Research Article

Received: 9 January 2023 Revised: 5 April 2023 Accepted article published: 12 April 2023 Published online in Wiley Online Library: 29 April 2023

(wileyonlinelibrary.com) DOI 10.1002/jsfa.12622

Biofortification of baby leafy vegetables using nutrient solution containing selenium

Alessandra Francini,[a] Emanuele Quattrini,[b] Francesco Giuffrida[c] and Antonio Ferrante[b*]

Abstract

BACKGROUND: Biofortification of vegetables is an important innovation technique in the horticultural sector. Vegetables can be a vector of different minor elements that have beneficial effects on human health. Selenium (Se) is an important element for human nutrition and plays a significant role in defence mechanisms. The aim of this work was to investigate the effect of Se in the nutrient solutions on the crop biofortification ability, yield, and quality parameters of four baby leafy vegetables destined to the minimally processed industry. Experiments were performed on lamb's lettuce, lettuce, wild rocket, and spinach. These crops were cultivated in the floating systems with nutrient solution enriched with 0, 2.6, 3.9, and 5.2 μmol L^{-1} Se provided as sodium selenate.

RESULTS: At harvest, Se concentrations, yield, nitrate concentration, sugars, and some mineral elements were measured. Data collected and analyses showed that yield, nitrate, sucrose, and reducing sugars were not affected by Se treatments, even if varied among species. Se concentrations linearly increased in leaves of different species by increasing the Se concentration in the nutrient solution. Rocket was the species with the highest accumulation ability and reached a concentration of 11 μg g^{-1} fresh weight Se in plants grown with 5.2 μmol L^{-1} Se.

CONCLUSION: A floating system with Se-enriched nutrient solution is an optimal controlled growing biofortification system for leafy vegetables. The accumulation ability decreased in different species in the order wild rocket, spinach, lettuce, and lamb's lettuce, highlighting a crop-dependent behaviour and their attitude to biofortification.

Keywords: fresh-cut; minimally processed; ready to eat; salads; sugars; yield; floating system; functional foods

INTRODUCTION

Leafy vegetables are primary source of antioxidants, vitamins, minerals, fibre, and other nutritional compounds in the human diet.[1] Leafy vegetables can be harvested and commercialized as adult salads or as baby leaves. The main differences between them are the harvesting stage and the nutritional composition. Baby leaves are harvested at the very young stage when they have three to five fully expanded leaves and at 10–15 cm in height. These vegetables are mainly commercialized as ready to eat or fresh-cut vegetables or minimally processed. Baby leaf vegetables are usually grown in greenhouses or tunnels in soil or hydroponic systems such as a floating system.[2] The composition of leafy vegetables, which should be a constant component in a healthy diet,[3] can be influenced by the nutrient availability. Some elements can be increased in the soil or in the nutrient solution to increase their concentration in the edible leaves of vegetables.[4] This procedure is also called biofortification and represents the enrichment of nutrients in food for improving human nutrition.

Selenium (Se) is a mineral involved in several biological functions in plants and animals. Se is also cofactor of important enzymes, such as thioredoxin reductase and glutathione peroxidase, which have a protection role against oxidative stresses. Se is a component of these enzymes as selenocysteine in plants,[5] but there are many others forms of organic Se, such as selenomethionine, selenomethylselenocysteine, selenocystathionine, selenomethylselenomethionine, dimethylselenopropionate, dimethylselenide, and dimethyldiselenide.[6] The main function of Se is as an antioxidant with tissue protection function in plants, humans, and animals.[7,8] The antioxidant ability of Se is particularly important in the protection of the cell membrane and in preventing cancer formation in humans.[9] However, the anticarcinogenic activity and its beneficial

---

* *Correspondence to: A Ferrante, Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, via Celoria 2, 20134, Milano, Italy, E-mail: antonio.ferrante@unimi.it*

a *Crop Science Research Center, Scuola Superiore Sant'Anna Pisa, Pisa, Italy*

b *Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, Milan, Italy*

c *Department of Agriculture, Food and Environment, Catania University, Catania, Italy*

effects on human health are obtained in a very narrow range of Se concentrations. Higher concentrations than the advisable daily intake can be dangerous for consumer health.[10-12] The Se concentration in the edible plant parts depends on its concentration in the soil and crop uptake ability. The Se available in soil depends on its forms, microflora, biological, chemical, and physical characteristics. In agricultural systems with low Se concentrations, it can be directly added to fertilizers.[13,14] However, this strategy must be carefully carried out, because high Se supply can induce accumulation in soil with the risk of excessive crop uptake and subsequent high Se concentration in the edible products.[15]

Se crop biofortification can also be achieved by foliar fertilization, avoiding accumulation in the soil.[16,17] High Se levels in soil are not only dangerous for human health but they can also have a negative effect on crop productivity with or without showing visual toxicity symptoms.[18]

Controlled cultivation, such as soilless systems, and in particular floating systems, can represent easy methods for modulating the Se availability, and thus uptake by the crop. Positive results have been reported for tomato grown with nutrient solution enriched with Se (0–4 μmol L^{-1} selenium dioxide) and administrated through fertigation in a soilless growing system.[19]

A floating system is the simplest hydroponic cultivation system, composed of a tank filled with a nutrient solution and floating panels. Vegetables are sown and grown on the nutrient solution. The addition of Se directly in the nutrient solution in a floating cultivation system could allow the production of enriched leafy vegetables while avoiding excessive concentration in leaves.[20,21] The stagnant nutrient solution is isolated in the cultivation tank, and it can allow a higher control level of Se uptake by modulating the Se concentration in the nutrient solution.

The aim of this work was to study the effect of Se concentration in the nutrient solutions on the crop biofortification ability, yield, and quality parameters of four baby leaf vegetables destined to the fresh-cut industry. The hypothesis of this work was that the application of Se by nutrient solution could increase the Se leaf content in leafy vegetables, which can be carriers of Se in the human diet. The accumulation ability of vegetables, at different Se concentrations in the nutrient solution, and the amount of leafy vegetables necessary to provide the recommended daily allowance (RDA) were investigated.

MATERIALS AND METHODS

Plant materials, growing conditions, and Se treatments

Lamb's lettuce (*Valerianella olitoria* L. [*Valerianella locusta* (L.) Laterr.] 'Trophy'), lettuce (*Lactuca sativa* L. 'Chiara'), wild rocket (*Diplotaxis tenuifolia* L. var. Frastagliata), and spinach (*Spinacia oleracea* L. 'Pungi F1') seedlings were grown under a greenhouse covered with plastic film at the Centre of Advanced Technologies in Greenhouse (CETAS, Tavazzano, Italy) of the University of Milan. Seeds were sown in polystyrene trays (32.5 cm × 51.5 cm) with 228 holes with perlite (seed density was about 4000–5360 m^{-2} based on about three or four seeds per hole). After germination, the trays were transferred in tanks containing 700 L with four trays for each tank.

The Se was directly added to the nutrient solution from the beginning of the cultivation by dissolving the sodium selenate ($\mathrm{Na_2SeO_4}$) salt. Se concentrations in the nutrient solution were 0, 2.6, 3.9, or 5.2 μmol L^{-1}.

The nutrient solution contained 6.5 mmol L^{-1} nitrate-nitrogen, 0.75 mmol L^{-1} phosphorus (P), 4 mmol L^{-1} potassium (K), 1.75 mmol L^{-1} calcium (Ca), 0.85 mmol L^{-1} magnesium (Mg), 4.7 mmol L^{-1} sodium (Na), 4.0 mmol L^{-1} chlorine (Cl), and 1.3 mmol L^{-1} sulfur (S), whereas micronutrients were provided in Hoagland's at the following concentrations (expressed in μmol L^{-1}): 20 μmol L^{-1} boron (B), 40 μmol L^{-1} iron (Fe), 1.5 μmol L^{-1} copper (Cu), 5 μmol L^{-1} zinc (Zn), and 10 μmol L^{-1} manganese (Mn).

The pH, electrical conductivity, and temperature of the nutrient solutions

During cultivation, the pH and electrical conductivity (EC) of the nutrient solution were constantly monitored (pH and EC meter, Hanna Instruments, Padua, Italy). The EC of nutrient solution was 1.6 dS m^{-1} at the beginning of the experiment, and the pH was adjusted to 6.0 with 0.11 mL L^{-1} (77 mL/700 L) sulfuric acid (9.6 g kg^{-1}; Sigma-Aldrich, Milan, Italy); during cultivation, water was not added. The pH showed variations and declined in all treatments until 4.65 at the end of the growing cycle (Fig. 1(A)). During the whole cultivation the EC was stable, with an increase at the end of the cycle, reaching 1.75 dS m^{-1} (Fig. 1(B)). The

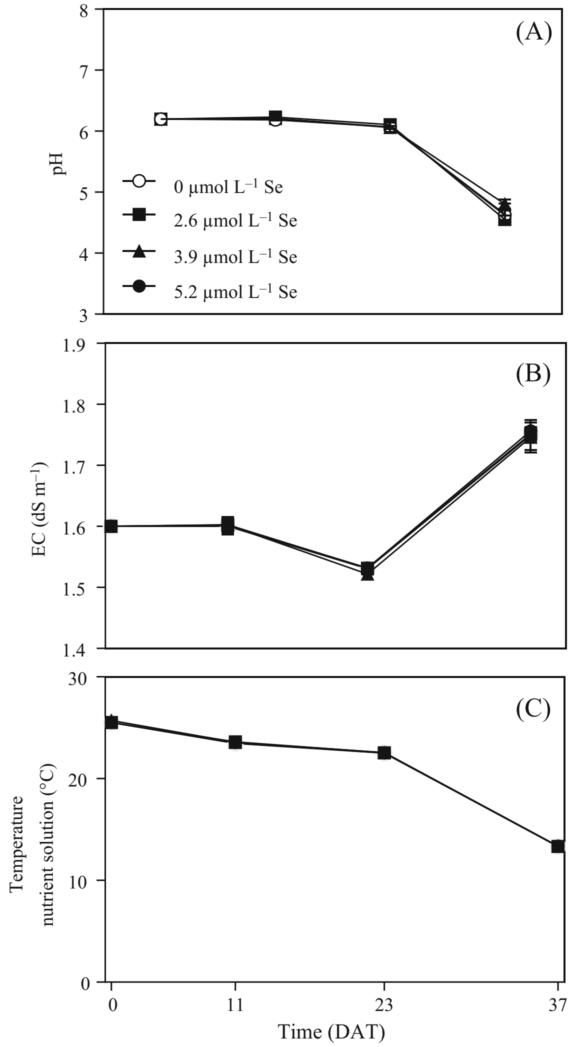

Figure 1. The pH (A), electrical conductivity (EC) (B), and temperature (C) of the nutrient solutions in the tanks during cultivation. The values are means (*n* = 3). Day After Transplant (DAT), days after transplant; Se, selenium.

temperature of the nutrient solution measured at 11 a.m. was in the range 13–25 °C, since the cultivations were in September–October (Fig. 1(C)).

Four cultivation tanks for four species were placed in four randomized blocks in the greenhouse. Oxygenation of the nutrient supply was performed by bubbling air using an air compressor. The leaves were collected at the commercial stage for baby leaves production. The temperature and the solar radiation outside the greenhouse were monitored during the whole experimental period (5 weeks), with temperature ranging from 12 to 27 °C and radiation from 3945 to 9707 kJ m^{-2} day^{-1}.

Yield, nitrate content, sucrose, and reducing sugars determination

At harvest, yield was determined and expressed as kilograms per metre squared of edible biomass. Leaf nitrate concentration was measured by spectrophotometry using the salicylic acid–sulfuric acid method. For each sample, 100 mg dry weight (DW) was ground and placed in Icon glass with 10 mL of distilled water. Dry leaf powder was shaken for 2 h at room temperature and then 10 mL was centrifuged for 15 min at 2800 x g. The supernatant was taken, and 0.2 mL was added to 0.8 mL 0.5 g kg^{-1} salicylic acid in sulfuric acid. Samples were placed on the stirring machine and 30 mL 1.5 mol L^{-1} sodium hydroxide was added. Cooled samples were read at 410 nm.[22] The nitrate concentration was determined using calibration standards containing 0, 1, 2.5, 5, 7.5, and 10 mmol L^{-1} potassium nitrate.

Sucrose and reducing sugars were determined; samples weighing about 2 g were homogenized in a mortar using water as buffer. The insoluble materials were separated by centrifugation for 5 min at 11200 x g.

The sucrose determination was carried out by adding 0.2 mL aqueous extract obtained from 2 g samples with 0.2 mL 2 mol L^{-1} sodium hydroxide and incubated in a water bath for 10 min at 100 °C; then, 1.5 mL hot resorcinol buffer was added and samples were incubated in a water bath at 80 °C for another 10 min. Resorcinol solution was prepared by mixing 250 mL hydrochloric acid 3 g kg^{-1}, 90 mg thiourea (Sigma, Milan, Italy), 35 mg resorcinol (Sigma), 25 mL acetic acid, and 10 mL distilled water. Sample readings were spectrophotometrically performed at 500 nm, using sucrose standard solutions containing 0, 0.5, 1, 1.5, and 2 mmol L^{-1}.

The reducing sugars analysis was performed using 0.2 mL aqueous extract obtained from 2 g samples that were added to 0.2 mL dinitrosalicylic acid. Reactions were heated at 100 °C for 5 min; then, 1.5 mL distilled water was added and absorbance readings were taken at 530 nm. Reducing sugars were measured and reported as glucose equivalent (standards: 0, 1, 2, 3, and 4 mmol L^{-1} glucose).

Se and mineral determinations

Fresh harvested leafy vegetables were placed in a ventilated oven at 40 °C until a constant weight. About 400 mg DW was mineralized in 5 mL 14.4 mol L^{-1} nitric acid, clarified with 1.5 mL 3.3 g kg^{-1} hydrogen peroxide. The mineralized material was solubilized in 5 mL 1 mol L^{-1} nitric acid and filtered on a 0.45 μm nylon membrane. Mineral concentrations (K, Ca, Na, Mg, B, aluminium (Al), chromium (Cr), Mn, Fe, cobalt (Co), nickel (Ni), Cu, Zn, cadmium (Cd), and lead (Pb)) were measured by inductively coupled plasma mass spectrometry (Varian 820-MS, USA).

Statistical analyses

The data reported in tables and figures are means with standard errors ($n = 4$). The significance of the effect of nutrient concentration was determined by two-way analysis of variance (ANOVA). Differences among treatments were determined by Tukey's multiple comparison test ($P = 0.05$).

Principal component analysis (PCA) was carried out to identify the mineral element distributions in baby leaf in relation to species. PCA with eigenvalues >2, explaining more than a single

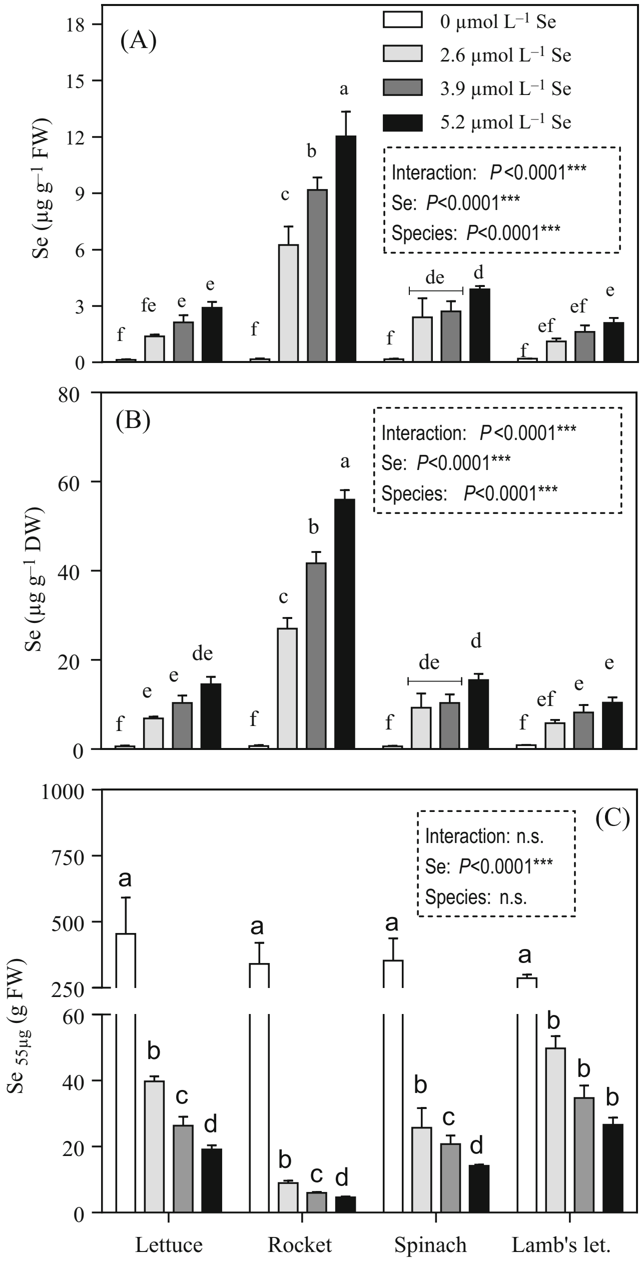

Figure 2. Selenium (Se) concentrations in the edible leaves in the different baby leaf vegetables on the fresh weight (A) or dry weight (B) basis. (C) The amount of baby leaves of each species required in a daily diet to satisfy the Se recommended daily allowance (55 μg Se). Values are means with standard errors ($n = 4$). Data were subjected to two-way analysis of variance. Differences among means were determined using Tukey's *post hoc* test. Different letters indicate statistically significant difference ($P = 0.05$). n.s., not significant.

parameter alone, were extracted. For these principal components, varimax rotation was applied on the factor obtained.

RESULTS

Se content in baby leaves, and the RDA

Leafy vegetables are mostly consumed as raw fresh vegetables, and Se concentrations are expressed on both a DW and a fresh weight (FW) basis (Fig. 2(A), (B)). Se content in the fresh biomass generally increased with the addition of Se in the nutrient solution. In lettuce, lamb's lettuce, and wild rocket the Se concentration in leaves increased. The linear regression analysis between Se concentrations in the nutrient solution and Se concentrations was significant in lettuce ($P = 0.0024$), wild rocket ($P = 0.0003$), spinach ($P = 0.0146$), and lamb's lettuce ($P = 0.0002$). Spinach plants grown in 2.6 and 3.9 μmol L^{-1} Se showed similar concentrations (2.5 μg g^{-1} FW Se). The highest Se concentration was found in wild rocket grown in 5.2 μmol L^{-1} Se (12 μg g^{-1} FW Se), followed by spinach, lettuce, and lamb's lettuce. The Se concentration in baby leaf vegetables expressed on a DW basis followed a similar trend as observed for FW. Se concentrations were higher in the baby leaves cultivated in nutrient solution containing 5.2 μmol L^{-1} Se in all species compared with the control. Rocket showed the highest Se concentration in 5.2 μmol L^{-1} with a value of 56 μg g^{-1} DW Se (Fig. 2(B)). Lettuce and spinach in the highest Se concentration showed an average of 15 μg g^{-1} DW Se, whereas lamb's lettuce was slightly lower at 10.5 μg g^{-1} DW Se (Fig. 2(B)).

The average amount of the baby leaf vegetables used in this study required to satisfy the Se RDA in non-biofortified treatments is about 358 g (Fig. 2(C)). The enrichment of Se greatly reduces the required amount. Rocket was able to take up a higher amount of Se at the different concentrations compared with other species. Therefore, wild rocket can satisfy the RDA just with 9 g per day if harvested from the 5.2 μmol L^{-1} Se treatment. At this concentration, the amount of the other baby leaves required for the satisfaction of the RDA was an average of 19.9 g on an FW basis.

Yield, nitrate, sucrose, and reducing sugars

Two-way ANOVA revealed that there was no significant interaction on yield among Se treatments and species (Table 1). Differences were not found among Se concentrations (Fig. 3(A)).

Nitrate data subjected to two-way ANOVA showed that the interaction among Se treatments and species was not significant, and no significant differences were found among Se concentrations. On the contrary, significant differences were observed among species. Spinach showed the lowest nitrate concentration, with a value of 818 mg kg^{-1} FW, whereas lettuce and lamb's lettuce had intermediate concentrations with 2099 mg kg^{-1} and 2145 mg kg^{-1} on average respectively. Rocket, instead, showed the higher concentration, with 3954 mg kg^{-1} on average for all Se treatments (Fig. 3(B)).

As reported for nitrate, the sucrose and reducing sugars were not statistically affected by Se concentrations. Significant differences were found among species, and the interaction Se × species was statistically significant (Table 1).

Se treatments in lettuce and spinach did not influence the concentration of reducing sugars; data ranged from 2.42 to 3.23 mg g^{-1} FW. In wild rocket, reducing sugars were about 1.9-fold higher in 3.9 μmol L^{-1} and 1.5-fold higher in 5.2 μmol L^{-1} than in the control. Lamb's lettuce Se showed a reduction of 41% in 3.9 μmol L^{-1} and 37% in 5.2 μmol L^{-1} compared with the control (Fig. 3(C)).

Sucrose concentration was affected by Se treatments only in spinach, which was higher in 5.2 μmol L^{-1} Se treatment than in the control by about 550% (Fig. 3(D)). No differences were observed at the increase of Se in the other species.

PCA and correlation analysis between measured variables

The PCA indicated that the mineral elements analysed had a distinct response pattern in wild rocket, lamb's lettuce, lettuce, and spinach. Two significant components were produced that, together, explained 69.39% of the total variance of data. Component 1, comprising Se, Ca, Na, Mg, Mn, and Zn, explained 43.5% of the total variance; K, B, Fe, and Cu were associated with the second component, explaining 25.8% of the variance (Fig. 4).

Table 1. Two-way analysis of variance data of the different parameters analysed

Treatment	Yield (g m^{-2} FW)	Nitrate (mg kg^{-1} FW)	Sucrose (mg g^{-1} FW)	Reducing sugars (mg g^{-1} FW)
Selenium (Se)				
Control	1409.8	2370.3	0.294	5.850
2.6 μmol L^{-1} Se	1324.3	2123.0	0.719	5.985
3.9 μmol L^{-1} Se	1502.5	2220.9	0.512	6.311
5.2 μmol L^{-1} Se	1349.7	2301.2	0.944	5.836
Species				
Lettuce	1405.3b	2098.7b	0.104c	2.873c
Wild rocket	1719.7c	3954.0a	0.260b	8.279a
Spinach	440.2d	817.6c	0.110c	7.186a
Lamb's lettuce	2021.2a	2145.1b	1.997a	5.645b
Significance				
Se treatment	0.061 ns	0.702 ns	0.053 ns	0.367 ns
Species	0.0001****	0.0001 ****	0.0001****	0.0001****
Interaction Se × species	0.837 ns	0.835 ns	0.0061**	0.0001****

Note: FW, fresh weight. Different letters indicate statistically significant difference ($P < 0.05$). ns, not significant; ** significant at $P < 0.005$; **** significant at $P < 0.0001$.

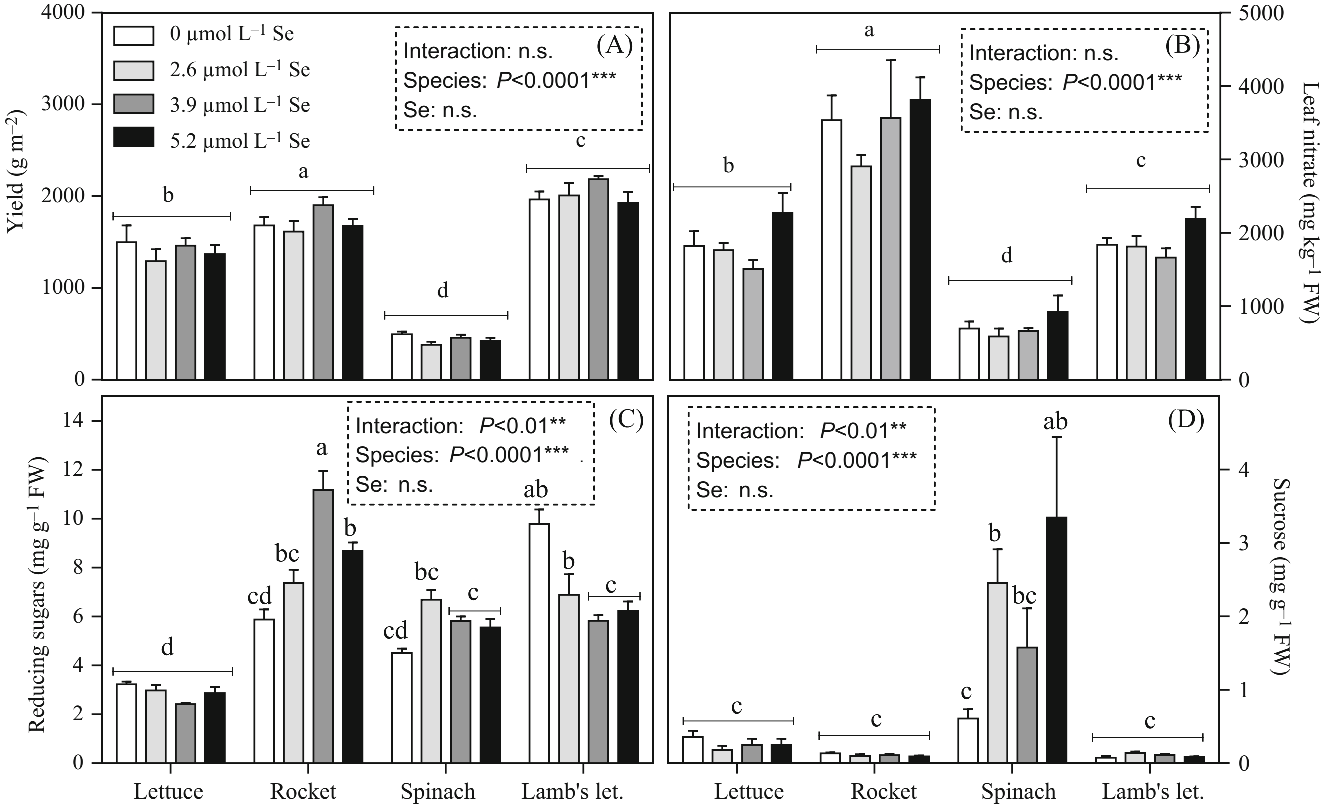

Figure 3. Yield (A), nitrate (B), reducing sugars (C), and sucrose (D) in lettuce, wild rocket, spinach, and lamb's lettuce grown in a floating system with nutrient solution containing 0, 2.6, 3.9, or 5.2 μmol L^{-1} selenium (Se). Values are means with standard errors ($n = 3$). Data were subjected to two-way analysis of variance. Statistical differences among treatments were determined using Tukey's test. Different letters indicate statistically significant difference ($P = 0.05$). n.s., not significant.

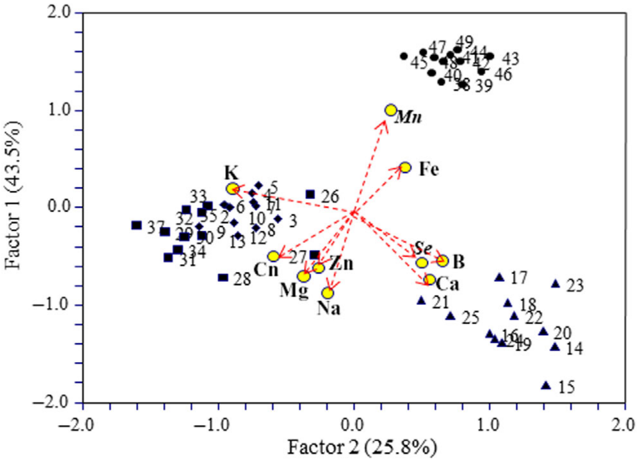

Figure 4. Biplot of principal component analysis results. Data correspond to wild rocket (▲), lamb's lettuce (●), lettuce (◆), and spinach (■) plants under selenium (Se) treatments. B, boron; Ca, calcium; Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Zn, zinc.

When the association among the parameters analysed and two main components were identified, the scores assigned to individual species with respect to each mineral element were analysed to identify common trends. Moreover, a scatter plot of scores of differently treated samples in a factor 1/factor 2 score space is reported in the Fig. 4. Scores are marked by sampling species to identify possible differences among the vegetables studied. In the PCA plot, a different distribution of the data with respect to species can be clearly observed. A net difference was detected among wild rocket and other species in relation to Se treatments (Fig. 4).

The correlation matrix revealed that significant correlations were observed by pooling together the data of all species. The Se was positively correlated with Ca, Na, B, and nitrate and negatively correlated with K and Mn (Table 2). It interesting that Se directly or indirectly affected the mineral elements uptake.

DISCUSSION

This work was planned to produce Se-enriched baby leaf vegetables for providing functional foods in the human diet. The biofortification of leafy vegetables with Se must be accurately performed to avoid high concentrations that could be phytotoxic for plants and dangerous to human health. In the open field, crop enrichment can be mainly obtained through fertilization using fertilizers containing Se applied to the soil or as a foliar spray.[14,23] In closed-loop hydroponics, such as a floating system, the Se can be included as a component of the nutrient solution and constantly monitored during cultivation. The use of nutrient solutions enriched with Se has been tested in a wide range of vegetables, showing promising practical applications.[20,24,25] The Se did not influence the chemical parameters of the nutrient solution, such as pH or EC.

Table 2. Full correlation matrix between different parameters analysed (mineral elements, reducing sugars (RS), sucrose (Suc.), nitrate, and yield) in all four species of leafy vegetables. Data reported in each cell represents the *r* value of the correlation between the two variables.

	K	Ca	Na	Mg	B	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	RS	Suc.	Nitrate	Yield
Se	**−0.391**	**0.675**	**0.397**	0.245	**0.505**	−0.167	−0.102	**−0.402**	−0.131	−0.088	−0.076	−0.014	0.219	−0.121	−0.036	0.247	−0.217	**0.430**	0.147
K		**−0.515**	−0.010	0.344	**−0.499**	−0.099	**−0.288**	**−0.051**	−0.239	−0.267	**−0.291**	**0.416**	0.008	**0.223**	−0.012	**−0.275**	**0.554**	**−0.631**	**−0.620**
Ca			**0.650**	**0.287**	**0.833**	−0.108	0.069	**−0.576**	0.016	0.059	0.003	**0.121**	**0.247**	−0.164	−0.140	0.346	−0.253	**0.795**	0.278
Na				**0.604**	0.335	−0.232	−0.158	**−0.904**	−0.219	−0.214	−0.129	**0.728**	**0.511**	0.099	−0.059	0.095	0.056	**0.367**	−0.293
Mg					0.290	**−0.466**	**−0.476**	**−0.766**	**−0.506**	**−0.473**	**−0.328**	**0.402**	**0.447**	0.084	**−0.388**	**0.344**	**0.532**	−0.131	**−0.767**
B						−0.057	0.088	**−0.321**	0.072	0.101	0.126	−0.129	0.251	−0.190	**−0.185**	**0.457**	**−0.167**	**0.665**	0.233
Al							**0.679**	**0.425**	**0.754**	**0.702**	**0.512**	−0.043	−0.181	0.153	**0.337**	−0.111	−0.231	0.066	**0.333**
Cr								**0.406**	**0.962**	**0.951**	**0.792**	−0.052	−0.280	−0.177	**0.124**	0.006	**−0.303**	**0.261**	**0.484**
Mn									**0.491**	**0.443**	**0.307**	−0.566	**−0.553**	−0.151	**0.173**	**−0.168**	−0.183	**−0.212**	**0.495**
Fe										**0.931**	**0.760**	−0.081	−0.342	−0.178	**0.165**	−0.034	**−0.302**	**0.220**	**0.510**
Co											**0.748**	−0.090	**−0.301**	−0.122	**0.158**	0.021	−0.198	**0.246**	**0.491**
Ni												−0.088	−0.106	−0.085	0.064	0.119	−0.231	**0.132**	**0.297**
Cu													**0.505**	**0.292**	0.174	**−0.207**	**0.201**	0.002	**−0.420**
Zn														**0.359**	0.073	0.058	**0.161**	0.161	**−0.458**
Cd															**0.413**	−0.166	**0.182**	**−0.291**	**−0.265**
Pb																**−0.362**	−0.192	−0.108	0.237
RS																	0.036	0.210	−0.071
Suc.																		**−0.398**	**−0.595**
Nitrate																			**0.557**
Yield																			

Note: Coloured *r* coefficients represent statistically significant for $P \leq 0.05$.

The Se concentrations found in the biofortified species studied were similar to those observed in analogous research studies reported in the literature. In cultivated rocket (*Eruca sativa* Mill.), the application every 2 days with a solution containing Se ranging from 5 to 3000 μmol L^{-1} confirmed that this species has a high accumulation ability. In treatment with 100 μmol L^{-1}, the Se in the shoots reached 6.5 mg g^{-1}. Cultivated rocket and wild rocket (*D. tenuifolia*) have different accumulation abilities, and the latter at the same Se concentration showed a higher accumulation in the range 10–20 μmol L^{-1}.[26] In lamb's lettuce, Se biofortification was studied for enhancing crop tolerance against high temperature stress. The application of 264 μmol L^{-1} (50 mg Se L^{-1} applied as Na_2SeO_4) improved the crop stress tolerance, and the Se concentration in shoots varied from 19 to 56 mg g^{-1} DW depending on the application method (foliar or soil).[27] A biofortification study carried out on different accessions of lettuce demonstrated that Na_2SeO_4 is the preferred uptake form, and Se accumulation ranged from 3.8 to 7.5 μg g^{-1} DW in treatment with 15 μmol L^{-1}.[28] Se concentrations found in leafy vegetables grown in the highest Se concentration were similar to those found in other vegetables species, such as lettuce or chicory (*Chicorium intybus* L.) cultivated with 0.5 mg L^{-1} Se.[20] Similar Se accumulations were also observed in edible parts of cabbage, garlic, onion, radish, or spinach.[29,30] Recently, biofortification has been successfully carried out on wild species, such as *Rumex acetosa* L., *Plantago coronopus* L., and *Portulaca oleracea* L.[31]

The RDA for Se has been defined to be 55 μg day^{-1} for adults.[32] In human nutrition, Se deficiency has been associated with diets with less than 0.1 mg Se kg^{-1} in foods. On the contrary, diets with Se concentration higher than 1 g Se kg^{-1} can cause toxicity and selenosis in humans. The higher limit of Se intake for adults has been set at 400 μg day^{-1}.[33] There are several geographical areas where the Se in soil is limited and can lead to the production of agricultural foods that lack this element.[34] In the human diet, the Se is better absorbed if incorporated in organic molecules, compared with a mineral Se supplement.[35] The floating system is an optimum hydroponic method for increasing Se concentration in leafy vegetables because the Se can be constantly monitored in the nutrient solution and in edible parts, avoiding excessive accumulation in plants. Biofortified baby leaves can easily provide the RDA value with progressively smaller amounts of vegetables with the increase of Se in the nutrient solution. The amount of baby leaves that can be used to satisfy the RDA depends on Se concentrations and the accumulation ability of the species used. Minimal-processing industries can use biofortified leafy vegetables for preparing bags with single leafy vegetable species or mixed ones that can satisfy the RDA of the consumer.

Se treatments affected the ionome of baby leaf species, with overall comparison and correlation analyses revealing that some mineral elements were positively or negatively correlated with Se. In particular, positive and significant correlations were observed between Se concentrations and Ca, Na, B, and nitrate. This positive correlation was also found in asparagus treated with sodium selenite.[36] A positive correlation was also found between Se and Na. Analogous results, were found in black gram (*Vigna mungo* L.) plants.[37] However, the interaction of Se on the uptake with other elements depends on the Se concentration applied. In cultivated rocket, the concentration of P, K, Mg, and Ca increased in shoots grown with 5 μmol L^{-1} selenate, whereas at 100 μmol L^{-1} selenate a significant reduction in P, K, and Mg concentrations was recorded. In tomato fruit, analogous results were observed at higher Se concentration.[38]

The effect of Se was studied on crop yield as well as on leafy vegetable quality parameters. The results demonstrated that Se treatments did not affect yield in any of the four species used. However, different species and diverse environmental conditions can lead to different results. An increase of yield in Se treatments was observed in chicory and lettuce,[20] whereas no yield change was observed in garlic and onion[39] or asparagus[36] foliar sprayed with Se.

In our study, besides the Se accumulation, the effects of Se treatments on quality parameters were also considered and studied. Among the quality parameters, the most important are represented by nitrate in leaves. The nitrate concentration in leafy vegetables must be below the commercialization limits that are defined by specific EU regulations. At the nutritional level, the nitrate intake from food can have negative and positive consequences on human health. A diet rich in nitrate can cause some physiological disorders and the increase of gastrointestinal cancers. For the free commercialization of leafy vegetables, independent from the nitrate effect on human health, limits imposed by the EU Regulation No. 1258/2011 must be respected.[40] The maximum concentrations allowed vary among leafy vegetable species and cultivation period.[41] Nitrate uptake and assimilation are regulated by environmental conditions, since nitrate reductase activity follows the circadian rhythm and its activity is strictly dependent on light intensity and photoperiod.[42] Since the experiment was carried out in the autumn, the high nitrate content may be explained by the lower light intensity and temperature. Our results are similar to analogous experiments carried out on lettuce and chicory leafy vegetables. In these vegetables, the nitrate concentration was mainly affected by seasons rather than Se concentration in the nutrient solution.[20] The nitrate leaf accumulation in plants grown under Se treatment did not show any significant effects.[21]

However, a reduction of nitrate concentration was found in lettuce treated with 10–120 μmol L^{-1} Se applied as selenite or selenite.[43] In that study, the activation of the main enzymes involved in the nitrogen assimilation, such as nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthase, was observed. In our experiment, a decline of nitrate with the increase of Se concentration in the nutrient solution was not observed. The difference in the results could be due to the Se application method. In our study, Se was added directly in the nutrient solution of the floating system, whereas in the Rios *et al.*[43] study the Se was distributed with nutrient solution in a pot cultivation. However, in our correlation data, a positive and significant correlation was observed. Since nitrate assimilation is affected by light intensity and duration, further studies are required. Negative and significant correlations were found between Se and K or Mn. In the literature there is no information regarding correlation data between Se and these two elements.

Reducing sugars and sucrose concentrations mainly depend on the crop photosynthesis activity. The concentration of sugars in leaves is important for the storage and shelf life of products. In treatments with Se concentrations the values were higher, but not statistically significant. The increase of sucrose and reducing sugars was also reported in mung, (*Vigna radiata* (L.) R.Wilczek), grown hydroponically with 2.6 μmol L^{-1} or 4.0 μmol L^{-1} (0.5 mg L^{-1} or 0.75 mg L^{-1} respectively) Na_2SeO_4.[44] In experiments performed in an open field on rapeseed (*Brassica napus* L.), Se treatments decreased the reducing sugars concentration. However, the Se concentrations employed in the rapeseed were from

12.7 to 51 μmol L^{-1} Se,[45] and so higher than those used in our experiments.

Low Se concentrations can also have a plant growth regulator function, influencing the antioxidant components, anti-senescence proprieties, abiotic stress regulator, and defensive ability against biotic stresses.[46,47]

CONCLUSION

This study confirms that a floating system is an optimal cultivation method for the biofortification of leafy vegetables, since the nutrient solution can be monitored anytime, and mineral composition can be essentially modified by crop uptake. Nutrient solution appropriately prepared can provide the exact amount of the required element in the vegetable that will be part of the consumer's diet. The enriched leafy vegetables can be used in the minimal-processing industries for producing commercial bags or packages of leafy vegetables with the correct amount of Se that can satisfy the RDA.[45] Our results demonstrated that knowing the accumulation ability of the different species enables optimization of the Se concentration in the nutrient solution for use in producing biofortified vegetables able to satisfy the dietary requirements.

The best crop performances were obtained with 2.6 or 3.9 μmol L^{-1} Se concentrations in the nutrient solution, although the four vegetable species used did not show any phytotoxic symptoms even at the highest Se concentration tested. This study provides original results on the correlation analysis among Se and other mineral elements, suggesting interesting new interactions among them.

ACKNOWLEDGEMENTS

The research work was funded by Lombardy Region – Project 'Biofortification of leafy vegetables grown in floating system—*Rete Colture Orticole*'. Open Access Funding provided by Universita degli Studi di Milano within the CRUI-CARE Agreement.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1 Kumar D, Kumar S and Shekhar C, Nutritional components in green leafy vegetables: a review. *J Pharmacogn Phytochem* **9**:2498–2502 (2020).
2 Petropoulos SA, Chatzieustratiou E, Constantopoulou E and Kapotis G, Yield and quality of lettuce and rocket grown in floating culture system. *Not Bot Horti Agrobot Cluj-Napoca* **44**:603–612 (2016).
3 Slavin JL and Lloyd B, Health benefits of fruits and vegetables. *Adv Nutr* **3**:506–516 (2012).
4 Buturi CM, Mauro RP, Fogliano V, Leonardi C and Giuffrida F, Mineral biofortification of vegetables as a tool to improve human diet. *Foods* **10**:2–23 (2021).
5 Chao W, Rao S, Chen Q, Zhang W, Liao Y, Ye J *et al.*, Advances in research on the involvement of selenium in regulating plant ecosystems. *Plants* **11**:2712 (2022).
6 Ellis DR and Salt DE, Plants, selenium and human health. *Curr Opin Plant Biol* **6**:273–279 (2003).
7 Fernández-Lázaro D, Fernandez-Lazaro CI, Mielgo-Ayuso J, Navascués LJ, Córdova Martínez A and Seco-Calvo J, The role of selenium mineral trace element in exercise: antioxidant defense system, muscle performance, hormone response, and athletic performance. A systematic review. *Nutrients* **12**:1790 (2020).
8 Hossain A, Skalicky M, Brestic M, Maitra S, Sarkar S, Ahmad Z *et al.*, Selenium biofortification: roles, mechanisms, responses and prospects. *Molecules* **26**:881 (2021).
9 Pyrzynska K and Sentkowska A, Selenium in plant foods: speciation analysis, bioavailability, and factors affecting composition. *Crit Rev Food Sci Nutr* **61**:1340–1352 (2021).
10 Consentino BB, Ciriello M, Sabatino L, Vultaggio L, Baldassano S, Vasto S *et al.*, Current acquaintance on agronomic biofortification to modulate the yield and functional value of vegetable crops: a review. *Horticulturae* **9**:219 (2023).
11 Liao SF, Brown KR, Stromberg AJ, Burris WR, Boling JA and Matthews JC, Dietary supplementation of selenium in inorganic and organic forms differentially and commonly alters blood and liver selenium concentrations and liver gene expression profiles of growing beef heifers. *Biol Trace Elem Res* **140**:151–169 (2011).
12 Kieliszek M and Błażejak S, Current knowledge on the importance of selenium in food for living organisms: a review. *Molecules* **21**:609 (2016).
13 Cheng C, Coldea TE, Yang H and Zhao H, Selenium uptake, translocation, and metabolization pattern during barley malting: a comparison of Selenate, selenite, and selenomethionine. *J Agric Food Chem* **71**:5240–5249 (2023). https://doi.org/10.1021/acs.jafc.3c00273.
14 Schiavon M, Nardi S, Dalla Vecchia F and Ertani A, Selenium biofortification in the 21st century: status and challenges for healthy human nutrition. *Plant Soil* **453**:245–270 (2020).
15 Shahid M, Niazi NK, Khalid S, Murtaza B, Bibi I and Rashid MI, A critical review of selenium biogeochemical behavior in soil–plant system with an inference to human health. *Environ Pollut* **234**:915–934 (2018).
16 Makela AL, Wang WC, Hamalainen M, Nanto V, Laihonen P and Kotilainen H, Environmental effects of nationwide selenium fertilization in Finland. *Biol Trace Elem Res* **36**:151–157 (1995).
17 Boldrin PF, Faquin V, Ramos SJ, Boldrin KVF, Ávila FW and Guilherme LRG, Soil and foliar application of selenium in rice biofortification. *J Food Compos Anal* **31**:238–244 (2013).
18 Hladun KR, Parker DR, Tran KD and Trumble JT, Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.). *Environ Pollut* **172**:70–75 (2013).
19 Sabatino L, La Bella S, Ntatsi G, Iapichino G, D'Anna F, De Pasquale S *et al.*, Selenium biofortification and grafting modulate plant performance and functional features of cherry tomato grown in a soilless system. *Sci Hortic* **285**:110095 (2021).
20 Malorgio F, Diaz KE, Ferrante A, Mensuali-Sodi A and Pezzarossa B, Effects of selenium addition on minimally processed leafy vegetables grown in floating system. *J Sci Food Agric* **89**:2243–2251 (2009).
21 Puccinelli M, Malorgio F and Pezzarossa B, Selenium enrichment of horticultural crops. *Molecules* **22**:933 (2017).
22 Cataldo DA, Haroon M, Sehrader LE and Youngs VL, Rapid colorimetric determination of nitrate in plant tissue by titration of salicylic acid. *Commun Soil Sci Plant Anal* **6**:71–80 (1975).
23 Germ M, Stibilj V, Osvald J and Kreft I, Effect of selenium foliar application on chicory (*Cichorium intybus* L.). *J Agric Food Chem* **55**:795–798 (2007).
24 Banuelos GS and Meek DW, Selenium accumulation in selected vegetables. *J Plant Nutr* **12**:1255–1272 (1989).
25 Puccinelli M, Malorgio F, Pintimalli L, Rosellini I and Pezzarossa B, Biofortification of lettuce and basil seedlings to produce selenium enriched leafy vegetables. *Horticulturae* **8**:801 (2022).
26 Dall'Acqua S, Ertani A, Pilon-Smits EA, Fabrega-Prats M and Schiavon M, Selenium biofortification differentially affects sulfur metabolism and accumulation of phytochemicals in two rocket species (*Eruca sativa* Mill. and *Diplotaxis tenuifolia*) grown in hydroponics. *Plants* **8**:68 (2019).
27 Hawrylak-Nowak B, Dresler S, Rubinowska K, Matraszek-Gawron R, Woch W and Hasanuzzaman M, Selenium biofortification enhances the growth and alters the physiological response of lamb's lettuce grown under high temperature stress. *Plant Physiol Biochem* **127**: 446–456 (2018).
28 Ramos SJ, Rutzke MA, Hayes RJ, Faquin V, Guilherme LRG and Li L, Selenium accumulation in lettuce germplasm. *Planta* **233**:649–660 (2011).
29 Silva MA, Ferreira de Sousa G, Bañuelos, G., Amaral, D., Brown, P. H., & Guimarães Guilherme, L. R. (2023). Selenium speciation in se-enriched soybean grains from biofortified plants grown under different methods of selenium application. *Foods* **12**(6), 1214.
30 Ferrarese M, Sourestani M, Quattrini E, Schiavi M and Ferrante A, Biofortification of spinach plants applying selenium in the nutrient solution of floating system. *Veg Crops Res Bull* **76**:127–136 (2012).

31 Puccinelli M, Pezzarossa B, Pintimalli L and Malorgio F, Selenium biofortification of three wild species, *Rumex acetosa* L., *Plantago coronopus* L., and *Portulaca oleracea* L., grown as microgreens. *Agronomy* **11**:1155 (2021).

32 Kuria A, Fang X, Li M, Han H, He J, Aaseth JO *et al.*, Does dietary intake of selenium protect against cancer? A systematic review and meta-analysis of population-based prospective studies. *Crit Rev Food Sci Nutr* **60**:684–694 (2020).

33 World Health Organization and the Food and Agriculture Organization of the United Nations, *Vitamin and Mineral Requirements in Human Nutrition*, 2nd edn. WHO/FAO, Geneva, Switzerland/Rome, Italy, p. 209 (2004).

34 Khan Z, Thounaojam TC, Chowdhury D and Upadhyaya H, The role of selenium and nano selenium on physiological responses in plant: a review. *Plant Growth Regul* (2023). [In Press]. https://doi.org/10.1007/s10725-023-00988-0.

35 Navarro-Alarcon M and Cabrera-Vique C, Selenium in food and the human body: a review. *Sci Total Environ* **400**:115–141 (2008).

36 Conversa G, Lazzizera C, Chiaravalle AE, Miedico O, Bonasia A, La Rotonda P *et al.*, Selenium fern application and arbuscular mycorrhizal fungi soil inoculation enhance Se content and antioxidant properties of green asparagus (*Asparagus officinalis* L.) spears. *Sci Hortic* **252**:176–191 (2019).

37 Jawad Hassan M, Ali Raza M, Khan I, Ahmad Meraj T, Ahmed M, Abbas Shah G *et al.*, Selenium and salt interactions in black gram (*Vigna mungo* L): ion uptake, antioxidant defense system, and photochemistry efficiency. *Plants* **9**:467 (2020).

38 Meucci A, Shiriaev A, Rosellini I, Malorgio F and Pezzarossa B, Se-enrichment pattern, composition, and aroma profile of ripe tomatoes after sodium selenate foliar spraying performed at different plant developmental stages. *Plants* **10**:1050 (2021).

39 Golubkina N, Amagova Z, Matsadze V, Zamana S, Tallarita A and Caruso G, Effects of arbuscular mycorrhizal fungi on yield, biochemical characteristics, and elemental composition of garlic and onion under selenium supply. *Plants* **9**:84 (2020).

40 EU Commission, Commission Regulation (EU) No 1258/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for nitrates in foodstuff. *Off J Eur Union Legis* **320**:15–17 (2011).

41 Cavaiuolo M and Ferrante A, Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. *Nutrients* **6**:1519–1538 (2014).

42 Podetta N, Antonacci S and Ferrante A, Diurnal pattern of nitrate reductase in leaves of rocket (*Diplotaxis tenuifolia* L.) plants. *Minerva Biotecnologica* **23**:40–41 (2011).

43 Rios JJ, Blasco B, Rosales MA, Sanchez-Rodriguez E, Leyva R, Cervilla LM *et al.*, Response of nitrogen metabolism in lettuce plants subjected to different doses and forms of selenium. *J Sci Food Agric* **90**:1914–1919 (2010).

44 Malik JA, Kumar S, Thakur P, Sharma S, Kaur N, Kaur R *et al.*, Promotion of growth in mungbean (*Phaseolus aureus* Roxb.) by selenium is associated with stimulation of carbohydrate metabolism. *Biol Trace Elem Res* **143**:530–539 (2011).

45 Sharma S, Bansal A, Dhillon SK and Dhillon KS, Comparative effects of selenate and selenite on growth and biochemical composition of rapeseed (*Brassica napus* L.). *Plant Soil* **329**:339–348 (2010).

46 Pyrzynska K, Selenium speciation in enriched vegetables. *Food Chem* **114**:1183–1191 (2009).

47 Guo Q, Ye J, Zeng J, Chen L, Korpelainen H and Li C, Selenium species transforming along soil–plant continuum and their beneficial roles for horticultural crops. *Hortic Res* **10**:uhac270 (2023).