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BIOTECHNOLOGICAL APPROACHES FOR BIOFORTIFICATION OF VEGETABLE CROPS: FROM NUTRIENT ENHANCEMENT TO PRECISION GENOME EDITING

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ABSTRACT

Micronutrient malnutrition, or “hidden hunger,” continues to affect more than two billion people worldwide, with iron, zinc, vitamin A, folate, and iodine deficiencies imposing the greatest public health burden. Vegetables are among the most concentrated dietary sources of vitamins, minerals, and health-promoting phytochemicals, yet their nutritional potential is often eroded by narrow cultivar selection, post-harvest losses, and cooking practices, and is rarely realised at population scale through diet diversification alone. Biofortification the deliberate enrichment of the edible portions of crops with bioavailable nutrients has therefore emerged as a complementary, sustainable strategy to conventional supplementation and industrial fortification. This review synthesises the conventional, agronomic, and biotechnological toolkit available for biofortifying vegetable crops, tracing the field’s progression from soil and foliar nutrient management and marker-assisted breeding through transgenic metabolic engineering to contemporary CRISPR/Cas-based precision genome editing. We examine the molecular mechanisms governing carotenoid, ascorbate, folate, tocopherol, anthocyanin, and mineral accumulation, and we compile documented genome-editing interventions in tomato, potato, carrot, Brassica vegetables, lettuce, pepper, cucumber, and eggplant that have altered nutritional or antinutritional traits. Complementary developments in multi-omics profiling, marker-assisted and genomic selection, tissue culture-based metabolite induction, and nanotechnology-enabled nutrient delivery are discussed alongside the trade-offs, regulatory heterogeneity, and consumer-acceptance barriers that continue to constrain deployment. We argue that the convergence of multiplexed genome editing, base and prime editing, and multi-omics-guided candidate gene discovery is positioning precision breeding to accelerate the second generation of biofortified vegetables, provided that regulatory harmonisation and public engagement keep pace with the underlying science.

KEYWORDS: Biofortification; Vegetable crops; CRISPR/Cas9; Genome editing; Micronutrient malnutrition; Carotenoid biosynthesis; Precision breeding; Metabolic engineering

1. INTRODUCTION

Micronutrient malnutrition remains one of the most persistent and least visible forms of undernutrition. Unlike acute caloric deficits, hidden hunger produces no immediate, dramatic symptoms, yet it compromises immune function, cognitive development, maternal health, and long-term productivity on a scale that rivals overt hunger [92,98]. Global estimates converge on a burden of at least two billion people affected by one or more micronutrient deficiencies, with iron, zinc, vitamin A, iodine, and folate consistently identified as the deficiencies of greatest public health significance [92,94,99,103]. Preschool children and women of reproductive age bear a disproportionate share of this burden: recent multi-country estimates place the prevalence of deficiency in at least one of iron, zinc, or vitamin A at approximately 56% among preschool-aged children and 69% among non-pregnant women of reproductive age [98]. Iron-deficiency anaemia alone contributes substantially to the global

burden of disease [95], vitamin A deficiency remains a leading preventable cause of childhood blindness and elevated infectious mortality [97], and inadequate zinc intake is estimated to affect roughly one-fifth of the world's population when assessed through national food-supply availability [96].

Vegetables occupy a distinctive position in this nutritional landscape. As a food group, they supply a dense and diverse array of provitamin A carotenoids, ascorbic acid, folate, vitamin E, and minerals such as iron, zinc, calcium, magnesium, and selenium, alongside non-nutrient bioactives including anthocyanins, flavonoids, and glucosinolates that confer additional antioxidant and chemoprotective properties [77,94]. Unlike staple cereals, which are the traditional targets of biofortification programmes because they dominate caloric intake in low- and middle-income countries, vegetables are typically consumed in smaller absolute quantities but at far higher nutrient density per unit fresh weight, and their diversity of pigments and secondary metabolites cannot be replicated by cereal biofortification alone. Diversifying diets to include more vegetables is frequently recommended as the most direct route to correcting micronutrient gaps, but this strategy is constrained in practice by affordability, seasonal and geographic availability, post-harvest spoilage, and entrenched dietary habits, particularly in resource-limited settings [92,103]. These constraints have motivated a complementary strategy: increasing the intrinsic nutrient density of the vegetables that are already being grown and consumed, so that a normal diet delivers more nutrition without requiring behavioural change.

Biofortification is the process of increasing the density of bioavailable vitamins, minerals, or beneficial phytochemicals in the edible portions of a crop through breeding, agronomic management, or biotechnological intervention [83,84]. The concept, first developed and scaled for staple cereals and pulses through international programmes such as HarvestPlus, has demonstrated measurable improvements in micronutrient status among consumers of biofortified crops [83,109]. Two decades of accumulated evidence indicate that biofortification is both biologically feasible and cost-effective as a component of a broader food-systems response to malnutrition [83]. Extending this logic to vegetable crops introduces both opportunities and complications: vegetables display far greater taxonomic and metabolic diversity than the handful of staple cereals that have historically dominated biofortification research, their edible tissues range from leaves and roots to fruits and inflorescences with correspondingly distinct nutrient biosynthesis and storage pathways, and many economically important vegetable species have historically been less amenable to the genetic transformation and regeneration protocols that underpin transgenic biofortification.

The biotechnological toolkit available to plant breeders has undergone a rapid and cumulative transformation over the past three decades. Conventional and agronomic biofortification, comprising soil and foliar nutrient management alongside phenotypic selection for existing genetic variation, remains the most immediately deployable strategy and continues to underpin most commercial mineral- and selenium-enriched vegetable products [31,32,38]. Marker-assisted selection and, more recently, genomic selection have allowed breeders to track and combine quantitative trait loci (QTLs) governing nutrient accumulation with a precision unattainable through phenotypic selection alone [61,65,66]. Transgenic metabolic engineering, exemplified by the introduction of the provitamin A biosynthetic pathway into rice endosperm to create Golden Rice, demonstrated that entire biosynthetic pathways absent from a tissue could be reconstituted through targeted gene introduction, a principle subsequently extended to tomato folate biosynthesis and multivitamin maize [25,26,87,89]. Most recently, CRISPR/Cas-based genome editing together with its derivative technologies, base editing and prime editing has enabled targeted, often transgene-free modification of endogenous metabolic and transport genes, offering a route to biofortification that can proceed without the regulatory and consumer-acceptance burdens historically associated with transgenic crops in many jurisdictions [1,4,52,53].

This review examines how these successive technological layers conventional and agronomic biofortification, tissue culture-based metabolite manipulation, transgenic and metabolic engineering, molecular and marker-assisted breeding, multi-omics-enabled candidate gene discovery, and CRISPR/Cas-based precision genome editing are converging to accelerate the biofortification of vegetable crops specifically. We first review the nutritional components for which vegetables are principal dietary sources and the biological rationale for targeting them. We then examine each biotechnological approach in turn, with particular emphasis on genome editing applications documented in tomato, potato, carrot, Brassica vegetables, lettuce, cucumber, eggplant, and pepper. We discuss the roles of omics technologies and nanotechnology in accelerating and complementing these approaches, and we conclude by considering the agronomic trade-offs, regulatory heterogeneity, and translational challenges that will determine whether these advances reach farmers and consumers.

2. Nutritional Importance of Vegetable Crops

Vegetables contribute a disproportionate share of dietary micronutrient intake relative to their caloric contribution, and different vegetable groups are enriched in distinct, complementary sets of nutrients, making the vegetable food group as a whole a particularly efficient target for biofortification.

Iron and zinc. Leafy vegetables, legume-associated vegetables, and root crops contribute variable but nutritionally meaningful amounts of iron and zinc, though the bioavailability of both minerals is frequently limited by co-occurring antinutrients such as phytate and oxalate [33,80]. Because plant-based iron is predominantly non-haem iron with comparatively low absorption efficiency, and because zinc status is difficult to assess directly, agronomic and genetic strategies that increase both the concentration and the bioavailable fraction of these minerals are of particular nutritional value [38,96].

Selenium. Selenium is not classified as an essential plant nutrient but is essential for human selenoenzyme function, and dietary selenium intake is almost entirely dependent on the selenium content of soils and, by extension, the crops grown on them [30,39]. Many agricultural soils are selenium-poor, and vegetables such as broccoli, lettuce, and Brassica greens have been widely investigated as selenium biofortification targets because of their capacity to accumulate and, in the case of Brassica species, metabolise selenium into organoselenium compounds with additional health relevance [36,81].

Calcium and magnesium. Leafy Brassica vegetables and other leafy greens can be substantial dietary sources of calcium and magnesium, although bioavailability again varies with the concentration of co-accumulated oxalate [78,79]. Natural genetic variation in shoot calcium and magnesium concentration within Brassica oleracea and Brassica rapa germplasm indicates that genetic biofortification for these two minerals is agronomically feasible [78].

Folate. Leafy vegetables and legumes are primary dietary sources of folate, and folate deficiency remains a global concern because of its association with neural tube defects and other adverse outcomes [25,26]. Folate content in many crops, including tomato fruit, is limited by the availability of pteridine and para-aminobenzoate precursors during fruit ripening, providing a defined metabolic bottleneck for biofortification [25,26].

Provitamin A carotenoids. Carrot, pumpkin, sweet potato leaves, and orange- or red-fleshed tomato and pepper cultivars are dense sources of β-carotene and other provitamin A carotenoids, and carrot alone is estimated to be one of the largest single dietary contributors of provitamin A carotenoids in many diets [22,23]. Carotenoid content and composition vary enormously across vegetable germplasm, providing extensive natural variation for both conventional breeding and biotechnological enhancement [20,21].

Vitamin C (ascorbic acid). Peppers, leafy Brassica vegetables, and tomato are major dietary sources of vitamin C, an essential water-soluble antioxidant vitamin synthesised in plants via the Smirnoff–Wheeler (L-galactose) pathway [34]. Because ascorbate is also central to non-enzymatic antioxidant defence in plant tissues, its biosynthetic pathway is a target for both nutritional and stress-tolerance improvement [34].

Vitamin E (tocopherols). Although seed oils remain the dominant dietary source of vitamin E, leafy vegetable tissues can accumulate tocopherols when chlorophyll-derived phytol is redirected toward the tocopherol biosynthetic pathway, and this route has been explored as a biofortification strategy in leafy crops [56,57].

Anthocyanins, carotenoid pigments, flavonoids, and glucosinolates. Beyond their roles as essential vitamins and minerals, vegetables are major dietary sources of non-essential but health-promoting phytochemicals. Anthocyanins, responsible for purple and red pigmentation in tomato, lettuce, cabbage, kale, and eggplant, have well-documented antioxidant properties and have become targets of both conventional breeding and genome editing [10,16,18,19]. Glucosinolates, characteristic of Brassica vegetables, are precursors of isothiocyanates with chemoprotective activity, and their profile can be manipulated agronomically and genetically to favour health-promoting derivatives over less desirable ones [77,80,81].

Taken together, this nutritional profile positions vegetable crops as targets for biofortification that are complementary to, rather than redundant with, staple cereal biofortification programmes: vegetables provide the carotenoid, vitamin C, folate, and phytochemical density that staple grains largely lack, while staple biofortification continues to address the mineral and caloric gaps that dominate diets in food-insecure populations.

Table 1. Nutritional Targets and Major Vegetable Crop Sources for Biofortification

Nutrient / bioactive compound	Major vegetable sources	Biological importance	Major deficiency / health relevance	Potential biofortification strategy
Iron	Leafy greens, legume-associated vegetables, root vegetables	Haemoglobin synthesis, oxygen transport, enzyme cofactor	Iron-deficiency anaemia; leading global micronutrient deficiency [95]	Enhance ZIP/NRAMP transporter expression; reduce phytate; co-enhance ascorbate for bioavailability [33,35]
Zinc	Leafy vegetables, legumes, root crops	Enzyme cofactor, immune function, linear growth	Affects an estimated one-fifth of the global population [96]	ZIP transporter overexpression; agronomic foliar/soil zinc application [38]
Selenium	Broccoli, lettuce, Brassica greens	Selenoenzyme (glutathione peroxidase) cofactor; antioxidant defence	Selenium-poor soils widespread; ~1 billion affected globally [31]	Foliar/soil selenate or selenite application; enhance sulfate-transporter-mediated uptake [30,39]
Calcium	Leafy Brassica	Bone mineralisation, cell signalling, cell wall structure	Inadequate intake widespread, compounded by	Genetic selection for high shoot Ca; nutrient

Nutrient / bioactive compound	Major vegetable sources	Biological importance	Major deficiency / health relevance	Potential biofortification strategy
	vegetables, leafy greens		oxalate co-accumulation [78]	solution/soilless production optimisation [79]
Magnesium	Leafy Brassica vegetables, leafy greens	Enzyme cofactor, chlorophyll structure, neuromuscular function	Suboptimal intake in many diets [78]	Genetic biofortification using natural Brassica variation [78]
Folate (vitamin B9)	Leafy vegetables, legumes, tomato	One-carbon metabolism, DNA synthesis, neural tube development	Deficiency linked to neural tube defects [25]	Overexpress GTP cyclohydrolase I and aminodeoxychorismate synthase; reduce γ-glutamyl hydrolase activity [25,26,29]
Provitamin A carotenoids	Carrot, orange tomato, orange/red pepper	Precursor of retinol; vision, immune function, epithelial integrity	Leading cause of preventable childhood blindness [97]	Redirect flux via lycopene β-cyclase; multiplex carotenoid pathway editing [5,20,22]
Vitamin C (ascorbate)	Pepper, Brassica vegetables, tomato	Antioxidant; collagen synthesis; enhances non-haem iron absorption	Contributes to compounded iron bioavailability deficits [34]	Engineer L-galactose (Smirnoff–Wheeler) pathway; edit uGGP genes [17,34]
Vitamin E (tocopherols)	Leafy vegetables (secondary source)	Lipid-soluble antioxidant; membrane protection	Chronic subclinical deficiency in some populations [61]	Couple chlorophyll-derived phytol release with VTE pathway overexpression [55,56,57]
Anthocyanins	Purple tomato, purple/red lettuce, red cabbage, kale, eggplant	Dietary antioxidants; anti-inflammatory activity	Associated with reduced chronic disease risk (epidemiological)	Activate/edit MYB–bHLH–WD40 regulators; promoter/base editing of structural genes [10,16,18,19]
Carotenoid pigments (lycopene, lutein, zeaxanthin)	Tomato, carrot, leafy greens	Antioxidants; macular health (lutein/zeaxanthin)	Suboptimal dietary intake in many populations	Multiplex carotenoid pathway editing; germplasm screening [5,17,23]
Flavonoids	Onion-adjacent alliums, leafy greens, tomato peel	Antioxidant and anti-inflammatory phytochemicals	Associated with reduced chronic disease risk (epidemiological)	MYB12 and related regulatory gene editing [10]
Glucosinolates / isothiocyanate precursors	Broccoli, cabbage, kale, Chinese cabbage	Chemoprotective bioactivity upon hydrolysis to isothiocyanates	Balance of beneficial vs. less-desirable glucosinolate classes varies by genotype	AOP2 allele selection/editing to favour glucoraphanin over progoitrin-derived compounds [77]

3. Concept and Principles of Biofortification

Biofortification is best understood not as a single technique but as a family of strategies unified by a common objective: increasing the density of bioavailable nutrients in the edible portion of a crop as it is normally grown and consumed, rather than adding nutrients after harvest [83,84]. Four broad strategic categories can be distinguished.

Agronomic biofortification increases nutrient concentration through soil or foliar application of mineral fertilisers, exploiting the plant's existing physiological capacity for nutrient uptake and translocation rather than altering its genotype [32,38]. This approach is agronomically flexible and can be deployed rapidly on existing cultivars, but its effectiveness depends on soil chemistry, application timing, and the crop's innate uptake efficiency, and it must generally be repeated each growing season [32].

Conventional (genetic) biofortification exploits naturally occurring genetic variation for nutrient accumulation within a crop's germplasm pool, using phenotypic selection or, increasingly, marker-assisted selection to combine favourable alleles into elite backgrounds [61,65]. Because it works within the existing gene pool of the crop and its sexually compatible relatives, conventional biofortification avoids the regulatory classification applied to transgenic crops in most jurisdictions, but it is constrained by the extent of naturally occurring variation and by linkage drag from wild donor parents [23,66].

Molecular breeding extends conventional breeding by using DNA markers linked to quantitative trait loci or candidate genes to accelerate selection, and by incorporating genomic selection models that estimate breeding values from genome-wide marker data rather than single markers [61]. This approach has been particularly important for polygenic nutritional traits, such as fruit mineral content in tomato, for which no single gene of major effect is available [65].

Biotechnological biofortification encompasses transgenic approaches, in which one or more genes — often from a different species — are introduced to reconstitute or amplify a biosynthetic pathway, and genome editing approaches, in which the crop's own genome is modified directly, typically without the stable introduction of foreign DNA once the editing reagents have segregated away [1,4]. Transgenic biofortification has produced landmark successes, most notably Golden Rice, but faces variable regulatory pathways and consumer acceptance across markets [87,88,89]. Genome editing, by contrast, is capable of achieving equivalent or related outcomes gene knockout, promoter modification, base substitution, or precise sequence replacement while in many cases avoiding the introduction of transgenic DNA into the final product, a distinction that has already begun to influence how several jurisdictions regulate the resulting crops [72,74,75].

These four strategic categories are not mutually exclusive. In practice, the most advanced biofortification programmes combine agronomic management with a genetic background pre-improved by conventional or molecular breeding, and increasingly layer genome editing onto elite, agronomically adapted cultivars rather than treating editing as a stand-alone breeding method. The remainder of this review considers each approach in turn as it applies specifically to vegetable crops.

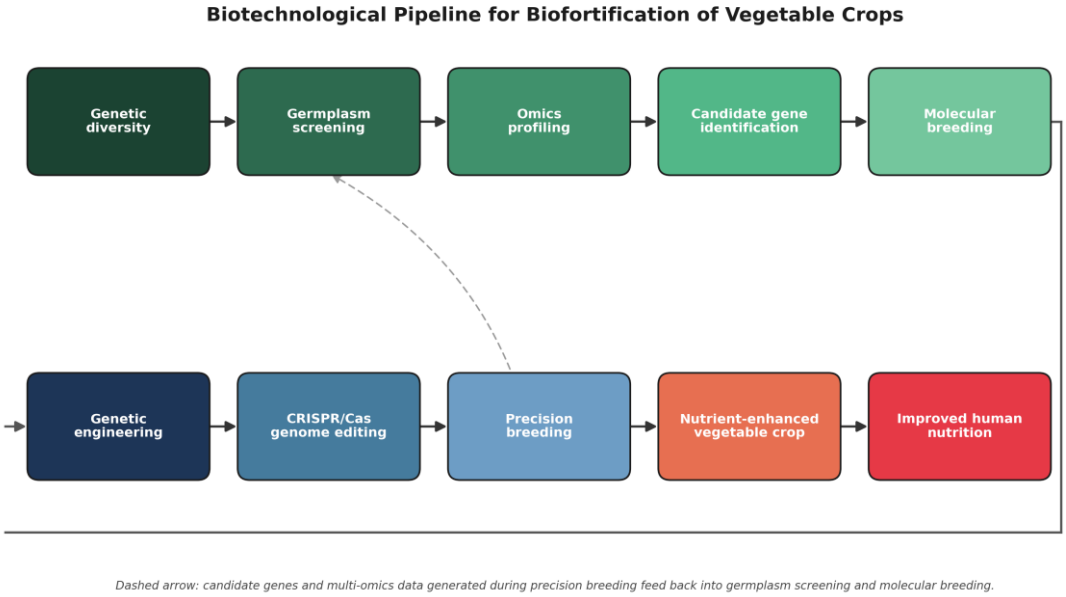

Figure 1. Biotechnological Pipeline for Biofortification of Vegetable Crops.

The pipeline progresses from natural genetic diversity through germplasm screening and multi-omics profiling to candidate gene identification, which feeds into molecular breeding, genetic engineering, and CRISPR/Cas genome editing. These converge in precision breeding programmes that generate nutrient-enhanced vegetable crops, ultimately supporting improved human nutrition. The dashed feedback arrow indicates that candidate genes and multi-omics data generated during precision breeding cycles are recursively used to inform subsequent rounds of germplasm screening and molecular breeding, making the pipeline iterative rather than strictly linear.

4. Conventional and Agronomic Approaches to Vegetable Biofortification

Agronomic biofortification remains the most immediately actionable strategy for vegetable nutrient enhancement because it requires no change to the crop genotype and can, in principle, be applied to any existing cultivar. Soil fertilisation with micronutrient-enriched formulations and foliar spraying of mineral solutions have both been used to increase selenium, zinc, and iodine concentrations in leafy vegetables, and foliar application is generally more efficient than soil application for elements such as selenium and zinc because it bypasses soil fixation reactions that reduce root uptake [32,38,39]. In lettuce, foliar selenium application at defined concentrations has been shown to substantially increase tissue selenium content without compromising yield [30]. Similarly, calcium biofortification of soilless "baby leaf" vegetable production systems has demonstrated that nutrient solution composition can be tuned to increase both calcium concentration and its bioaccessible fraction [79].

Genetic variability within existing germplasm collections provides the raw material for conventional breeding-based biofortification. Systematic germplasm screening across wild relatives, landraces, and breeding lines has repeatedly revealed far greater natural variation in mineral and vitamin content than is expressed in modern commercial cultivars, a pattern documented in tomato fruit mineral content [65,66], carrot carotenoid content [22,23], and Brassica shoot calcium and magnesium concentration [78]. This variation provides breeders with donor sources for introgression into elite backgrounds, although the process is often complicated by the polygenic nature of nutrient accumulation traits and by unfavourable linkage between nutrient content and undesirable agronomic characteristics inherited from wild donors.

Marker-assisted selection (MAS) addresses the inefficiency of purely phenotypic selection for nutrient traits, which are typically laborious and expensive to measure directly and are strongly influenced by environment. By identifying molecular markers tightly linked to quantitative trait loci or candidate genes governing nutrient accumulation, MAS allows breeders to select at the seedling stage without waiting for mature fruit or root phenotypes [61,62]. In tomato, dense genetic maps and an increasing catalogue of QTLs for fruit mineral content, carotenoid accumulation, and related quality traits have made MAS a practical tool for pyramiding favourable alleles from multiple sources [65,66,67]. Comparable resources have been developed for pepper, eggplant, and other Solanaceous vegetables, and a systematic review of genomics-assisted vegetable improvement across tomato, pepper, eggplant, lettuce, spinach, cucumber, and chicory has documented the progressively increasing density of genetic maps and markers available for MAS across this crop group [61].

The principal advantages of conventional and agronomic biofortification are regulatory simplicity, immediate deployability, and broad consumer acceptance, since no novel genetic material is introduced. Their principal limitations are the constraints imposed by existing natural variation, the transience of agronomic interventions that must be repeated each season, and the frequent trade-offs between nutrient accumulation and yield or other agronomic traits that emerge when nutrient-dense donor alleles are introgressed from wild or unadapted germplasm.

5. Plant Tissue Culture and Micropropagation in Biofortification

Plant tissue culture technologies contribute to vegetable biofortification through several complementary mechanisms that operate largely independently of, but can be combined with, genetic modification. In vitro cell, callus, and organ culture systems allow the biosynthesis of secondary metabolites to be manipulated under controlled physical and chemical conditions that are difficult to replicate in the field, including precisely defined light quality, elicitor exposure, and hormonal balance [68,69,71]. Because many of the phytochemicals of interest in vegetable biofortification anthocyanins, flavonoids, and other polyphenols among them are synthesised via inducible secondary metabolic pathways, elicitation strategies applied to cell or organ cultures can substantially increase their accumulation relative to field-grown tissue, a principle well established for medicinal and horticultural plant systems [68,71].

In vitro selection exploits the capacity to screen large populations of somatic cells or regenerated plantlets for altered metabolite profiles under culture conditions that would be impractical to apply at the whole-plant, field scale. Somaclonal variation the heritable genetic and epigenetic variation that arises spontaneously during tissue culture and regeneration, particularly following extended callus phases provides an additional, non-transgenic source of novel variation that has historically been exploited for stress tolerance and quality traits and remains a candidate route for generating novel nutrient-accumulation phenotypes, though its unpredictability limits its use as a targeted biofortification strategy compared with genome editing [70].

Micropropagation itself does not directly alter nutrient content, but it plays a supporting role in biofortification programmes by enabling the rapid, genetically uniform multiplication of nutrient-enhanced lines developed through breeding, mutagenesis, transgenic, or genome-editing approaches, and by providing the regeneration step that is a prerequisite for the recovery of edited or transformed plants from transformed cells or protoplasts in species such as potato, pepper, and eggplant [12,15,45]. The efficiency of regeneration protocols is frequently a limiting step in the application of genetic engineering and genome editing to vegetable crops, particularly in species recalcitrant to tissue culture such as many pepper genotypes, and continued optimisation of tissue culture protocols therefore remains directly relevant to the pace at which biotechnological biofortification can be extended across vegetable taxa [45,46].

6. Genetic Engineering and Transgenic Approaches

Transgenic approaches to biofortification introduce one or more genes sourced from the same species, a related species, a microorganism, or a distantly related plant to reconstitute a missing biosynthetic pathway, amplify a rate-limiting step, or suppress a competing or degradative pathway. Agrobacterium-mediated transformation remains the predominant method for stable gene transfer into vegetable crops, owing to its comparatively precise, low-copy-number integration relative to particle bombardment, and it underlies the majority of documented vegetable biofortification transgenics [11,14].

The most widely cited proof of concept for pathway-level transgenic biofortification is Golden Rice, in which the provitamin A biosynthetic pathway absent from rice endosperm was reconstituted through the introduction of phytoene synthase and carotene desaturase genes, enabling β-carotene accumulation in a tissue that does not naturally synthesise it [89,90]. Although Golden Rice targets a cereal rather than a vegetable, the underlying principle pathway reconstitution through the introduction of rate-limiting enzymes has been directly extended to vegetable crops. In tomato fruit, fruit-specific overexpression of GTP cyclohydrolase I, the first committed

enzyme of the pteridine branch of folate biosynthesis, increased fruit pteridine content by up to 140-fold and folate content approximately two-fold, and subsequent combination with overexpression of aminodeoxychorismate synthase which supplies the complementary para - aminobenzoate precursor raised folate content up to 25-fold relative to controls [25,26,27]. Comparable transgenic folate engineering strategies have since been extended to common bean seed, where seed-specific overexpression of Arabidopsis GTP cyclohydrolase I increased pteridine levels up to 150-fold and folate levels approximately three-fold [29], and to biofortified rice, in which combined engineering of the pteridine and para-aminobenzoate branches has been used to increase folate stability during storage as well as concentration at harvest [28].

Carotenoid transgenics in vegetable crops have similarly targeted specific enzymatic steps in the pathway. Overexpression or heterologous expression of lycopene β-cyclase and related carotenogenic genes has been used to shift the balance between lycopene and downstream β-carotene in tomato and other fruit crops, while transgenic manipulation of anthocyanin regulatory transcription factors, such as the maize Delila and Rosea1 genes expressed in tomato, has been used to activate anthocyanin biosynthesis in a species that does not normally accumulate anthocyanins in ripe fruit [67]. Comparable transgenic strategies have also been applied to potato, where overexpression of genes operating in the phenylpropanoid, ascorbic acid, carotenoid, and steroidal glycoalkaloid biosynthetic pathways has been used to increase tuber anthocyanin, vitamin, and carotenoid content while modulating the accumulation of glycoalkaloid antinutrients [12].

The primary benefit of transgenic biofortification is its capacity to introduce entirely novel biosynthetic capacity reconstituting pathways absent from the target tissue rather than merely amplifying existing flux a feat that neither conventional breeding nor most forms of genome editing can achieve, since editing can only modify sequences already present in the genome. This capability comes with corresponding limitations: transgenic crops are subject to case-by-case biosafety assessment and, in many jurisdictions, distinct regulatory pathways from conventionally bred or genome-edited crops, and public acceptance of transgenic vegetable products has historically lagged behind that of edited or conventionally bred alternatives in several major markets [72,74,84]. Nevertheless, decades of accumulated safety assessment of transgenic crops already in commercial production, together with the demonstrated nutritional impact of programmes such as Golden Rice, support the continued relevance of transgenic approaches for biofortification targets such as pathway reconstitution that cannot currently be achieved through gene editing of the native genome alone [84,87].

7. Metabolic Engineering for Nutrient Enhancement

Metabolic engineering for biofortification targets the enzymatic steps that limit flux through a nutrient biosynthetic pathway, using knowledge of pathway structure and regulation to identify the intervention points most likely to increase end-product accumulation without disrupting essential plant physiology.

Carotenoid biosynthesis proceeds through the isoprenoid pathway, in which geranylgeranyl diphosphate is condensed by phytoene synthase to form phytoene, which is subsequently desaturated and isomerised to lycopene before branching toward α-carotene and lutein on one side and β-carotene, zeaxanthin, and violaxanthin on the other, with lycopene β-cyclase and lycopene ε-cyclase governing the branch point [5,21]. Because lycopene itself has no provitamin A activity while β-carotene does, engineering interventions that redirect flux away from the ε-cyclase branch and toward the β-cyclase branch, or that knock down downstream conversion of lycopene entirely to favour its own accumulation as an antioxidant, represent two distinct and sometimes competing biofortification objectives depending on whether the goal is provitamin A enhancement or lycopene enrichment [5,20].

Anthocyanin biosynthesis is a branch of the broader phenylpropanoid and flavonoid pathway, in which chalcone synthase, flavanone 3-hydroxylase, dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase act sequentially to produce anthocyanidins that are subsequently glycosylated and stabilised, with the entire pathway under the transcriptional control of MYB, basic helix-loop-helix, and WD40 regulatory complexes [16,18,19]. In many vegetable species, the structural genes of the anthocyanin pathway are present but transcriptionally silent in commercial cultivars, so engineering or editing strategies frequently target the regulatory transcription factors activating them to induce anthocyanin accumulation in tissues that do not normally pigment, or knocking them out to test pathway function rather than the structural enzymes themselves [10,18,19].

Vitamin C (ascorbate) biosynthesis in plants proceeds predominantly through the Smirnoff–Wheeler, or L-galactose, pathway, in which GDP-mannose is converted through GDP-L-galactose and L-galactose to L-galactono-1,4-lactone before final oxidation to ascorbate, with GDP-mannose pyrophosphorylase, GDP-mannose epimerase, and GDP-L-galactose phosphorylase representing key regulatory nodes [34]. Because ascorbate metabolism intersects with sugar and starch metabolism at the level of shared precursors, metabolic engineering interventions in this pathway must account for pleiotropic effects on fruit carbohydrate composition, a consideration illustrated by studies showing that manipulation of pyrophosphate metabolism in tomato fruit alters both ascorbate and starch content simultaneously [34].

Folate biosynthesis in plants is compartmentalised across the cytosol, plastid, and mitochondrion: the pteridine moiety is synthesised in the cytosol beginning with GTP cyclohydrolase I, para-aminobenzoate is synthesised in plastids from chorismate via aminodeoxychorismate synthase, and the two precursors are condensed and glutamylated in the mitochondrion to yield the mature polyglutamylated folate pool [25,26,27]. Because the two precursor branches are synthesised in different subcellular compartments and can each independently limit total folate accumulation, effective metabolic engineering of folate content generally requires coordinated intervention at both branches rather than amplification of a single step [26].

Mineral uptake and transport is governed by families of membrane transporters, most extensively characterised for zinc and iron. The ZIP (zinc-regulated transporter, iron-regulated transporter-like protein) family and the NRAMP (natural resistance-associated macrophage protein) family mediate metal uptake from soil and intracellular trafficking, while YSL (yellow stripe-like) transporters and nicotianamine-dependent chelation systems govern long-distance translocation from root to shoot and, ultimately, to edible tissues [35,36,37]. Overexpression of specific ZIP family members under tissue-specific promoters has been explored as a route to increasing zinc accumulation in edible tissues, although translocation to and sequestration within the specific edible organ rather than uptake alone is frequently the rate-limiting step for mineral biofortification of vegetable crops [34,37].

Antioxidant metabolism, encompassing the interlinked ascorbate–glutathione cycle, tocopherol biosynthesis, and phenolic antioxidant pathways, is both a nutritional target in its own right and a modulator of the stability of other biofortified traits, since oxidative degradation during storage and processing can substantially erode the nutritional gains achieved at harvest [56,60]. Tocopherol biosynthesis, which converts homogentisate and phytyl diphosphate into α-, β-, γ-, and δ-tocopherols via homogentisate phytyltransferase and subsequent methylation and cyclisation steps, is constrained in vegetative tissue by the limited availability of phytyl diphosphate derived from chlorophyll turnover, a constraint that has been directly addressed by engineering strategies that couple enhanced chlorophyll-derived phytol release with tocopherol pathway overexpression [55,56,57].

Collectively, these pathway-level insights illustrate a recurring principle in biofortification metabolic engineering: single-gene overexpression is often necessary but rarely sufficient to achieve substantial nutrient enhancement, because plant metabolic networks are governed by multiple, distributed control points, feedback regulation, and cross-pathway competition for shared precursors. The most successful metabolic engineering studies in vegetable biofortification, from tomato folate biofortification to lettuce carotenoid and ascorbate enhancement, have generally combined interventions at two or more pathway steps, or paired pathway engineering with storage or stability improvements, to achieve nutritionally meaningful increases in end-product accumulation [17,25,26].

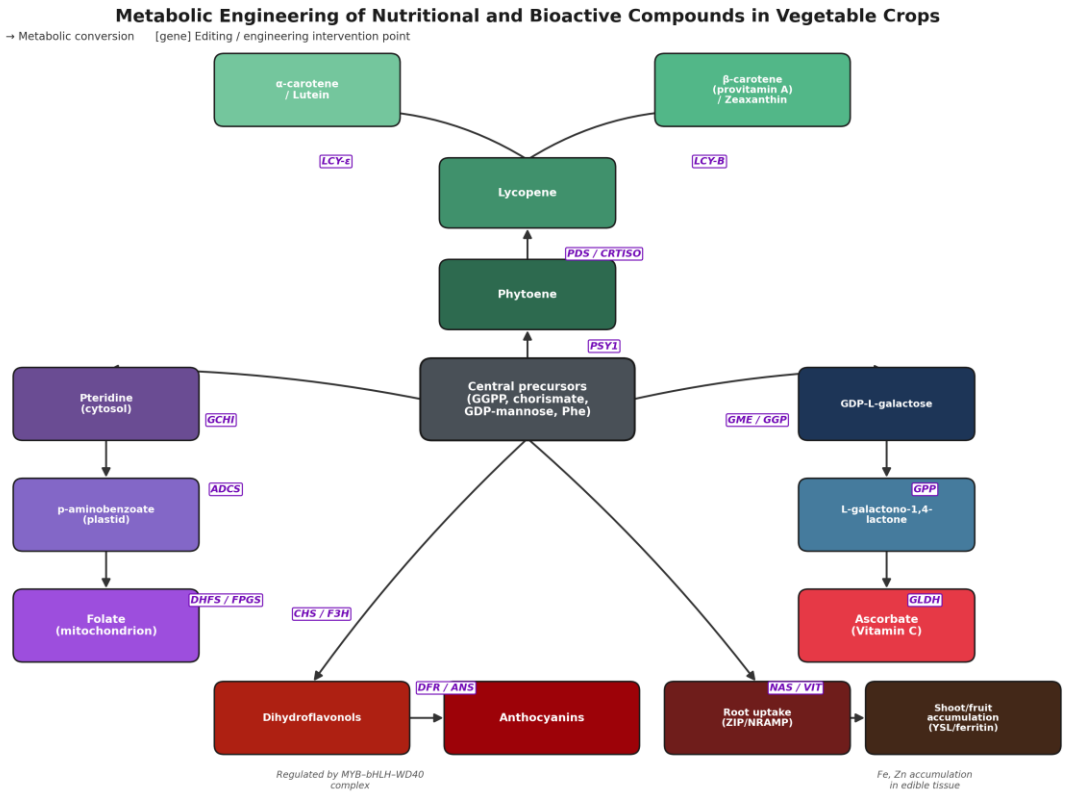

Figure 2. Metabolic Engineering of Nutritional and Bioactive Compounds in Vegetable Crops. Central isoprenoid, shikimate, and sugar-nucleotide precursors feed five nutritionally important branch pathways: the carotenoid pathway (phytoene → lycopene → α-carotene/lutein or β-carotene/zeaxanthin, provitamin A), the ascorbate (vitamin C) pathway via GDP-L-galactose, the folate pathway spanning the cytosol, plastid, and mitochondrion, the anthocyanin branch of the phenylpropanoid/flavonoid pathway regulated by the MYB–bHLH–WD40 transcriptional complex, and mineral (Fe, Zn) uptake and translocation via ZIP/NRAMP and YSL/ferritin transporter systems. Boxed gene names in purple and red indicate documented genetic engineering or genome-editing intervention points discussed in the text (e.g., PSY1, PDS/CRTISO, LCY-ε, LCY-B, GME/GGP, GPP, GLDH, GCHI, ADCS, DFR/ANS, NAS/VIT).

8. Molecular Breeding and Marker-Assisted Biofortification

Molecular breeding for nutritional traits in vegetable crops relies on the identification of DNA markers linked to the genomic regions controlling nutrient accumulation, followed by their deployment in marker-assisted selection

or genomic selection schemes. Quantitative trait locus (QTL) mapping using biparental populations including recombinant inbred lines, backcross populations, and near-isogenic lines has been the primary tool for dissecting the genetic architecture of nutrient traits in tomato, pepper, carrot, and pea. In tomato, QTL mapping of interspecific populations derived from crosses with wild relatives such as Solanum pimpinellifolium has identified multiple loci controlling fruit mineral content, several of which are flanked by co-dominant markers suitable for marker-assisted breeding [65,66]. Comparable QTL mapping efforts in carrot have resolved loci controlling root colour and carotenoid content that underlie the classic transition from white or yellow to orange, high-carotenoid carrot germplasm [22,23], while QTL analyses in pea have identified numerous loci governing seed mineral concentration and content, providing markers for mineral biofortification in a crop where seed mineral variation has been comparatively understudied [67].

Marker-assisted selection has a particularly long history in tomato breeding, where dense genetic maps and, more recently, whole-genome resequencing of large germplasm panels have enabled the fine mapping and, in several cases, positional cloning of genes underlying fruit weight, quality, and composition traits [61,62,64]. The systematic characterisation of metabolite diversity across large tomato collections sometimes described as "rewiring the fruit metabolome" through comparative analysis of cultivated and wild accessions has additionally revealed that modern breeding for yield and shelf life has inadvertently eroded some favourable metabolite alleles present in wild and landrace germplasm, providing both a cautionary lesson and a roadmap for reintroducing these alleles through marker-assisted introgression [63,64].

Genomic selection, which estimates breeding values from genome-wide marker data rather than from a small number of markers linked to individual QTLs, has become increasingly relevant as multi-omics datasets encompassing genomic, transcriptomic, proteomic, and metabolomic layers have expanded the pool of predictive markers available for complex, polygenic nutritional traits [44,45]. Because most nutrient accumulation traits in vegetable crops are quantitative and influenced by numerous loci of small effect, as well as by genotype-by-environment interaction, genomic selection models that integrate multiple data layers are increasingly favoured over single-marker or single-QTL approaches for traits such as fruit mineral content and carotenoid accumulation, where genomics-with-metabolomics data combinations have been shown to improve prediction accuracy for quality traits specifically [45].

Transcriptomics, proteomics, and metabolomics each contribute distinct and complementary information to candidate gene discovery for biofortification. Transcriptomic profiling of contrasting genotypes such as high- and low-anthocyanin non-heading Chinese cabbage lines has been used to identify differentially expressed regulatory genes, including basic helix-loop-helix transcription factors, that subsequently serve as functionally validated candidates for marker development or targeted editing [16,19]. Metabolomic profiling complements this approach by directly quantifying the pathway end-products of interest, allowing metabolite quantitative trait loci to be mapped alongside conventional agronomic QTLs and enabling the identification of loci that affect metabolite composition independently of visible phenotype [44,63]. The integration of these multiple omics layers into a single analytical framework multi-omics integration is discussed further in Section 12, but its foundational role in molecular breeding for biofortification, particularly in narrowing large QTL intervals to a small number of testable candidate genes prior to functional validation by transgenic or genome-editing approaches, warrants emphasis here as a bridge between conventional molecular breeding and precision genome editing.

Table 2. Biotechnological Approaches for Biofortification of Vegetable Crops

Approach	Principle	Target trait	Suitable vegetable crops	Major advantages	Major limitations	Representative evidence
Agronomic biofortification (soil/foliar application)	Exogenous mineral application exploiting existing plant uptake physiology	Zn, Se, I, Ca concentration	Lettuce, broccoli, leafy Brassica, tomato	Rapid deployment on existing cultivars; no genetic change; broad regulatory and consumer acceptance	Must be repeated each season; efficacy depends on soil chemistry and timing; limited to elements amenable to foliar/soil delivery	[30,32,38,39,79]
Conventional breeding / germplasm screening	Phenotypic selection on natural genetic variation	Mineral and vitamin content, carotenoid/anthocyanin profile	Tomato, carrot, Brassica vegetables, pepper	Avoids GMO regulatory classification; broad consumer acceptance	Limited by extent of natural variation; linkage drag from wild donors; slow multi-generation process	[23,65,66,78]

Approach	Principle	Target trait	Suitable vegetable crops	Major advantages	Major limitations	Representative evidence
Marker-assisted selection (MAS) / genomic selection	DNA markers linked to QTLs/candidate genes guide selection	Polygenic nutrient and quality traits	Tomato, pepper, carrot, pea	Accelerates selection; enables early-stage (seedling) screening; combinable with all other approaches	Requires pre-existing marker–trait associations; genomic selection needs large training populations	[61,65,67]
Plant tissue culture / in vitro elicitation	Controlled culture conditions and elicitors induce secondary metabolite pathways	Anthocyanins, flavonoids, other phytochemicals	Broad applicability across vegetable taxa	Enables biosynthesis under standardised, scalable conditions; complements genetic approaches	Metabolite yields can be variable; scale-up to whole-plant nutrition not always straightforward	[68,69,71]
Transgenic / metabolic engineering	Introduction or overexpression of pathway gene(s), often reconstituting an absent pathway	Folate, provitamin A, ascorbate, anthocyanin, tocopherol pathways	Tomato, potato, common bean	Can introduce entirely novel biosynthetic capacity; large nutrient increases achievable (e.g., up to ~25-fold folate)	Case-by-case biosafety regulation; variable consumer acceptance; long product-development timelines	[12,25,26,29,67,89]
CRISPR/Cas nuclease editing (knockout/knock-in)	Site-specific double-strand break and repair disrupts or replaces target sequence	Antinutrient reduction (SGAs, PPO), pathway gene knockout	Tomato, potato, eggplant, pepper, lettuce	Precise; can be transgene-free (RNP delivery); rapid relative to conventional breeding	Off-target risk requires validation; regeneration/transformation efficiency varies by species	[1,5,6,12,14]
Base editing	Deaminase-mediated single-base conversion without double-strand breaks	Precise allele installation/correction in pathway regulators	Tomato	Avoids DSB-associated unpredictability; enables precise allele engineering	Narrower range of editable outcomes than nuclease or prime editing; sequence-context dependent efficiency	[7,50,51,54]
Prime editing	Nickase–reverse transcriptase fusion installs defined	Any base substitution or small indel at defined loci	Emerging across major crops, including	Broadest editing precision; no DSB or donor template required	Currently lower and more variable efficiency in plants than base/nuclease editing	[49,52,53]

Approach	Principle	Target trait	Suitable vegetable crops	Major advantages	Major limitations	Representative evidence
	edits via pegRNA		vegetables			
Multiplex genome editing	Simultaneous targeting of multiple genes/sites in one event	Pathway-level redirection (e.g., carotenoid, GABA pathways)	Tomato, lettuce	Addresses polygenic pathway traits in a single transformation event	Increased design and off-target-screening complexity with more simultaneous targets	[5,6,17]
Multi-omics-guided candidate discovery	Integration of genomic, transcriptomic, proteomic, metabolomic data	Candidate gene identification for downstream editing/breeding	Tomato, Brassica vegetables	Narrows QTL intervals to testable candidates; captures post-transcriptional regulation	Requires substantial data generation and bioinformatic infrastructure	[19,44,46,47]
Nanotechnology-enabled nutrient delivery	Engineered nanoscale carriers for controlled nutrient release/uptake	Zn, Fe, Se concentration	Broccoli microgreens, leafy vegetables	Potentially improved nutrient-use efficiency and reduced input losses	Long-term environmental and food-safety evaluation still maturing; uptake mechanisms not fully characterised	[40,41,43]

9. CRISPR/Cas and Genome Editing

CRISPR/Cas-based genome editing has, within roughly a decade, become the dominant technology platform for precision modification of plant genomes, and its application to nutrient enrichment in crops including vegetable crops has expanded rapidly as transformation and regeneration protocols have matured across an increasing number of species [1,4,52]. The core CRISPR/Cas9 system uses a single guide RNA to direct the Cas9 nuclease to a complementary genomic sequence adjacent to a protospacer-adjacent motif, generating a site-specific double-strand break that is repaired predominantly by error-prone non-homologous end joining, producing small insertions or deletions that frequently disrupt gene function, or, less efficiently, by homology-directed repair when a donor template is supplied [4,52]. This basic architecture has been extended in several directions relevant to biofortification.

CRISPR/Cas12a (formerly Cpf1) recognises a distinct protospacer-adjacent motif and generates staggered rather than blunt double-strand breaks, properties that have made it a useful complement to Cas9 for multiplex editing and for applications in species or sequence contexts where Cas9 target site availability is limiting; Cas12a-based multiplex editing has already been used to generate field-tested, agronomically improved genome-edited rice lines, illustrating the platform's readiness for deployment at scale [1,3]. *CRISPR/Cas13* systems target RNA rather than DNA and have found application in multiplexed viral interference in crops such as potato, offering a route to indirectly protecting nutrient-related traits from pathogen-induced losses rather than directly editing nutrient biosynthesis genes [3].

Base editing couples a catalytically impaired Cas9 nickase to a cytidine or adenine deaminase, enabling the direct, irreversible conversion of one base pair to another at a target site without generating a double-strand break, and therefore without relying on the error-prone repair pathways that produce variable indel outcomes [50,51,54]. Because base editing can install or correct single-nucleotide substitutions with high precision, it is particularly well suited to introducing specific, previously characterised beneficial alleles for example, alleles identified through QTL mapping or association studies directly into elite backgrounds, and it has already been used to modify carotenoid pathway regulatory genes in tomato through targeted base substitutions [7]. *Prime editing* extends this precision further by using a fusion of a Cas9 nickase and a reverse transcriptase, guided by a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit, to achieve "search-and-replace" editing capable of installing any base substitution as well as small insertions and deletions without requiring a double-strand break or donor repair template [49,52,53]. Prime editing in plants has historically been constrained by comparatively low and variable efficiency relative to base editing and conventional CRISPR/Cas9

nuclease editing, and a substantial share of ongoing methodological research is directed at improving pegRNA design, prime editor architecture, and delivery to raise editing efficiency to levels compatible with routine crop breeding use [49,52].

Gene knockout remains the most straightforward and widely deployed CRISPR/Cas application in vegetable biofortification, exploiting the fact that many antinutritional or undesirable metabolic branches can be eliminated by disrupting a single structural gene. Examples directly relevant to vegetable nutritional quality include the knockout of polyphenol oxidase genes in eggplant to reduce enzymatic browning [14], and the knockout of steroidal glycoalkaloid pathway genes in potato to reduce the accumulation of toxic secondary metabolites in tubers [12]. *Gene activation*, using catalytically dead Cas9 fused to transcriptional activator domains (CRISPR activation, or CRISPRa), offers a complementary route to up-regulating endogenous biosynthetic genes without altering their coding sequence, a strategy of particular relevance to pathways such as anthocyanin biosynthesis in which the structural genes are frequently present but transcriptionally silent in commercial cultivars [18,19]. *Promoter editing*, in which CRISPR/Cas9 is used to introduce indels or targeted modifications within cis-regulatory elements rather than coding sequences, provides a means of fine-tuning gene expression level rather than producing a binary knockout, an approach that has been used to generate a continuous range of fruit size and inflorescence architecture phenotypes in tomato through editing of cis-regulatory regions upstream of quantitative trait genes, and that is conceptually well suited to tuning, rather than switching off, nutrient biosynthetic gene expression [8].

Multiplex genome editing, in which multiple guide RNAs are delivered simultaneously to target several genes or several sites within a single gene in one transformation event, has proven essential for manipulating polygenic and pathway-level nutritional traits, since single-gene edits are frequently insufficient to produce nutritionally meaningful shifts in complex biosynthetic pathways. The multiplex editing of five carotenoid pathway genes in tomato to redirect metabolic flux toward lycopene accumulation, achieving an approximately 5.1-fold increase in fruit lycopene content, exemplifies the pathway-level precision achievable through multiplexed CRISPR/Cas9 approaches that would be difficult to replicate through sequential single-gene transformation [5]. Multiplex editing of independent genes within the GABA shunt pathway has similarly been used to increase γ-aminobutyric acid accumulation in tomato fruit, illustrating the applicability of the same multiplexing principle to a distinct metabolic pathway of nutritional interest [6].

Together, these tools nuclease-based knockout, base editing, prime editing, transcriptional activation, promoter editing, and multiplexing constitute a modular toolkit that can be matched to the specific molecular architecture of a given nutritional trait: simple antinutrient elimination is generally well served by straightforward knockout, fine-tuning of pathway flux is increasingly addressed through promoter editing or base editing, and complex, multi-gene pathway redirection is addressed through multiplexed approaches, often deployed in combination.

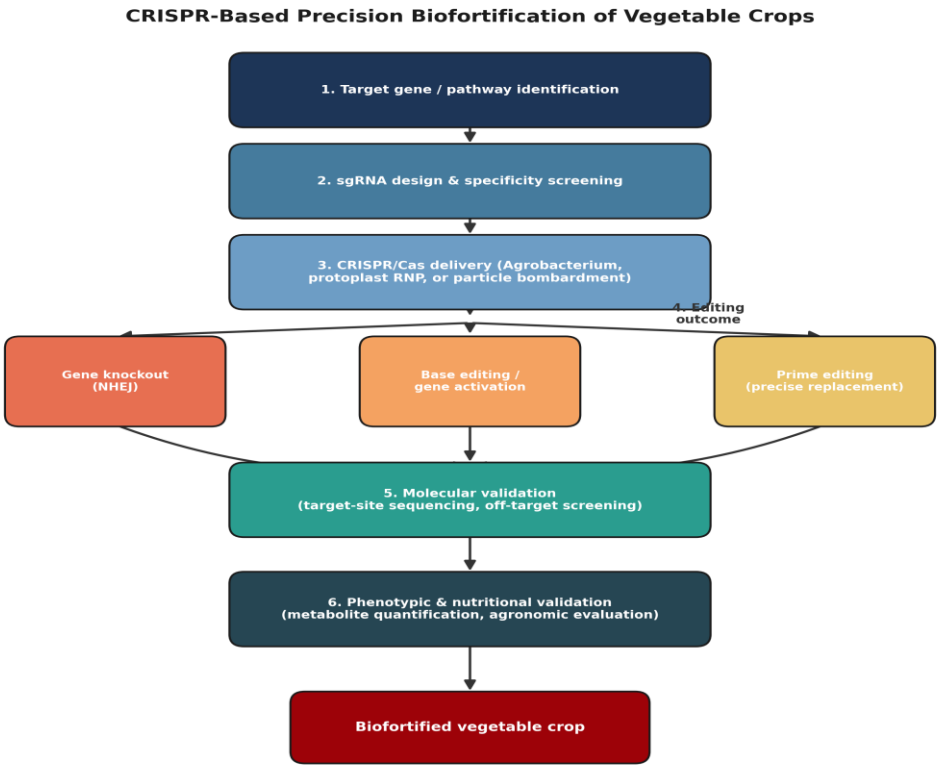

Figure 3. CRISPR-Based Precision Biofortification of Vegetable Crops. The workflow begins with identification of a target gene or pathway and progresses through sgRNA design and specificity screening, delivery of CRISPR/Cas reagents (via Agrobacterium-mediated transformation, protoplast ribonucleoprotein delivery, or particle bombardment), and generation of one of several editing outcomes gene knockout via non-

homologous end joining, base editing or transcriptional activation, or prime editing for precise sequence replacement. All editing outcomes converge on molecular validation (target-site sequencing and off-target screening) followed by phenotypic and nutritional validation (metabolite quantification and agronomic evaluation), culminating in a validated biofortified vegetable crop.

10. Precision Genome Editing of Vegetable Crops

This section reviews documented CRISPR/Cas and related genome-editing applications with direct relevance to nutritional or quality traits across the major vegetable crops in which such editing has been reported. Table 3 summarises representative examples in tabular form; the narrative below expands on the biological context and significance of selected cases.

Tomato (Solanum lycopersicum). Tomato is the most extensively genome-edited vegetable crop, reflecting its efficient transformation and regeneration protocols, well-annotated reference genome, and status as a model species for fleshy fruit biology. Multiplex CRISPR/Cas9 editing of five carotenoid pathway genes SGR1, LCY-E, Blc, LCY-B1, and LCY-B2 redirected carbon flux toward lycopene, increasing fruit lycopene content approximately 5.1-fold while homozygous edits were stably transmitted across generations [5]. Targeted knockout of the two GABA-transaminase and related genes in the GABA shunt pathway using CRISPR/Cas9 achieved substantial increases in fruit γ-aminobutyric acid content, a non-protein amino acid associated with antihypertensive and neuromodulatory bioactivity [6]. Base editing of DE-ETIOLATED1 (SlDET1), DDB1, and SlCYC-B using a cytidine base editor produced graded increases in carotenoid, lycopene, and β-carotene content without inducing double-strand breaks, illustrating the precision achievable with base editing relative to conventional nuclease-based knockout [7]. Editing of the carotenoid isomerase (CRTISO) and phytoene synthase 1 (PSY1) genes using geminiviral replicon-delivered CRISPR/Cas9 produced orange- and yellow-fruited tomato lines by blocking distinct steps of the carotenoid pathway [9]. Editing of the MYB12 transcriptional regulator produced pink-fruited tomato by reducing flavonoid accumulation in the fruit peel, altering the perceived colour without directly manipulating the underlying carotenoid content [10]. TALEN- and CRISPR/Cas9-mediated targeted insertion of a strong promoter upstream of the SlANT1 anthocyanin regulatory gene activated anthocyanin biosynthesis in tomato fruit, a species that does not naturally accumulate anthocyanins in ripe fruit tissue, demonstrating promoter engineering as a route to activating a silent but structurally intact pathway [11]. More recently, CRISPR/Cas9-mediated homology-directed repair has been used to restore functional AN2-like regulatory alleles at their native locus, directly reintroducing anthocyanin biosynthetic capacity lost during domestication [16]. Genome editing has additionally been applied to tomato fruit weight and yield-associated cis-regulatory regions, illustrating that the same multiplex, promoter-level editing principles developed for nutritional traits are being applied concurrently to agronomic traits within the same crop [8].

Potato (Solanum tuberosum). Potato biofortification through genome editing has concentrated on reducing antinutritional steroidal glycoalkaloids (SGAs) while, in parallel efforts, modifying carotenoid and pigment pathways. CRISPR/Cas9 editing of the sterol side chain reductase 2 gene (StSSR2) reduced total tuber SGA content, with the most strongly affected mutant lines showing SGA concentrations reduced to roughly half of wild-type levels in peel tissue [12]. TALEN-mediated disruption of CYP88B1 (also known as PGA3 or GAME4) reduced SGAs to undetectable levels while correspondingly increasing the accumulation of non-toxic steroidal saponins, illustrating that disruption of a shared branch-point enzyme can simultaneously reduce an antinutrient and increase a related, potentially beneficial metabolite class. CRISPR/Cas9 knockout of phytoene desaturase (PDS) in diploid potato, although primarily used as a visible marker for editing efficiency because it produces an albino phenotype, has additionally revealed that PDS disruption triggers transcriptomic reorganisation extending beyond the carotenoid pathway itself, a finding relevant to interpreting the pleiotropic consequences of carotenoid pathway edits more broadly. RNP-mediated, DNA-free editing of the susceptibility gene StSR4 to confer resistance to Phytophthora infestans illustrates a complementary strategy relevant to nutritional quality only indirectly, by protecting tuber yield and quality from late blight-associated losses, but underscores the rapid maturation of protoplast- and RNP-based, transgene-free editing platforms in potato that are equally applicable to direct nutritional trait editing [12].

Carrot (Daucus carota). As the dietary vegetable most closely associated with provitamin A carotenoid intake, carrot has been the subject of extensive QTL mapping of the loci controlling the transition from white or yellow to high-carotenoid orange storage roots, including the Y and Y2 loci and DcLCYB1, whose overexpression or silencing directly increases or decreases chlorophyll and carotenoid content in both leaves and storage roots [20,21,22,23]. While direct, published CRISPR/Cas9 editing of carotenoid biosynthetic genes specifically in carrot storage root remains comparatively less extensively documented than in tomato or lettuce, the detailed characterisation of the underlying carotenogenic gene network including DcPAR1, a positive regulator of phytoene synthase expression whose antisense suppression dramatically reduces carotenoid accumulation and impairs taproot development has established clear, functionally validated candidate targets for future genome-editing intervention, and the availability of a complete carrot reference genome and transcriptomic database provides the infrastructure necessary to translate these candidates into edited germplasm [23,24].

Brassica vegetables (cabbage, kale, broccoli, Chinese cabbage, and relatives). Genome editing in Brassica oleracea and Brassica rapa vegetables has focused predominantly on anthocyanin pathway genes controlling the widely valued purple pigmentation of ornamental and culinary Brassica cultivars. Map-based cloning combined with CRISPR/Cas9 editing identified BoNA1 homologous to a dihydroflavonol-4-reductase-like gene as the

causal gene underlying the no-anthocyanin-accumulation phenotype in certain curly kale varieties, with independent loss-of-function mutations in BoNA1 confirmed across multiple naturally occurring green kale accessions [18]. In non-heading Chinese cabbage, transcriptome-guided identification of the basic helix-loop-helix transcription factor BcTT8 as a regulator of anthocyanin biosynthesis demonstrated that silencing BcTT8 substantially decreased total anthocyanin content and down-regulated downstream structural genes, while ectopic overexpression in Arabidopsis produced the converse pigmentation phenotype, together validating BcTT8 as an editing target for tuning anthocyanin content in leafy Brassica vegetables. Fine mapping of the R2R3-MYB regulatory gene BrMYB2 as the determinant of the purple-head trait in Chinese cabbage has similarly established a validated candidate for targeted activation or allele engineering [19]. Beyond anthocyanins, Brassica oleracea harbours substantial natural variation in glucosinolate composition, including loss-of-function variation at AOP2 that determines the balance between health-promoting glucoraphanin and less desirable progoitrin-derived compounds in broccoli, a variation pattern that has been explicitly proposed as a target for CRISPR/Cas9-based manipulation to favour beneficial glucosinolate profiles [77].

Lettuce (Lactuca sativa). Lettuce has emerged as an increasingly tractable platform for genome editing of pigment and vitamin traits, aided by its relevance to controlled-environment and plant-factory production systems where nutritional traits can be evaluated under standardised conditions. CRISPR/Cas9 knockout of dihydroflavonol 4-reductase (DFR) in red leaf lettuce produced a complete loss of anthocyanin accumulation and a visible shift from red-purple to green leaf colour, accompanied by an increase in total flavonol content, without detectable growth penalties, illustrating that pathway redirection rather than simple growth-compromising knockout can result from targeted anthocyanin pathway editing [16]. In a more directly biofortification-oriented application, simultaneous CRISPR/Cas9 editing of lycopene ε-cyclase (LCY-ε), the ascorbate pathway genes uGGP1 and uGGP2, and the carotenoid cleavage dioxygenase CCD4a has been used to increase provitamin A carotenoid, zeaxanthin, and ascorbate content concurrently in lettuce, demonstrating that multiplexed editing across independent pathways can be combined within a single leafy vegetable genotype to address several nutrient targets simultaneously [17].

Cucumber, eggplant, and pepper (Capsicum). Genome editing in these three Solanaceous and Cucurbitaceous crops has progressed at differing rates, reflecting differences in transformation efficiency and genomic resources. In eggplant (Solanum melongena), simultaneous CRISPR/Cas9 editing of three polyphenol oxidase genes (SmelPPO4, SmelPPO5, and SmelPPO6) reduced enzymatic browning of cut fruit flesh, a quality trait with direct relevance to the retention of polyphenolic antioxidant compounds that would otherwise be oxidatively degraded following tissue damage, and deep sequencing confirmed the absence of detectable off-target mutation at predicted potential off-target loci [14]. In pepper (Capsicum annuum), genome-wide identification of highly specific CRISPR/Cas9 target sites in the reference genome has established the technical foundation for precision editing across the genus, while ribonucleoprotein (RNP)-based, DNA-free editing of the CaMLO2 gene in both hot and sweet pepper cultivars validated first in protoplasts before extension to whole-plant regeneration — illustrates the maturation of transgene-free editing platforms applicable to future nutritional trait editing in this historically recalcitrant crop [43,44]. CRISPR/Cas9 editing of phytoene desaturase (PDS) in chilli pepper, though again used primarily as a visible marker of editing efficiency, confirms the functional tractability of the carotenoid pathway to editing in Capsicum, a genus of substantial nutritional interest given its high native vitamin C and carotenoid content [2]. Published, nutrition-specific genome editing in cucumber (Cucumis sativus) remains comparatively limited relative to the other crops discussed here, reflecting the crop's historically greater transformation recalcitrance, though improvements in Agrobacterium-mediated transformation efficiency and the increasing availability of high-quality reference genomes are expected to narrow this gap in coming years.

Across all crops reviewed in this section, several patterns recur. First, carotenoid and anthocyanin pathway genes are disproportionately represented among documented editing targets, reflecting both the strong visual phenotype associated with pigment pathway disruption which facilitates screening and the well-characterised, linear structure of these pathways relative to more complex, multi-branch pathways such as mineral transport. Second, multiplex editing of several pathway genes simultaneously is increasingly favoured over single-gene editing for nutritionally meaningful trait shifts, consistent with the broader principle that nutrient accumulation is rarely governed by a single rate-limiting step. Third, the transition toward RNP- and protoplast-based, transgene-free editing platforms is progressing across nearly every vegetable crop discussed, driven by the regulatory and consumer-acceptance advantages of avoiding stable transgene integration, even in species that remain comparatively difficult to transform and regenerate.

Table 3. Genome Editing and Molecular Approaches for Nutritional Improvement in Vegetable Crops

Crop	Target gene / pathway	Editing / molecular approach	Target nutritional / quality trait	Reported outcome	Reference
Tomato	SGR1, LCY-E, Blc, LCY-B1, LCY-B2 (carotenoid pathway)	Multiplex CRISPR/Cas9 knockout	Lycopene enrichment	~5.1-fold increase in fruit lycopene content	[5]

Crop	Target gene / pathway	Editing / molecular approach	Target nutritional / quality trait	Reported outcome	Reference
Tomato	GABA shunt genes	Multiplex CRISPR/Cas9 targeted mutagenesis	GABA (γ-aminobutyric acid) accumulation	Substantial increase in fruit GABA content	[6]
Tomato	SlDET1, DDB1, SlCYC-B	Cytidine base editing (Target-AID)	Carotenoid, lycopene, β-carotene content	Graded increases in carotenoid and β-carotene accumulation	[7]
Tomato	CRTISO, PSY1	Geminiviral replicon-delivered CRISPR/Cas9	Fruit colour / carotenoid composition	Orange- and yellow-fruited lines via distinct pathway blocks	[9]
Tomato	MYB12	CRISPR/Cas9 knockout	Peel flavonoid content / fruit colour	Pink-fruited phenotype via reduced peel flavonoids	[10]
Tomato	SlANT1	TALEN/CRISPR/Cas9 promoter insertion	Anthocyanin biosynthesis activation	Anthocyanin accumulation induced in normally non-pigmented fruit	[11]
Tomato	AN2-like (SlAN2-like)	CRISPR/Cas9 homology-directed repair	Restoration of anthocyanin biosynthesis	Functional allele restored at native locus, reactivating pathway	[16]
Potato	StSSR2 (steroidal glycoalkaloid pathway)	CRISPR/Cas9 knockout	Reduction of antinutritional glycoalkaloids	Tuber SGA content reduced to ~54% of wild type in edited lines	[12]
Potato	CYP88B1 (GAME4/PGA3)	TALEN-mediated disruption	Antinutrient reduction; saponin accumulation	SGAs reduced to undetectable levels; steroidal saponins accumulated	[12]
Potato	Phytoene desaturase (PDS)	CRISPR/Cas9 knockout	Carotenoid pathway functional validation	Albino phenotype confirming pathway disruption; broader transcriptomic reorganisation observed	[12]
Lettuce	DFR (dihydroflavonol 4-reductase)	CRISPR/Cas9 knockout	Anthocyanin / flavonoid pathway redirection	Loss of anthocyanin accumulation; increased total flavonol content; no growth penalty	[16]
Lettuce	LCY-ε, uGGP1/uGGP2, CCD4a	Multiplex CRISPR/Cas9 editing	Provitamin A, zeaxanthin, ascorbate content	Concurrent increases in provitamin A carotenoid, zeaxanthin, and ascorbate	[17]
Curly kale (Brassica oleracea)	BoNA1 (DFR-like gene)	Map-based cloning validated by CRISPR/Cas9 knockout	Anthocyanin accumulation	Loss-of-function confirmed as cause of no-anthocyanin (green) phenotype	[18]
Chinese cabbage (Brassica rapa)	BrMYB2	Fine mapping / candidate gene validation	Purple-head anthocyanin pigmentation	Regulatory allele identified as determinant of purple-head trait	[19]
Eggplant	SmelPPO4, SmelPPO5, SmelPPO6	Multiplex CRISPR/Cas9 knockout	Reduced flesh browning;	Reduced enzymatic browning of cut fruit	[14]

Crop	Target gene / pathway	Editing / molecular approach	Target nutritional / quality trait	Reported outcome	Reference
	(polyphenol oxidase)		polyphenol retention	flesh; no detectable off-target mutations	
Pepper (Capsicum annuum)	CaMLO2	RNP-based CRISPR/Cas9 / Cas12a protoplast editing	Platform validation (disease resistance-linked; extensible to quality traits)	Differential editing efficiency achieved across hot and sweet cultivars via DNA-free RNPs	[43,44]
Chilli pepper (Capsicum annuum)	Phytoene desaturase (PDS)	CRISPR/Cas9 knockout	Carotenoid pathway functional validation	Confirmed genome editing tractability of carotenoid pathway in Capsicum	[2]
Carrot	DcLCYB1, DcPAR1	Overexpression / antisense silencing (candidate validation)	Carotenoid and chlorophyll accumulation in root and leaf	Altered carotenoid/chlorophyll levels confirm functional roles; validated targets for future editing	[23,24]

11. Biofortification of Specific Nutrients and Bioactive Compounds

11.1 Iron. Iron biofortification of vegetable crops is constrained less by the plant's capacity to accumulate iron than by the bioavailability of that iron to human consumers, since plant iron is predominantly non-haem iron whose absorption is strongly inhibited by co-occurring phytate and, to a lesser extent, polyphenols [33,38]. Biotechnological strategies have therefore pursued two complementary objectives: increasing iron concentration in edible tissue through enhanced uptake and translocation, chiefly by modulating ZIP, NRAMP, and YSL transporter gene expression [35,36,37], and increasing iron bioavailability by reducing phytate content or by increasing the concentration of promoter compounds such as ascorbate that enhance non-haem iron absorption when co-consumed [33]. The dual dependence of iron nutrition on both plant-side accumulation and diet-level bioavailability underscores why iron biofortification strategies are most effective when combined with parallel enhancement of ascorbate or other bioavailability-enhancing compounds within the same crop.

11.2 Zinc. Zinc biofortification has benefited from a more mature mechanistic understanding of transport genetics than most other minerals, owing largely to research originally conducted in cereal crops such as wheat, where ZIP family transporters and nicotianamine synthase have been directly linked to grain zinc accumulation [35,38]. Transloctation of this knowledge to vegetable crops has been slower, but the conserved nature of the ZIP transporter family across plant taxa provides a strong rationale for targeted overexpression or promoter editing of vegetable orthologues, particularly in leafy and root vegetables where zinc bioaccessibility is less constrained by phytate than in cereal grain [36,37].

11.3 Selenium. Because selenium is not an essential plant nutrient, its accumulation in vegetable tissue is governed largely by non-specific uptake through sulfate transporters, given selenate's chemical similarity to sulfate, followed by assimilation into selenocysteine and selenomethionine or, in Brassica species specifically, further metabolism into organoselenium compounds such as selenoglucosinolates [30,81]. This mechanistic dependence on sulfate transport pathways explains why selenium biofortification has proven more tractable through agronomic foliar or soil application than through genetic engineering, and why Brassica vegetables with their capacity to further metabolise selenium into bioactive organoselenium derivatives have received particular research attention as selenium biofortification targets [39,81,82].

11.4 Provitamin A and Carotenoids. Provitamin A biofortification targets the accumulation of α-carotene, β-carotene, and β-cryptoxanthin, the carotenoids capable of enzymatic conversion to retinol in humans, and requires either increasing total carotenoid flux through the pathway or redirecting existing flux away from non-provitamin A carotenoids such as lutein and toward the β-carotene branch via lycopene β-cyclase activity [20,21]. Carrot, orange-fleshed tomato, and orange or red pepper cultivars represent the vegetable crops with the greatest natural variation and the most extensively characterised carotenogenic gene networks for this purpose, and the CRISPR-based editing successes documented in tomato and lettuce in Section 10 illustrate the pathway's tractability to both multiplex knockout and base-editing approaches [5,7,17].

11.5 Vitamin C. Ascorbate biofortification proceeds through manipulation of the L-galactose pathway, most commonly by overexpressing GDP-mannose pyrophosphorylase, GDP-L-galactose phosphorylase, or related enzymes to increase precursor flux, or, as demonstrated in lettuce, by editing the uGGP genes directly via CRISPR/Cas9 [17,34]. Because ascorbate also functions as a central redox buffer in plant stress physiology,

ascorbate biofortification strategies must be evaluated not only for nutritional impact but for potential effects on the plant's own stress tolerance and yield stability.

11.6 Vitamin E. Vitamin E biofortification of vegetable tissue is constrained by the limited availability of phytyl diphosphate, the isoprenoid side-chain precursor for tocopherol synthesis, which in green tissue is derived predominantly from chlorophyll turnover rather than de novo isoprenoid synthesis [55,56]. Strategies that couple enhanced phytol release from chlorophyll degradation with overexpression of homogentisate phytyltransferase and downstream tocopherol cyclase and methyltransferase genes have achieved substantial increases in leaf tocopherol content in model systems, establishing a template for extension to leafy vegetable crops where tocopherol content is not naturally maximised [55,56,57,58].

11.7 Folate. As detailed in Section 7, folate biofortification requires coordinated engineering of both the pteridine and para-aminobenzoate biosynthetic branches, since amplification of either branch alone typically produces a smaller folate increase than combined engineering of both, as demonstrated in transgenic tomato and common bean [25,26,29]. Beyond transgenic pathway engineering, natural genetic variation for folate retention for example, reduced γ-glutamyl hydrolase activity associated with elevated folate levels in certain tomato accessions offers a conventional breeding-compatible route to folate biofortification that avoids the regulatory classification of transgenic approaches.

11.8 Anthocyanins and Other Polyphenols. Anthocyanin biofortification has advanced further through genome editing than perhaps any other bioactive compound class reviewed in this article, owing to the strong, easily screened visual phenotype associated with pathway activation or disruption and the relatively well-conserved MYB–bHLH–WD40 regulatory architecture across vegetable taxa [10,16,18,19]. This regulatory conservation means that transcription factor targets validated in one Brassica or Solanaceous species frequently provide a template for homologous targeting in related crops, accelerating the pace at which anthocyanin biofortification can be extended across vegetable taxa relative to nutrients governed by more taxonomically divergent biosynthetic machinery.

11.9 Other Health-Promoting Phytochemicals. Glucosinolates in Brassica vegetables, flavonoids across nearly all vegetable taxa, and carotenoid-derived apocarotenoid signalling compounds represent additional bioactive compound classes with documented or proposed genome-editing targets, including AAOP2-mediated glucosinolate profile modification in broccoli and CCD4a-mediated carotenoid cleavage modulation in lettuce [17,77]. The nutritional and health relevance of these compounds, combined with their frequently well-defined single- or few-gene genetic control, positions them as promising near-term targets for continued precision breeding effort, even where their contribution to human health remains, in several cases, better established through epidemiological association than through mechanistic dose-response evidence.

12. Role of Omics Technologies and Systems Biology

The accelerating pace of biofortification research in vegetable crops has been enabled substantially by the parallel maturation of genomic, transcriptomic, proteomic, metabolomic, and phenomic technologies, and by the increasing integration of these layers into unified multi-omics and systems biology frameworks [44,46,47]. Genomics provides the reference sequences and structural variant catalogues necessary to design guide RNAs, identify candidate genes within QTL intervals, and assess off-target risk prior to genome editing; the availability of high-quality reference genomes for tomato, potato, carrot, and increasingly cucumber, eggplant, and pepper has been a direct enabling factor for the editing applications reviewed in Section 10 [23,24]. Transcriptomics allows the identification of differentially expressed candidate genes underlying contrasting nutrient phenotypes, exemplified by the identification of BcTT8 as a regulator of anthocyanin biosynthesis in Chinese cabbage through comparative transcriptome analysis of purple and green genotypes [19]. Proteomics and metabolomics extend this analysis to the protein and metabolite levels respectively, providing more direct readouts of pathway flux than transcript abundance alone, and metabolomic profiling in particular has become central to identifying metabolite quantitative trait loci that would not be detectable through phenotypic or transcriptomic analysis alone [44,45,47]. The integration of these distinct omics layers into multi-omics frameworks addresses a key limitation of single-omics analyses: transcript, protein, and metabolite abundances are frequently poorly correlated with one another because of post-transcriptional and post-translational regulation, so conclusions drawn from any single layer risk mischaracterising the true regulatory bottleneck governing a nutritional trait [46,47]. Systematic multi-omics integration frameworks, classified according to increasing levels of analytical complexity from simple co-expression analysis through pathway-level mapping to full genome-scale mathematical integration, provide a structured methodology for combining these data layers, and machine learning approaches are increasingly being applied to multi-omics datasets to improve the prediction accuracy of complex, polygenic nutritional and quality traits beyond what classical genomic selection models achieve, with genomics-metabolomics combinations showing particular promise for quality-trait prediction [45,48]. Phenomics the high-throughput, often image-based quantification of plant phenotypes at scal complements these molecular omics layers by providing the large phenotypic datasets necessary to train and validate genomic and multi-omics prediction models, and its integration with digital and machine-learning-based analysis is discussed further as an emerging direction in Section 15.

For vegetable biofortification specifically, the principal value of multi-omics and systems biology approaches lies in accelerating the transition from a statistically significant QTL interval to a functionally validated, editable candidate gene a transition that, in earlier decades of molecular breeding, often required years of positional cloning effort but that can now frequently be substantially shortened when genomic, transcriptomic, and metabolomic

evidence converge on a small number of high-confidence candidates, as illustrated by the BcTT8, BrMYB2, and BoNA1 examples discussed in Section 10 [18,19].

13. Nanotechnology and Emerging Biotechnological Approaches

Nanotechnology-enabled approaches to biofortification represent a comparatively recent but rapidly expanding complement to the genetic and molecular strategies discussed above, operating principally at the level of nutrient delivery rather than plant genetics. Nanofertilisers engineered nanoscale formulations of essential mineral nutrients have been developed for zinc, iron, and selenium, among other elements, with the objective of improving nutrient use efficiency, controlling release kinetics, and enhancing foliar or root uptake relative to conventional bulk fertiliser formulations [40,41,42]. Foliar application of zinc oxide nanoparticles, for example, has been shown to increase zinc accumulation in treated tissue while, in some formulations, reducing the total mass of fertiliser required relative to conventional zinc sulfate application, and combined biofertiliser–nanofertiliser treatments have been used to simultaneously increase glucosinolate, zinc, and iron content in broccoli microgreens [43,80].

Nano-enabled nutrient delivery systems, including mesoporous silica nanoparticles, layered double hydroxides, and chitosan-based nanocarriers, are additionally being explored as platforms for the controlled, slow release of micronutrients synchronised with crop developmental stages, potentially reducing the leaching and volatilisation losses that limit the efficiency of conventional fertiliser application in field conditions [41,42]. Because nanoparticle behaviour in planta depends strongly on particle size, surface functionalisation, and composition, translocation efficiency and tissue-specific accumulation remain active areas of investigation, and a recent review specifically addressing nanoparticle-mediated biofortification of iron, zinc, and selenium in edible plants has synthesised current evidence on nanoparticle uptake mechanisms, translocation pathways, and the factors governing bioavailability in the resulting biofortified tissue [40].

Beyond nutrient delivery, nanotechnology intersects with precision genome editing through its emerging role as a delivery vehicle for CRISPR/Cas reagents themselves, potentially offering a route to transgene-free, tissue-culture-independent genome editing were nanoparticle-mediated delivery of editing ribonucleoproteins or RNA to be validated at practical efficiency in vegetable crops; this application remains substantially more exploratory than agronomic nanofertilisation and should be clearly distinguished from the latter, established use case when interpreting the current evidence base. It is important to emphasise that, while agronomic nanofertiliser applications have accumulated a reasonably substantial body of field-tested evidence across multiple crops, several proposed applications of plant nanobiotechnology including nanoparticle-based genetic reagent delivery and nanosensor-integrated precision nutrient management remain at earlier stages of development, and the distinction between established, field-validated nanofertiliser use and more speculative nanobiotechnological applications should be maintained when evaluating the near-term versus longer-term contribution of nanotechnology to vegetable biofortification [41,42].

14. Challenges and Limitations

Despite substantial technical progress, the translation of biofortification research into widely cultivated, consumer-accepted vegetable varieties continues to face a set of interconnected biological, agronomic, regulatory, and economic constraints.

Nutrient trade-offs and yield penalties. Because nutrient biosynthesis and accumulation draw on cellular resources and, in the case of mineral transporters, can interact with pathways governing toxic metal accumulation or ionic homeostasis, biofortification interventions can carry agronomic costs. Redirecting carbon flux toward carotenoid or anthocyanin accumulation, for instance, competes with flux toward other isoprenoid- or phenylpropanoid-derived compounds, including some involved in defence signalling, and heavy-metal stress susceptibility has been documented as an unintended consequence of lycopene pathway editing in tomato in at least one study, underscoring that pathway-level interventions require careful multi-trait evaluation rather than assessment of the target nutrient in isolation [5]. Yield penalties associated with nutrient-dense donor alleles introgressed from wild relatives remain a persistent obstacle in conventional breeding-based biofortification and must be actively selected against during subsequent breeding cycles [65,66].

Antinutritional compounds and bioavailability. Increasing the raw concentration of a target nutrient does not guarantee a corresponding increase in bioavailable nutrition if antinutritional compounds phytate, oxalate, or, in the case of potato, steroidal glycoalkaloids are simultaneously elevated or remain unaddressed. Iron biofortification efforts, in particular, must contend with the strong inhibitory effect of phytate on non-haem iron absorption, meaning that simultaneous or sequential reduction of antinutrient content is frequently as important as increasing the target mineral's absolute concentration [33,38].

Nutrient stability and environmental interaction. Many biofortified nutrients, including folate, vitamin C, and certain carotenoids, are susceptible to degradation during post-harvest storage, processing, and cooking, meaning that nutrient content measured at harvest can substantially overstate the nutrition actually delivered to the consumer; stability-focused engineering, as demonstrated for folate in biofortified rice, is therefore an important but sometimes overlooked complement to concentration-focused biofortification [28]. Genotype-by-environment interaction further complicates the deployment of biofortified vegetable varieties across diverse agroecological zones, since nutrient accumulation phenotypes validated under controlled research conditions do not always reproduce reliably across the range of soil, temperature, and light conditions experienced in commercial production.

Consumer acceptance. Visual, textural, and flavour changes associated with biofortification altered fruit or leaf colour resulting from carotenoid or anthocyanin pathway manipulation being the most obvious example can influence consumer acceptance independently of the underlying nutritional improvement, and acceptance patterns differ substantially across markets and cultural contexts. Public perception of the underlying technology, particularly the distinction drawn by many consumers and regulators between transgenic and genome-edited crops, further shapes market access even when the nutritional outcome is functionally equivalent [72,74,84].

Regulatory issues and biosafety. Perhaps the most significant near-term barrier to the deployment of genome-edited biofortified vegetables is the continued heterogeneity of the global regulatory landscape. Jurisdictions broadly fall into two regulatory philosophies: process-based systems that classify genome-edited organisms as functionally equivalent to genetically modified organisms regardless of whether foreign DNA is present in the final product, and product-based systems that regulate genome-edited crops according to the characteristics of the resulting product, often exempting edits that could plausibly have arisen through conventional mutagenesis from GMO-equivalent regulation [72,74,75]. The United States Department of Agriculture's revised, more risk-proportionate framework for gene-edited crops and the European Union's evolving proposals for a tiered regulatory framework distinguishing mutagenesis-equivalent, cisgenic, intragenic, and transgenic edits illustrate the ongoing, incomplete convergence between these two regulatory philosophies [72]. This heterogeneity creates substantial uncertainty for breeding programmes intending to commercialise edited vegetable varieties across multiple export markets, and artificial-intelligence-assisted regulatory frameworks have recently been proposed as a means of harmonising risk assessment and accelerating consistent classification across jurisdictions, though such frameworks remain at an early, largely conceptual stage of development [75,76].

Off-target effects and technical limitations. Although modern guide RNA design tools and genome-wide specificity analyses have substantially reduced the practical risk of off-target CRISPR/Cas9 mutagenesis, rigorous off-target assessment typically through targeted deep sequencing of computationally predicted candidate sites, as performed in eggplant PPO gene editing — remains an essential quality control step, particularly as multiplexed editing of increasing numbers of simultaneous target sites is adopted [14,15]. Prime editing efficiency in particular remains a significant technical constraint, with editing outcomes in plants generally lower and more variable than in mammalian systems, limiting its routine application to biofortification targets that would benefit most from precise, single-nucleotide-resolution editing [49,52].

Transformation efficiency, cost, and access. Transformation and regeneration efficiency continue to vary enormously across vegetable species and even across genotypes within a species, with pepper representing a particularly well-documented example of a genus that has historically been recalcitrant to stable transformation, constraining the pace at which genome editing can be extended across its diverse cultivated forms [45,46]. The cost of establishing genome-editing pipelines including guide RNA design, transformation infrastructure, and regulatory compliance testing remains substantial relative to the resources available to public breeding programmes in many low- and middle-income countries, where the nutritional need for biofortified vegetables is often greatest, raising a persistent equity concern regarding differential access to these technologies between well-resourced private breeding programmes and public-sector or smallholder-oriented breeding efforts.

Intellectual property. The proliferation of patented CRISPR/Cas components, delivery methods, and specific editing applications has created a complex intellectual property landscape that can constrain the freedom to operate of public and smaller private breeding programmes, particularly in the absence of the humanitarian and royalty-free licensing arrangements that were eventually negotiated for foundational transgenic technologies such as those underlying Golden Rice [88,89]. Navigating this landscape, or securing comparable licensing arrangements for biofortification-relevant CRISPR applications, will likely remain an important non-technical determinant of how quickly genome-edited biofortified vegetables reach public breeding programmes and, ultimately, farmers in the regions of greatest nutritional need.

15. Future Perspectives

Several converging trends are likely to shape the next phase of vegetable biofortification research and deployment.

Precision breeding and multiplex, multi-trait editing. As guide RNA design, delivery, and validation pipelines continue to mature, the field is expected to move increasingly from single-gene proof-of-concept edits toward routine multiplex editing of entire pathways, and eventually toward simultaneous editing of nutritional traits alongside agronomic traits such as yield, disease resistance, and shelf life within a single elite background, reducing the number of breeding cycles required to combine these traits relative to sequential introgression [8,53].

Base and prime editing maturation. Continued optimisation of base editor and prime editor architecture, codon usage, and delivery including plant-specific improvements to pegRNA design and prime editor expression is expected to narrow the current efficiency gap between these technologies and conventional CRISPR/Cas9 nuclease editing, extending their applicability to a wider range of nutritionally relevant single-nucleotide and small-sequence modifications that cannot currently be achieved reliably through nuclease-based knockout alone [49,51,52,53].

AI-assisted crop improvement. Machine learning approaches are increasingly being integrated into multi-omics data analysis for candidate gene discovery and trait prediction, into guide RNA design and off-target prediction for genome editing, and, more speculatively, into regulatory risk assessment frameworks intended to harmonise the classification of genome-edited crops across jurisdictions [45,48,75,76]. While the application of artificial intelligence to regulatory decision-making in particular remains at a conceptual and largely untested stage, its

application to technical aspects of genome editing guide design, off-target prediction, and multi-omics integration is already delivering measurable improvements in prediction accuracy and is likely to become a routine component of biofortification breeding pipelines.

Multi-omics-guided candidate discovery. The continued expansion and integration of genomic, transcriptomic, proteomic, and metabolomic datasets across a broader range of vegetable taxa, beyond the comparatively well-resourced model crops of tomato and Brassica, is expected to substantially expand the catalogue of functionally validated candidate genes available for targeted editing in currently under-researched vegetable crops such as cucumber, eggplant, and many leafy Asian vegetables [44,46,47].

Speed breeding and synthetic biology. Speed breeding protocols, which use extended photoperiods and optimised environmental control to compress the generation time of breeding cycles, are increasingly being combined with genome editing and marker-assisted selection to accelerate the multi-generational process of introgressing and stabilising biofortification traits in elite backgrounds. Synthetic biology approaches, extending beyond single-pathway metabolic engineering toward the design and introduction of entirely novel biosynthetic modules, represent a longer-term but potentially transformative direction for introducing nutrient biosynthetic capacities that do not exist in any sexually compatible relative of the target crop, building on the precedent established by pathway reconstitution in Golden Rice [87,89].

Digital phenotyping and the integration of nutrition and agronomy. High-throughput, often image- or sensor-based phenomics platforms are increasingly being integrated with genomic and multi-omics data to accelerate the phenotypic validation of candidate biofortification lines at a scale previously achievable only for simple, visually scored traits such as pigmentation, extending rapid, non-destructive assessment toward more complex traits such as mineral and vitamin content through the development and validation of spectral and other proxy phenotyping methods [45,48].

Climate-resilient biofortified vegetables. As climate change alters the temperature, water availability, and pest and disease pressure experienced by vegetable production systems, breeding programmes are increasingly seeking to combine biofortification traits with climate resilience traits within the same genetic background, recognising that a nutritionally enhanced variety that cannot maintain yield stability under future climate conditions will fail to deliver its intended nutritional benefit at scale; multi-omics and genome-editing platforms developed initially for single-trait biofortification are directly applicable to this combined breeding objective given their capacity for multiplex, multi-gene intervention [47].

Translational and commercial prospects. The eventual impact of the biotechnological advances reviewed in this article will depend as much on regulatory harmonisation, public engagement, and equitable access to breeding technology as on further scientific progress. Continued convergence between process-based and product-based regulatory philosophies, transparent public communication regarding the distinction between transgenic and genome-edited crops, and licensing arrangements that extend access to CRISPR/Cas technology to public and humanitarian breeding programmes will together determine whether the scientific advances documented in this review translate into biofortified vegetable varieties available to the populations with the greatest nutritional need [72,74,84,88].

16. CONCLUSION

Vegetable crops occupy a nutritionally distinctive and largely complementary position relative to the staple cereals that have historically dominated biofortification research, supplying a dense and diverse array of provitamin A carotenoids, vitamin C, folate, tocopherols, minerals, and health-promoting phytochemicals that cannot be replicated by cereal biofortification alone. The biotechnological toolkit available for enhancing these nutritional attributes has expanded and matured considerably: agronomic and conventional breeding-based biofortification remain the most immediately deployable strategies and continue to underpin most commercially available mineral- and vitamin-enriched vegetable products; marker-assisted and, increasingly, genomic selection have accelerated the introgression of favourable nutritional alleles identified through QTL mapping; transgenic metabolic engineering has demonstrated that entire biosynthetic pathways can be reconstituted or substantially amplified where native genetic variation is insufficient; and CRISPR/Cas-based genome editing, together with its base-editing and prime-editing derivatives, now offers a precise, frequently transgene-free route to modifying endogenous nutrient biosynthesis, transport, and antinutrient pathways across an expanding range of vegetable species. Documented editing successes in tomato, potato, lettuce, Brassica vegetables, pepper, and eggplant illustrate that carotenoid, anthocyanin, ascorbate, and antinutrient pathways are now demonstrably tractable to precision genome editing, even as cucumber and several other vegetable taxa await comparable transformation and regeneration advances. Multi-omics profiling and systems biology approaches are increasingly compressing the time required to move from a statistically identified quantitative trait locus to a functionally validated, editable candidate gene, while nanotechnology-enabled nutrient delivery offers a complementary, non-genetic route to improving nutrient use efficiency in the field. The principal barriers to realising the full potential of this technological convergence are no longer predominantly technical: nutrient trade-offs, bioavailability constraints, post-harvest stability, consumer acceptance, and, above all, the continued heterogeneity of the global regulatory landscape for genome-edited crops will together determine the pace and equity with which biofortified vegetable varieties reach the populations that stand to benefit most from them. Continued scientific progress in precision genome editing, matched by parallel progress in regulatory harmonisation and equitable technology access, will

be necessary to translate the biological potential documented throughout this review into a measurable reduction in the global burden of micronutrient malnutrition.

REFERENCES

1. Das P, Dutta A, Das A. CRISPR/Cas9 applications for nutritional enhancement in major oilseed, vegetable, and cereal crops: a comprehensive review. Plant Mol Biol Rep. 2025;43:1732-1751.
2. Sardar A. Corrigendum: Genetic amelioration of fruit and vegetable crops to increase biotic and abiotic stress resistance through CRISPR genome editing. Front Plant Sci. 2024. doi:10.3389/fpls.2024.1418620.
3. Kushwaha SB, Nagesh CR, Lele SS, Viswanathan C, Rama Prashat G, Goswami S, Kumar RR, Kunchge N, Gokhale JS, Vinutha T. CRISPR/Cas technology in vegetable crops for improving biotic, abiotic stress and quality traits: challenges and opportunities. Sci Hortic. 2025;344:113957.
4. Kumar D, Yadav A, Ahmad R, Dwivedi UN, Yadav K. CRISPR-based genome editing for nutrient enrichment in crops: a promising approach toward global food security. Front Genet. 2022;13:932859.
5. Li X, Wang Y, Chen S, Tian H, Fu D, Zhu B, Luo Y, Zhu H. Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Front Plant Sci. 2018;9:559.
6. Nonaka S, Arai C, Takayama M, Matsukura C, Ezura H. Efficient increase of ω-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. Sci Rep. 2017;7:7057.
7. Hunziker J, et al. Multiple gene substitution by Target-AID base-editing technology in tomato. Plant Biotechnol J. 2020;18(12):2515-2523.
8. Rodriguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB. Engineering quantitative trait variation for crop improvement by genome editing. Cell. 2017;171(2):470-480.
9. Dahan-Meir T, Filler-Hayut S, Melamed-Bessudo C, et al. Efficient in planta gene targeting in tomato using geminiviral replicons and the CRISPR/Cas9 system. Plant J. 2018;95(1):5-16.
10. Deng L, et al. Efficient generation of pink-fruited tomatoes using CRISPR/Cas9 system. J Genet Genomics. 2018;45(1):51-54.
11. Cermak T, Baltes NJ, Cegan R, Zhang Y, Voytas DF. High-frequency, precise modification of the tomato genome. Genome Biol. 2015;16:232.
12. Zheng Z, et al. Editing sterol side chain reductase 2 gene (StSSR2) via CRISPR/Cas9 reduces the total steroidal glycoalkaloids in potato. All Life. 2021;14(1):401-413.
13. Bhatta BP, et al. Genome editing for vegetable crop improvement: challenges and future prospects. Front Genome Ed. 2022;4:1029216.
14. Maioli A, et al. Simultaneous CRISPR/Cas9 editing of three PPO genes reduces fruit flesh browning in Solanum melongena L. Front Plant Sci. 2020;11:607161.
15. Li G, Zhou Z, Liang L, et al. Genome-wide identification and analysis of highly specific CRISPR/Cas9 editing sites in pepper (Capsicum annuum L.). PLoS One. 2020;15(12):e0244515.
16. Nagamine A, et al. CRISPR/Cas9-mediated knockout of DFR alters pigmentation and shifts flavonoid accumulation in red leaf lettuce without detectable growth penalties. Front Genome Ed. 2026;8:1755922.
17. Livneh O, et al. CRISPR/Cas9 editing of LCY-ε, uGGP1/uGGP2 and CCD4a enhances provitamin A, ascorbate and zeaxanthin content of lettuce. Plant Biotechnol J. 2025.
18. Yuan K, Zhao X, Sun W, Yang L, Zhang Y, Wang Y, Ji J, Han F, Fang Z, Lv H. Map-based cloning and CRISPR/Cas9-based editing uncover BoNA1 as the causal gene for the no-anthocyanin-accumulation phenotype in curly kale (Brassica oleracea var. sabellica). Hortic Res. 2023;10(8):uhad133.
19. Yu Z, Wu R, Wang Y, et al. The novel gene BrMYB2, located on chromosome A07, with a short intron 1 controls the purple-head trait of Chinese cabbage (Brassica rapa L.). Hortic Res. 2021;8:74.
20. Yuan H, Zhang J, Nageswaran D, Li L. Carotenoid biofortification of crops in the CRISPR era. Trends Biotechnol. 2021;39(6):536-538.
21. Morelli L, Rodriguez-Concepcion M. Open avenues for carotenoid biofortification of plant tissues. Plant Commun. 2023;4(1):100466.
22. Ma S, Cao J, Zhang Y, et al. Identification of QTLs for root color and carotenoid contents in Japanese orange carrot F2 populations. Sci Rep. 2022;12:8479.
23. Simon PW, Iorizzo M, Senalik D, Ellison S. Carrot carotenoid genetics and genomics. Theor Appl Genet. 2019;132:2493-2508.
24. Xu ZS, Tan HW, Wang F, Hou XL, Xiong AS. CarrotDB: a genomic and transcriptomic database for carrot. Database (Oxford). 2014;bau096.
25. Diaz de la Garza R, Quinlivan EP, Klaus SMJ, Basset GJC, Gregory JF, Hanson AD. Folate biofortification in tomatoes by engineering the pteridine branch of folate synthesis. Proc Natl Acad Sci U S A. 2004;101(38):13720-13725.
26. Diaz de la Garza RI, Gregory JF, Hanson AD. Folate biofortification of tomato fruit. Proc Natl Acad Sci U S A. 2007;104(10):4218-4222.
27. Basset GJC, Quinlivan EP, Ziemak MJ, et al. Folate synthesis in plants: the p-aminobenzoate branch is initiated by a bifunctional PabA-PabB protein that is targeted to plastids. Proc Natl Acad Sci U S A. 2004;101(6):1496-1501.

28. Blancquaert D, Van Daele J, Strobbe S, et al. Improving folate (vitamin B9) stability in biofortified rice through metabolic engineering. Nat Biotechnol. 2015;33(10):1076-1078.
29. Ma Y, Kaur N, Bhattacharya S, et al. Metabolic engineering of folate and its precursors in Mexican common bean (Phaseolus vulgaris L.). Plant Biotechnol J. 2016;14(11):2170-2184.
30. Zhu YG, Pilon-Smits EAH, Zhao FJ, Williams PN, Meharg AA. Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. Trends Plant Sci. 2009;14(8):436-442.
31. D'Amato R, Regni L, Falcinelli B, et al. Current knowledge on selenium biofortification to improve the nutraceutical profile of food: a comprehensive review. J Agric Food Chem. 2020;68(14):4075-4097.
32. Ros GH, van Rotterdam AMD, Bussink DW, Bindraban PS. Selenium fertilization strategies for crop biofortification and quality improvement: a review. Nutr Cycl Agroecosyst. 2016;108:1-16.
33. Ricachenevsky FK, Punshon T, Lee S, et al. Elemental biofortification of cereals: from soil to seed. Front Plant Sci. 2015;6:359.
34. Ramesh SA, Choimes S, Schachtman DP. Over-expression of an Arabidopsis zinc transporter in Hordeum vulgare increases short-term zinc uptake after zinc deprivation and seed zinc content. Plant Mol Biol. 2004;54:373-385.
35. Guerinot ML. The ZIP family of metal transporters. Biochim Biophys Acta. 2000;1465(1-2):190-198.
36. Milner MJ, Seamon J, Craft E, Kochian LV. Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. J Exp Bot. 2013;64(1):369-381.
37. Singh SP, Vogel-Mikuš K, Arčon I, et al. Zinc-regulated transporter, iron-regulated transporter-like protein (ZIP) family in plants: a comprehensive review. Int J Biol Macromol. 2020;158:685-696.
38. Cakmak I, Pfeiffer WH, McClafferty B. Biofortification of durum wheat with zinc and iron. Cereal Chem. 2010;87(1):10-20.
39. Zuo Y, Zhang F. Soil and crop management strategies to prevent iron deficiency in crops. Plant Soil. 2011;339:83-95.
40. Rathi S, Verma S, Vashishth A, et al. Nanotechnology-driven biofortification of Fe, Zn, and Se in edible plants: mechanisms, methods, and prospects. J Nanobiotechnology. 2025;23.
41. Ahmed T, Noman M, Manzoor N, et al. Biofortification of crops with nutrients by application of nanofertilizers for effective agriculture. Environ Res. 2024.
42. Verma KK, Song XP, Joshi A, et al. Smart nutrient delivery: the promise of nano-fertilizers for sustainable crop production. Discov Appl Sci. 2026.
43. Elemike EE, Uzoh IM, Onwudiwe DC, Babalola OO. The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. Appl Sci. 2019;9(3):499.
44. Zhang R, Zhang C, Yu C, Dong J, Hu J. Integration of multi-omics technologies for crop improvement: status and prospects. Front Bioinform. 2022;2:1027457.
45. Yang Y, Saand MA, Huang L, et al. Applications of multi-omics technologies for crop improvement. Front Plant Sci. 2021;12:563953.
46. Loo EP, Durán-Nebreda S, et al. Systematic multi-omics integration (MOI) approach in plant systems biology. Front Plant Sci. 2020;11:944.
47. Song X, Fan BL, Hong X, Su P, Sun M. Unveiling plant metabolic diversity: integrating metabolomics with multi-omics approaches for crop improvement. Plants (Basel). 2026;15(5):846.
48. Tian S, Yao L, Zhang Y, Rao X, Zhu H. Prime editing for crop improvement: a systematic review of optimization strategies and advanced applications. Genes (Basel). 2025;16(8):965.
49. Molla KA, Sretenovic S, Bansal KC, Qi Y. Improvement of base editors and prime editors advances precision genome engineering in plants. Plant Physiol. 2022;188(4):1795-1810.
50. Kang BC, Bae SJ, Lee S, et al. Base editing in plants: applications, challenges, and future prospects. Front Plant Sci. 2021;12:664997.
51. Gallo D, Meunier AC, Périn C. A long journey towards genome editing technologies in plants: a technical and critical review of genome editing technologies. Front Genome Ed. 2025;7:1663352.
52. Li C, Zong Y, Wang Y, et al. Plant base editing and prime editing: the current status and future perspectives. J Integr Plant Biol. 2023;65(2):444-467.
53. Zhao Y, Xu M, et al. Plant base editing: a decade of progress and future applications. Trends Genet. 2025.
54. Merchant Z, Ravichandran A, Nawade B, et al. Genome-wide association identifies a missing hydrolase for tocopherol synthesis in plants. Proc Natl Acad Sci U S A. 2022;119(2):e2113488119.
55. Almeida J, Perez-Fons L, Fraser PD. Vitamin E biofortification: enhancement of seed tocopherol concentrations by altered chlorophyll metabolism. Metab Eng. 2023.
56. Perez-Fons L, Ohana-Levi N, Fraser PD, et al. Nutritional enrichment of plant leaves by combining genes promoting tocopherol biosynthesis and storage. Metabolites. 2023;13(2):193.
57. Rani P, Kumar V, Ahmad F, et al. Vitamin E in plants: biosynthesis pathways, biofortification strategies, and regulatory dynamics. Antioxidants (Basel). 2025;14.
58. Fritsche S, Wang X, Jung C. Recent advances in our understanding of tocopherol biosynthesis in plants: an overview of key genes, functions, and breeding of vitamin E improved crops. Antioxidants (Basel). 2017;6(4):99.
59. Cahoon EB, Hall SE, Ripp KG, et al. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat Biotechnol. 2003;21(9):1082-1087.

60. DellaPenna D. Current strategies for vitamin E biofortification of crops. Curr Opin Biotechnol. 2017;44:169-174.
61. Foolad MR, Panthee DR. Marker-assisted selection in tomato breeding. Crit Rev Plant Sci. 2012;31(2):93-123.
62. Sacco A, Ruggieri V, Parisi M, et al. Exploring a tomato landraces collection for fruit-related traits by the aid of a high-throughput genomic platform. PLoS One. 2015;10(9).
63. Zhu G, Wang S, Huang Z, et al. Rewiring of the fruit metabolome in tomato breeding. Cell. 2018;172(1-2):249-261.
64. Foolad MR. Genome mapping and molecular breeding of tomato. Int J Plant Genomics. 2007;2007:64358.
65. Rambla JL, et al. QTL mapping of fruit mineral contents provides new chances for genetic improvement of tomato nutritional value. Theor Appl Genet. 2017;130(10):2019-2036.
66. Xu J, Ranc N, Muños S, et al. Phenotypic diversity and association mapping for fruit quality traits in cultivated tomato and related species. Theor Appl Genet. 2013;126(3):567-581.
67. Ma Y, McGee RJ, Coyne CJ, et al. Genome-wide SNP identification, linkage map construction and QTL mapping for seed mineral concentrations and contents in pea (Pisum sativum L.). BMC Plant Biol. 2017;17:43.
68. Fazili MA, Bashir I, Ahmad M, Yaqoob U, Geelani SN. In vitro strategies for the enhancement of secondary metabolite production in plants: a review. Bull Natl Res Cent. 2022;46:35.
69. Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav. 2011;6(11):1720-1731.
70. Espinosa-Leal CA, Puente-Garza CA, García-Lara S. In vitro plant tissue culture: means for production of biological active compounds. Planta. 2018;248(1):1-18.
71. Karam NS, Jawad FM, Arikat NA, Shibli RA. Growth and rosmarinic acid accumulation in callus, cell suspension, and root cultures of wild Salvia fruticosa. Plant Cell Tissue Organ Cult. 2003;73:117-121.
72. Ahmad A, Hoffman NE, Jones MGK, Zhang B. Editorial: Frontiers in global regulatory landscape of CRISPR-edited plants. Front Plant Sci. 2024;15:1367698.
73. Menz J, Modrzejewski D, Hartung F, Wilhelm R, Sprink T. Genome edited crops touch the market: a view on the global regulatory and patent landscape of gene-edited crops. Front Plant Sci. 2020;11:586027.
74. Ahmad A. An outlook on global regulatory landscape for genome-edited crops. J Cell Physiol. 2022;237(1):27-37.
75. Zhu L, Huang J, Xie C. AI-enhanced framework for optimizing CRISPR-Cas gene editing in crop biotechnology addressing regulatory challenges and opportunities in global agricultural practices. Front Plant Sci. 2026;17:1770472.
76. Zhu F, Liu Z, Zheng Z. An AI-driven framework for enhancing regulatory precision and efficiency in CRISPR-Cas gene-edited crops: challenges, opportunities, and global harmonization. Front Plant Sci. 2026;16:1693105.
77. Traka MH, Saha S, Rollins F, et al. Brassica vegetables—an undervalued nutritional goldmine. Hortic Res. 2024;12(2):uhae302.
78. Broadley MR, White PJ, Bryson RJ, et al. Biofortifying Brassica with calcium (Ca) and magnesium (Mg). Plant Soil. 2010;335:401-412.
79. D'Imperio M, Renna M, Cardinali A, et al. Calcium biofortification and bioaccessibility in soilless "baby leaf" vegetable production. Food Chem. 2016;213:149-156.
80. Guardiola-Márquez CE, García-Sánchez CV, Sánchez-Arellano ÓA, Bojorquez-Rodríguez EM, Jacobo-Velázquez DA. Biofortification of broccoli microgreens (Brassica oleracea var. italica) with glucosinolates, zinc, and iron through the combined application of bio- and nanofertilizers. Foods. 2023;12(20):3826.
81. Avato P, Migoni D, Argentieri MP, et al. Selenium biofortification effect on glucosinolate content of Brassica oleracea var. italica and Eruca vesicaria. Molecules. 2013;18(11).
82. Bachiega P, Salgado JM, de Carvalho JE, et al. Floret biofortification of broccoli using amino acids coupled with selenium under different surfactants: a case study of cultivating functional foods. Foods. 2023;12(6):1234.
83. Bouis HE, Saltzman A. Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Glob Food Sec. 2017;12:49-58.
84. White PJ, Broadley MR. Biofortification of crops with seven mineral elements often lacking in human diets—iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol. 2009;182(1):49-84.
85. White PJ, Broadley MR. Biofortifying crops with essential mineral elements. Trends Plant Sci. 2005;10(12):586-593.
86. Bhullar NK, Gruissem W. Nutritional enhancement of rice for human health: the contribution of biotechnology. Biotechnol Adv. 2013;31(1):50-57.
87. Naqvi S, Zhu C, Farre G, et al. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci U S A. 2009;106(19):7762-7767.
88. Beyer P. Golden Rice and 'Golden' crops for human nutrition. N Biotechnol. 2010;27(5):478-481.
89. Ye X, Al-Babili S, Klöti A, et al. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science. 2000;287(5451):303-305.

90. Ricroch A, Clairand P, Harwood W. Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture. Emerg Top Life Sci. 2017;1(2):169-182.
91. FAO. Human vitamin and mineral requirements / global prevalence of hidden hunger. FAO/WHO Expert Consultation, 2002.
92. Adesogan AT, Havelaar AH, McKune SL, Eilittä M, Dahl GE. Animal source foods: sustainability problem or malnutrition and sustainability solution? Perspective matters. Glob Food Sec. 2020;25:100325.
93. Sarwar S, Khan MW, Aslam N, et al. Beyond calories: addressing micronutrient deficiencies in the world's most vulnerable communities—a review. Nutrients. 2025;17.
94. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;123(5):615-624.
95. Harika R, Faber M, Samuel F, et al. Micronutrient status and dietary intake of iron, vitamin A, iodine, folate and zinc in women of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa: a systematic review of data from 2005 to 2015. Nutrients. 2017;9(10):1096.
96. Wessells KR, Brown KH. Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS One. 2012;7(11):e50568.
97. Stevens GA, Bennett JE, Hennocq Q, et al. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013. Lancet Glob Health. 2015;3(9):e528-e536.
98. Micronutrient Forum/GAIN. New global estimates for hidden hunger. Advocacy Brief; 2022.
99. Saltzman A, Birol E, Bouis HE, Boy E, De Muñoz FF, Islam Y, Pfeiffer WH. Biofortification: progress toward a more nourishing future. Glob Food Sec. 2013;2(1):9-17.
100. de Steur H, Blancquaert D, Strobbe S, et al. Status and market potential of transgenic biofortified crops. Nat Biotechnol. 2015;33(1):25-29.
101. Dwivedi SL, Sahrawat KL, Puppala N, Ortiz R. Plant prebiotics and human health: biotechnology to breed prebiotic-rich nutritious food crops. Electron J Biotechnol. 2014;17(5):238-245.
102. Zsögön A, Čermák T, Naves ER, et al. De novo domestication of wild tomato using genome editing. Nat Biotechnol. 2018;36(12):1211-1216.
103.