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Review

Precision Selenium Biofortification of Leafy Vegetables in Controlled-Environment Agriculture: Mechanisms, Production Strategies, Safety, and Research Priorities

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Highlights

What are the main findings?

- Hydroponic selenate at 2–16 μM raised leaf Se 10- to 100-fold with little yield loss.
- Many published "optimal" doses supply 90–1,000% of the adult RDA per serving.

What are the implications of the main findings?

- Production should target a serving-based window, not maximum accumulation.
- Studies should report fresh- and dry-weight Se, speciation, and batch variability.

Abstract

Selenium (Se) is an essential micronutrient whose dietary supply varies widely because Se concentrations in plant foods depend on geography, substrate chemistry, crop species, and production practices. Agronomic biofortification can raise Se concentrations in edible tissues, but open-field outcomes are often inconsistent because fertilizer fate and plant uptake are governed by soil properties and weather. Controlled-environment agriculture (CEA) allows Se form, concentration, exposure duration, nutrient interactions, and aerial conditions to be regulated more precisely. This review examines the physiological basis of Se uptake and assimilation, the suitability of CEA platforms for Se delivery, the influence of nutrient-solution and environmental factors, effects on yield and phytochemical quality, food-safety considerations, and priorities for commercial deployment. In hydroponic studies, selenate supplied at roughly 2–16 μM typically raised leaf Se by one to two orders of magnitude with little or no yield penalty, whereas higher concentrations reduced biomass in some cultivars. Responses differ among species, cultivars, Se forms, and experimental systems, so universal application rates cannot yet be recommended. We propose that CEA biofortification be designed around a serving-based target window, and that future work prioritize harmonized fresh- and dry-weight reporting, Se speciation, solution mass balance, batch variability, economic evaluation, and commercial-scale validation.

Keywords: agronomic biofortification; hydroponics; leafy vegetables; nutrient solution; selenium speciation; vertical farming; food safety

1. Introduction

Selenium is required for the synthesis and function of human selenoproteins, including the glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases, which participate in redox regulation, thyroid-hormone metabolism, and immune function [1–3]. Both inadequate and excessive intake are undesirable, and the margin between nutritional adequacy and chronic excess is

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narrower than for most other mineral nutrients. In the United States, the adult Recommended Dietary Allowance (RDA) is 55 μg Se day^{-1} and the Tolerable Upper Intake Level (UL) is 400 μg day^{-1} (Institute of Medicine, 2000). In Europe, the adequate intake for adults is 70 μg day^{-1} [4] , and the UL was revised downward in 2023 to 255 μg day^{-1} [5]. The ratio between recommended intake and upper limit is therefore only about 4 to 7, which means that biofortification outcomes are most useful when expressed not only as tissue Se concentration, but also as the amount of Se supplied by a realistic serving of the edible crop.

The Se content of plant-derived foods varies widely because plant uptake depends on Se availability in the root environment. Populations relying heavily on crops produced on low-Se soils can have low dietary intakes, as documented in parts of southern Africa and on the Tibetan Plateau [6, 7] , and people following predominantly plant-based diets may also be at greater risk of low intake [8] . Nevertheless, individual status is shaped by food choice, processing, and the contribution of animal products. Biofortification seeks to increase both the amount and, ideally, the nutritionally useful chemical forms of Se in foods before consumption [9, 10] . It can be achieved through agronomic practices or genetic modification. Agronomic biofortification uses fertilizers or nutrient solutions, whereas genetic biofortification relies on selection or breeding for greater uptake, translocation, or assimilation. Food-based Se biofortification can improve human status: in a randomized controlled trial in Malawi, consumption of maize grown with Se fertilizer increased Se status in women and children [11].

Leafy vegetables are attractive vehicles for agronomic biofortification because they grow rapidly, are commonly consumed fresh, and accumulate Se in edible shoot tissue [12, 13]. Their short production cycle also permits stage-specific dosing. High accumulation, however, is not automatically desirable. The production target should be a reproducible concentration that provides a useful dietary contribution without compromising yield, sensory quality, or consumer safety.

Several reviews have addressed Se biofortification in field crops, but fewer have examined how the specific capabilities and constraints of CEA alter the problem. This review therefore asks a focused question: how can controlled-environment systems be used to deliver predictable, safe, and nutritionally meaningful Se enrichment of leafy vegetables? To answer it, we (i) summarize the physiological basis of Se uptake and assimilation; (ii) compare CEA platforms and delivery routes; (iii) synthesize quantitative evidence on enrichment, yield, and quality from representative CEA studies; (iv) translate published tissue concentrations into serving-based dietary exposure; and (v) identify the research and quality-assurance steps required before commercial deployment.

2. Materials and Methods

This article is a narrative review of Se biofortification in controlled-environment crop production. Literature searches were conducted between January and June 2026 in the Web of Science Core Collection and Google Scholar. Search terms were used alone and in combination and included “selenium biofortification,” “selenate,” “selenite,” “hydroponic selenium,” “controlled-environment agriculture,” “vertical farming,” “leafy vegetables,” “lettuce,” “spinach,” “kale,” “microgreens,” “nutrient solution,” “selenium speciation,” and “phytonutrients.” Reference lists of relevant reviews and primary studies were also examined to identify additional publications. Dietary reference values were obtained directly from national and European health agencies, and one industry source was consulted for current CEA construction costs.

Although the databases, search period, and search terms are reported here for transparency, the search was exploratory rather than systematic. No pre-registered protocol, PRISMA screening workflow, formal duplicate-removal procedure, or quantitative risk-of-bias assessment was applied, and no restriction was placed on publication year. Publications were selected by the authors on the basis of their relevance to the review objectives, with preference given to peer-reviewed primary research and reviews that addressed Se uptake, application methods, plant responses, CEA production factors, nutritional quality, or safety. Studies on field crops and non-leafy species were retained only where they clarified mechanisms or delivery strategies that are not yet documented for

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CEA leafy vegetables, and this is indicated in the text. Studies published after June 2026 are not included.

The evidence base is heterogeneous in crop species, cultivar, production system, Se source, treatment concentration, exposure duration, analytical method, and the basis on which tissue Se is reported. These differences limit direct comparison. Throughout, this review distinguishes nutrient-solution concentrations (μM) from tissue concentrations, and fresh-weight (FW) from dry-weight (DW) values.

3. Controlled-Environment Agriculture as a Biofortification Platform

Controlled-environment agriculture includes production systems in which one or more aspects of the root-zone and aerial environment are actively managed. Relevant systems include the nutrient-film technique (NFT), deep-water or floating culture, drip-fertigated substrates, aeroponics, greenhouses, growth chambers, and multilayer indoor farms [14, 15]. These systems differ in capital intensity and environmental control, but they share the capacity to regulate nutrient delivery more closely than conventional field production [16, 17]. For Se biofortification, the principal advantage of CEA is control of exposure. The grower can select the chemical form of Se, introduce it at a defined concentration, vary the treatment by growth stage, monitor solution pH and electrical conductivity (EC), and terminate exposure before harvest. By contrast, in soil, a large share of applied Se can remain in the soil or be immobilized, and recovery by the crop varies with soil type, organic matter, and microbial activity [18, 19]. Recirculating systems can improve resource efficiency, but they may also allow Se to accumulate if solution replacement, plant uptake, and water loss are not accounted for. Closed-loop production therefore requires both agronomic precision and environmental safeguards, as CEA does not eliminate biological variability. Crop genotype, growth rate, transpiration, root-zone oxygenation, competing ions, and microbial activity can still alter uptake. In addition, indoor systems generally have higher energy and infrastructure costs than field production. The strongest justification for Se biofortification in CEA is therefore not that CEA is universally more sustainable, but that it can support reproducible dosing, rapid treatment adjustment, and batch-level quality control for high-value vegetables.

3.1. Relevant CEA Systems

Hydroponic systems provide the most direct route for Se delivery because the root system is exposed to a nutrient solution of known composition [20]. NFT and deep-water or floating culture are especially relevant for lettuce, spinach, rocket, endive, basil, and other leafy crops, and most quantitative CEA evidence comes from these two configurations (Table 1). Drip-fertigated substrate systems provide a buffered root environment, but sorption and retention in the substrate can make the effective Se dose less predictable. Aeroponics provides highly oxygenated roots and precise mist delivery and has been used for nutrient biofortification of lettuce [21], although published evidence for Se biofortification in aeroponics remains limited. Vertical farms add fine control of light, temperature, and humidity [22], but the associated energy and monitoring requirements must be included in any sustainability or economic assessment.

3.2. Representative Evidence from CEA Leafy-Vegetable Studies

Table 1 summarizes representative studies in which leafy vegetables or culinary herbs were biofortified under controlled or soilless conditions. Four patterns emerge. First, leaf Se increased steeply with solution concentration in every study, often by one to two orders of magnitude relative to controls. Second, selenate supplied at roughly 2–16 μM produced substantial enrichment with neutral or even positive effects on yield, whereas yield penalties appeared at higher concentrations in some cultivars [23, 24]. Third, accumulation differed several-fold among species grown under identical conditions [13, 25]. Fourth, reporting practice varied, as some studies reported only DW concentrations, others FW, and few reported Se speciation or solution depletion over time.

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Table 1. Representative studies of Se biofortification of leafy vegetables and herbs in controlled or soilless systems.

Study	Crop (cultivar)	System and duration	Se form, route and solution concentration	Leaf Se (basis as reported)	Growth and yield response	Quality and other outcomes
[23]	Lettuce (cv. Justyna)	Hydroponic nutrient solution	Selenite 2–30 µM and selenate 2–60 µM (toxicity screening); 2–15 µM for biofortification; root-zone	Shoot Se higher with selenate; translocation factor 0.69–2.47 for selenate vs < 0.15 for selenite	Shoot FW reduced from 15 µM selenite and 20 µM selenate; while root and/or shoot FW increased at 6 µM selenate and 2–10 µM selenite	15 µM selenite increased lipid peroxidation and shoot S more than doubled at 15 µM selenate
[24]	Butterhead lettuce (green and red Salanova®)	Closed NFT with continuous recirculation	Selenate 0–40 µM; root-zone	Up to approx. 128 mg kg^{-1} DW; red cultivar accumulated 57% more than green	Green: approx. 9% lower fresh yield at all doses; red: 11% and 21% lower only at 32 and 40 µM	At 16 µM, phenolic acids increased and anthocyanins rose 184% in red lettuce; nitrate below EU limits
[25]	Microgreens (coriander, green basil, purple basil, tatsoi)	Soilless capillary-mat trays	Selenate 0, 8, 16 µM; root-zone	26–150 µg g^{-1} DW at 16 µM, depending on species	Yield increased 44% (coriander) and 18% (tatsoi) at 16 µM; basil unaffected	Total phenols +21% (coriander) and +95% (tatsoi); nitrate reduced by approx. 24% on average
[26]	Curly endive	Open floating system	Selenate 0–8 µM; root-zone vs foliar	0.71–17.61 mg kg^{-1} DW (root-zone); 0.72–12.67 mg kg^{-1} DW (foliar)	Head weight +42.6% (4 µM, root-zone) and +27.8% (8 µM, foliar)	Ascorbic acid approx. +33%; total phenolics +55–59%; leaf N reduced at the highest doses
[27]	Sweet basil	Hydroponic, greenhouse; 28 days	Selenate 2, 5, 10 µM; root-zone vs foliar	Up to 20.23 µg g^{-1} DW (root-zone) and 10.74 µg g^{-1} DW (foliar)	No significant change in yield or leaf biomass	Essential oil, hydroxycinnamic acids, total phenolics and antioxidant activity increased at 2 µM (root-zone) and 5 µM (foliar); flavonoids unaffected
[13]	Baby leaf: lamb's lettuce, lettuce, wild rocket, spinach	Floating system; approx. 5 weeks to baby-leaf harvest	Selenate 0, 2.6, 3.9, 5.2 µM; root-zone	Linear increase with dose; wild rocket up to 11 µg g^{-1} FW (approx. 56 µg g^{-1} DW)	Yield unaffected; no phytotoxic symptoms	Nitrate and sugars unaffected; accumulation order: rocket to spinach to lettuce to lamb's lettuce

Note: Values are taken directly from the cited studies. DW, dry weight; FW, fresh weight; NFT, nutrient-film technique.

4. Selenium Uptake, Transport, and Assimilation in Plants

Selenium is not generally considered essential for higher plants, but at low concentrations it can influence antioxidant metabolism and stress responses [28–30]. Plant uptake occurs largely because inorganic Se species resemble essential sulfur- or phosphorus-containing ions. Selenate is chemically analogous to sulfate and is taken up through sulfate transporters. It is relatively mobile in the xylem and is efficiently transported from roots to shoots. Selenite is generally less mobile, is rapidly transformed in roots, and its uptake may involve phosphate transporters and other pathways depending on species and root-zone conditions [28, 31, 32]. These differences are clearly visible in hydroponic lettuce, where the shoot-to-root Se ratio ranged from 0.69 to 2.47 under selenate but was below 0.15 under selenite [23].

After uptake, Se enters the sulfur-assimilation pathway and forms organic compounds such as selenocysteine and selenomethionine. These compounds are nutritionally important because organic Se forms are generally better retained in human metabolism than inorganic forms [9]. However, nonspecific replacement of sulfur amino acids by their Se analogues can disrupt protein function at excessive exposure. Plants may also convert Se into methylated or volatile forms, such as methylselenocysteine and dimethylselenide, which can reduce tissue accumulation [31]. Selenate also

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interacts directly with sulfur metabolism: in hydroponic lettuce, 15 μM selenate more than doubled shoot S concentration, whereas selenite tended to reduce it [23].

The relative proportions of inorganic and organic Se in edible tissue depend on Se source, concentration, species, tissue type, exposure duration, and analytical method. Total tissue Se alone therefore provides an incomplete measure of biofortification quality. A crop with moderate total Se but a high proportion of nutritionally desirable organic forms may be preferable to a crop with higher total Se dominated by residual selenate or selenite. Speciation studies in cereals and legumes show that the organic fraction varies widely with Se source and application route [33–35]. In contrast, none of the representative CEA leafy-vegetable studies in Table 1 reported Se speciation. This is a substantial gap, because selenate-fed crops can retain part of the absorbed Se as unassimilated selenate [31], and the nutritional value of a product cannot be inferred from total Se alone.

5. Production Factors Controlling Selenium Biofortification

5.1. Selenium Form, Concentration, and Exposure Duration

Selenate and selenite are the most widely studied inorganic Se sources. Selenate is usually translocated more readily to shoots, whereas selenite is more strongly retained or assimilated in roots [23]. The most effective form therefore depends on whether the production objective is high shoot accumulation, a greater organic-Se fraction, minimal growth inhibition, or a combination of these. Organic Se compounds, chelated formulations, and nano-Se are also being investigated [36–38], but their cost, stability, behavior in recirculating solutions, and regulatory acceptance require further evaluation.

The plant response to Se follows a dose-dependent pattern. Low concentrations can produce substantial enrichment with little effect on biomass, whereas higher concentrations inhibit growth, cause chlorosis, alter root development, and disturb nutrient metabolism. In hydroponic lettuce, shoot fresh mass declined significantly from 15 μM selenite and 20 μM selenate [23]; in NFT-grown red lettuce, yield declined only at 32 and 40 μM selenate [24]; and four baby-leaf species showed no phytotoxicity at 5.2 μM selenate [13]. These thresholds are not directly comparable, because cultivar, exposure duration, and solution management differed among studies. Exposure duration is as important as nominal concentration because tissue accumulation reflects both uptake rate and time. Late-cycle or short-duration treatments may enrich edible tissue while limiting prolonged phytotoxic exposure, but the effectiveness of this approach varies by crop and Se form and has rarely been tested directly.

5.2. Nutrient-Solution Composition, pH, and Competing Ions

Nutrient-solution chemistry can substantially alter Se availability and uptake. Because selenate and sulfate share chemical and transport similarities, high sulfate availability can reduce selenate uptake or change its assimilation [28, 31]. Selenite interactions with phosphate are also important, although the magnitude and direction of competition depend on concentration and species. Nitrogen form and supply may influence amino-acid synthesis and the incorporation of Se into organic compounds. Consequently, Se treatments should be interpreted in the context of the complete nutrient formulation rather than as isolated additions, and studies should report the sulfate and phosphate concentrations of the base solution together with the Se treatment.

Solution pH can affect Se speciation, root-surface reactions, nutrient availability, and overall root function. EC influences osmotic conditions and may modify transpiration and nutrient uptake. In recirculating systems, evaporation and selective plant uptake change the concentration of Se and competing ions over time. Few of the CEA studies in Table 1 reported how Se concentration in the solution changed during the cycle. Studies that report only the initial treatment concentration provide less useful guidance than those that monitor the solution throughout production, and the sulfate-to-selenate molar ratio is likely to be a more transferable variable than the selenate concentration alone.

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5.3. Light, Temperature, Humidity, and Transpiration

Light spectrum, intensity, and photoperiod influence biomass production, sulfur metabolism, antioxidant pathways, and the dilution or concentration of Se in shoot tissue. Red and blue light combinations are widely used in indoor production, but evidence that a particular spectrum consistently increases Se uptake in leafy vegetables remains limited. Light treatments may change tissue Se indirectly by altering growth rate, transpiration, root activity, or the expression of nutrient-transport and assimilation genes. Studies that manipulate light and Se together should therefore report both tissue concentration and total Se per plant, so that changes in uptake can be distinguished from changes in biomass dilution.

Temperature and atmospheric humidity influence enzymatic activity and transpiration. High relative humidity can reduce transpirational flow, whereas low humidity may increase water and nutrient movement but also impose stress. Vapor-pressure deficit (VPD) is therefore a more informative variable than relative humidity alone. The importance of interacting environmental factors is illustrated by vertical-farming optimization studies on lettuce and basil [22], although such studies have not yet included Se. Studies evaluating environmental effects on Se biofortification should report air temperature, root-zone temperature, humidity or VPD, light regime, and airflow.

5.4. Species, Cultivar, and Harvest Stage

Leafy vegetables differ markedly in Se accumulation because of variation in root architecture, transporter activity, sulfur demand, growth rate, and capacity to assimilate or volatilize Se. Under identical floating-system conditions, accumulation decreased in the order wild rocket to spinach to lettuce and lamb's lettuce [13], and among microgreens grown at 16 μM selenate, leaf Se ranged from approximately 26 μg g^{-1} DW in coriander to 150 μg g^{-1} DW in green basil [25]. Differences are also large within species: a red lettuce cultivar accumulated 57% more Se than a green cultivar under the same NFT treatment [24], and leaf Se in one *Brassica juncea* variety was almost twice that of three others [39]. Comparable genotypic variation has been reported in wheat [40] This variability creates opportunities for selecting cultivars that combine high edible-tissue enrichment with stable yield, but it also limits the transferability of application rates between genotypes. The harvest stage affects both biomass and tissue concentration. Younger tissues may show high concentrations but low total yield, while rapid later growth can dilute accumulated Se. Treatment timing should therefore be designed around the intended market stage and serving size. For microgreens and baby leaves, short production cycles permit precise exposure, but small serving sizes and very high tissue concentrations require careful dose calculation.

6. Effects on Crop Performance and Nutritional Quality

6.1. Biomass, Yield, and Physiological Performance

The effect of Se on crop growth is not uniformly positive. Low doses may have neutral or stimulatory effects, particularly when plants experience oxidative or abiotic stress [29, 30]. In CEA leafy vegetables, increases in yield or head weight of 18–44% have been reported at moderate selenate doses [25, 26], while other studies found no yield change [13, 27] or small reductions [24]. At higher exposure, Se interferes with sulfur metabolism, generates malformed Se-containing proteins, disturbs nutrient balance, increases lipid peroxidation, and reduces photosynthetic performance [23, 28]. A credible biofortification study should therefore report marketable fresh mass, dry mass, root and shoot responses, visible injury, and, where feasible, physiological indicators alongside tissue Se concentration. The apparent increases in tissue concentration should be carefully interpreted, especially when biomass declines. Growth inhibition can produce a concentration effect without a proportional increase in total Se accumulated per plant. Conversely, biomass gains can dilute Se, as shown for wheat in soil [19]. Reporting both concentration and total Se content per plant helps distinguish enhanced uptake from biomass dilution or concentration.

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6.2. Phenolics, Flavonoids, Carotenoids, Vitamins, and Antioxidant Activity

Selenium treatment has been associated with changes in phenolic compounds, flavonoids, anthocyanins, ascorbic acid, and antioxidant-assay values in several leafy crops and herbs. In NFT-grown red lettuce, 16 μM selenate increased phenolic acids and raised anthocyanin content by 184% [24]. Total phenols increased by 21% in coriander and 95% in tatsoi microgreens [25], and ascorbic acid and total phenolics increased by approximately one-third and more than one-half, respectively, in Se-treated curly endive [26]. In basil, low root-zone or foliar doses increased essential oil, hydroxycinnamic acids, total phenolics, and antioxidant activity, but flavonoid content did not change [27]. In field-grown Indian mustard, foliar Se and iodine each roughly doubled leaf flavonoid content [41]. These responses are frequently attributed to mild Se-induced oxidative signaling and activation of antioxidant or phenylpropanoid pathways. However, the direction and magnitude of response vary with dose, crop, tissue, environment, and assay method, and not every compound class responds; for example, Se did not alter flavonoid content in basil [27]. The evidence does not support the broad conclusion that Se consistently increases every class of phytonutrient. The antioxidant capacity measured in plant extracts should not be presented as direct evidence of improved human health. Chemical assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP), and Oxygen Radical Absorbance Capacity (ORAC) estimate different aspects of reducing capacity and are not interchangeable because they rely on distinct chemical mechanisms, react with different target radicals, and operate under unique experimental conditions. Stronger nutritional interpretation requires identification of individual compounds, assessment of bioaccessibility or bioavailability, and consideration of the amount consumed in a realistic serving.

6.3. Interactions with Nitrate and Other Mineral Nutrients

Selenium can interact with nitrogen, sulfur, phosphorus, zinc, iron, and other mineral nutrients. Several studies report lower nitrate following Se supplementation, including an average reduction of about 24% in microgreens [25] and a fourfold reduction in field-grown Indian mustard [41], whereas nitrate was unaffected in baby-leaf vegetables [13]. Reductions in leaf total N at the highest Se doses have also been reported [26]. These interactions are agronomically important because a biofortified crop should not achieve higher Se at the expense of lower concentrations of other essential nutrients or higher concentrations of antinutritional compounds. Multi-nutrient biofortification, including combinations of Se with iodine, zinc, or iron, may address several dietary deficiencies simultaneously. However, combined treatments require factorial experiments because nutrients interact during uptake, transport, and metabolism. In Indian mustard, separate foliar application of Se or iodine stimulated growth, whereas their joint application did not, and the joint treatment reduced nitrate up to 27-fold [41]. Transcriptomic work in mung bean likewise shows that Se and zinc share overlapping effects on essential-nutrient metabolism [42]. Equivalent factorial evidence for hydroponic leafy vegetables is scarce, and results from single-nutrient trials cannot be assumed to apply when several nutrients are added together.

7. Selenium Delivery Strategies in CEA

7.1. Root-Zone Delivery

Adding Se directly to the nutrient solution is the most controllable delivery method in hydroponic and aeroponic systems. It permits defined root exposure and can be integrated into automated dosing. The method is especially useful for comparing Se forms and concentrations, but it also requires accurate solution preparation, periodic monitoring, and responsible management of residual solution. Root-zone selenate generally produces higher leaf Se than foliar application at the same nominal concentration [26, 27], whereas root-zone selenite is largely retained in roots [23].

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7.2. Foliar Application

Foliar application delivers Se directly to the leaf surface and may rapidly increase shoot Se while reducing the amount entering a recirculating root solution. In curly endive and basil, foliar selenate achieved substantial enrichment and comparable quality benefits, although at lower tissue concentrations than root-zone delivery [26, 27]. Uptake depends on droplet retention, surfactant use, leaf age, cuticle properties, temperature, humidity, and the interval between treatment and harvest. Isotope-labelling and speciation studies in field crops show that foliar Se is redistributed within the plant over time and can be converted into organic forms [35, 43, 44]. For leafy vegetables, which are harvested shortly after treatment and consumed whole, foliar treatments must also be evaluated for residue uniformity and for the proportion of surface-deposited inorganic Se that remains at harvest.

7.3. Seed Treatment, Biostimulants, and Emerging Formulations

Seed priming, microbial inoculants, organic Se sources, and nano-Se formulations have been proposed as alternative delivery strategies [36, 38, 45, 46]. These approaches may alter uptake efficiency or stress responses, but evidence in commercial CEA leafy-vegetable production remains limited. Nano-Se therefore cannot be described as inherently safer or more sustainable without data on particle characterization, transformation, accumulation, worker exposure, food safety, and disposal.

Table 2. Comparison of Se delivery routes for leafy vegetables in CEA.

Delivery route	Dose precision	Main uptake and transport behavior	Organic-Se formation	Principal risks	Analytical needs	Readiness for commercial CEA
Root-zone selenate	High; dose set by solution concentration and duration	Sulfate transporters; efficient root-to-shoot translocation	Partial; residual selenate can remain in leaves	Over-accumulation; Se build-up in recirculating solution; sulfate competition	Solution Se and sulfate; tissue Se (FW and DW); speciation	High; most CEA evidence
Root-zone selenite	High	Phosphate and other transporters; strong root retention	Rapid in roots; less shoot enrichment	Root toxicity at lower concentrations than selenate	As above, plus root Se	Moderate; less efficient for shoot enrichment
Foliar spray	Moderate; depends on coverage and retention	Leaf cuticle and stomata; limited phloem redistribution in short cycles	Variable; time-dependent	Residue non-uniformity; short pre-harvest interval	Tissue Se after washing; residue checks	Moderate; useful when solution must remain Se-free
Seed priming	Low to moderate	Early seedling uptake	Uncertain	Limited enrichment of mature leaves	Seed and tissue Se	Low; most relevant to microgreens and sprouts
Organic or chelated Se	Moderate	Form-dependent; amino-acid uptake possible	Pre-formed organic Se	Cost; stability in solution; microbial degradation	Speciation of product and tissue	Low; limited CEA data
Nano-Se	Uncertain	Particle size and coating dependent; biotransformation in plant	Reported in some crops	Particle fate, worker exposure, regulatory status	Particle characterization; speciation	Low; experimental

8. Food Safety and Definition of a Target Biofortification Window

Selenium biofortification must be designed around a target window rather than maximum accumulation (Figure 1). The lower boundary is the concentration required to make a meaningful dietary contribution. The upper boundary is set by crop tolerance, variability among plants and batches, serving size, background dietary intake, and the risk of chronic excessive consumption. Because leafy vegetables are consumed in variable amounts and may be marketed to health-conscious consumers who also use supplements, serving-based exposure calculations are essential.

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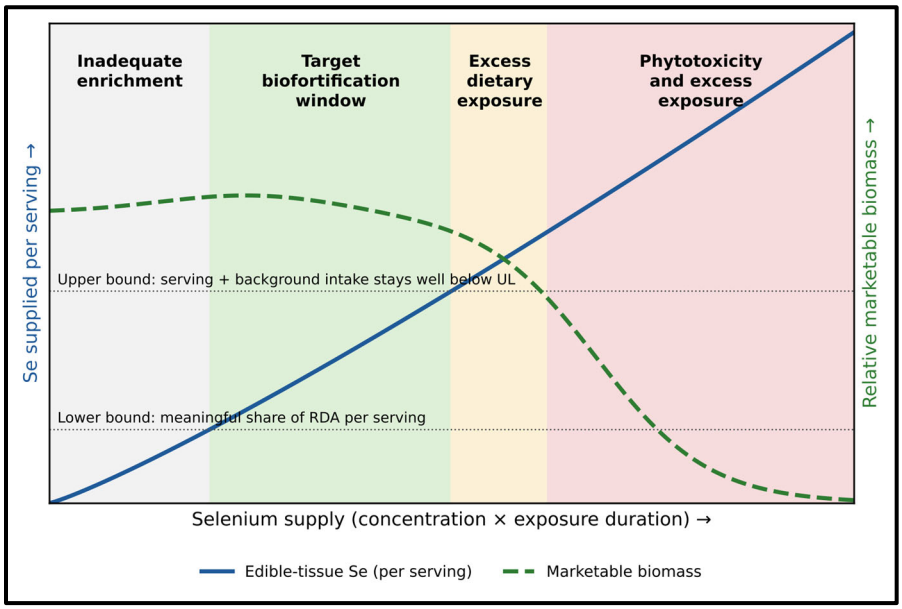


Figure 1. Conceptual biofortification window for Se-enriched leafy vegetables. Edible-tissue Se rises with Se supply (concentration by exposure duration), while marketable biomass is maintained or slightly increased at low supply and declines beyond a crop-specific phytotoxicity threshold. Because the dietary upper bound can be reached before visible phytotoxicity, a crop that grows normally is not necessarily a safe product.

Dietary reference values differ among jurisdictions and population groups. The U.S. adult RDA is 55 µg day^{-1}, rising to 60 µg in pregnancy and 70 µg during lactation, and the UL is 400 µg day^{-1} [47, 48]. The European Food Safety Authority set an adult adequate intake of 70 µg day^{-1} [4] and a UL of 255 µg day^{-1}, based on alopecia as an early sign of selenosis [5]. A product-development study should therefore identify its intended consumers, jurisdiction, and portion size, and then calculate expected intake from the measured FW concentration.

Table 3 applies this approach to published CEA treatments. The results are striking. At the doses highlighted in these studies, a single serving supplied between about 90% and 1,000% of the adult RDA. One serving of red lettuce grown at 16 µM selenate would supply 42% of the EFSA UL, and a 50 g serving of wild rocket grown at 5.2 µM would exceed both the U.S. and EFSA ULs, although the plants showed no phytotoxic symptoms [13]. Plant-focused definitions of an "optimal" dose, based on maximum accumulation without yield loss, therefore tend to overshoot a nutritionally appropriate target. For many CEA leafy crops, lower solution concentrations or shorter exposure than those commonly tested are likely to be sufficient.

Table 3. Serving-based Se exposure calculated from published CEA biofortification studies.

Study and treatment	Leaf Se (FW)	Serving	Se per serving (µg)	% of U.S. RDA (55 µg)	% of U.S. UL (400 µg)	% of EFSA UL (255 µg)
Green butterhead lettuce, NFT, 16 µM selenate [24]	1.0 µg g^{-1} [a]	50 g	50	91	13	20
Red butterhead lettuce, NFT, 16 µM selenate [24]	2.1 µg g^{-1} [a]	50 g	106	193	27	42
Green basil microgreens, 8 µM selenate [25]	approx. 13.8 µg g^{-1} [b]	10 g	~ 138	250	34	54
Curly endive, foliar 8 µM selenate [26]	approx. 1.2 µg g^{-1} [c]	100 g	~ 116	211	29	45
Wild rocket baby leaf, floating, 5.2 µM selenate [13]	11 µg g^{-1}	50 g	550	1,000	138	216
Proposed target (this review)	0.3–0.6 µg g^{-1} (30–60 µg per 100 g)	50 g	15–30	27–55	4–8	6–12

Notes: [a] Derived from the Se supply per 50 g FW serving reported by the authors. [b] Derived from the authors' statement that 4 g FW meets the RDA. [c] Derived from the authors' statement that 47.4 g

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FW day^{-1} satisfies requirements, assuming a 55 µg reference. Values are single-serving contributions and exclude background dietary intake. Percentages are rounded.

On this basis, we propose a provisional product target of 15–30 µg Se per labelled serving, equivalent to 30–60 µg per 100 g FW for a 50 g salad portion. This range supplies roughly one-quarter to one-half of the adult RDA, keeps a single serving below about 12% of the EFSA UL, and exceeds the per-100 g threshold for a "high in selenium" nutrition claim in the European Union, which is 30% of the 55 µg nutrient reference value, or 16.5 µg per 100 g [49, 50]. The target should be adjusted for products with very different portion sizes, such as microgreens and herbs, and for markets with different reference values. It is intended as a design objective for research and product development rather than a regulatory limit. Tissue concentration must be reported with an unambiguous mass basis. Confusion between micrograms per kilogram FW, milligrams per kilogram DW, and nutrient-solution concentrations can lead to errors of one order of magnitude or more, because leafy vegetables typically contain 90–95% water. Moisture content should be reported whenever values are converted between FW and DW. Batch variability should also be quantified, because a safe mean concentration may conceal excessively enriched individual plants or lots.

Plant toxicity thresholds depend on species, Se form, exposure duration, and on whether the concentration refers to the nutrient solution or to plant tissue. A universal tissue threshold for phytotoxicity in leafy vegetables is therefore not scientifically defensible. For example, wild rocket tolerated leaf concentrations of about 56 µg g^{-1} DW without symptoms [13], while lettuce cultivars showed yield reductions at different solution concentrations [23, 24]. Toxicity thresholds should instead be determined for each crop and system and expressed in terms of both solution concentration and exposure duration. Other contaminants, such as pesticide and heavy-metal residues, should be monitored within the same quality-assurance program, as is already done when hydroponic and conventionally grown vegetables are compared [51].

9. Commercial Feasibility, Quality Assurance, and Sustainability

Commercial adoption requires more than proof that plants can accumulate Se. Growers need reproducible recipes, compatible fertilizers, monitoring procedures, harvest specifications, and a market willing to pay for a nutritionally differentiated product. Quality assurance should include verification of fertilizer concentration, nutrient-solution records, crop identity, tissue sampling, analytical method, lot variability, and serving-based labelling.

Direct real-time measurement of Se in a production nutrient solution is more difficult than monitoring pH or EC. EC reflects total dissolved ions and cannot determine Se concentration specifically. Where online Se sensors are unavailable or insufficiently validated, routine operation may require mass-balance calculations supported by periodic laboratory analysis using appropriate elemental methods, such as inductively coupled plasma mass spectrometry (ICP-MS) or hydride-generation atomic absorption or fluorescence spectrometry. Imaging and plant-health sensors may detect stress responses, but they should not be assumed to quantify edible-tissue Se without crop- and system-specific calibration.

The environmental performance of CEA depends on system design and energy source. Recirculating production can reduce water and nutrient discharge, but residual Se solutions require controlled reuse, treatment, or disposal. Indoor farms may reduce land requirements and enable year-round local production, while increasing electricity demand. Sustainability claims should therefore be supported by system-level water, nutrient, energy, and waste accounting rather than by CEA status alone. Consumer perceptions of CEA, including how its sustainability is framed, are mixed [52, 53].

Economic assessment should take a partial-budget approach. Added costs include Se fertilizer, dosing and storage equipment, laboratory analysis, worker training, discarded or non-compliant batches, regulatory compliance, packaging, and consumer communication. Because Se salts are used at micromolar concentrations, fertilizer cost is likely to be small relative to analytical and compliance costs. These costs should be evaluated against the potential price premium and market size.

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Consumer willingness to purchase Se- and iodine-biofortified produce has been explored in discrete choice experiments [54], but evidence for commercial profitability of Se-biofortified CEA vegetables is currently much weaker than evidence for physiological feasibility.

10. Emerging Technologies and Research Priorities

10.1. Sensors, Automation, and Decision Support

CEA infrastructure can support automated dosing, environmental logging, imaging, and decision support [55–57]. A practical control system could combine reservoir volume, nutrient additions, plant water use, solution replacement, and periodic tissue analysis to estimate Se mass balance (Figure 2). Machine-learning models may eventually predict crop Se status from environmental and management data, but robust models will require diverse calibration datasets and independent validation across cultivars and production systems.

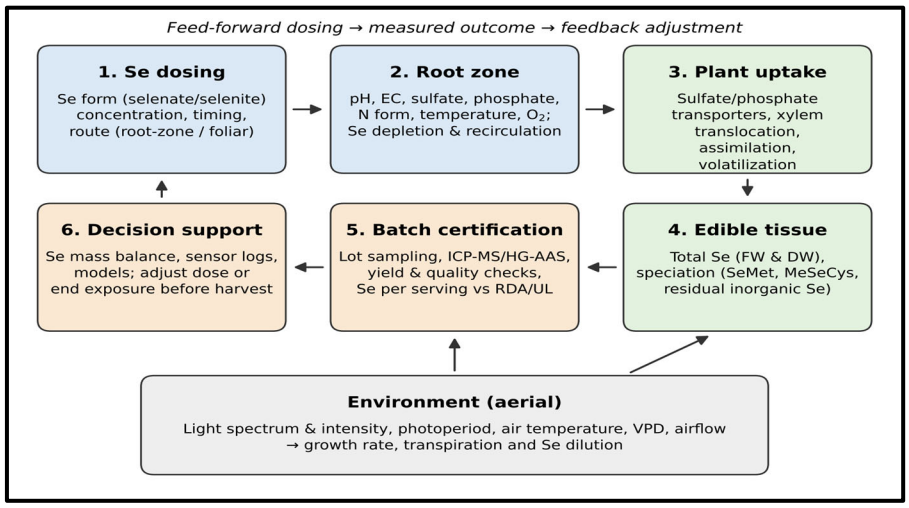


Figure 2. Closed-loop framework for precision Se biofortification in CEA. Dosing decisions (1) act through root-zone chemistry (2) and plant uptake (3) to determine edible-tissue Se and speciation (4). Batch certification (5) feeds a decision-support layer (6) that adjusts dose or ends exposure before harvest. The aerial environment modulates growth rate, transpiration, and Se dilution throughout. EC, electrical conductivity; HG-AAS, hydride-generation atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; MeSeCys, methylselenocysteine; SeMet, selenomethionine; VPD, vapor-pressure deficit.

Multispectral or hyperspectral imaging may detect physiological responses to excessive Se, particularly when toxicity affects pigments or canopy structure [58]. However, spectral responses are not specific to Se and can be confounded by nitrogen status, water stress, disease, and lighting. Imaging should therefore be treated as a screening tool unless validated against direct chemical analysis.

10.2. Genetics and Crop Improvement

Genetic variation in uptake, shoot translocation, Se tolerance, and organic-Se formation could be used to select cultivars suited to biofortification [24, 40, 59]. Gene editing may help clarify transporter and assimilation pathways [60], but commercial deployment will depend on trait stability, food safety, regulation, consumer acceptance, and the avoidance of undesirable nutrient trade-offs. Near-term progress is more likely to come from screening existing germplasm under standardized CEA conditions than from immediate commercialization of edited cultivars.

10.3. Priority Research Questions

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Table 4 summarizes the principal research gaps, the limitations of current evidence, and the study designs needed to close them.

Table 4. Research gaps and deployment readiness for Se-biofortified leafy vegetables in CEA.

Research question	Current evidence	Main limitation	Recommended study design	Commercial relevance
What Se concentration and exposure duration deliver a useful serving without yield loss in each major crop?	Dose-response data for lettuce, endive, basil, rocket, spinach and microgreens (Table 1)	Doses chosen for accumulation, not for serving-based targets; units inconsistent	Dose × duration factorials with FW and DW reporting and serving-based calculation	High: defines production recipes
How do selenate, selenite, organic Se and new formulations differ in organic-Se fraction and residual inorganic Se?	Speciation data mainly from cereals and legumes	Almost no speciation data for CEA leafy vegetables	Speciation (HPLC-ICP-MS) across forms and doses	High: determines nutritional quality and labeling
How do sulfate, phosphate, N form, pH, EC and recirculation modify uptake?	Mechanistic understanding of transporter competition	Few studies report base-solution composition or solution depletion	Factorial sulfate: selenate ratios with solution monitoring	High: enables transferable recipes
Can late-cycle dosing improve enrichment while reducing phytotoxicity and residual losses?	Limited	Rarely tested directly	Timing trials comparing continuous and late-cycle exposure	High: reduces Se use and waste
How stable is tissue Se across plants, batches, seasons and facilities?	Single-site, single-cycle experiments	No data on lot variability	Multi-cycle, multi-site trials with lot-level sampling	High: prerequisite for certification
How do washing, storage, cooking and digestion affect Se retention and bio-accessibility?	Very limited for leafy vegetables	Post-harvest fate unknown	In vitro digestion and storage trials; human feeding studies	Medium to high: supports health claims
What analytical frequency is needed for reliable batch certification?	None specific to CEA	No sampling plans or acceptance limits	Statistical sampling designs based on observed variability	High: determines QA cost
What are the energy, water, nutrient, waste and economic consequences of commercial production?	General CEA analyses; consumer willingness-to-pay studies	No Se-specific partial budgets or life-cycle assessments	Partial-budget analysis and life-cycle assessment at pilot or commercial scale	High: determines adoption

11. Conclusions

Controlled-environment agriculture offers a technically promising platform for Se biofortification because it allows greater control over Se form, root-zone concentration, exposure duration, nutrient interactions, and harvest timing. This control can improve the reproducibility of enrichment compared with systems in which Se availability is strongly influenced by heterogeneous soil conditions and weather.

The existing evidence does not, however, support a single application rate or universal production method. Crop species and cultivar, Se form, nutrient composition, environmental conditions, and treatment duration jointly determine tissue accumulation, organic-Se formation, yield, and quality. When published CEA results are expressed per serving, many treatments described as optimal supply well above the adult recommended intake, and some exceed tolerable upper limits without any visible effect on the crop. The central production challenge is therefore to maintain a narrow biofortification window, which we provisionally define as 15–30 μg Se per serving, that delivers a meaningful amount of Se without phytotoxicity, nutritional imbalance, or excessive dietary exposure.

Future studies should move beyond demonstrating increased total Se. They should report FW and DW concentrations, Se speciation, marketable yield, serving-based intake, solution mass balance, batch variability, and treatment economics. Commercial-scale validation and clear quality-assurance protocols are also needed. With these safeguards, CEA could support the development of standardized Se-enriched leafy vegetables as functional foods.

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Abbreviations

The following abbreviations are used in this manuscript:

CEA	Controlled Environment Agriculture
RDA	Recommended Dietary Allowance
NFT	Nutrient-Film Technique

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