation is most plausibly related to the dose and duration of supply, although the transport route into the fruit has not been resolved; either way, organ-specific limits appear to be quantitative rather than categorical.

Iron presents the most intractable agronomic constraint. It is readily rendered insoluble in soils and moves poorly into edible organs, and fertilisation has generally failed to raise iron concentrations in vegetables to a useful degree (Buturi *et al.*, 2021). In Andean potato cultivars, neither soil nor foliar iron fertilisation increased tuber iron, and foliar iron caused toxicity symptoms, whereas zinc applied by either route raised tuber zinc markedly (Kromann *et al.*, 2017). For carotenoids, the limiting factor is often the capacity of the harvested tissue to store the product rather than the flux through the biosynthetic pathway. The cauliflower *Orange* (*Or*) mutation, caused by insertion of a long terminal repeat retrotransposon, triggers the differentiation of plastids into carotenoid-sequestering chromoplasts in tissues that normally accumulate little carotenoid, which demonstrates that creating a metabolic sink can be as effective as enhancing synthesis (Lu *et al.*, 2006). Genetic variation for mineral traits also exists within vegetable gene pools, and shoot calcium and magnesium concentrations varied about two-fold among 376 *Brassica oleracea* L. accessions, were highly heritable and mapped to quantitative trait loci (Broadley *et al.*, 2008).

3.4 A Typology of Techniques

The techniques examined in this review can be arranged along two axes, namely whether the change resides in the environment of the crop or in its genome, and how far each has progressed from proof of concept to demonstrated nutritional benefit. Table 1 summarises this typology. Agronomic techniques act quickly and can be adjusted to local deficiencies, but they depend on recurrent inputs and on elemental chemistry that is favourable for selenium, iodine and zinc and unfavourable for iron. Genetic techniques embed the trait in the crop and can target vitamins and phytochemicals that fertilisation cannot supply, but they require longer development and, for transgenic and genome-edited products, regulatory approval and public acceptance. The final column of the table indicates that only conventional breeding of provitamin A sweetpotato and, to a lesser extent, agronomic selenium and iodine enrichment have reached human outcome evidence, whereas most other techniques remain at the stage of concentration data from controlled environments.

Table 1 should be read as a map of evidence maturity rather than a ranking of technical merit. The agronomic rows show that techniques relying on soluble, phloem-mobile elements are the most dependable, whereas those relying on proprietary formulations, including many microbial and nanoscale products, have not yet been compared rigorously with conventional inputs. The genetic rows show an inverse relationship between technical precision and translational depth: genome editing achieves the most specific metabolic changes but has the thinnest evidence on bioavailability and field performance, whereas conventional breeding is slow but is the only route with efficacy data in humans.

Table 1. Typology of biofortification techniques applied to vegetable crops, principal constraints and maturity of the supporting evidence

Technique	Principal mechanism	Representative vegetable applications	Main advantages	Main limitations	Furthest evidence stage reached	Supporting references
Soil and fertigation mineral supply	Increased availability of the element in the root zone	Selenium and iodine in carrot; zinc in potato	Simple; adjustable to local deficiency; compatible with existing fertiliser supply chains	Recurrent cost; soil fixation, especially of iron; losses to leaching and volatilisation	Population-level selenium status (national fertiliser policy); human iodine status (small trial)	Alfthan *et al.* (2015); Kromann *et al.* (2017); Smoleń *et al.* (2016); Tonacchera *et al.* (2013)
Foliar application	Uptake through leaf surfaces and redistribution to edible organs	Zinc in potato; iodine and selenium in leafy vegetables	Bypasses soil fixation; low doses	Limited phloem mobility of some elements; leaf injury and yield loss at excessive rates	Field concentration data; retention after cooking	Buturi *et al.* (2021); Kromann *et al.* (2017); White *et al.* (2017)
Soilless nutrient-solution enrichment	Precise control of element supply in hydroponic or substrate culture	Iodine in lettuce; zinc and iron in microgreens	High precision; enrichment or depletion for specific consumer groups	Confined to high-input systems; limited reach to deficient populations	Controlled-environment concentration and bioaccessibility data	Di Gioia *et al.* (2019); Renna *et al.* (2022); Voogt *et al.* (2010)
Microbial and biostimulant-assisted enrichment	Enhanced nutrient mobilisation and uptake by mycorrhizal fungi or bacteria	Mycorrhizal inoculation of lettuce	Potential co-benefits for growth and stress tolerance	Strong dependence on nutrient regime and cultivar; inconsistent field performance	Controlled-environment quality data	Baslam *et al.* (2011); Schiavon *et al.* (2020)

Technique	Principal mechanism	Representative vegetable applications	Main advantages	Main limitations	Furthest evidence stage reached	Supporting references
Nanoscale fertilisers	Altered solubility, release and uptake of nutrients formulated at nanoscale	Zinc and selenium nanoparticles in vegetables	Possible dose reduction	Modest median gain over conventional products; frequent lack of adequate controls; unresolved environmental fate; little field evaluation	Mostly laboratory and greenhouse data	Carmona *et al.* (2024); Kah *et al.* (2018)
Conventional breeding	Selection within natural genetic variation	Provitamin A sweetpotato; iron-dense potato; anthocyanin-rich potato and sweetpotato	No recurrent input cost; broad acceptability	Long development time; genotype × environment interaction; possible yield–density trade-offs	Human efficacy (sweetpotato) and absorption (potato, sweetpotato)	Burgos *et al.* (2023); Low *et al.* (2017); Mattoo *et al.* (2022)
Marker-assisted breeding	Use of diagnostic markers for known alleles	*Or* allele in tropical cauliflower	Faster, more precise introgression	Depends on major-effect alleles; few validated markers for nutrient traits	Breeding-line development	Lu *et al.* (2006); Singh *et al.* (2025)
Transgenic metabolic engineering	Introduction of foreign or modified pathway genes or transcription factors	Carotenoids and folate in potato; anthocyanins and folate in tomato	Large gains; access to traits absent from the gene pool	Regulatory cost; public acceptance; limited bioavailability and field data	Event characterisation; animal studies; regulatory review	Butelli *et al.* (2008); De Lepeleire *et al.* (2018); Díaz de la Garza *et al.* (2007); Diretto *et al.* (2007)
Genome editing	Targeted modification of endogenous genes or regulatory elements	GABA, lycopene and provitamin D3 in tomato; ascorbate in lettuce	Precision; potential absence of foreign DNA; rapid trait stacking	Requires detailed pathway knowledge; pleiotropy; transformation bottlenecks; divergent regulation	Event characterisation; one marketed product	Ezura (2022); J. Li *et al.* (2022); X. Li *et al.* (2018); Nonaka *et al.* (2017); H. Zhang *et al.* (2018)

Note. GABA = γ-aminobutyric acid; Or = Orange gene of cauliflower. The evidence stage refers to the most advanced level reached by any study of the technique in vegetables and does not imply that all applications have reached that stage

4. Agronomic Biofortification: Effectiveness, Calibration and Trade-offs

Agronomic biofortification is the most immediate route to enriched vegetables because it requires no new cultivar and can be implemented within a single season. Its effectiveness, however, depends on the chemistry of the target element in the growing medium, its mobility within the plant and the chemical form applied, and the evidence differs sharply among elements (Buturi *et al.*, 2021; White & Broadley, 2009). This section examines selenium and iodine, for which enrichment is dependable, then zinc and iron, whose responses diverge, before considering soilless platforms and the newer microbial and nanoscale inputs.

4.1 Selenium: The Most Mature Agronomic Model

Selenium provides the clearest demonstration that agronomic enrichment can shift the nutritional status of a population. In Finland, the addition of selenium to multinutrient fertilisers, applied nationally from 1984, raised selenium concentrations throughout the food supply and moved the population from deficiency to an optimal selenium status, with the programme accompanied by systematic monitoring of foods, animals and people (Alfthan *et al.*, 2015). Comparable programmes or regional initiatives have since been described in the United Kingdom, New Zealand, Malawi, China and Brazil, against an estimated global burden of about one billion people with inadequate selenium intake (Schiavon *et al.*, 2020). The Finnish outcome, however, was achieved through fertilisation across the agricultural system, with effects documented in foods and in animal as well as human health, and it cannot be read as evidence that enrichment of vegetables alone would produce a comparable effect.

For horticultural crops specifically, the chemical form is the principal determinant of success. Selenate is less phytotoxic than selenite and supports higher biomass production, and it is translocated more readily to shoots, whereas selenite is more readily converted to organic forms and retained in roots (Gupta & Gupta, 2017; Puccinelli *et al.*, 2017). The speciation of selenium in the edible product also differs among families. In onion (*Allium cepa* L.), γ-glutamyl-Se-methylselenocysteine has been reported to account for about 63% of total selenium, and in broccoli [*Brassica oleracea* var. *italica* Plenck] Se-methylselenocysteine accounts for about 45% (Puccinelli *et al.*, 2017). Organic selenium species are generally more bioavailable to humans than inorganic forms, and plants are the main dietary source of selenium (D'Amato *et al.*, 2020). These properties make brassica and allium crops efficient vehicles, but they also expose a trade-off. Because selenate competes with sulfate for uptake and assimilation, selenium supply interacts with sulfur-containing glucosinolates in brassicas. The direction of the effect appears to be dose-dependent: in greenhouse broccoli, selenium fertilisation increased glucosinolates and sulforaphane up to a point, beyond which glucosinolate production declined (Robbins *et al.*, 2005), and selenate fertilisation has been associated with lower glucosinolate concentrations in other brassica studies (Wiesner-Reinhold *et al.*, 2017). The apparent disagreement is therefore better interpreted as a non-linear response than as a genuine contradiction, but it means that selenium rates optimised for accumulation may be suboptimal for other quality attributes. The possibility that adjusting the nitrogen-to-sulfur ratio could allow simultaneous synthesis of glucosinolates and selenoglucosinolates has been proposed but not yet validated at field scale (Wiesner-Reinhold *et al.*, 2017).

The narrow interval between adequate and excessive intake is the defining constraint of selenium biofortification. The European Food Safety Authority set a tolerable upper intake level of 255 µg per day for adults, derived from alopecia as the critical effect (EFSA Panel on Nutrition, Novel Foods and Food Allergens, 2023). Against this threshold, some experimental protocols clearly overshoot. In the carrot field study cited earlier, selenium applied alone produced roots of which a 100 g portion exceeded the recommended allowance several-fold (Smoleń *et al.*, 2016). Such results do not indicate a risk under normal consumption of mixed diets, but they show that application rates designed to maximise accumulation are inappropriate for public-health programmes, which require dose–response data expressed per portion. The confidence that can be placed in current conclusions is therefore high for the feasibility and predictability of selenium enrichment, moderate for its contribution to status when vegetables are the sole vehicle, and low for the chemopreventive benefits often attributed to selenium-enriched brassicas, for which human evidence remains limited and analytical characterisation of minor selenium species is difficult (Wiesner-Reinhold *et al.*, 2017).

4.2 Iodine: Reliable Enrichment with a Thin Human Evidence Base

Iodine is the nutrient with the highest estimated prevalence of inadequate intake worldwide (Passarelli *et al.*, 2024), and iodine-enriched vegetables have been proposed as a complement to iodised salt rather than a replacement for it (Tonacchera *et al.*, 2013). Iodine biofortification has been demonstrated in leafy vegetables, fruit vegetables and storage organs through soil, nutrient-solution and foliar supply (Gonzali *et al.*, 2017; Medrano-Macías *et al.*, 2016). The chemical form again determines the balance between accumulation and phytotoxicity. In soilless culture, iodide is more phytotoxic than iodate, and concentrations in the micromolar range can be beneficial for leafy vegetables whereas higher concentrations inhibit growth (Medrano-Macías *et al.*, 2016; Voogt *et al.*, 2010). Under soil conditions, the relative merit of the two forms can reverse. In field-grown carrot, potassium iodide produced higher iodine accumulation in storage roots than potassium iodate without a significant yield penalty (Smoleń *et al.*, 2016). This divergence is consistent with differences in sorption, microbial transformation and volatilisation of iodine in soils, which reviews identify as a principal reason for inconsistent results among studies (Medrano-Macías *et al.*, 2016).

Interactions with other elements add a further layer of variability. Simultaneous application of iodine and selenium to carrot reduced the uptake of each element compared with single applications (Smoleń *et al.*, 2016), whereas a review of joint selenium and iodine enrichment found synergistic, antagonistic and neutral interactions depending on species and cultivar, together with a small evidence base that omits several major vegetables, including tomato, onion, garlic and pepper (Golubkina *et al.*, 2021). Multi-element formulations may therefore require crop-specific calibration rather than transfer of single-element protocols.

Human evidence remains limited to a single small study. Fifty healthy volunteers who consumed one 100 g serving per day of iodine-enriched potato, cherry tomato, carrot or salad for two weeks, each serving supplying about 45 µg iodine, showed an increase in urinary iodine concentration from 98.3 µg/L to 117.5 µg/L, which declined to 85 µg/L one week after consumption ceased (Tonacchera *et al.*, 2013). The authors described the effect as mild but significant. The design was uncontrolled and short, urinary iodine reflects recent intake rather than thyroid function, and the modest increment matches the modest dose, so the study establishes plausibility rather than efficacy. Retention during preparation is more encouraging. In biofortified potato, carrot and tomato, iodine was preserved under all the domestic cooking procedures tested, whereas potato and carrot boiled with iodised salt did not take up the added iodine (Comandini *et al.*, 2013). This finding suggests that, for cooked vegetables, iodine incorporated during growth may be delivered more reliably than iodine added at the cooking stage, although it derives from a single laboratory study. The evidence thus supports iodine enrichment as technically reliable and as a potentially useful complement to salt iodisation, but its public-health contribution has not been tested in populations with documented deficiency.

4.3 Zinc and Iron: Divergent Responses

Zinc and iron are often grouped together as targets, but the agronomic evidence for vegetables shows that they behave very differently. Zinc responds to fertilisation in potato. In Andean cultivars, foliar zinc at high rates increased tuber zinc 2.51-fold and soil application increased it 1.91-fold, with no concomitant yield reduction (Kromann *et al.*, 2017). In Scottish trials with four genotypes, foliar zinc as oxide, sulfate or nitrate raised tuber zinc concentrations, and cooked tubers retained 2.36 to 3.58 times more zinc than controls, but excessive applications reduced yield in all genotypes and tuber phytate increased (White *et al.*, 2017). The two studies agree on the direction and approximate magnitude of the response but disagree on yield penalties, a discrepancy that is most plausibly explained by differences in the range of rates tested, formulation, environment and cultivar. The increase in

phytate matters because phytate inhibits zinc absorption, which means that concentration gains may overstate gains in absorbable zinc. In cereals, soil and foliar zinc fertilisation has been reviewed extensively and is regarded as a practical and cost-effective route to higher grain zinc (Cakmak & Kutman, 2018), whereas the vegetable evidence rests on a small number of crop-specific trials.

Iron has proven largely resistant to agronomic enrichment in field-grown vegetables. In the Andean potato trials, tuber iron did not respond to soil or foliar iron fertilisation, and foliar iron damaged leaves (Kromann *et al.*, 2017). A broader review of mineral biofortification in vegetables reached a similar conclusion, attributing the failure to insolubilisation of iron in soils and poor translocation to edible organs and noting yield reductions of up to 25% in some iron studies (Buturi *et al.*, 2021). The principal exception involves soilless production of microgreens, in which iron supplied in the nutrient solution at 10–20 mg/L increased iron concentrations by 64% in arugula [*Eruca vesicaria* subsp. *sativa* (Mill.) Thell.] and by up to 278% in red cabbage (*B. oleracea* var. *capitata* L.), although 40 mg/L was phytotoxic and reduced fresh yield in all species tested (Di Gioia *et al.*, 2019). The contrast implies that agronomic iron enrichment is realistic only where short cycles and direct contact with a controlled solution circumvent soil chemistry, and that field vegetables are better targeted through genetic approaches. Calcium can be raised effectively in leafy vegetables grown in soilless systems, but its value is constrained by oxalate, which binds calcium and lowers its bioavailability, and some calcium treatments reduced yield by up to 32% (Buturi *et al.*, 2021).

4.4 Soilless Systems and Microgreens as Precision Platforms

Soilless cultivation allows the composition of the nutrient solution to be controlled closely, and it has broadened the concept of biofortification to include tailoring vegetables for particular consumer groups. Examples include increasing calcium, silicon and boron together, and reducing potassium by 40–60% in vegetables intended for people with chronic kidney disease (Renna *et al.*, 2022). Microgreens, harvested at the seedling stage, extend this logic by combining very short cycles with direct exposure of seedlings to enriched solutions; zinc supplied at 5–10 mg/L increased zinc concentration by 75–281% relative to controls in brassica microgreens, with responses that were genotype-specific (Di Gioia *et al.*, 2019).

The precision of these systems is also their principal limitation from a public-health perspective. Soilless production is capital- and energy-intensive and serves mainly urban and higher-income consumers, who are not the populations with the greatest prevalence of deficiency. The scoping review of biofortification in high-income countries found that the evidence base for vegetables was small and concentrated on bioavailability and nutrient stability, with almost no work on safety or sensory acceptance (Gulyas *et al.*, 2025). Soilless platforms are nonetheless valuable as experimental systems for establishing dose–response relationships, speciation and interactions under reproducible conditions, and as a route to specialised foods. Their outputs should be interpreted as proof of concept for element behaviour in plants rather than as evidence of population benefit. The distinction between bioaccessibility, the fraction released during digestion, and bioavailability, the fraction absorbed and used, is especially relevant here because most soilless studies stop at concentration or, at best, in vitro bioaccessibility (Renna *et al.*, 2022).

4.5 Microbial and Nanoscale Inputs: Promise Ahead of Evidence

Microbial inoculants, including arbuscular mycorrhizal fungi and plant growth-promoting bacteria, are proposed as low-input means of enhancing nutrient uptake, and microbial-integrated approaches are listed among emerging priorities for selenium biofortification (Schiavon *et al.*, 2020). The vegetable evidence remains largely confined to controlled environments. In greenhouse lettuce, mycorrhizal improvement of nutritional quality depended on the source of phosphorus supplied, which illustrates how strongly microbial effects are conditioned by the nutrient regime (Baslam *et al.*, 2011). Such context dependence implies that results obtained under one fertilisation regime in pot or greenhouse experiments may not transfer to commercial vegetable production, where nutrient supply is usually managed differently.

Nanoscale fertilisers present a similar pattern in which technological promise has outpaced comparative evaluation. A critical analysis of 78 studies comparing nanoformulated agrochemicals with conventional analogues found a median efficacy gain of about 20–30%, noted that many studies lacked nano-specific quality assurance and adequate controls, and found no comprehensive field study evaluating both efficacy and environmental impact (Kah *et al.*, 2018). A more recent synthesis of nanomaterial-based biofortification reported enrichment of various crops with zinc, iron, selenium and other elements, together with improvements in physiological performance, antioxidant compounds and yield, and described nanofertilisers as a promising delivery route (Carmona *et al.*, 2024). Such syntheses document that enrichment occurs, but they do not resolve the comparative question of whether nanoscale formulations outperform equivalent doses of conventional salts, nor the absence of field-scale evaluation identified earlier (Kah *et al.*, 2018). For vegetables, which are often eaten fresh and with minimal processing, the fate of engineered particles in edible tissues is a substantive safety question rather than a formality. Until controlled comparisons with equivalent doses of conventional salts are available under field conditions, claims that nanoscale inputs are superior for vegetable biofortification should be regarded as preliminary.

Table 2 summarises representative agronomic studies discussed in this section. Read together, the entries show a consistent hierarchy of reliability: selenium and iodine respond predictably, zinc responds in potato but with emerging trade-offs involving yield and phytate, and iron responds only in soilless microgreen systems. The table also shows that almost every study reports concentration outcomes, that few report portion-based contributions against both recommended intake and upper limits, and that human outcome data exist only for iodine in a small uncontrolled study and for selenium at the level of a national food system.

Table 2. Representative agronomic biofortification studies in vegetables: principal outcomes and critical caveats

Crop and setting	Element and form or input	Application route	Principal reported outcome	Critical caveat	Supporting references
National food system, Finland	Selenium added to multinutrient fertilisers	Soil, nationwide from 1984	Population moved from deficiency to optimal selenium status	Benefit mediated through the whole food system, not vegetables alone; requires sustained monitoring	Alfthan *et al.* (2015)
Broccoli and other brassicas	Selenium, mainly selenate	Soil and nutrient solution	Efficient accumulation of organic selenium species	Glucosinolates increased at moderate and declined at high selenium supply; limited human data	Robbins *et al.* (2005); Wiesner-Reinhold *et al.* (2017)
Carrot, field	Iodine (KI, KIO_3) and selenium (selenate, selenite), alone and combined	Soil, pre-sowing and top-dressing	KI more effective than KIO_3; no significant yield penalty	Combined supply reduced uptake of each element; selenium alone exceeded the recommended allowance several-fold per 100 g	Smoleń *et al.* (2016)
Tomato, greenhouse	Iodine (KI, KIO_3)	Root application from fruit set	Fruit iodine 1.5–10 mg/kg fresh weight with KI; no significant yield loss at non-phytotoxic doses	Single study system; bioavailability not measured	Kiferle *et al.* (2013)
Lettuce, water culture	Iodide and iodate	Nutrient solution	Form- and concentration-dependent uptake and growth responses	Controlled environment only	Voogt *et al.* (2010)
Mixed vegetables, human study	Iodine-enriched potato, tomato, carrot, salad (about 45 µg per 100 g)	Consumption for 2 weeks	Urinary iodine rose from 98.3 to 117.5 µg/L	Uncontrolled, short, small sample; biomarker of recent intake only	Tonacchera *et al.* (2013)
Potato, Andean highlands	Zinc and iron fertilisers	Soil and foliar	Tuber zinc increased 1.91-fold (soil) and 2.51-fold (foliar); iron unresponsive	Foliar iron caused leaf toxicity	Kromann *et al.* (2017)
Potato, Scotland, four genotypes	Zinc oxide, sulfate, nitrate	Foliar	Tuber zinc increased; 2.36–3.58-fold higher after cooking	Excessive rates reduced yield; tuber phytate increased	White *et al.* (2017)
Brassica microgreens	Zinc sulfate; iron sulfate	Nutrient solution	Zinc +75–281%; iron +64% to +278%	Iron at 40 mg/L phytotoxic; genotype-specific responses; bioavailability untested	Di Gioia *et al.* (2019)
Lettuce, greenhouse	Arbuscular mycorrhizal fungi	Inoculation	Improved nutritional quality	Effect conditioned by phosphorus source	Baslam *et al.* (2011)

Note. KI = potassium iodide; KIO_3 = potassium iodate. Values are those reported by the cited studies and are not directly comparable because doses, cultivars, growing conditions and reporting bases differ

5. Genetic Biofortification: From Natural Variation to Genome Editing

Genetic approaches alter the capacity of the crop itself, which removes the need for recurrent inputs and allows vitamins and phytochemicals to be targeted as well as minerals. They differ, however, in the source of the genetic change, the time needed to deliver a cultivar, the regulatory pathway and the depth of evidence available. This section appraises conventional breeding, marker-assisted introgression of major alleles, transgenic metabolic engineering and genome editing in turn, and then considers how regulatory change is reshaping the relative merits of these routes.

5.1 Exploiting Natural Variation through Conventional Breeding

Conventional breeding depends on usable, heritable variation within the crop or its crossable relatives, and the vegetable evidence confirms that such variation exists for several targets. In a diversity panel of *B. oleracea*, shoot calcium and magnesium concentrations varied about two-fold and were highly heritable (Broadley *et al.*, 2008). Screening of 250 plants from 77 accessions of ten *Solanum* species revealed a ten-fold range in tuber folate, with some individuals of *S. tuberosum* subsp. *andigenum* and of the wild species *Solanum vernei* Bitter & Wittm. and *Solanum boliviense* Dunal producing more than twice the folate of the commercial cultivar Russet Burbank, whereas modern cultivars supply only about 6% of the recommended daily folate intake (Robinson *et al.*, 2015). For iron and zinc, three cycles of recurrent selection in diploid Andean potato achieved broad-sense heritability of about 0.81 and genetic gains of more than 29% for iron and 26% for zinc, although dry matter declined by 2% and 5% in later cycles and cycle-by-location interactions were significant (Amoros *et al.*, 2020). These results support the early argument that micronutrient density can be bred into crops by exploiting variation already present in plant genomes (Welch & Graham, 2004), but the small decline in dry matter in potato is a reminder that density and other quality attributes must be selected jointly, in line with the dilution effect observed among high-yielding cultivars (Marles, 2017).

OFSP remains the reference case for successful vegetable biofortification through breeding. Since the mid-1990s, an integrated agriculture and nutrition effort in sub-Saharan Africa, where most sweetpotato varieties grown were white-fleshed and lacked β-carotene, has bred 42 OFSP varieties adapted to farmer needs and consumer preferences, and a multi-partner initiative launched in 2009 had reached 2.8 million households by the time of that review (Low *et al.*, 2017). Multi-site evaluation in Zimbabwe detected significant genotype-by-environment interaction, yet the four best introduced cultivars outyielded the preferred local check by 135–184%, all introduced cultivars had dry matter above 25%, and farmers accepted their taste and texture (Gasura *et al.*, 2021). The success of OFSP plausibly reflects a favourable combination of attributes: a nutrient with strong genetic control, a visible colour change that serves as a proxy for the trait, a crop that is eaten in substantial quantities, and sustained investment in delivery. Few other vegetable–nutrient combinations share all of these attributes, which limits the extent to which the OFSP model can be generalised.

Anthocyanin-rich cultivars illustrate both the reach and the limits of breeding. Pigmented potato and sweetpotato cultivars, including 'Kufri Neelkanth' potato and 'Bhu Krishna' sweetpotato in India, were obtained through conventional crossing, whereas substantial anthocyanin accumulation in tomato fruit required introgression of the *Aft* and *atv* genes from wild relatives, as in 'Sun Black', or genetic engineering (Mattoo *et al.*, 2022). A comparable pattern has emerged for GABA in tomato. An introgression line carrying a high-GABA locus from *Solanum pennellii* Correll also carried a linked small-fruit trait, and six generations of marker-assisted screening were needed to recover recombinant lines combining normal fruit size with high GABA (Oi *et al.*, 2026). The same nutritional target was reached far more quickly by editing an autoinhibitory domain of an endogenous glutamate decarboxylase gene (Nonaka *et al.*, 2017). The comparison captures the central trade-off between breeding and editing: breeding is slower and can be encumbered by linkage drag, but it produces material that faces no special regulatory barrier in most jurisdictions.

5.2 Marker-Assisted Introgression of Major Alleles: The Orange Gene

Where a single allele of large effect is known, molecular markers can accelerate breeding substantially. The cauliflower *Or* allele is the clearest vegetable example, because its effect operates through the creation of a carotenoid sink rather than through increased pathway flux (Lu *et al.*, 2006). The allele is semi-dominant, so phenotypic selection cannot readily distinguish homozygous from heterozygous plants. Co-dominant gene-based markers developed from the *Or* sequence resolved this difficulty and were used to introgress the allele into tropical cauliflower backgrounds of early, mid-early and mid-late maturity, yielding homozygous orange-curd progenies adapted to Indian growing conditions (Singh *et al.*, 2025). The same regulator has been explored in fruit crops. Ectopic expression of an *Arabidopsis thaliana* (L.) Heynh. *OR* variant in tomato initiated chromoplast formation in very young fruit and increased carotenoids at all developmental stages, but it also promoted flowering, fruit set and ethylene production and altered the expression of ripening-associated genes (Yazdani *et al.*, 2019). The pleiotropy observed in tomato implies that sink-creating alleles may have developmental consequences beyond carotenoid content, which should be evaluated in each crop before deployment. No published human bioavailability data for orange cauliflower were identified in this review, so its nutritional contribution remains inferred from composition rather than demonstrated.

5.3 Transgenic Metabolic Engineering

Transgenic engineering provides access to traits that are absent from, or too rare in, the crossable gene pool. Early work in potato showed that tuber-specific expression of a three-gene bacterial pathway raised total carotenoids about 20-fold and β-carotene about 3600-fold, to 47 μg per gram dry weight (Diretto *et al.*, 2007). Folate engineering in tomato revealed a characteristic pattern of metabolic bottlenecks. Overexpression of GTP cyclohydrolase I increased pteridine production up to 140-fold but raised folate only two-fold, because the fruit became depleted of *p*-aminobenzoate; combining this trait with overexpression of aminodeoxychorismate synthase raised folate up to 25-fold in vine-ripened fruit and up to 15-fold in fruit ripened by ethylene treatment, as in commercial practice, so that less than one standard serving could meet the adult daily requirement (Díaz de la Garza *et al.*, 2007). In potato,

tuber-specific expression of four folate biosynthesis genes increased folate to levels considered nutritionally relevant and maintained folate stability during long-term tuber storage (De Lepeleire *et al.*, 2018). The attention paid to storage stability in the potato work addresses a problem that has constrained other biofortified crops. Provitamin A in biofortified maize, for example, declined by about 70% after six months of storage (Van Der Straeten *et al.*, 2020).

Anthocyanin-enriched tomato is the most advanced transgenic vegetable with a health-oriented trait. Fruit-specific expression of the *Delila* and *Rosea1* transcription factors from snapdragon (*Antirrhinum majus* L.) produced intensely purple fruit with anthocyanin levels comparable to those of blackberries and blueberries and a three-fold increase in hydrophilic antioxidant capacity, and cancer-susceptible *Trp53*-deficient mice fed a diet supplemented with these tomatoes showed a significant extension of lifespan (Butelli *et al.*, 2008). Anthocyanin accumulation also doubled shelf life by delaying over-ripening and reduced susceptibility to *Botrytis cinerea* Pers. (Y. Zhang *et al.*, 2013), a trait with direct commercial value that may matter as much for adoption as the nutritional claim. The United States regulator concluded in 2022 that the purple tomato was unlikely to pose an increased plant pest risk and was not subject to regulation under its biotechnology rules (APHIS, 2022). The health evidence for this product, however, rests on animal models and on the general association between anthocyanin intake and chronic disease, which remains largely correlational (Mattoo *et al.*, 2022). Reviews of plant metabolic engineering for nutrition argue that nutritionally enhanced foods could help protect against chronic disease when made widely accessible and examine the evidence for that proposition (Martin & Li, 2017); for the purple tomato, the published evidence cited for health benefit derives from animal models.

Across transgenic vegetables, three weaknesses recur. Most events have been characterised in greenhouses or small field plots, although plants tested under controlled conditions may perform less well under the nutrient limitations and stresses of farmers' fields (Cakmak & Kutman, 2018). Bioavailability studies of engineered vegetables are scarce, even though bioavailability studies have demonstrated efficacy in maintaining vitamin A status for several provitamin A 'golden' crops (Giuliano, 2017). Regulatory approval and public acceptance remain costly and uncertain, particularly when the product offers no agronomic benefit to growers (Van Der Straeten *et al.*, 2020). The anthocyanin tomato is instructive precisely because it combines a nutritional claim with a shelf-life advantage.

5.4 Genome Editing

Genome editing with clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated nucleases enables targeted knockout, regulatory modification and, with base and prime editors, precise nucleotide substitution in endogenous genes (Zhu *et al.*, 2020). In vegetables, it has been used mainly to redirect existing metabolic flux. Multiplex editing of five genes at six target sites in tomato, designed to promote lycopene synthesis while blocking its conversion to α- and β-carotene, increased fruit lycopene about 5.1-fold, with stable inheritance of homozygous mutations (X. Li *et al.*, 2018). Removal of the autoinhibitory domain of glutamate decarboxylase increased GABA 7–15-fold in red tomato fruit. The choice of paralogue was decisive, because *SlGAD3* mutants retained normal growth and fruit set whereas *SlGAD2* mutants showed reduced plant size, delayed flowering and lower fruit production associated with high GABA accumulation in leaves (Nonaka *et al.*, 2017). A high-GABA tomato developed with this technology was notified to the Japanese government in December 2020, seedlings were distributed to about 4,200 home gardeners from May 2021, and online sales of fruit began in September 2021 (Ezura, 2022).

Genome editing has also created nutritional traits that breeding could not easily provide. Knockout of the tomato *Sl7-DR2* gene, which converts 7-dehydrocholesterol to cholesterol, caused accumulation of the provitamin D3 precursor in leaves and green fruit without detectable effects on growth, development or yield; after exposure to ultraviolet-B light, one fruit could contain vitamin D3 equivalent to two medium-sized eggs or 28 g of tuna (J. Li *et al.*, 2022). The nutritional value of this trait therefore depends on post-harvest or pre-harvest ultraviolet exposure, and no human study has yet examined its bioavailability. In lettuce, editing an upstream open reading frame (uORF) that represses translation of GDP-L-galactose phosphorylase increased ascorbate by about 150% and improved tolerance of oxidative stress (H. Zhang *et al.*, 2018). A subsequent study in a commercial lettuce cultivar combined knockout of lycopene ε-cyclase, which raised β-carotene 2.7-fold and zeaxanthin to 4.3 μg per gram fresh weight, with uORF editing that raised ascorbate 6.9-fold; the edited plants grew normally but showed reduced non-photochemical quenching capacity under high light (Livneh *et al.*, 2025). That photoprotective cost would be invisible in growth-chamber assessments and may matter under field irradiance, which illustrates why edited lines require evaluation beyond controlled environments. Editing without integration of foreign DNA is feasible in lettuce through delivery of preassembled Cas9 ribonucleoprotein complexes into protoplasts (Woo *et al.*, 2015), which has regulatory significance in jurisdictions that classify products according to the presence of introduced sequences.

The constraints on nutritional genome editing are nonetheless substantial. Editing presupposes detailed knowledge of the pathway and its regulation, is vulnerable to pleiotropic effects, as the *SlGAD2* and *OR* examples show, and depends on efficient transformation and regeneration, which remain bottlenecks in many vegetable crops (Kumar *et al.*, 2022; Zhu *et al.*, 2020). Editing is also best suited to knockouts and regulatory modifications that redistribute existing flux, whereas traits requiring enzymatic steps absent from the species still require transgenesis. The most promising strategic direction may be the stacking of several nutritional traits, and of agronomic traits that give growers a reason to adopt the variety, in single elite backgrounds (Van Der Straeten *et al.*, 2020).

5.5 Regulatory Divergence as a Determinant of Technique Choice

Regulation now shapes the practical merit of genetic techniques as much as biology does. By 2020, Argentina, Brazil and other Latin American countries had adopted case-by-case consultation systems that generally exclude from genetically modified organism regulation those edited products lacking foreign DNA, the United States had exempted many edited plants under a product-focused rule, Japan had introduced a notification system for edits without introduced sequences, and the European Union had been bound by

Table 3. Representative genetic biofortification outputs in vegetables: reported compositional change, development status and principal limitations

Crop	Target	Approach	Reported change	Development status	Principal limitation	Supporting references
Sweetpotato (*Ipomoea batatas*)	Provitamin A (β-carotene)	Conventional breeding	Orange-fleshed cultivars; top introduced cultivars outyielded local check by 135–184% with acceptable dry matter	Released and disseminated in sub-Saharan Africa	Genotype × environment interaction; carotenoid losses during storage of dried products	Bechoff *et al.* (2010); Gasura *et al.* (2021); Low *et al.* (2017)
Potato (*Solanum tuberosum*)	Iron and zinc	Recurrent selection	Genetic gains above 29% (iron) and 26% (zinc) over three cycles	Advanced clones; absorption tested in humans	Slight decline in dry matter; lower fractional iron absorption	Amoros *et al.* (2020); Burgos *et al.* (2023)
Potato	Folate	Germplasm screening	Ten-fold range; some wild and primitive accessions above twice a commercial cultivar	Pre-breeding	Not yet transferred to cultivars	Robinson *et al.* (2015)
Cauliflower (*Brassica oleracea* var. *botrytis*)	β-Carotene	Spontaneous *Or* mutation; marker-assisted introgression	Orange curd through chromoplast formation; homozygous lines in three maturity groups	Breeding lines	No human bioavailability data identified	Lu *et al.* (2006); Singh *et al.* (2025)
Tomato (*Solanum lycopersicum*)	GABA	Wild-species introgression with markers	High-GABA lines with normal fruit size after six generations	Breeding lines	Linkage drag; long timeline	Oi *et al.* (2026)
Potato	Provitamin A carotenoids	Transgenic (bacterial mini-pathway)	β-Carotene about 3600-fold higher (47 µg/g dry weight)	Research event	Regulatory cost; no field or bioavailability data	Diretto *et al.* (2007)
Tomato	Folate	Transgenic (two pathway genes, combined by crossing)	Folate up to 25-fold higher	Research event	Flux constraint at dihydropteroate synthesis; no human data	Díaz de la Garza *et al.* (2007)
Potato	Folate	Transgenic (four pathway genes)	Nutritionally relevant folate with storage stability	Research event	Regulatory status; no human data	De Lepeleire *et al.* (2018)
Tomato	Anthocyanins	Transgenic (*Del*/*Ros1* transcription factors)	Anthocyanins comparable to blackberries; doubled shelf life	Regulatory review completed in the United States	Health evidence from animal models only	APHIS (2022); Butelli *et al.* (2008); Y. Zhang *et al.* (2013)
Tomato	Lycopene	Multiplex genome editing	About 5.1-fold higher lycopene	Research lines	Field and consumer evaluation lacking	X. Li *et al.* (2018)
Tomato	GABA	Genome editing (*SlGAD3*)	GABA 7–15-fold higher	Marketed in Japan from 2021	Health relevance of GABA less firmly established than for essential nutrients	Ezura (2022); Nonaka *et al.* (2017)
Tomato	Provitamin D3	Genome editing (*Sl7-DR2*)	One fruit after UVB equivalent to two eggs in vitamin D3	Research lines	Requires ultraviolet conversion; no human data	J. Li *et al.* (2022)
Lettuce (*Lactuca sativa*)	Ascorbate, β-carotene, zeaxanthin	Genome editing (uORF, lycopene ε-cyclase)	Ascorbate up to 6.9-fold; β-carotene 2.7-fold	Research lines	Reduced photoprotective capacity under high light	Livneh *et al.* (2025); H. Zhang *et al.* (2018)

Note. GABA = γ-aminobutyric acid; Or = Orange; Del = Delila; Ros1 = Rosea1; UVB = ultraviolet-B; uORF = upstream open reading frame. Status reflects the most advanced stage documented in the cited sources

a 2018 court ruling that treated targeted mutagenesis as genetic modification (Menz *et al.*, 2020). Japan's framework relies on pre-submission consultation, notification and prompt public disclosure, with transparency presented as the basis of consumer trust (Kondo & Taguchi, 2022). In April 2026 the Council of the European Union formally adopted a regulation, subject to final adoption by the European Parliament before publication, distinguishing category 1 plants, considered equivalent to conventional plants and exempt from labelling except for seed and reproductive material, from category 2 plants, which remain subject to authorisation, traceability and labelling under genetically modified organism rules; herbicide tolerance is excluded from category 1, and most provisions are expected to apply from 2028 (Council of the European Union, 2026). For nutritional traits that arise from small edits, such as those in GABA, lycopene, provitamin D3 and ascorbate-enriched lines, these changes reduce the regulatory distance between editing and breeding. Transgenic products, including the purple tomato, remain in a separate and costlier category in most markets.

Table 3 brings together representative genetic biofortification outputs in vegetables. Its central message is that the technical feasibility of large compositional change is now well established across a wide range of nutrients, whereas deployment and human evidence remain concentrated in conventionally bred root and tuber crops. The table also shows that pleiotropic or agronomic side-effects, where they have been examined, are specific to the gene and allele rather than to the technique, which argues for trait-by-trait rather than technique-by-technique evaluation.

6. From Concentration to Nutrition: Retention, Bioavailability and Human Evidence

The preceding sections show that the composition of vegetables can be changed substantially by several techniques. Whether those changes improve nutrition depends on three further conditions, namely that the nutrient survives storage and preparation, that it is released and absorbed from the food matrix, and that the enriched food is eaten in sufficient quantity by people whose intake is inadequate. This section examines the evidence at each of these stages, which together constitute the translational gap between biofortification research and public-health benefit.

6.1 Matrix Effects: Inhibitors, Enhancers and the Limits of Concentration Data

The bioavailability of iron, zinc and provitamin A carotenoids from plant foods is typically low, and a review of biofortified staple crops concluded that, although biofortified foods achieve higher total absorption than conventional varieties, antinutrients and processing strongly modify the amount absorbed; the authors recommended breeding simultaneously for higher micronutrient density and lower concentrations of inhibitors (La Frano *et al.*, 2014). The vegetable evidence reinforces this conclusion in ways that concentration studies cannot reveal. In the Peruvian potato trial, the biofortified cultivar contained about twice as much iron as the conventional cultivar and more ascorbic acid, but fractional iron absorption was lower, at 12.1% compared with 16.6%, so that total iron absorbed per meal was 45.8% higher rather than doubled (Burgos *et al.*, 2023). In an earlier pair of studies, fractional absorption from biofortified purple-fleshed potato was less than half that from regular yellow potato, at 13.3% compared with 28.4%, and total absorbed iron did not differ between varieties, whereas biofortified OFSP achieved similar fractional absorption to the regular variety and therefore delivered 1.9-fold more absorbed iron (Jongstra *et al.*, 2020). The authors attributed the low absorption from the enriched potatoes largely to polyphenols.

These findings are consequential because they show that the same breeding strategy can succeed in one genetic background and fail in another, depending on the co-selection of inhibitors such as chlorogenic acid and anthocyanins. They also imply a potential conflict between two nutritional objectives that are often promoted together. Anthocyanin-rich vegetables are bred for their putative health benefits, yet phenolic compounds can diminish iron absorption from the same foods (Mattoo *et al.*, 2022). A similar interaction arises in agronomic zinc enrichment, where foliar zinc raised tuber phytate (White *et al.*, 2017), and in calcium-enriched leafy vegetables, where oxalate limits calcium bioavailability (Buturi *et al.*, 2021). The implication is that concentration targets should be accompanied by targets or at least monitoring for inhibitors and enhancers, and that the choice of genetic background matters as much as the magnitude of enrichment.

6.2 Post-harvest Retention and Food Preparation

Retention differs markedly among nutrients and processing pathways. For OFSP, carotenoid losses during solar or sun drying were generally 15% or less, but dried chips lost about 70% of their carotenoids after four months of storage at ambient temperature, irrespective of packaging type, and retention differed among cultivars (Bechoff *et al.*, 2010). Storage rather than processing therefore emerged as the principal constraint for dried products. Minerals are generally more stable. Cooked zinc-biofortified potatoes retained 2.36–3.58 times more zinc than cooked controls (White *et al.*, 2017), and iodine incorporated into potato, carrot and tomato during cultivation was preserved under the domestic cooking procedures tested (Comandini *et al.*, 2013). For vitamins produced by metabolic engineering, stability is a design consideration in its own right. The four-gene folate potato was engineered with long-term storage stability as an explicit objective (De Lepeleire *et al.*, 2018), whereas the provitamin D3 tomato requires ultraviolet-B exposure to convert the accumulated precursor into vitamin D3 (J. Li *et al.*, 2022). The latter means that nutritional value will depend on handling practices outside the breeder's control. Retention evidence for most agronomically enriched leafy vegetables and for most genome-edited lines remains unavailable.

6.3 Human Absorption, Status and Efficacy

The strongest human evidence for any biofortified vegetable concerns OFSP. In South African primary school children, consumption of β-carotene-rich OFSP improved vitamin A status assessed with the modified relative dose response test, a measure of liver retinol stores (van Jaarsveld *et al.*, 2005). In rural Mozambique, an integrated agriculture and nutrition intervention that

introduced OFSP increased vitamin A intake and serum retinol concentrations in young children (Low *et al.*, 2007), and a larger introduction in rural Uganda increased vitamin A intakes among children and women and improved vitamin A status among children (Hotz *et al.*, 2012). These studies used different designs, ranging from a controlled feeding study to community-level programme evaluations, and they measured different outcomes, but their consistency across settings provides the most secure evidence base in this field. Their generalisability is nonetheless bounded by the characteristics of sweetpotato, which is consumed in substantial quantities in these settings.

For iron, stable-isotope studies in women provide direct but short-term evidence on absorption, as described above, but no efficacy trial of an iron-biofortified vegetable measuring change in iron status over months has been identified in this review (Burgos *et al.*, 2023; Jongstra *et al.*, 2020). The contrast with iron-biofortified beans is instructive. In a randomised controlled feeding trial in Rwandan women, consumption of iron-biofortified common bean (*Phaseolus vulgaris* L.) for 128 days increased iron status (Haas *et al.*, 2016). Beans, however, are consumed daily and in large quantities in that setting, and their matrix differs from that of tubers, so the pulse evidence cannot be transferred to vegetables without an equivalent trial. Evidence for iodine is limited to the small uncontrolled study showing a modest increase in urinary iodine (Tonacchera *et al.*, 2013), and evidence for selenium comes from a national food-system intervention rather than from vegetables alone (Alfthan *et al.*, 2015). For the vitamins, carotenoids and phytochemicals introduced by transgenic or genome-editing approaches in tomato, potato and lettuce, no human bioavailability or efficacy data were identified. This pattern is consistent with the scoping review of high-income countries, which found that vegetable biofortification studies were few and that safety, sensory and modelling studies were almost absent (Gulyas *et al.*, 2025).

The confidence that can be placed in the translational value of vegetable biofortification therefore varies widely across crop–nutrient combinations. It is high for provitamin A from OFSP, moderate for absorbed iron from biofortified potato and sweetpotato, with the important qualification that gains in absorbed iron can be much smaller than gains in concentration, low for iodine and for selenium when vegetables are the sole vehicle, and absent for most engineered traits. Table 4 summarises the human and absorption evidence and its main methodological limitations.

Table 4. Human evidence on absorption, status and efficacy for biofortified vegetables, with a pulse comparator

Crop and nutrient	Design and population	Principal finding	Main methodological limitation	Supporting references
Orange-fleshed sweetpotato; provitamin A	Controlled feeding in primary school children, South Africa	Improved vitamin A status (modified relative dose response)	Single setting; school-feeding context	van Jaarsveld *et al.* (2005)
Orange-fleshed sweetpotato; provitamin A	Integrated agriculture–nutrition intervention, young children, Mozambique	Increased vitamin A intake and serum retinol	Effect of crop not separable from nutrition promotion	Low *et al.* (2007)
Orange-fleshed sweetpotato; provitamin A	Large-scale introduction, children and women, Uganda	Higher vitamin A intakes; improved status in children	Programme evaluation; improvement in status reported for children only	Hotz *et al.* (2012)
Orange-fleshed sweetpotato; iron	Stable-isotope multiple-meal crossover, iron-depleted women, Malawi (n = 24)	Similar fractional absorption; 1.9-fold higher total iron absorbed	Two-week exposure; absorption rather than status	Jongstra *et al.* (2020)
Potato; iron	Stable-isotope multiple-meal crossover, iron-depleted women, Peru (n = 35)	Fractional absorption 13.3% versus 28.4%; no difference in total iron absorbed	Polyphenol-rich biofortified genotype; short exposure	Jongstra *et al.* (2020)
Potato; iron	Randomised single-blind crossover with stable isotopes, women, Peruvian highlands (n = 28)	Fractional absorption 12.1% versus 16.6%; total iron absorbed 45.8% higher	Absorption rather than status; single genotype pair	Burgos *et al.* (2023)
Mixed vegetables; iodine	Uncontrolled before–after study, healthy volunteers (n = 50), two weeks	Urinary iodine rose by 19.6%	No control group; biomarker of recent intake	Tonacchera *et al.* (2013)
Whole food system; selenium	National fertiliser policy with population monitoring, Finland	Population selenium status raised from deficient to optimal	Vegetables not isolated as vehicle; observational time series	Alfthan *et al.* (2015)
Common bean (pulse comparator); iron	Randomised controlled feeding trial, women, Rwanda, 128 days	Increased iron status	Pulse rather than vegetable; high daily intake	Haas *et al.* (2016)

Note. Fractional absorption values are those reported by the original studies for biofortified versus conventional varieties. The pulse study is included only as a comparator for the type of efficacy evidence not yet available for vegetables

Table 4 shows that the human evidence for biofortified vegetables is concentrated in a handful of crops and dominated by short-term absorption or intake outcomes, with functional outcomes largely unmeasured. The limitations column indicates that small samples, short exposure periods, single study sites and heterogeneous biomarkers limit comparability across studies, and that even the best-supported case, OFSP, rests on programme evaluations whose effects cannot be attributed solely to the crop.

7. Cross-cutting Risks, Trade-offs and Conditions for Implementation

7.1 Safety Margins and Unintended Effects

Biofortification is sometimes presented as intrinsically safe because it works through foods rather than supplements, but that assumption holds only when enrichment is calibrated. Selenium has been described as an essential element with a narrow margin between adequacy and toxicity (Schiavon *et al.*, 2020), and the current European tolerable upper intake level of 255 µg per day for adults (EFSA Panel on Nutrition, Novel Foods and Food Allergens, 2023) is readily approached by some experimental products when portion-based intake is calculated (Smoleń *et al.*, 2016). Iodine enrichment has so far been tested in humans only at modest doses, and the authors of that study concluded that, in combination with iodised salt, it would improve iodine nutrition without risk of excess (Tonacchera *et al.*, 2013); that conclusion should not be extrapolated to more intensively enriched products or to populations with high background intake. For nanoscale inputs, the environmental fate of formulations has not been evaluated comprehensively under field conditions (Kah *et al.*, 2018), and the persistence of engineered particles in edible tissues of vegetables eaten raw has not been addressed systematically in the literature reviewed here. For genetic approaches, the unintended effects documented in vegetables are pleiotropic rather than toxicological, as in the growth penalties of *SlGAD2* mutants, the developmental changes associated with ectopic *OR* expression and the reduced photoprotective capacity of carotenoid-edited lettuce (Livneh *et al.*, 2025; Nonaka *et al.*, 2017; Yazdani *et al.*, 2019). Such effects are gene-specific and can often be avoided by choosing a different target, but they show that nutritional editing is not free of agronomic risk.

7.2 Yield, Quality and Nutrient Trade-offs

Trade-offs between enrichment and other traits appear in every technique family. Agronomic enrichment has been associated with yield reductions of up to 32% for calcium and 25% for iron in vegetables (Buturi *et al.*, 2021), foliar zinc reduced potato yields at excessive rates (White *et al.*, 2017), and iron became phytotoxic to microgreens at the highest concentration tested (Di Gioia *et al.*, 2019). Selenium affects glucosinolate synthesis in a dose-dependent way (Robbins *et al.*, 2005), and selection for iron and zinc density in potato was accompanied by a slight decline in dry matter (Amoros *et al.*, 2020). These trade-offs are not uniformly negative. Selenium can delay fruit ripening by reducing ethylene biosynthesis (Puccinelli *et al.*, 2017), and anthocyanin accumulation doubled the shelf life of tomato (Y. Zhang *et al.*, 2013), which suggests that some nutritional traits can be paired with post-harvest advantages that growers and retailers value. A broader lesson from the food composition literature is that selection for one bioactive component may reduce others (Robbins *et al.*, 2005) and that selection for yield can dilute mineral concentrations (Marles, 2017). Biofortification programmes that measure only the target nutrient therefore risk overlooking losses elsewhere in the nutritional profile.

7.3 Acceptance, Adoption and Delivery

The acceptance literature is dominated by staples and by OFSP. A systematic review of 72 studies in low- and middle-income countries found that sensory acceptance of biofortified crops was generally good, that information about health benefits was an important determinant of acceptance and adoption, and that changes in colour did not act as an obstacle; it also noted that few studies had examined crops after wide-scale dissemination (Talsma *et al.*, 2017). A later review of 24 farmer-level studies identified socioeconomic, institutional, agronomic and psychological determinants of adoption, but most studies were cross-sectional and relied on stated intentions such as willingness to plant or pay (Samuel *et al.*, 2024). For vegetables grown in commercial markets, adoption will also depend on yield, shelf life and appearance, which is why agronomic co-benefits may be decisive. The first genome-edited tomato was introduced through free distribution of seedlings to home gardeners before retail sales, a strategy intended to build familiarity with the product (Ezura, 2022), and Japan's regulatory framework emphasises transparency as the basis of consumer trust (Kondo & Taguchi, 2022). Evidence on consumer responses to nutritionally edited vegetables in other markets remains sparse, and the high-income evidence base contains almost no sensory or acceptance studies of biofortified vegetables (Gulyas *et al.*, 2025).

7.4 Economic Logic and Choice of Technique

The economic case for biofortification was built on ex ante assessments showing favourable cost-effectiveness for staple crops, including sweetpotato, under assumptions about adoption, retention and efficacy (Meenakshi *et al.*, 2010). Agronomic enrichment offers an immediate route to higher micronutrient concentrations but incurs recurrent costs, whereas genetic biofortification may be more cost-effective in the long run (de Valença *et al.*, 2017). For vegetables, the recurrent cost of agronomic enrichment falls on growers, who will bear it only if a price premium, a regulatory requirement or a public programme compensates them. The Finnish selenium programme shows that national fertiliser policy can overcome this constraint when the nutrient is supplied through general fertilisers rather than through crop-specific enrichment (Alfthan *et al.*, 2015). No comparable economic analysis specific to biofortified vegetables other than sweetpotato was identified in this review.

Integrating the evidence across Sections 4 to 7 suggests that the choice of technique should follow from three properties of the target, rather than from the novelty of the method. The first is the chemistry and mobility of the nutrient. Selenium and iodine, which are taken up readily and accumulate in leaves, are well suited to calibrated agronomic enrichment of brassicas, alliums and

leafy vegetables, whereas iron in field vegetables is not, and genetic routes with explicit monitoring of absorption inhibitors are more promising for it. The second is the availability of genetic variation. Where heritable variation or major alleles exist, as for provitamin A in sweetpotato and cauliflower, iron and zinc in potato and folate in potato germplasm, breeding offers durable gains without special regulatory hurdles; where the gene pool lacks the trait, as for provitamin D3 in tomato fruit or high anthocyanins in cultivated tomato, editing or transgenesis is required. The third is the delivery context. Vegetables that function as staples in low-income settings justify investment in bred cultivars with human efficacy trials, whereas vegetables produced in protected cultivation for higher-income markets are better suited to agronomic enrichment tailored to specific consumer needs. This framework is derived from the present synthesis rather than from comparative trials, and its principal value is in identifying the evidence each combination still requires.

8. Future Directions

The research agenda that follows from this synthesis is ordered by the degree to which each gap limits interpretation of existing evidence. The immediate priorities concern measurement and calibration, because without them the large body of agronomic and genetic studies cannot be translated into nutritional terms. The longer-term priorities concern human efficacy, field performance of genetically improved lines and the conditions for delivery.

The first immediate priority is standardised, portion-based reporting. Current studies report concentrations on dry-weight or fresh-weight bases, as fold changes or as percentages of recommended intake, and rarely relate the enriched product to the tolerable upper intake level (Buturi *et al.*, 2021; Smoleń *et al.*, 2016). Agronomic studies should report fresh-weight concentrations, the chemical form and speciation of the element in the edible product, and the contribution of a realistic portion to both the recommended intake and the upper level for defined age and sex groups. Factorial dose–response trials across at least two seasons and several sites would allow application rates to be calibrated to nutritional targets rather than to maximum accumulation, particularly for selenium and iodine. Journals and funders could encourage this by adopting minimum reporting standards, which would make future syntheses quantitatively tractable.

The second immediate priority is to measure absorption and inhibitors alongside concentration. The potato and sweetpotato isotope studies show that concentration gains can be partly or wholly offset by polyphenols (Burgos *et al.*, 2023; Jongstra *et al.*, 2020), and foliar zinc can raise phytate (White *et al.*, 2017). Breeding programmes for iron and zinc should therefore include chlorogenic acid, other polyphenols, phytate and ascorbate among selection or monitoring traits, as proposed for staples (La Frano *et al.*, 2014), and stable-isotope absorption studies should be conducted for zinc-enriched potato and for iron-enriched genotypes from different genetic backgrounds before cultivars are promoted for nutritional purposes. In vitro digestion models can screen candidates, but they should be validated against human absorption data for each vegetable matrix.

The third immediate priority concerns post-harvest retention and preparation. Carotenoid losses during storage of dried OFSP (Bechoff *et al.*, 2010) and the ultraviolet requirement of the provitamin D3 tomato (J. Li *et al.*, 2022) illustrate that nutritional value can be lost or left unrealised after harvest. Retention studies should follow enriched products through typical storage, retail and household preparation for each target population, using fresh-weight losses and nutrient speciation as outcomes, and should be completed before human efficacy trials are designed.

Among the longer-term priorities, adequately powered efficacy trials are the most important. With the exception of OFSP, no biofortified vegetable has been shown to improve a nutritional status biomarker over a period sufficient to alter body stores. Cluster-randomised or individually randomised trials lasting several months in populations with documented deficiency, using ferritin and soluble transferrin receptor for iron, urinary iodine together with thyroid function markers for iodine, and plasma selenium or selenoprotein biomarkers for selenium, are needed for iron-biofortified potato, iodine-enriched vegetables and selenium-enriched brassicas. The pulse trial in Rwanda offers a methodological template (Haas *et al.*, 2016). Such trials would determine whether vegetable biofortification merits inclusion in national nutrition strategies.

A second longer-term priority is multi-environment evaluation of genetically improved lines. Transgenic and genome-edited vegetables have mostly been characterised in controlled environments, where pleiotropic effects on photoprotection, flowering or ripening may be missed or misjudged (Livneh *et al.*, 2025; Yazdani *et al.*, 2019). Multi-location field trials that record yield, stress tolerance, nutrient stability and genotype-by-environment interaction are needed before nutritional claims for these lines can be judged, and they should be designed to detect trade-offs as well as gains. Changing climate adds a further dimension, because soil selenium availability is projected to decline in some agricultural regions (Schiavon *et al.*, 2020), which would alter the baseline against which biofortification is assessed.

A third longer-term priority is the integration of multiple nutrients and techniques. Stacking several nutritional traits in elite backgrounds, and combining them with agronomic traits valued by growers, has been advocated for staples (Van Der Straeten *et al.*, 2020) and has begun in lettuce (Livneh *et al.*, 2025). Joint agronomic enrichment with selenium and iodine produces species-specific interactions that remain poorly characterised for major vegetables (Golubkina *et al.*, 2021). Factorial experiments combining bred or edited cultivars with calibrated agronomic enrichment would clarify whether gains are additive and would identify the most efficient combinations for each crop.

A fourth longer-term priority concerns the newer inputs. Nanoscale fertilisers and microbial inoculants should be tested against equivalent doses of conventional salts under field conditions, with nano-specific characterisation, environmental fate assessment and residue analysis in edible tissues (Kah *et al.*, 2018), and microbial effects should be evaluated across the range of fertilisation regimes used in commercial vegetable production (Baslam *et al.*, 2011). Until such comparisons exist, these inputs should be regarded as experimental.

Table 5. Prioritised research needs for vegetable biofortification

Priority and timescale	Unresolved problem	Recommended design or improvement	Key outcome measures	Supporting references
Portion-based reporting and calibration (immediate)	Results reported on inconsistent bases; enrichment not calibrated to recommended and upper intakes	Multi-season, multi-site factorial dose–response trials with minimum reporting standards	Fresh-weight concentration; speciation; portion contribution to recommended intake and upper level	Buturi *et al.* (2021); EFSA Panel on Nutrition, Novel Foods and Food Allergens (2023); Smoleń *et al.* (2016)
Absorption and inhibitors (immediate)	Concentration gains offset by polyphenols and phytate	Stable-isotope absorption studies across genetic backgrounds; inhibitor monitoring in breeding	Fractional and total absorption; polyphenol, phytate and ascorbate levels	Burgos *et al.* (2023); Jongstra *et al.* (2020); La Frano *et al.* (2014); White *et al.* (2017)
Retention through storage and preparation (immediate)	Losses during storage; unrealised precursors	Food-chain retention studies under local practices	Nutrient retention on a fresh-weight basis; conversion of precursors	Bechoff *et al.* (2010); Comandini *et al.* (2013); J. Li *et al.* (2022)
Human efficacy (longer term)	No status-based efficacy evidence for vegetables other than sweetpotato	Randomised trials of several months in deficient populations	Ferritin, soluble transferrin receptor, urinary iodine, thyroid function, selenium biomarkers	Haas *et al.* (2016); Hotz *et al.* (2012); Tonacchera *et al.* (2013)
Field performance of genetically improved lines (longer term)	Pleiotropy and genotype × environment effects unassessed	Multi-location field trials of edited and transgenic lines	Yield; stress tolerance; nutrient stability; interaction effects	Cakmak and Kutman (2018); Livneh *et al.* (2025); Yazdani *et al.* (2019)
Multi-nutrient and integrated approaches (longer term)	Interactions among nutrients and techniques poorly characterised	Factorial trials combining improved cultivars with calibrated agronomic enrichment	Additivity of gains; element interactions	Golubkina *et al.* (2021); Van Der Straeten *et al.* (2020)
Nanoscale and microbial inputs (longer term)	Superiority over conventional inputs unproven	Field comparisons at equivalent doses with nano-specific characterisation	Relative efficacy; environmental fate; residues in edible tissues	Baslam *et al.* (2011); Kah *et al.* (2018)
Implementation and economics (longer term)	Adoption, acceptance and cost-effectiveness unknown for most vegetables	Longitudinal adoption studies; vegetable-specific economic evaluation	Adoption; consumption; cost per unit of nutritional benefit	Meenakshi *et al.* (2010); Samuel *et al.* (2024); Talsma *et al.* (2017)

Note. Immediate priorities are those addressable within existing research programmes; longer-term priorities require multi-site or multi-year investment

The final priority is implementation research. Adoption and acceptance studies need to move beyond cross-sectional surveys of intention towards longitudinal assessment after dissemination (Samuel *et al.*, 2024; Talsma *et al.*, 2017), and economic evaluations specific to vegetables other than sweetpotato are needed to test whether the favourable ex ante results obtained for staples extend to horticultural value chains (Meenakshi *et al.*, 2010). Regulatory change in the European Union and elsewhere will create natural experiments in the introduction of nutritionally edited vegetables (Council of the European Union, 2026), and consumer responses and market uptake in these settings should be monitored systematically.

Table 5 condenses these priorities. It distinguishes immediate needs, which could be addressed within existing research programmes at modest cost, from longer-term needs that require multi-site trials and cross-disciplinary collaboration. The table also shows that most priorities concern the translational stages of evidence rather than the generation of new enriched material, which is a reversal of the emphasis evident in the current literature.

9. Conclusions

This review set out to determine which biofortification techniques produce nutritionally meaningful, bioavailable, safe and deployable gains in vegetables. The most defensible conclusion is that the technical capacity to change vegetable composition now far exceeds the evidence that such changes improve nutrition. Agronomic enrichment reliably raises selenium and iodine concentrations in a wide range of vegetables, raises zinc in potato and is largely ineffective for iron in field-grown crops. Conventional breeding has delivered the only vegetable with consistent evidence of improved nutritional status, orange-fleshed sweetpotato, and has produced iron-dense potato whose absorption advantage is real but smaller than its concentration advantage would suggest. Transgenic engineering and genome editing have generated large gains in carotenoids, folate, anthocyanins, ascorbate, provitamin D3 and γ-aminobutyric acid, yet almost none of these lines has been evaluated for bioavailability, retention or performance across environments.

The evidence is strong for the feasibility of selenium and iodine enrichment, for the provitamin A efficacy of orange-fleshed sweetpotato and for the principle that absorption inhibitors can offset concentration gains. It is moderate for the contribution of iron-biofortified potato and sweetpotato to absorbed iron and for the stability of minerals during cooking. It is weak or absent for the population benefits of iodine- and selenium-enriched vegetables used as sole vehicles, for microbial and nanoscale inputs, and for the human health effects of engineered phytochemical and vitamin traits. Several tensions cut across these conclusions, including the narrow margin between adequate and excessive selenium intake, the dose-dependent effects of selenium on glucosinolates, the co-selection of polyphenols that reduce iron absorption, and pleiotropic effects of some edited or introduced genes.

The broader significance of the synthesis is that vegetable biofortification should be judged by the contribution of a realistic portion to a defined nutrient gap, measured after storage and preparation and confirmed by absorption or status outcomes, rather than by the magnitude of the concentration increase. When evaluated in this way, the most promising combinations are calibrated selenium and iodine enrichment of accumulating vegetables in managed production systems, bred provitamin A and iron-dense cultivars of vegetables that serve as staples, and edited traits that supply nutrients absent from the crop's gene pool, provided that these traits are subjected to the same translational scrutiny as conventionally bred crops. Changing regulation of genome-edited plants is likely to increase the number of such traits reaching markets, which makes the development of consistent standards for nutritional evidence more urgent rather than less.

10. Limitations

This review has the intrinsic limitations of a narrative design. Although the search strategy, eligibility criteria and appraisal principles were stated explicitly, studies were selected purposively and interpreted through expert judgement, so selection and interpretive bias cannot be excluded. The emphasis on field studies, portion-based reporting and human evidence may have led to under-representation of the large number of controlled-environment enrichment studies, which were cited selectively as examples rather than exhaustively.

The literature search relied on openly accessible scholarly databases, citation searching and institutional sources, and subscription-only bibliographic databases were not searched, so some relevant studies, particularly those indexed only in commercial agricultural databases, may have been missed. The restriction to English-language publications may have excluded relevant work published in other languages, including national literature from countries with active vegetable biofortification programmes. The evidence base itself is uneven, being concentrated in a small number of crops, notably sweetpotato, potato, tomato, lettuce and brassicas, and in a small number of countries, which limits the generalisability of conclusions to other vegetables and settings.

Heterogeneity in study designs, doses, chemical forms, cultivars, growing conditions, analytical methods and reporting bases precluded quantitative synthesis, and comparisons of the magnitude of effects across studies should therefore be regarded as indicative. Human evidence was sparse, frequently short-term and in some cases uncontrolled, and publication bias favouring positive enrichment results is plausible in a field dominated by small experimental studies. Finally, the field is developing rapidly, particularly in genome editing and its regulation, and developments published after the search end date are not reflected in this synthesis.

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 no competing interests exist.

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