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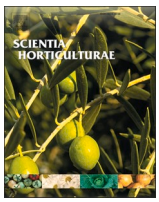

Biofortification of kale microgreens with selenate-selenium using two delivery methods: Selenium-rich soilless medium and foliar application

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ABSTRACT

Selenium (Se) is essential for human health as it is involved in various fundamental biological functions. This study aimed to assess the effects of Se enrichment in kale microgreens through biofortification in a soilless cultivation system. Two Se (as sodium selenate) application methods were assessed, including supplementation into the nutrient solution or as a foliar spray at four concentrations: 0, 10, 20 and 40 μM Se in a completely randomized design considering triplicates. For this purpose, minerals, nitrate and ammonium content, as well as fresh yield and dry matter of kale microgreens, were recorded after a 14-day growing period in an environmentally controlled vertical farm. Results showed that kale microgreens successfully accumulated up to 893.3 and 24 μg Se/ kg dry matter under the nutrient solution and foliar treatments, respectively, while yield remained unaffected. Selenium (Se) enrichment of the nutrient solution at 20 μM Se concentration resulted in the optimum treatment for fresh consumption purposes and supplying this element in human diets in the future, providing adequate dietary Se in less than five grams of the fresh kale microgreens.

1. Introduction

In recent years, there has been an increasing interest in microgreens as consumers have gained more awareness about their nutritional benefits. Microgreens are an emerging class of salad crops grown from the seeds of various crops. Microgreens add intense flavours, vitamins, antioxidants, and minerals to meals (Z. Xiao et al., 2016; Z. L. Xiao et al., 2012). With the germination process reducing the concentration of certain antinutrients such as phytate (Liang et al., 2009), microgreens may offer a better bioavailability and higher absorption of mineral elements than adult vegetables, which are often high in such absorption inhibitors (K. Khoja, Buckley, F. Aslam, A. Sharp, and Latunde-Dada, 2020). Furthermore, the short growing cycle (7–21 days), minimal space requirement (vertical farming), and high nutritional value make microgreens the crop of choice grown within an urban and peri-urban farming framework and individual households providing nutrients for urban dwellers while minimizing food miles (K. Khoja et al., 2020).

Selenium (Se) is an essential micronutrient for human health, required for the proper function of several physiological and metabolic activities such as thyroid hormones metabolism, immune function, and antioxidant defence. Insufficient dietary selenium intake can result in diseases such as poor immune function, cognitive decline, and the endemic kashin-beck disease (KBD), also known as the ‘‘Big Bone Disease’’, which severely affects the health of bones and joints in children and adolescents. The recommended dietary intake of Se is 55 μg. day-1 and Se intake of up to 200 μg. day-1 is believed to be associated with additional health benefits such as cancer prevention (EXAMINE.com). Based on the Nutrient Reference Values for Australia and New Zealand developed by the national health and medical research council (NHMRC) (Capra, 2006), the upper level of intake (UL) for Se is considered to be 400 μg/ day. Sustained Se intake above the UL can cause selenium poisoning or selenosis (Malagoli et al., 2015). Data about selenosis is limited, but the most prevalent symptoms include gastrointestinal disturbance, fatigue, hair loss and nails brittleness (MacFarquhar et al., 2010; Yang et al., 1983).

Selenium deficiency is estimated to affect one billion people around the globe especially in areas where this element is lacking from the soil such as parts of China, New Zealand, Australia and Finland (Oldfield,

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2002; Schiavon and Pilon-Smits, 2017). Selenium content in vegetables and microgreens can be improved using biofortification techniques. Biofortification is the process of increasing the concentrations of vitamins and minerals in a crop or food product that would otherwise not be present (Bouis and Saltzman, 2017). Biofortification of some vegetables with selenium (Se) has been shown to be effective at increasing the Se intake in human diets and satisfying the recommended dietary allowance (RDA) of 55 μg/ day (Kipp et al., 2015). However, despite their evident potential, microgreens have not been studied extensively and only a limited number of studies have investigated selenium biofortification in microgreens.

One of the recent examples of successful selenium biofortification in hydroponically grown microgreens was reported by Newman et al. (2021). In this study, basil, cilantro and scallion microgreens were grown in a Se-rich nutrient solution containing 2.5 or 5.0 mg/ L sodium selenate. As scallions are a member of the Se-accumulator Allium family, a stronger treatment of 10 mg/ L sodium selenate was applied to the scallion microgreens only which was taken up successfully but decreased its yield. They concluded that, the 5 mg/ L sodium selenate treatment increased selenium content in all the three species without compromising on yield of the microgreens. In another study by Germ et al. (2019), buckwheat microgreens were examined for a joint iodine and selenium enrichment. To achieve this, a pre-sowing seed treatment was applied, through which buckwheat seeds were soaked in a selenium and iodine containing solution for four hours and were then grown to microgreen stage. By exhibiting a selenium content of up to 0.24 μg. g^{-1} dry weight, this study demonstrated another successful example of selenium biofortification in microgreens (Germ et al., 2019). A. A. Pannico et al. (2020) also reported successful selenium biofortification in basil, coriander and tatsoi microgreens by adding sodium selenate to the hydroponic nutrient solution at 8, and 16 μM levels. A slightly different approach to selenium biofortification was demonstrated in M. Puccinelli, F. Malorgio, I. Rosellini, and B. Pezzarossa (2019)'s study in which they applied selenium treatments to the mother plants and used their seeds to grow microgreens. Interestingly, basil microgreens grown from those seeds showed an elevated selenium content and antioxidant activity. Similar studies on wheat microgreen extract (M. Z. Islam, B. J. Park, H. M. Kang, and Y. T. Lee, 2020) mizuna and arugula microgreens (Mezeyova et al., 2022) and a variety of wild species grown as microgreens (M. Puccinelli et al., 2021) have also been reported.

This study investigated kale (*Brassica oleracea* var. *acephala*) microgreens for their Se uptake and accumulating capabilities. Kale was chosen since it is gaining popularity amongst consumers for its health-related benefits. More importantly, kale belongs to the *Brassicaceae* family, a family of natural Se-bio-accumulators, exhibiting the great potential to uptake Se and tolerate high Se environmental conditions. Although several studies have focused on fortifying other vegetables such as sprouts (Arscott and Goldman, 2012; Lintschinger et al., 2000), buckwheat, wheat and basil microgreens (Germ et al., 2019; M. Z. Islam, B.-J. Park, H.-M. Kang, and Y.-T. Lee, 2020; Martina Puccinelli, Fernando Malorgio, Irene Rosellini, and Beatrice Pezzarossa, 2019) and several adult crops (Kopsell et al., 2009; Malorgio et al., 2009; Ramos et al., 2011; Šindelářová et al., 2015), to the best of authors' knowledge, Se biofortification in kale microgreens has not been investigated previously.

2. Materials and methods

2.1. Experimental site, growing conditions, and cultivation

This experiment was conducted on an environmentally controlled vertical farm at the Dookie campus belonging to The Faculty of Veterinary and Agricultural Sciences (FVAS), The University of Melbourne. The crop growing conditions were maintained at air temperature and relative humidity of around 22.5 °C and 65–70 %, respectively. For each tray, 20 g of Toscano black kale (*B. acephala*) (Fairbank Seeds Pty. Ltd.) seeds (300 seeds/ gram) were sown evenly on saturated PureGrown Hemp-felt mats (High Sun Pty Ltd, QLD, Australia), which were placed into perforated plastic trays of 34 × 28 × 5 cm dimensions. Each perforated tray was put into a solid bottomed tray containing distilled water to create a wicking-type hydroponic system. The growing trays were filled with distilled water until the emergence of cotyledonary leaves on day four after sowing. An example of a growing tray representing one experimental replicate is shown in Fig. 1. Trays were kept in the dark for three days to allow uniform seed germination and then exposed to LED lighting (Valoya, Finland) on day four under an 18/6 h photoperiod until day 14 after sowing. Visible irradiation intensity at the leaf cover was 109.30 μmol/m^2s, measured using a handheld spectrometer (AsenseTek, Taipei, Taiwan). The spectral output of the LED lights is shown in Table 1.

On day four, after sowing, 700 mL of half-strength nutrient solution (NS) (NPK – 23: 3.95: 14) (Manutec Pty Ltd, SA, Australia) was applied to all trays. Treatments included the application of selenium (Se) in the form of sodium selenate (Na_2SeO_4; Sigma-Aldrich, St. Louis, MO, USA) using two application methods, including foliar and NS applications, at four Se concentrations, including 0 (control), 10, 20, and 40 μM. The Se concentrations were considered based on preliminary experiments conducted with 0, 10, 20, 40, 200, 400 and 1266 μM selenate-Se in NS, which showed that 200, 400 and 1266 μM treatments are less safe in terms of Se toxicity for consumers.

For the NS application method, NS was modified with Se at 0 (control), 10, 20, and 40 μM and applied in triplicates (trays). For the foliar application method, kale microgreens were sprayed with 15 mL of a solution prepared by adding sodium selenate to distilled water at concentrations of 0 (control), 10, 20 and 40 μM in triplicates. Before applying the foliar treatments, an empty tray was set up on an allocated marked spot on a lab bench. To optimize the spraying position, the marked spot was sprayed with water to examine if all droplets fell within the region of interest. The height and position from where the initial spray was done were then marked with a tripod stand and maintained throughout the experiment. Foliar treatments were applied uniformly on the cotyledonary leaves of microgreens on days five, seven and ten after sowing using a handheld sprayer. Each replicate tray was placed on an electronic balance and zeroed on each spraying day. The spray was then applied until the balance registered 15 g, which accounts for 15 mL of the treatment solution as the density of the solution was considered to be equal to water (1 g/ mL).

In both foliar and NS application methods, sodium sulphate (Na_2SO_4) (Sigma-Aldrich, St. Louis, MO, USA) was applied in triplicates to a parallel trial at 10, 20 and 40 μM (same concentration as sodium selenate) to determine the potential effects of sodium, which was present in sodium selenate solution, on the outcome of this study.

Microgreens were harvested from each tray on day 14 after sowing. Fresh yield (FW) and dry matter content (DM) of microgreens, harvested from randomly selected 10 × 10 cm areas away from the edges of the container to avoid the edge effect, were measured before and after oven desiccation at 70 °C for 72 h.

2.2. Mineral analysis and green vegetables hazard quotient (HQgv)

Microgreen shoots were randomly picked from various locations within each tray, mixed and washed with distilled water, then dried at 70 °C for 72 h. Dried samples were ground to a fine powder, from which a 500 mg sample was taken for mineral analysis. Methods used for mineral analysis are summarised in Table 2.

The green vegetables' hazard quotient (HQgv) of Se was calculated based on a protocol developed by the United States Environmental Protection Agency (USEPA) using the below equation (Iris., 2011):

$$HQgv = (ADD / RfD) \quad (1)$$

Where ADD is the average daily dose of Se (μg Se/ day), and RfD

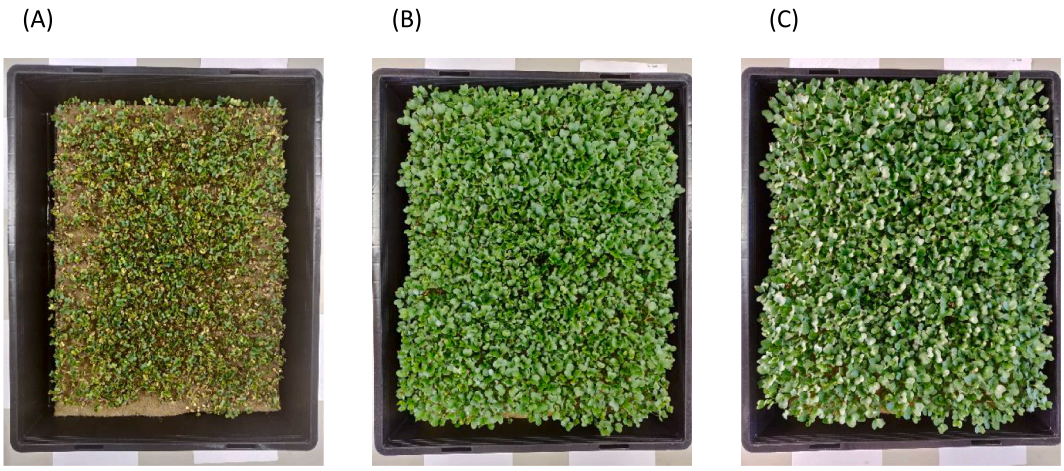

Fig. 1. Example of a growing tray (considered as one experimental replicate). Images were taken from the same tray on day seven (A), 11 (B) and 13 (C) after sowing.

Table 1
The spectral output of the LED lights used in growing microgreens.

Parameter	Wavelength	Intensity
	nm	μmol/ m²s
Visible	400~700	109.30
Infrared	701~780	11.945
Red	600~700	75.238
Green	500~599	20.776
Blue	400~499	13.275

Table 2
Reference methods and equipment used for plant tissue analysis for mineral analysis.

Analyte	Method	Analysis Equipment	Reference
Total nitrogen (TN)	Combustion	Combustion	(Schmitter and Rihs, 1989)
Nitrate nitrogen (NO_3^-)	Soluble nitrate	FIA	(Baker and Thompson, 1992)
Ammonium nitrogen (NH_4^+)	Soluble Ammonium nitrogen	FIA	(Baethgen and Alley, 1989)
Chloride (Cl)	Soluble chloride	FIA	(Watson and Isaac, 1990)
Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K)	HNO_3 and H_2O_2 digestion	ICP-OES	(Hansen et al., 2013)
Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Sodium (Na), Sulphur (S), Zinc (Zn)	HNO_3 and H_2O_2 digestion	ICP-OES	(Hansen et al., 2013)
Selenium (Se)	HNO_3 and H_2O_2 digestion	ICP-MS	(Hansen et al., 2013)

represents the recommended upper limit of dietary tolerable Se consumption (considered as 400 μg Se/ day), quantifying the health risk to a 70 kg adult consuming a 10 g portion of fresh microgreens (one serving). HQgv values smaller than 1.00 show that the vegetable is safe for the adult consumer.

2.3. Statistical analysis

Data were analysed using ANOVA with the General Linear Model procedures in Minitab 21 software (Minitab Inc., State College, PA, USA), considering effects of Se concentration for each application method and the potential interaction effects of application method and Se concentrations. *Post hoc* multiple comparison tests were performed using Tukey's honest significant difference (HSD) test ($\alpha = 0.05$).

The data collected for nutritional, mineral and growth parameters were analysed using multivariate analysis techniques using a customized code written in MATLAB® R020b (MathWorks Inc., Natick, MA, USA) for principal component analysis (PCA) to assess the relationships amongst variables and associations between samples and variables.

3. Results

3.1. Microgreens fresh yield (FW), dry matter content (DM) and shoot length (SL)

Fig. 2A shows FW results in different NS and foliar application methods at different Se concentrations. In NS treatments, fresh microgreen yield ranged from a minimum of 858 g/ m^2 in control to a maximum of 1062.3 g/ m^2 in 20 μM Se concentration. For the foliar treatments, it ranged from a minimum of 981 g/ m^2 in 10 μM Se concentration to a maximum of 1212.3 g/ m^2 in 40 μM Se concentration. Overall, FW was not significantly affected by the Se concentration in either NS or the foliar application methods. Microgreens fresh yield (FW) was significantly affected by the application method showing an average of 16% FW in the foliar method compared with the NS application method ($p < 0.05$).

Results for DM are shown in Fig. 2B for NS and foliar application methods. In the NS application method, microgreens DM ranged from a minimum of 3.50 g/ 100 g FW in 40 μM to a maximum of 4.59 g/ 100 g FW in 20 μM Se concentration. The foliar application method ranged from a minimum of 4.21 g/ 100 g FW in 10 μM to a maximum of 5.45 g/ 100 g FW in 40 μM Se concentration (Fig. 2B). No significant differences were found across different Se concentrations for DM. An approximately 2-fold increase in DM was found at 40 μM Se concentration in the foliar application method as compared with the DM in the same Se concentration in the NS application method ($p < 0.05$p) but DM was generally unaffected by the application method showing an average of 4.00 g/ 100 g FW in the NS and 4.80 g/ 100 g FW in the foliar application methods.

Results for SL are shown in Fig. 2C for NS and foliar treatments. Although not affected by Se concentration alone, an interaction was observed between Se concentration and the application method for SL ($p < 0.05$), showing that NS-treated microgreens at 20 μM Se concentration were%14 taller than foliarly treated microgreens at 40 μM Se concentration. Shoot length (SL) in NS treatments ranged from a minimum of 32 mm in the control treatment to a maximum of 35.2 mm in 20 μM Se concentration (Fig. 2C), and in foliar treatments ranged from a minimum of 30.8 mm in 40 μM Se concentration to a maximum of 33.9 mm

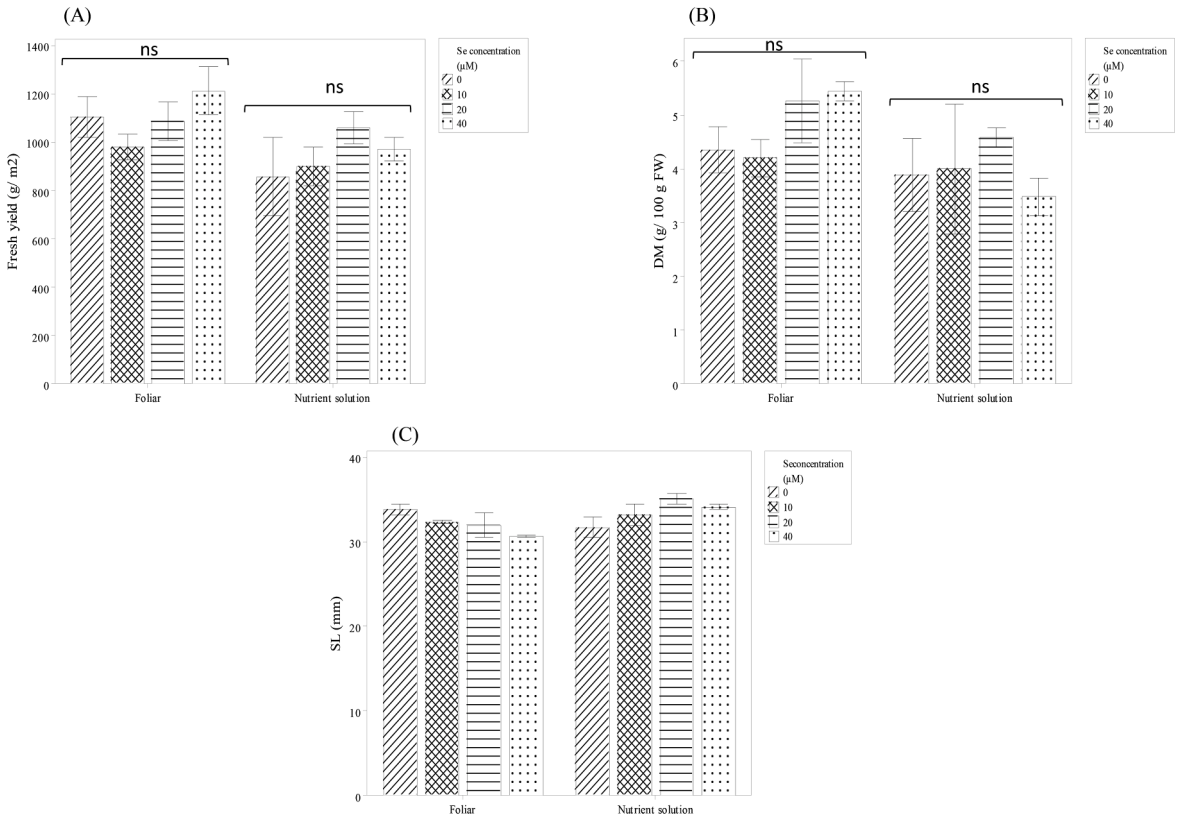

Fig. 2. Fresh yield (A), dry matter (DM) content (B), and shoot length (SL) (C) subjected to different Se treatments. Treatments included addition of Se either to the nutrient solution (NS) or as a foliar spray at 0 (control), 10, 20 and 40 μM. Each bar represents mean of three replicates, and error bars represent standard error. ns = not statistically significant (Tukey test with 95 % confidence).

in control (C). Although not statistically significant, in the foliar application method, 40 μM Se concentration reduced SL by 9% compared to the control. In contrast, in the NS application method, SL was improved by 11% at 20 μM Se concentration compared to the control (not statistically significant). Shoot length (SL) was significantly affected by the Se application method showing that foliarly treated microgreens were 4% shorter than their NS-treated counterparts (Fig. 2C).

3.2. Selenium content in kale microgreens and green vegetables' hazard quotient (HQgv)

Results for Se content are shown in Table 3 for NS and foliar application methods at different Se concentrations. A significant interaction between Se concentration and application method was observered for Se content, Se content per serving,%RDA and HQgv in microgreens ($p < 0.05$). In the NS application method, the Se content in kale microgreens was 89-, 151- and 487-folds higher than the control treatment at 10, 20 and 40 μM Se concentrations, respectively. In the Foliar application method, Se content in Se-treated kale microgreens was 34-folds higher than the control treatment. Kale microgreens accumulated Se up to 24 μg/ g DW in foliar treatments at 40 μM Se concentration and up to 893.33 μg/ g DW in NS treatments at 40 μM Se concentration. The addition of Se to the NS was significantly more effective in increasing the Se content of kale microgreens than the foliar application method at an equal Se concentration ($p < 0.05$). Selenium (Se) content in the NS-treated microgreens were 8, 20, and 37-folds higher than the foliar treatments at 10, 20, and 40 μM Se treatment levels, respectively (Table 3).

Results for Se content per serving,%RDA and HQgv values are shown in Table 3. Selenium (Se) content per serving ranged from 0.12 to a maximum of 13.26 μg/ 10 g FW in the foliar application method and from a minimum of 0.48 to a maximum of 318.5 μg/ 10 g FW in NS

Table 3
Microgreens' selenium (Se) content (on dry weight (DW) basis), dietary Se intake in one serving (10 g) of fresh microgreens (Se per serving), per cent of the recommended dietary allowance (RDA) and the hazard quotient (HQgv) values subjected to different biofortification treatments. Treatments included the addition of Se either to the nutrient solution (NS) or as a foliar spray at 0 (control), 10, 20 and 40 μM.

Parameter		Method	Se concentration (μM)				Se conc. × Method
			0.0 (Control)	**10**	**20**	**40**	***p-value***
Se content	μg/ g DW	NS	1.83 ^{c} ± 0.64	163.30 ^{b} ± 13.30	276.70 ^{b} ± 64.90	893.30 ^{a} ± 86.70	0.00
		Foliar	0.57 ^{b} ± 0.13	20.67 ^{a} ± 3.48	13.67 ^{ab} ± 1.2	24.00 ^{a} ± 5.77	
Se per serving	μg/ 10 g FW	NS	0.75 ^{b} ± 0.34	64.40 ^{b} ± 17.10	127.20 ^{b} ± 29.30	318.50 ^{a} ± 58.40	0.00
		Foliar	0.25 ^{b} ± 0.07	8.54 ^{ab} ± 1.09	7.31 ^{ab} ± 1.57	13.26 ^{a} ± 3.56	
RDA	%	NS	1.36 ^{b} ± 0.63	117.10 ^{b} ± 31.20	231.20 ^{b} ± 53.40	579.00 ^{a} ± 106.00	0.00
		Foliar	0.46 ^{b} ± 0.13	15.54 ^{ab} ± 1.98	13.30 ^{ab} ± 2.85	24.10 ^{a} ± 6.48	
HQgv		NS	0.00 ^{b} ± 0.00	0.16 ^{b} ± 0.04	0.32 ^{b} ± 0.07	0.79 ^{a} ± 0.15	0.00
		Foliar	0.00 ^{b} ± 0.00	0.02 ^{ab} ± 0.00	0.02 ^{ab} ± 0.00	0.03 ^{a} ± 0.01	

application method (Table 3). The%RDA also increased in response to an increase in Se concentration; in the NS application method, it increased by 86-, 170- and 427-folds at 10, 20 and 40 μM Se concentrations compared to the control respectively. In the foliar application method,% RDA increased by 34-, 29- and 52-folds at 10, 20 and 40 μM Se concentrations compared with the control, respectively. In NS treatments, no statistically significant differences were found between 10 and 20 μM Se concentration and the control ($p > 0.05$), whereas 40 μM Se concentration showed significantly higher%RDA in comparison with the control ($p < 0.05$). Green vegetables' hazard quotient (HQgv) remained below the unity across all treatments (Table 3).

Values are expressed as mean ± standard error, $n = 3$. Different letters in the same row indicate significant differences at $p < 0.05$ for each application method, an application method marked as "ns" indicates no statistical significance (Tukey test with 95% confidence). *p-values* for the interaction effects of the application method and Se concentrations are presented in the last column for each parameter.

3.3. Nitrate, ammonium, and total nitrogen (TN)

Nitrate content was not significantly affected by the Se concentration in either foliar or NS application methods. In the case of foliar application, nitrate ranged from a minimum of 766.7 μg/ g DW in 20 μM to a maximum of 3600 μg/ g DW in the control treatment. In NS treatments, nitrate ranged from a minimum of 1480 in 20 μM to a maximum of 3233 in 40 μM. The application method only made a significant difference at 40 μM, where the NS method resulted in almost 3-folds higher nitrate content than the foliar method (Table 4).

Ammonium content was unaffected by the Se concentration in both NS and the foliar application methods (Table 4). In terms of the application method effect, ammonium content was only affected at 10 μM Se concentration where NS-treated microgreens showed a 5-fold higher ammonium content than the foliar application method ($p < 0.05$). Total nitrogen (TN) was not significantly affected by the Se concentration in the foliar application method. Across the NS application method, TN decreased by 9% at 20 μM Se concentration compared with the control but it was not statistically significant (Table 4). Total nitrogen (TN) was significantly affected by the application method, with the NS application method showing an average of 17% higher TN content than its other counterpart ($p < 0.05$) (Table 4).

3.4. Mineral composition

Results of mineral composition in Se-treated microgreens are shown in Table 5. Chloride content was not significantly affected by the Se concentration in neither of the NS and foliar application methods. However, the application method affected chloride content, where the foliar application method showed a 2- and 7-folds decrease compared to the NS application method at the control and 20 μM Se concentrations, respectively.

In the foliar application method, magnesium content ranged from 0.40 g/ 100 g DW in 20 μM Se concentration to a maximum of 0.49 g/ 100 g DW in control without statistically significant differences. In the NS application method, magnesium content was reduced by 14% at 20 μM Se concentration than the control (0.59 g/ 100 g DW, $p > 0.05$). The foliar application method reduced the magnesium content at 10 and 40 μM Se concentrations by about 21 and 24%, respectively, when compared to the NS application method ($p < 0.05$) (Table 5).

In the NS application method, potassium did not show any significant variations, ranging from a minimum of 2.2 g/ 100 g DW in 10 μM to a maximum of 3.2 g/ 100 g DW in 40 μM Se concentration. In the foliar treatments, potassium content was reduced by an average of 40% at all Se concentrations in comparison with the control but this reduction was not statistically significant (Table 5).

A significant interaction between Se concentration and application method was observered for sodium content ($p < 0.05$). In NS application method, sodium content increased as the Se concentration increased, with 40 μM Se concentration showing 2.3 folds higher sodium content than the control (Table 5). Unlike NS application method, foliar treatments did not significantly increase the sodium content (Table 5).

No significant differences were observed for sulphur content in terms of treatment levels within the NS and foliar application methods. However, sulphur content was significantly affected by the application method as it showed an average decrease of 36% in the foliar application method as compared with the NS application method ($p < 0.05$) (Table 5).

Boron ranged from a minimum of 32 μg/ g DW in control to a maximum of 26 μg/ g DW in 10 μM Se foliar treatment and from 23 μg/ g DW (10 μM Se) to 30 μg/ g DW (20 μM Se) in NS treatments. Overall, boron was not notably affected by se concentration in both application methods.

Zinc content was significantly affected by the application method. It showed a 19% decrease in the foliar application method at 20 μM Se concentration than the NS application method of the same concentration ($p < 0.05$). Zinc content in the NS application method ranged from 89.7 μg/ g DW in control to a maximum of 113.3 μg/ g DW in 20 μM Se concentration. In foliar treatments, it ranged from 91 μg/ g DW to a maximum of 105 μg/ g DW in 10 μM and control, respectively (Table 5).

Iron content slightly increased in the NS application method by almost 32% at 20 μM Se concentration while unaffected by the different foliar Se concentrations. Regarding the application methods, iron content was significantly higher in the foliar treatments by 57% and 33% at the control and 10 μM Se concentrations, respectively, compared with the same treatments in the NS application method ($p < 0.05$ in control and 10 μM).

The manganese content in the foliar treatments ranged from a minimum of 95.67 μg/ g DW in 10 μM to a maximum of 107.33 μg/ g DW in 40 μM. In NS treatments, manganese content ranged from a minimum of

Table 4
Nitrate, ammonium and total nitrogen content (on dry weight (DW) basis) subjected to different biofortification treatments. Treatments included the addition of selenium (Se) either to the nutrient solution (NS) or as a foliar spray at 0 (control), 10, 20 and 40 μM.

Parameter		Method	Se concentration (μM)				Se conc. × Method
			0.0 (Control)	**10**	**20**	**40**	***p-value***
Nitrate	mg/ 100 g FW	NS ^{ns}	10.76 ± 3.5	8.28 ± 3.32	6.84 ± 2.2	11.53 ± 2.16	0.54
		Foliar ^{ns}	17.3 ± 10.6	4.03 ± 1.11	4.135 ± 0.882	6.91 ± 1.1	
Ammonium	mg/ 100 g FW	NS ^{ns}	8.62 ± 1.86	7.78 ± 2.19	5.68 ± 1.98	3.547 ± 0.963	0.43
		Foliar ^{ns}	5.27 ± 2.82	1.793 ± 0.38	1.863 ± 0.584	3.127 ± 0.445	
TN	mg/ 100 g FW	NS ^{ns}	22.48 ± 4.02	22.82 ± 6.71	24.04 ± 0.884	20.36 ± 2.34	0.62
		Foliar ^{ns}	20.98 ± 2.27	19.86 ± 2.37	24.11 ± 4.11	26.23 ± 1.72	

Values are expressed as mean ± standard error, $n = 3$. Different letters in the same row indicate significant differences at $p < 0.05$ for each application method, an application method marked as "ns" indicates no statistical significance (Tukey test with 95% confidence). *p-values* for the interaction effects of the application method and Se concentrations are presented in the last column for each parameter.

Table 5
Macro- and micro-elemental content in kale microgreens (on dry weight (DW) basis) subjected to different biofortification treatments. Treatments included the addition of selenium (Se) either to the nutrient solution (NS) or as a foliar spray at 0 (control), 10, 20 and 40 μM.

	Parameter		Method	Se concentration (μM)				Se conc. × Method
				0.0 (Control)	**10**	**20**	**40**	**p-value**
Macroelements	Chloride	g/ 100 g DW	NS ^{ns}	0.27 ± 0.01	0.20 ± 0.09	0.43 ± 0.12	0.33 ± 0.11	0.28
			Foliar ^{ns}	0.15 ± 0.05	0.04 ± 0.03	0.06 ± 0.03	0.05 ± 0.01	
	Calcium	g/ 100 g DW	NS ^{ns}	1.40 ± 0.06	1.43 ± 0.15	1.33 ± 0.07	1.27 ± 0.03	0.34
			Foliar ^{ns}	1.23 ± 0.03	1.23 ± 0.03	1.20 ± 0.06	1.30 ± 0.00	
	Magnesium	g/ 100 g DW	NS ^{ns}	0.59 ± 0.04	0.57 ± 0.02	0.51 ± 0.02	0.55 ± 0.00	0.94
			Foliar ^{ns}	0.49 ± 0.01	0.45 ± 0.03	0.40 ± 0.04	0.42 ± 0.02	
	Phosphorus	g/ 100 g DW	NS ^{ns}	1.04 ± 0.11	0.97 ± 0.02	0.93 ± 0.05	1.06 ± 0.04	0.63
			Foliar ^{ns}	1.01 ± 0.10	0.88 ± 0.05	0.86 ± 0.08	0.85 ± 0.09	
	Potassium	g/ 100 g DW	NS ^{ns}	2.47 ± 0.50	2.17 ± 0.30	2.77 ± 0.67	3.20 ± 0.15	0.23
			Foliar ^{ns}	1.80 ± 0.31	1.06 ± 0.04	1.03 ± 0.10	1.17 ± 0.18	
	Sodium	g/ 100 g DW	NS	0.03 ^{c} ± 0.00	0.04 ^{bc} ± 0.00	0.05 ^{ab} ± 0.01	0.07 ^{a} ± 0.00	0.00
			Foliar ^{ns}	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	
	Sulphur	g/ 100 g DW	NS ^{ns}	1.80 ± 0.12	1.70 ± 0.10	1.60 ± 0.10	1.83 ± 0.07	0.95
			Foliar ^{ns}	1.17 ± 0.09	1.07 ± 0.08	0.97 ± 0.08	1.12 ± 0.10	
Microelements	Boron	mg/ kg DW	NS	25.33 ^{ab} ± 2.19	23.33 ^{b} ± 1.45	29.67 ^{a} ± 0.33	29.00 ^{ab} ± 0.58	0.13
			Foliar ^{ns}	32.00 ± 1.15	26.00 ± 0.58	29.33 ± 2.73	30.00 ± 0.58	
	Copper	mg/ kg DW	NS ^{ns}	7.97 ± 0.64	11.60 ± 2.75	18.76 ± 10.10	10.97 ± 1.76	0.49
			Foliar ^{ns}	7.07 ± 1.07	6.13 ± 0.26	6.07 ± 0.41	6.43 ± 0.38	
	Iron	mg/ kg DW	NS	146.67 ^{ab} ± 6.67	140.00 ^{b} ± 10.00	193.33 ^{a} ± 14.50	170.00 ^{ab} ± 10.00	0.06
			Foliar ^{ns}	230.00 ± 26.50	186.67 ± 3.33	190.00 ± 11.50	206.67 ± 18.60	
	Manganese	mg/ kg DW	NS ^{ns}	97.67 ± 0.33	113.33 ± 28.60	101.33 ± 8.67	76.67 ± 4.18	0.36
			Foliar ^{ns}	98.00 ± 11.90	95.67 ± 7.17	106.67 ± 14.50	107.33 ± 8.19	
	Zinc	mg/ kg DW	NS ^{ns}	89.67 ± 15.60	107.33 ± 22.00	113.33 ± 6.67	94.33 ± 3.18	0.38
			Foliar ^{ns}	105.00 ± 10.40	91.00 ± 3.21	91.67 ± 3.28	98.00 ± 13.30	
	Molybdenum	mg/ kg DW	NS ^{ns}	0.90 ± 0.06	0.89 ± 0.06	1.30 ± 0.21	1.20 ± 0.06	0.02
			Foliar ^{ns}	0.87 ± 0.17	0.52 ± 0.02	0.56 ± 0.05	0.62 ± 0.03	

Values are expressed as mean ± standard error, $n = 3$. Different letters in the same row indicate significant differences at $p < 0.05$ for each application method, an application method marked as "ns" indicates no statistical significance (Tukey test with 95% confidence). *p-values* for the interaction effects of the application method and Se concentrations are presented in the last column for each parameter.

76.7 μg/ g DW in 40 μM to a maximum of 113.3 μg/ g DW in 10 μM. None of the Se concentrations affected manganese content compared to the control treatment.

This study showed a significant interaction between Se concentration and application method for molybdenum ($p < 0.05$). In NS treatments, molybdenum ranged from a minimum of 0.89 μg/ g DW in 10 μM to a maximum of 1.3 μg/ g DW in 20 μM Se concentration. In foliar treatments, molybdenum ranged from a minimum of 0.52 μg/ g DW in 10 μM Se concentration to a maximum of 0.87 μg/ g DW in the control. No significant differences were detected in molybdenum content in response to different Se concentrations.

Calcium, phosphorus, and copper concentrations were neither affected by the Se concentrations nor by the application methods from all the mineral elements analysed.

3.5. Multivariate data analysis

The PCA results from both biofortification methods, including NS and foliar application methods, are shown in 3. The PCA explained a total of 93.96 % (PC1 = 55.15 %; PC2 = 38.81 %) of data variability for the NS application method (Fig. 3A) and 85.28 % (PC1 = 63.94 %; PC2 = 21.34 %) for the foliar application method (Fig. 3B).

According to the factor loadings (FL) for the NS application method, variables such as iron (Fe) (FL= 0.26), molybdenum (Mo) (FL= 0.28), fresh yield (FY) (FL= 0.26) and SL (FL= 0.26) represented the PC1 on the positive side of the axis; while ammonium (NH4) (FL= −0.27),

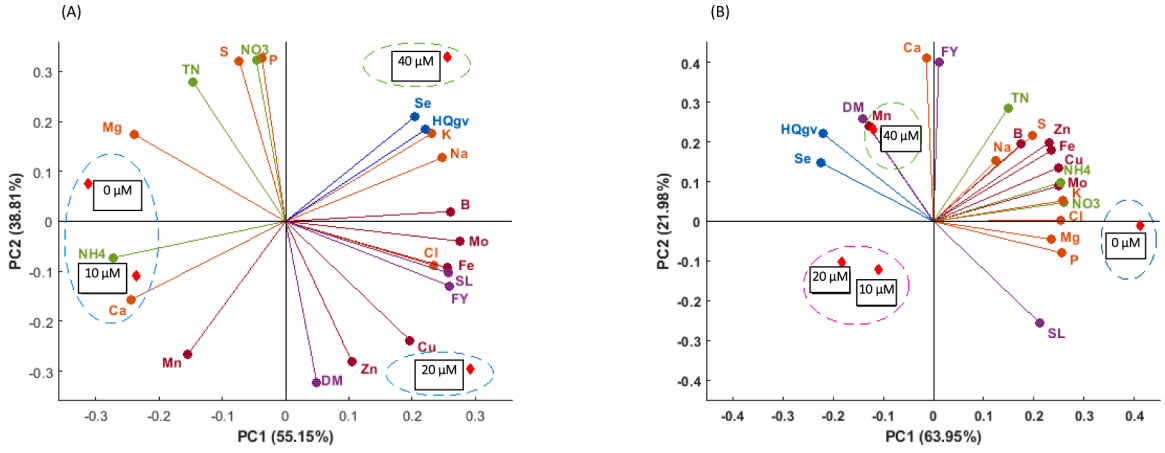

Fig. 3. Multivariate data analysis showing principal components analysis (PCA) biplot for the studied parameters including microgreens fresh yield (FY), dry matter content (DM), shoot length (SL), selenium content (Se), hazard quotient (HQgv), macroelements (potassium (K), sodium (Na), chloride (Cl), calcium (Ca), magnesium (Mg), sulfur (S) and phosphorous (P)) and microelements (boron (B), molybdenum (Mo), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn)) derived from (A)-nutrient solution (NS) and (B)- foliar biofortification methods. In graph (A) and (B) labels represent selenium treatments at 0 (control), 10, 20 and 40 μM.

calcium (Ca) (FL= −0.24) and magnesium (Mg) (FL= −0.24) characterized it on the negative side. On the other hand, PC2 was represented by nitrate (NO3) (FL= 0.33), sulphur (S) (FL= 0.32) and phosphorous (P) (FL= 0.32) on the positive side and by manganese (Mn) (FL= −0.27), zinc (FL= −0.28) and DM (FL= −0.32) on the negative side of the axis (Fig. 3A). Figure A also shows three distinctive groups for the NS treatments; 0 (control) and 10 μM Se concentrations were positively associated with Ca, NH_4 and Mg and negatively associated with Se, HQgv, K and Na. The second group, represented only by 20 μM Se concentration, was positively associated with Cu, Zn, DM, and FY and negatively associated with Mg, TN, S, NO_3, and P. On the other hand, the third group was represented by sample 40 μM Se concentration, which was positively associated with Se, HQgv, K and Na and negatively associated with Mn, NH_4 and Ca.

Based on the FL for the foliar application method, variables such as nitrate (NO3) (FL= 0.26), sulphur (S) (FL= 0.20), copper (Cu) (FL= 0.25), iron (Fe) (FL= 0.24), molybdenum (Mo) (FL= 0.25), ammonium (NH4) (FL= 0.25) and chloride (Cl) (FL= 0.25) represented the PC1 on the positive side of the axis; while Se (FL= −0.22), HQgv (FL= −0.22) and DM (FL= −0.14) characterized it on the negative side. On the other hand, PC2 was represented by Se (FL= 0.15), total nitrogen (TN) (FL= 0.29), manganese (Mn) (FL= 0.24) and calcium (Ca) (FL= 0.41) on the positive side while SL (FL= −0.26) represented it on the negative side of the axis. Fig. 3B also shows three separate groups for the foliar method. The control treatment (0 μM) had a positive association with P, Mg, Cl, NO3, K, Mo and NH4 and a negative association with Se. The 10 and 20 μM Se concentrations formed the second group and were negatively associated with Cu, Fe, Zn, S, B, Na, and TN. Sample 40 μM Se concentration was positively associated with Mn and DM and negatively associated with P (Fig. 3B).

The correlation matrix for both foliar and NS application methods showed that Se content was strongly and significantly positively correlated with Se/ serve ($r = 0.97$ and 0.99, respectively) (Fig. 4). A similar correlation was found between the Na and Se content in the NS application method ($r = 0.96$) (Fig. 4A). In contrast, no statistically significant correlation was found in the foliar application method for these two traits (Fig. 4B). Molybdenum (Mo) was positively correlated with Fe content in both foliar and NS application methods ($r = 0.98$ and 0.97 respectively) (Fig. 4A and B) and was only positively correlated with NO_3 ($r = 0.98$), NH_4 ($r = 0.98$), Cu ($r = 0.98$), Zn ($r = 0.96$), K ($r = 0.98$) and Cl ($r = 0.98$) in the foliar application method (Fig. 4B). Microgreens fresh yield (FY) showed a positive correlation with SL ($r = 0.97$), Fe ($r = 0.95$) and Mo ($r = 0.95$) content in the NS application method (Fig. 4A). In contrast, it did not show any negative or positive correlations with other parameters in the foliar application method (Fig. 4B). In the foliar application method, SL showed a negative correlation with Se/ serve (r= −0.97), HQgv, (r= −0.97) and%RDA (r= −0.97) (Fig. 4B).

4. Discussion

Several studies have investigated the effects of Se biofortification on different aspects of plant growth and yield. Previous studies have shown that yield was neither affected in fully grown basil plants grown in a hydroponic solution containing 4, 8 or 12 mg Se/ L (Martina Puccinelli et al., 2017) nor in the basil microgreens grown from Se-enriched seeds collected from the aforementioned basil plants (Martina Puccinelli et al., 2019). Similar results were reported for green and purple basil microgreens grown in a modified solution enriched with sodium selenate at 8 and 16 μM concentrations (Antonio A. Pannico et al., 2020). These findings are consistent with the results of this study, as shown in Fig. 2 and the correlation matrix (Fig. 4), which did not show any correlations between Se content and microgreens fresh yield, DM or SL.

In this study, kale microgreens showed a dose-dependant increase in their Se content in response to all Se containing treatments, leading to an elevated Se-content in their edible parts (Table 3). Due to the chemical structure similarity between Se and S, plant roots can uptake Se through their sulphate transporters and transfer it to the shoots through the xylem (Malagoli et al., 2015). It has been shown that mineral Se converts to its organic forms via the chloroplasts' regular S metabolic pathways (P J White et al., 2004). Selenium (Se) in the form of selenate appears to be transported via shared pathways with sulphate, whereas selenite is internalized by either sulphate, phosphate or possibly silicate transporters. Consequently, uptake of Se in the presence of phosphate and sulphate may be supressed while low availability of them might enhance the Se uptake by increasing the expression of sulphate and phosphate transporter genes (Li et al., 2008; Winkel et al., 2015). Being applied as sodium selenate in this study, selenium caused a high sodium content in the microgreens at 20 and 40 μM Se in the NS application. The latter explains the positive correlation between sodium and Se as shown in Fig. 4A and the significant interaction between Se concentration and the

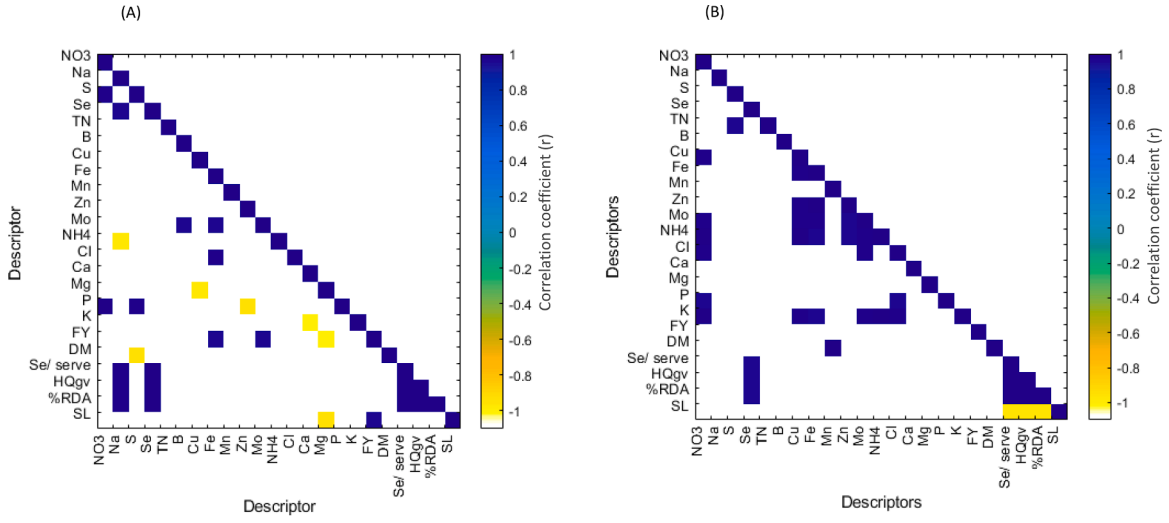

Fig. 4. Correlation matrix for the studied parameters including microgreens fresh yield (FY), dry matter content (DM), shoot length (SL), selenium content (Se), recommended daily allowance (%RDA), hazard quotient (HQgv), Se/ serve, macroelements (potassium (K), sodium (Na), chloride (Cl), calcium (Ca), magnesium (Mg), sulfur (S) and phosphorous (P)) and microelements (boron (B), molybdenum (Mo), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn)) derived from (A)-nutrient solution (NS) and (B)- foliar biofortification methods. Treatments included selenium (Se) biofortification at 0 (control), 10, 20 and 40 μM either through NS application (graph (A)) or foliar application (graph (B)). The colour bar shows correlation coefficients in the range of −1 to 1, where the blue side represents positive correlations and the yellow side denotes negative correlations.

application method for sodium content as shown in Table 5.

For a Se biofortification program to be considered successful, the target crop must be able to accumulate Se in its edible parts (Philip J White and Broadley, 2009). As compared to a recent study by Antonio A. Pannico et al. (2020), NS-treated kale microgreens in the current study performed exceptionally well, showing nine-, six-, two-, and three-folds higher Se content than coriander, tatsoi, green and purple basil microgreens grown in a modified NS containing 8 or 16 μM Se. Although not as successful as the NS-treated microgreens, foliar-treated microgreens also accumulated Se to a reasonable degree, reaching 39-folds higher Se content than the Se content reached in buckwheat microgreens grown from seeds enriched with Se by soaking in a solution containing 10 mg/ L of Se in either form of sodium selenate or sodium selenite for four hours (Germ et al., 2019). Less than five grams of fresh NS-treated kale microgreens at 20 μM Se concentration would be enough to meet the RDA requirement for an adult, whereas 42 g of the foliar-treated kale microgreens at 40 μM Se concentration would be required to meet the daily 55 μg Se intake (Table 3). Since microgreens are considered speciality crops, a small portion size of less than 10 g is more prevalent amongst the consumers. In addition, microgreens are often characterized by intense flavors, which may be considered overpowering when consumed in large quantities. Therefore, a 5–10 g serving size of the 20 μM Se NS-treated kale microgreens would be more suitable for day-to-day fresh consumption.

This study showed that, as with mature plants (Colla et al., 2018), the accumulation of anti-nutritional compounds, such as nitrate, occurs in kale microgreens (Table 4). Excessive nitrate consumption can harm human health, causing illnesses such as methemoglobinemia and blue baby syndrome (Ranasinghe and Marapana, 2018). Nitrate in the human diet is often sourced from fresh fruits and vegetables, particularly leafy greens. The PCA also showed that nitrate content was negatively associated with Se treatments, particularly in NS application at 20 μM (Fig. 3). Nitrate content in foliar Se treatments, at all concentrations, showed an average 71% reduction compared to the control treatment. NS-treated kale microgreens also showed an average 30% reduction in their nitrate content at 10 and 20 μM Se concentrations compared to the untreated microgreens. These findings follow the results reported by Antonio A. Pannico et al. (2020). The latter study showed that Se application at 8 and 16 μM in the NS reduced nitrate content by 24% in coriander, tatsoi, green, and purple basil microgreens. Se-prompted nitrate reduction has also been reported in green Salanova lettuce grown in soilless conditions where nitrate content was reduced by 15, 16 and 32% at 8, 32 and 40 μM Se supplied through the nutrient solution (Pannico et al., 2019). The reduction of nitrate content induced by Se biofortification can be explained by the antagonistic relation of nitrate and selenate (Juan J Rios et al., 2010) as well as enhanced activity of the nitrate reductase enzyme in response to Se (Nowak et al., 2004).

Macro- (K, P, Na, Ca and Mg) and micro- (Mn, Br, Fe, Cu and Zn) elements are essential for human health, and plant-based food is considered a good source of minerals that are essential for human health (Tiwari et al., 2012).

Results from this study showed that potassium was the most abundant macro element across all the treatments (Table 5), consistent with a previous study by Z. Xiao et al. (2016), which reported that potassium was the most abundant mineral element in 30 different varieties of *brassica* microgreens, including kale. Contrary to the results of the current study, which showed that different Se concentrations in both application methods had no significant effects on the potassium content, Antonio A. Pannico et al. (2020) showed that potassium content in green basil, grown in a nutrient solution containing 8 or 16 μM Se, increased by 39% and 40%, respectively. The same researchers showed that potassium content in tatsoi microgreens, grown in a nutrient solution containing 8 μM Se, decreased the potassium content by 30%. This accords with an average 40% decrease in potassium content observed in the foliar Se treatments in this study (Table 5). Similarly, a 9% reduction in potassium content has been previously reported for green butterhead lettuce as affected by 8.5 μg Se/ dm^3 NS treatment combined with iodine (Smoleń et al., 2016).

The 32% increase in the iron content at 20 μM Se NS treatments found in this study (Table 5) is consistent with the 60% increase in the iron content of lettuce also treated with 20 μM Se as selenate (Juan Jose Rios et al., 2013) and the 24% increase in coriander microgreens treated with selenate at 16 μM Se concentration (Antonio A. Pannico et al., 2020).

In both biofortification methods, the PCA biplot categorized the control treatment in a distinctive group from the Se treatments, further explaining the effectiveness of the Se biofortification methods employed in this study. In the PCA for the NS application method, 20 μM Se concentration showed a notably high positive association with fresh yield and most of the mineral elements tested while showing a highly negative association with nitrate content. This is in accordance with the findings of Antonio A. Pannico et al. (2020), who showed that overall macro-elemental concentrations and yield was improved in basil microgreens in response to Se biofortification at 8 μM. In contrast, Martina Puccinelli et al. (2020) showed elevated nitrate content in fully grown basil leaves enriched with Se at 12 mg/ L as NS application.

Also, no negative correlations were found between Se and other studied parameters in both foliar and NS application methods, indicating that Se biofortification did not compromise yield and mineral concentrations.

In the current study, selenization of microgreens was more effective in NS treatments than foliar treatments (Table 3). The latter may be explained by the small kale cotyledon foliar surface, limiting the Se receiving area and/or due to the foliar spray running off the target area. Foliar Se application has been known as a more efficient Se biofortification method than Se fertilization in soil-grown crops due to lower enrichment rates in the edible parts of the plant and the environmental concerns associated with the long-term use of Se fertilizers. Besides, Se might be less available in soil due to various parameters affecting its mobility and bioavailability, such as soil pH and ionic composition (Winkel et al., 2015). In contrast, having more control over the various parameters in a closed soilless growing system, including pH and ionic composition, the availability of Se to the plants can be optimized even to overtake the foliar biofortification method. Besides, the NS enrichment method is a one-time application, whereas the foliar method is more laborious and may require multiple applications to be as effective as the other counterpart.

5. Conclusion

Biofortification methods offer immediate benefits to overcome microelement deficiencies in populations with insufficient dietary intakes. In this study, kale microgreens proved to be great vehicles for carrying Se to human diets without compromising the fresh yield and quality of the kale microgreens. Enrichment of the NS with sodium selenate at 40 μM resulted in the highest Se content in the microgreens. However, the fortification at 20 μM was the most efficient treatment for increasing Se in kale microgreens with the collateral benefit of avoiding high sodium content. Although not as effective, foliar treatments also offered a reasonable increase in Se content. Improving the foliar delivery method by adding a surfactant to increase leaf surface adherence would deserve further research. The findings of this study could be beneficial for the development of new fortified products such as ready-to-eat salads and extracts, amongst others. Further research should be done to investigate the Se speciation, its bioavailability, and the effects of Se biofortification on sensory characteristics of kale microgreens.

Author contributions

MT, DG, GB, SF, and AP conceived and designed the experiment. MT and BW conducted the experiment, collected and analysed the data. MT wrote the first draft of the manuscript. DG, GB, AP, CV and SF assisted

with the data analysis and edited the manuscript. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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