

Original Research

Effects of selenium application on biochemical characteristics and biofortification level of kohlrabi (*Brassica oleracea* L. var. *gongylodes*) produce

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1. Abstract

Background: Biofortification of vegetables with selenium (Se) greatly depends on species tolerance to Se supply. Due to the scant information regarding kohlrabi Se biofortification, the aim of the present work was the evaluation of foliar sodium selenate application on yield and

biochemical characteristics of three kohlrabi cultivars. **Material and methods:** A two years field experiment was conducted in Moscow region (Russia) on 3 kohlrabi cultivars using foliar biofortification with Na₂SeO₄ solutions (50, 75 and 100 mg/L) and subsequent biochemical analysis of roots, stems and leaves. **Results:** Out of the three con-

centrations tested (50, 75 and 100 mg/L) plus an untreated control, the Se 75 dose demonstrated the strongest growth stimulation effect resulting in the increase of stem weight (by 1.35–1.61 times), yield (1.37–1.66 times), monosaccharide (1.59–2.24 times), ascorbic acid (1.54–2.01 times) and total phenolic levels (by 1.23–1.37 times), compared to the untreated control. The biofortification values varied from 69.4 (White Vienna 1390) to 59.9 (Dobrynya F₁ hybrid) and 43.6 (Sonata F₁ hybrid) under the Se dose of 100 mg/L. The maximum Se content in kohlrabi stems reached 4.40 mg/kg d.w. for Sonata F₁, 3.53 mg/kg d.w. for Dobrynya F₁ hybrids and 5.20 mg/kg d.w. for cultivar White Vienna 1390. Significant correlations were revealed between Se and total phenolics (0.720; $p < 0.002$), ascorbic acid (0.842; $p < 0.001$), monosaccharides (0.898; $p < 0.001$) and total sugar (0.764; $p < 0.001$). No significant changes in nitrate levels and dry matter content were recorded as the result of Se supply. **Conclusion:** The outcomes of the present research demonstrated the high benefits of Se application in improving kohlrabi yield and nutritional quality.

2. Introduction

Improvement of functional food yield is one of the most urgent aims of modern agriculture. In this respect, the practice of vegetable biofortification with essential macro- and microelements has been gaining an increasing popularity [1] due to the possibility to optimize human nutrition and stimulate plant growth and development. Among trace elements, Se is one of the most attractive ones, due to its significant beneficial effects on human health, providing a protection against viral and cardiovascular diseases, and cancer [2], also showing the ability to improve plant resistance to different forms of biotic and abiotic stresses [3]. Another benefit of agrochemical biofortification with Se is connected with the ability of plants to convert the most toxic inorganic Se salts to biologically active organic derivatives with remarkable health improving properties [3]. Furthermore, the significant sensitivity of plants to high Se levels makes them act as a buffer preventing the occurrence of human Se toxicosis. Such an approach is not simple, because each plant species has a certain tolerance degree to high levels of Se [4]. Being a chemical analog of sulfur, Se freely substitutes this element in biological systems, which makes Brassicaceae family a positive target of Se biofortification, due to the plant ability to accumulate high levels of sulfur. Furthermore, *Brassica* species are capable to synthesize powerful anti-carcinogens: selenomethyl selenocystein and γ -glutamyl selenomethyl selenocystein from inorganic Se forms [5, 6] and Se derivatives of glucosinolates [5]. Glucosinolates are considered to be biomarkers of *Brassica* vegetable species, demonstrating powerful antioxidant and anticancer properties [7]. Great differences of various Brassicaceae representatives in tolerance degree to Se supplementation were revealed for sprouts of kale,

Savoy, white and red cabbage, cauliflower, kohlrabi, turnip and broccoli [8], with the latter species showing a particularly positive reaction to Se biofortification [9]. Though scant data are available regarding the Se biofortification of kohlrabi, among Brassicaceae sprouts this species showed low tolerance to Se supply [8]. In hydroponic conditions, kohlrabi biofortification with Se revealed a high dependence on the sulfur content in nutritive medium [10]. An excellent investigation of selenium-iodine interaction in conditions of foliar supplementation, achieved with low dose of Se (23.6 mg/L) [11] resulted in the production of kohlrabi stems providing from 6.0 to 8.5% of recommended daily Se consumption level per 100 g of product. In the latter conditions, no significant changes in the kohlrabi yield were reported. Overall, up to date no information about the optimal Se doses and broad biochemical characteristics of kohlrabi subjected to Se supplementation is available.

The aim of the present research was the evaluation of the effects of different Se dose applications on yield and biochemical characteristics of three kohlrabi cultivars.

3. Materials and methods

3.1 Growing conditions and experimental protocol

A research was conducted in 2018 and 2019, from April to July, at the experimental field of the Federal Scientific Center of Vegetable Production, Moscow region, Russia (Moscow region, 55°39.51'N, 37°12.23'E), in a loam sod podzolic soil, pH 6.2, 2.12% organic matter, 1.32 mg-eq/100 g hydrolytic acidity, 18.5 mg/kg mineral nitrogen, 21.3 mg/kg ammonium nitrogen, sum of absorbed bases as much as 93.6%, 402 mg/kg mobile phosphorous, 198 mg/kg exchangeable potassium. The mean values of temperature (°C) and relative humidity (%) are presented in Table 1.

On 23 April, kohlrabi (*Brassica oleracea* var. *gongylodes*) seeds of the hybrids Sonata F₁, Dobrynya F₁, and White Vienna 1390 (a selection of the aforementioned Research Center) were sown in a peat substrate multi-cell tray. Four weeks after germination (29 May), plants with four true leaves were transplanted in open field, with 25 cm spacing between the plants along the rows which were 70 cm apart. The trials were carried out using a split-plot design for the treatment distribution in the field, with 4 replications, and the experimental unit contained 40 plants on a 7 m² surface area. Mineral fertilizers (N₁₆P₁₆K₁₆) were supplied twice: on 17 May (10-leaf stage) and on 3 June. At the beginning of May (stage of stem formation), on 1 June and at the stage of 6–8 leaves the plants were foliar sprayed with: (1) water (control), (2) 50 mg/L solution of sodium selenate (Se 50), (3) 75 mg/L solution of sodium selenate (Se 75), and (4) 100 mg/L solution of sodium selenate (Se 100), using the manual knapsack sprayer Solo with a spray rate of 300 L/ha. The latter treatments were practiced in the evening, in order to avoid leaf burning and solution evapo-

Table 1. Mean temperature and total rainfall in 2018 and 2019.

Month	2018		2019	
	Mean temperature (°C)	Rainfall (mm)	Mean temperature (°C)	Rainfall (mm)
May	16.2	61	16.3	57
June	17.3	56	19.6	64
July	20.5	92	16.8	69

ration from the leaf surface. At the harvest of kohlrabi, performed in the last week of July, in each experimental plot 10 plants were randomly sampled for biochemical analysis.

3.2 Marketable yield

Marketable yield was determined of undamaged and regular-shaped stems not exceeding 600 g weight.

3.3 Sample preparation

After harvesting and removing soil particles from roots and stems, the two latter plant parts were separated from each other, washed with water and dried with filter paper, and then individually weighed as well as the leaves. Samples were homogenized and fresh homogenates of stems were used for the determination of nitrates, total sugar, monosaccharides and ascorbic acid, while leaf homogenates were used only for the determination of ascorbic acid concentration. Sample aliquots of roots, stems and leaves were dried at 70 °C to constant weight and used for the determination of total polyphenols content (TP), total antioxidant activity (AOA) and selenium.

3.4 Dry matter

The dry matter was assessed gravimetrically by drying the samples in an oven at 70 °C until constant weight. The percentage of dry matter content was calculated according to the following formula:

$$\text{DM (\%)} = 100 \times W_2/W_1,$$

where W_1 is the fresh weight, W_2 dry weight, and 100 is the conversion of the results to percentage.

3.5 Water soluble proteins (WSP)

Water soluble protein levels were detected spectrophotometrically using the Bradford method based on utilization of Coomassie Brilliant Blue 250 and 0.05 M Tris buffer, at pH 8 [12]. Half a g of homogenized stem powder was accurately ground in a mortar with 15 mL of freshly prepared Tris buffer and left at room temperature for phases separation (about 1 h). One hundred μL of the resulting supernatant was mixed with 0.9 mL of Tris buffer and 3 mL of Coomassie reagent and the reaction mixtures were subjected to spectrophotometer determination of absorption value at 595 nm. Bovine albumin (Sigma) with concentrations of 0, 10, 20, 30 and 40 mg/mL was used to plot the calibration curve.

3.6 Nitrates

Nitrates were assessed using ion selective electrode on ionomer Expert-001 (Econix, Moscow, Russia). The results were expressed in mg/kg fresh weight.

3.7 Monosaccharides (SS)

The monosaccharides were determined using the ferricyanide colorimetric method based on the reaction of monosaccharides with potassium ferricyanide [13]. The total sugars were analogically determined after acidic hydrolysis of water extracts with 20% hydrochloric acid. The disaccharides content was calculated as a difference between total sugar and monosaccharides contents. Fructose was used as an external standard. The results were expressed in %.

3.8 Ascorbic acid

The ascorbic acid content was determined by visual titration of leaf and stem extracts in 3% trichloroacetic acid with sodium 2,6-dichlorophenol indophenolate solution (Tillmans reagent) [14]. Roots were not taken into consideration due to low ascorbic acid content. Three grams of fresh stem/leaves homogenates were homogenized in a porcelain mortar with 5 mL of 3% trichloroacetic acid and quantitatively transferred to a measuring cylinder. The volume was brought to 60 mL using trichloroacetic acid, and the mixture was filtered through filter paper 15 min later. The concentration of ascorbic acid was determined from the amount of Tillmans reagent that went into the sample titration.

3.9 Total polyphenols (TP)

Total polyphenols were determined in 70% ethanol extract using the Folin–Ciocalteu colorimetric method as previously described [15]. One gram of dry kohlrabi homogenates was extracted with 20 mL of 70% ethanol at 80 °C for 1 h. The mixture was cooled down and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL. The mixture was filtered through filter paper, and 1 mL of the resulting solution was transferred to a 25 mL volumetric flask, to which 2.5 mL of saturated Na_2CO_3 solution and 0.25 mL of diluted (1:1) Folin–Ciocalteu reagent were added. The volume was brought to 25 mL with distilled water. One hour later the solutions were analyzed through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA), and the concentration of polyphenols was calculated according to the absorption

of the reaction mixture at 730 nm. As an external standard, 0.02% gallic acid was used. The results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g d.w.).

3.10 Antioxidant activity (AOA)

The antioxidant activity of kohlrabi roots, stems and leaves was assessed using a redox titration method [15] via titration of 0.01 N KMnO_4 solution with ethanolic extracts of dry samples, produced as described in the 3.9 section. The reduction of KMnO_4 to colorless Mn^{2+} in this process reflects the quantity of antioxidants dissolvable in 70% ethanol. The values were expressed in mg gallic acid equivalents (mg GAE/g d.w.).

3.11 Selenium

Se was analyzed using the fluorimetric method previously described for tissues and biological fluids [16]. About 0.1 g of dried homogenized samples were digested via sequential heating with a mixture of 1.5 mL nitric-perchloric acids (10 : 7, v/v) at 120 °C (1 h), 150 °C (1 h) and 180 °C (1 h). To eliminate traces of nitric acid, the samples were heated during 10 min at 150 °C with 2 drops of 30% H_2O_2 . Subsequent reduction of selenate (Se^{+6}) to selenite (Se^{+4}) was achieved via heating of samples with 1 mL solution of 6 N HCl at 120 °C during 10 min. The formation of a complex between Se^{+4} and 2,3-diaminonaphthalene (DAN) was elicited at 53 °C (30 min) using 1 mg/mL solution of DAN in 1% HCl. After cooling, the obtained piazoselenol solution was extracted with 3 mL of hexane and the extracts were subjected to fluorescence analysis at 519 nm λ emission and 376 nm λ excitation (Fluorimeter 02-4M, Lumex marketing, St. Petersburg, Russia). Each determination was done in triplicate. The precision of the results was verified using a reference standard—lyophilized cabbage in each determination with Se concentration of 0.150 mg/kg d.w.

3.12 Biofortification level

Biofortification level (BL) was calculated according to the equation:

$$\text{BL} = C_1/C_2,$$

where C_1 — Se concentration in Se treated plants; C_2 — Se concentration in control plants.

3.13 Statistical analysis

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SPSS software version 21 (Armonk, NY, USA). Data expressed as a percentage were subjected to angular transformation before performing statistical processing: $Y = \arcsin \sqrt{p}$, where p is the original value and Y is the result of the transformation.

4. Results and discussion

4.1 Stem yield and plant biomass

Improvement of yield is considered to be a paramount issue in vegetable production. Out of the three kohlrabi cultivars tested, White Vienna 1390 was characterized by the highest stem biomass, while Dobrynya F_1 demonstrated the lowest one, despite the total plant biomass did not differ between the varieties (Table 2). The latter result suggests that the hybrids Sonata F_1 and Dobrynya F_1 showed significantly larger leaf biomass compared to White Vienna 1390. Marketable yield had a decreasing trend from White Vienna 1390 to Sonata F_1 and to Dobrynya F_1 , reaching 27.8 t/ha for cultivar White Vienna 1390, whereas the percentage of stem marketability was the lowest in White Vienna 1390.

Table 2. Interaction between selenium dose and cultivar on Kohlrabi biomass and yield.

Parameter	Treatment	Sonata F_1	Dobrynya F_1	White Vienna 1390
Total plant biomass (kg)	control	1.02d	0.98d	1.09d
	Se 50	1.18cd	1.16cd	1.24c
	Se 75	1.64a	1.48ab	1.76a
	Se 100	1.35bc	1.27c	1.41b
Mean stem weight (kg)	control	0.46e	0.39f	0.60cd
	Se 50	0.50de	0.42ef	0.67abc
	Se 75	0.74ab	0.61bcd	0.81a
	Se 100	0.53d	0.45c	0.67abc
Marketable yield (t/ha)	control	21.1efg	18.3g	27.8cd
	Se 50	23.5ef	19.9fg	31.2bc
	Se 75	35.0ab	29.0cd	38.1a
	Se 100	25.1cd	20.9fg	31.4b
Total yield (t/ha)	control	21.7ef	18.8f	28.7d
	Se 50	23.9e	20.2ef	31.8bc
	Se 75	35.3ab	29.2cd	38.4a
	Se 100	25.4de	21.2ef	31.9bc

For each parameter, values with the same letters do not differ significantly according to Duncan test at $p < 0.05$.

An outstanding feature of kohlrabi plants was the close relationship between stem yield and Se dose, the latter being applied at higher concentrations in the present research, compared to that used (23.6 mg/L) to kohlrabi sprouts by Golob *et al.* [11], i.e., 50–100 mg Na_2SeO_4 per L.

The data reported in Table 2 indicate that Se biofortification resulted in statistically significant increase of kohlrabi biomass, stem total and marketable yield and a significant decrease of non-marketable stem fraction (Table 2). In particular, the highest beneficial effect was recorded at the Se concentration level of 75 mg/L leading to stem weight increase of 61.0% for Sonata F_1 , 56.4% for Dobrynya F_1 and 35.0% for cultivar White Vienna 3390; the

Table 3. Quantitative parameters of kohlrabi plant parts as affected by cultivar.

Parameter	Plant part	F ₁ Sonata	F ₁ Dobrynya	White Vienna 1390
Dry matter (%)	Stem	7.84 ± 0.18b	8.78 ± 0.18a	9.19 ± 0.42a
	Leaves	12.93 ± 0.36b	14.5 ± 0.30a	14.10 ± 0.35a
	Roots	27.70 ± 1.60a	25.25 ± 1.15a	27.63 ± 1.53a
WSP (% d.w.)	Stem	2.20 ± 0.04a	2.09 ± 0.04b	2.12 ± 0.07ab
Nitrates (mg/kg f.w.)	Stem	190.0 ± 20.5a	162.3 ± 13.7a	207.5 ± 19.0a
	Leaves	142.5 ± 10.0a	131.7 ± 11.7ab	122.5 ± 7.0b

WSP, water soluble proteins; f.w., fresh weight; d.w., dry weight. Along each line, values with the same letters do not differ significantly according to Duncan test at $p < 0.05$.

Table 4. Interaction between Se dose and cultivar on sugar content in kohlrabi stems.

Parameter	Treatment	Sonata F ₁	Dobrynya F ₁	White Vienna 1390
Total sugars (% f.w.)	control	36.5a	30.1b	33.9ab
	Se 50	38.6a	34.9ab	36.2a
	Se 75	39.3a	36.2a	37.6a
	Se 100	40.4a	41.0a	39.5a
Monosaccharides (% f.w.)	control	16.4d	16.7d	13.8e
	Se 50	16.4d	19.7b	15.8de
	Se 75	17.7cd	22.2b	28.4a
	Se 100	28.2a	26.5a	30.9a
Disaccharides (% f.w.)	control	20.1a	13.4c	20.1a
	Se 50	22.2a	16.5b	20.4a
	Se 75	21.7a	14bc	9.2d
	Se 100	12.2c	14.5b	8.6d

For each parameter, values with the same letters do not differ significantly according to Duncan test at $p < 0.05$.

three cultivars showed the decrease of non-marketable yield by 2, 2.5 and 3.0 times respectively.

Though foliar application of 100 mg/L solution of sodium selenate resulted in lower kohlrabi yield than those produced by Se 75 solution supply, the values were higher than those obtained for control plants. Between the three cultivars tested, Sonata F₁ and Dobrynya F₁ showed similar changes both in total plant biomass and stem weight in Se-fortified plants. Contrary, White Vienna 1390 under Se 75 supply increased the total plant biomass by 61.0% and the stem weight only by 35.0%, whereas the aforementioned Se treatment increased the leaf biomass of White Vienna 1390 by almost 100%, and of Sonata F₁ and Dobrynya F₁ by 47.0% and 60.7% respectively.

The growth stimulation effect of Se has been described for many agricultural crops, indicating significant species and varietal differences in plant tolerance to high levels of Se [3]. The growth-promoting response to Se was demonstrated for some *Brassica* species, such as broccoli [17], canola [18], Indian mustard [19]. Foliar application of sodium selenate to broccoli [17] elicited a yield increase by 39% under 10 mg/L selenate solution and by 25% with 100 mg/L application. Foliar biofortification of Indian mustard with sodium selenate 50 mg/L resulted in 54% yield increase [19]. In the present research, the highest yield increase was recorded for Sonata F₁ and, to a lesser extent,

Dobrynya F₁ hybrids. Overall, the results indicate for the first time the high prospects of sodium selenate utilization for enhancing kohlrabi yield.

4.2 Content of dry matter, nitrates, water soluble proteins and sugars

Interestingly, Se application had no significant effects on dry matter content of stems, leaves and roots (Table 3), which is in accordance with the results previously obtained in broccoli treated with 0 to 100 mg Na₂SeO₄/L solutions [15–17]. Differently, Se biofortification often led to a significant dry matter increase in various plant species [20].

Se is closely connected with nitrogen metabolism, by stimulating the amino acid biosynthesis and increasing the nitrate reductase activity [21]. Indeed, selenate supply decreased nitrate levels and enhanced nitrate reductase activity in sunflower [22], Indian mustard [19], lettuce [23, 24], potato [25] and wheat [26]. Contrary, in the present research Se biofortification of kohlrabi did not affect nitrate levels (Table 3). The controversial aforementioned outcomes may be referred to the fact that nitrate accumulation under Se supply may greatly vary depending on plant hormonal status [27, 28]. In the latter respect, nitrate levels in spinach plants under sodium selenate treatment were reduced in female Se-fortified plants and increased in male ones [28], and the leaves/stems nitrate distribution

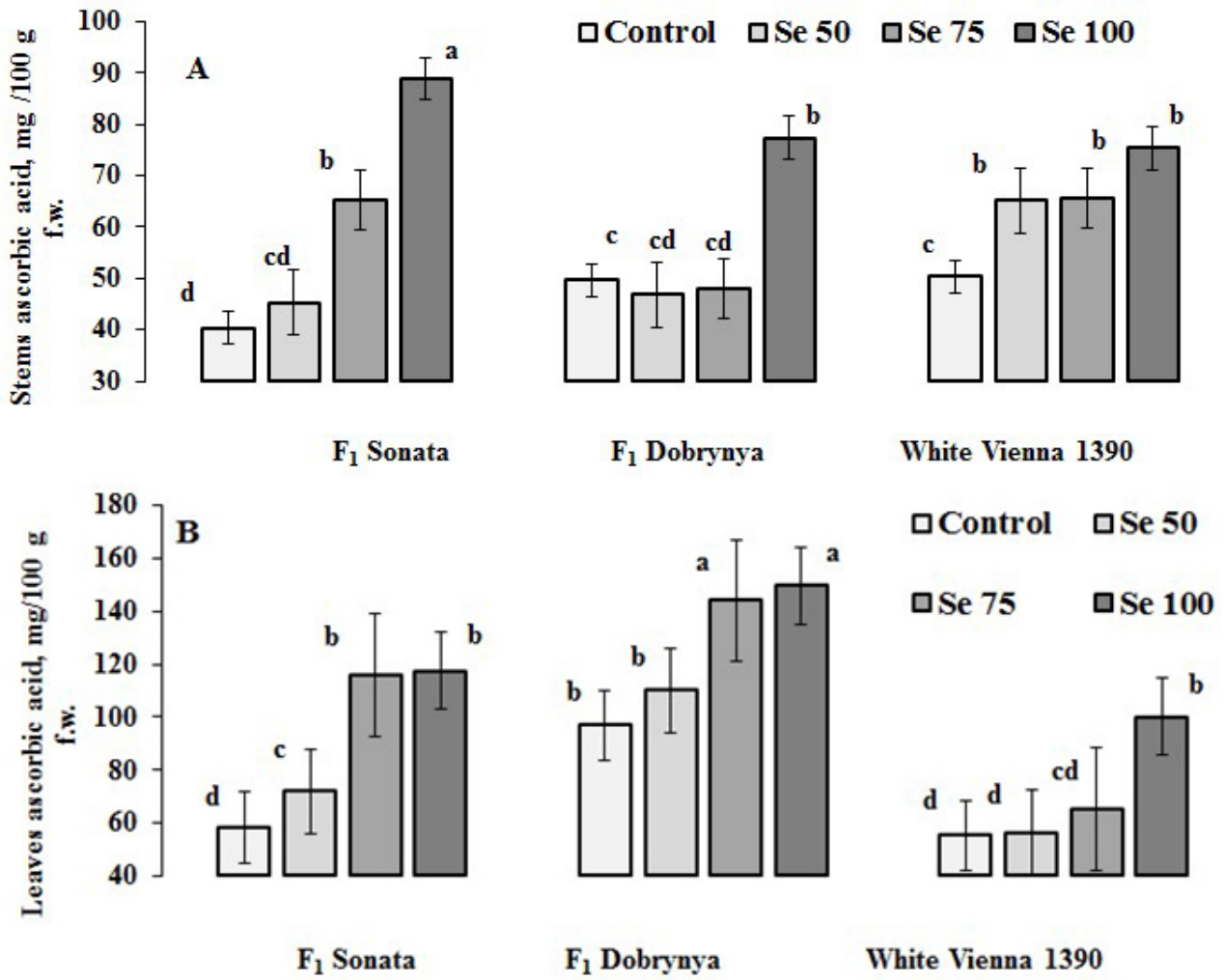


Fig. 1. Interaction between Se dose and cultivar on ascorbic acid content in kohlrabi. (A) Ascorbic acid content in stems. (B) Ascorbic acid content in leaves. Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$. Data are expressed as mean \pm SEM.

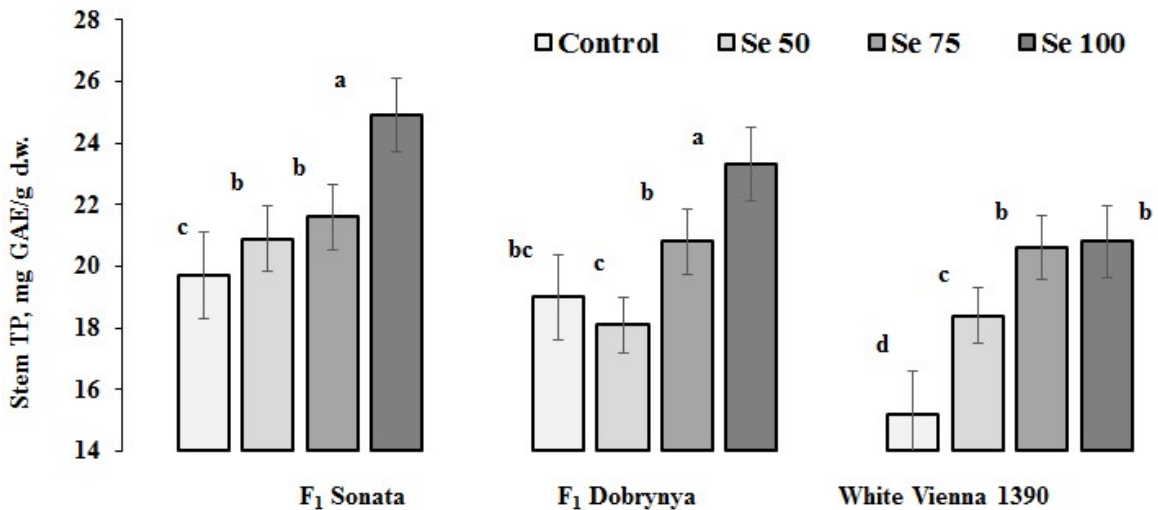


Fig. 2. Effect of Se dose and cultivar on total phenolics content in kohlrabi stems. Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$. Data are expressed as mean \pm SEM.

Table 5. Antioxidant status of kohlrabi plants as affected by cultivar (but not by Se).

Parameter	Plant part	F ₁ Sonata	F ₁ Dobrynya	White Vienna 1390
AOA (mg GAE/g d.w.)	Stems	22.0 ± 1.7a	22.5 ± 1.7a	19.4 ± 1.6a
	Leaves	42.3 ± 2.7a	32.3 ± 0.8b	31.8 ± 2.4b
	Roots	15.9 ± 1.0b	19.2 ± 1.8a	14.6 ± 1.0b
Total phenolics (mg GAE/g d.w.)	Leaves	22.7 ± 0.8b	20.0 ± 0.6c	23.8 ± 0.3a
	Roots	11.9 ± 0.6a	12.0 ± 0.4a	9.4 ± 0.9b

Along each line, values with the same letters do not differ significantly according to Duncan test at $p < 0.05$.

was rather similar to that recorded with the hybrids Sonata F₁ and Dobrynya F₁, i.e., 1.23–1.33, but was significantly higher in the cultivar White Vienna 1390 (1.69).

Furthermore, despite the described relationship between Se and amino acids metabolism [19–21] no significant effect of Se biofortification was recorded on water soluble protein accumulation in kohlrabi stems (Table 2), which was about 30% of the total protein level reported in literature [29].

The results of the present study indicate that in the open field conditions of European Russia the di/monosaccharides ratio in kohlrabi stems is in the range from 1.8 to 2.5, which is in agreement with the data of Ben Sassi *et al.* [29]. The beneficial effect of Se on carbohydrate metabolism was revealed earlier in potato [30], wheat [31] and canola [32]. Up to date, scant information is available on the effect of Se on carbohydrate accumulation in Brassicaceae species. The present results indicate the high stimulating effect of Se on sugar accumulation in kohlrabi stems (Table 4): at 100 Se dose the monosaccharides content increased by 1.72 times in Sonata F₁, 1.59 times in Dobrynya F₁ and 2.24 times in White Vienna 1390. At the highest Se dose, the disaccharides content decreased in Sonata F₁ and White Vienna 1390, contrary to the significant monosaccharides increase, which suggests that high concentrations of sodium selenate were able to partially stimulate the disaccharides hydrolysis in kohlrabi stems. Differently, Dobrynya F₁ hybrid showed an anomalous stability of disaccharides content under Se supply.

4.3 Plant antioxidant status

Several studies have shown that the appropriate concentration of Se reinforced the antioxidant defense system of plants [33–35]. In the latter respect, in broccoli the Se growth promoting effect is supposed to be related to the encouragement of plant protective ability against oxidative stress [17]. In the present research, among the antioxidants studied only the ascorbic acid (AA) levels were significantly enhanced by Se supply (Fig. 1A,B). Indeed, at the highest dose of Se (Se 100) the stem AA concentration increased by 2.2 folds in Sonata F₁, 1.56 folds in Dobrynya F₁ and 1.5 folds in White Vienna 1390.

Kohlrabi leaves showed higher levels of AA than stems, but the effect of Se application did not significantly

Table 6. Interaction between Se dose and cultivar on Se content in kohlrabi stems, leaves and roots (in mg/kg d.w.).

Plant part	Treatment	Sonata F ₁	Dobrynya F ₁	White Vienna 1390
Stems	Control	0.101g	0.059g	0.075g
	Se 50	1.085f	1.414e	0.789f
	Se 75	2.321d	1.703e	2.319d
	Se 100	4.400b	3.532c	5.206a
Leaves	Control	0.104e	0.083e	0.061e
	Se 50	2.921c	1.764d	2.689c
	Se 75	4.570b	2.131d	4.176b
	Se 100	6.911a	4.190b	7.399a
Roots	Control	0.132g	0.119g	0.108g
	Se 50	0.539f	1.331d	0.921e
	Se 75	1.086e	1.784c	1.859c
	Se 100	2.482b	2.672b	4.140a

For each plant part, values with the same letters do not differ significantly according to Duncan test at $p < 0.05$.

differ between leaves and stems (Fig. 1A). Indeed, a statistically significant increase in AA biosynthesis in Sonata F₁ and Dobrynya F₁ leaves was recorded in plants subjected to the treatments Se 75 and Se 100, by 2.0 and 1.54 times respectively, whereas White Vienna 1390 was able to increase the AA leaf level only upon Se 100 supply, by 1.8 times. These results are in agreement with the Se beneficial effect on ascorbic acid biosynthesis recorded in shallot [36] and broccoli sprouts [37]. Differently, no beneficial effect of Se on AA accumulation was recorded in mature broccoli [9], Indian mustard [19] and sprouts of alfalfa, radish and white mustard sprouts [38]. From the present research a significant Se beneficial effect on AA biosynthesis in kohlrabi arose, which is characteristic of this species with a decreasing intensity from Sonata F₁ to Dobrynya F₁ to White Vienna 1390.

Contrary, unexpected low changes in total antioxidant activity and total polyphenols content were recorded in stems, leaves and roots of kohlrabi plants (Table 5), and indeed only the total polyphenols in kohlrabi stems were significantly affected (Fig. 2).

4.4 Selenium accumulation

Se biofortified Brassicaceae plants provide two important types of utilization: (i) as natural sources of highly bioavailable organic Se; (ii) as products with high

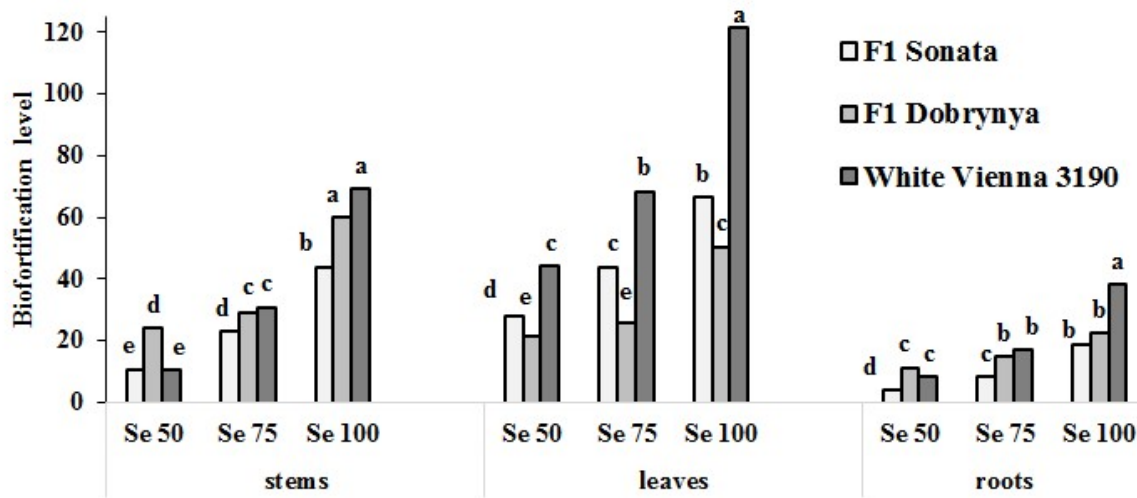


Fig. 3. Interaction between Se dose and cultivar on Se biofortification levels (BL) of kohlrabi plant parts. BL- biofortification level, indicating the intensity of Se accumulation by plant (see section 3.12). Within stems, leaves and roots, values with the same letters do not differ significantly according to Duncan test at $p < 0.05$.

Table 7. Correlations between the analyzed biochemical parameters of kohlrabi stems (n = 12).

	DM	NO ₃	Se	Weight	AOA	TP	AA	MS	TS
NO ₃	-0.437	1							
Se	-0.243	-0.176	1						
Weight	0.333	0.243	0.276	1					
AOA	-0.406	-0.206	-0.096	-0.438	1				
TP	-0.558	-0.234	0.720 ^d	0.016	0.385	1			
AA	-0.077	-0.297	0.842 ^a	0.330	-0.093	0.663 ^c	1		
MS	-0.021	-0.311	0.898 ^a	0.270	-0.181	0.659 ^c	0.728 ^d	1	
TS	-0.472	0.068	0.764 ^a	0.324	0.164	0.748 ^a	0.657 ^c	0.598 ^b	1
WSP	-0.141	0.064	-0.017	0.158	-0.032	0.274	0.225	-0.081	0.384

DM, dry matter; NO₃, nitrates; AOA, total antioxidant activity; TP, total phenolics; AA, ascorbic acid; MS, monosaccharides; TS, total sugar; WSP, water soluble proteins; ^a, $p < 0.001$; ^b, $p < 0.05$; ^c, $p < 0.02$; ^d, $p < 0.002$.

levels of powerful anti-carcinogens: methylated derivatives of Se containing amino acids and Se containing glucosinolates [5, 39]. Within the Brassicaceae family, broccoli and kohlrabi are among the most and the least studied species respectively [5]. Based on the comparison between the Se accumulation ability of different Brassicaceae seedlings, kohlrabi sprouts showed low Se tolerance, ranking the eighth based on the ability to accumulate Se after kale, white cabbage, red cabbage, cauliflower, savoy cabbage, broccoli and Brussels [8]. Contrary, the present results indicate high prospects of kohlrabi biofortification with Se, and the data presented in Table 6 and Fig. 3 suggest high Se accumulation levels in kohlrabi along with the Se growth stimulation effect (Table 2). Generally, the highest level of Se was recorded in kohlrabi leaves and the lowest in roots.

The consumption of 100 g of selenium biofortified kohlrabi fresh stems associated to the Se 100 treatment may provide from 31 to 48 μg Se which corresponds to 44.3–68.6% of the recommended daily Se intake of 70 $\mu\text{g}/\text{d}$ in Europe, according to the European Food Safety Authority [40]; the products obtained upon Se 75 application will provide from 26 to 30.4% of the adequate Se consumption level. These results indicate that both the aforementioned Se doses may be successfully applied to kohlrabi plants, thus providing a valuable functional food with high Se content, which may be highly useful in the program of the human Se status optimization. The varietal differences in Se accumulation shown in Fig. 3 demonstrate the significantly greater ability of cultivar White Vienna 3190 to concentrate Se, compared to the other two hybrids. The latter finding may be connected with the highest leaf biomass increase in White Vienna 3190 due to Se biofortification (Table 1), the latter promoting both the absorption and transition of Se from leaves to stems.

The leaves of biofortified kohlrabi may be used as Se-supplement to humans, due to higher antioxidant activity, ascorbic acid and Se content compared to stems, whereas biofortified kohlrabi roots may be suitable as a green Se containing fertilizer.

4.5 Correlations

Summarizing the results of kohlrabi Se biofortification, special peculiarities of kohlrabi plants can be highlighted (Tables 2,7): (i) the highly positive correlations between Se and monosaccharides, ascorbic acid, TP and TS contents; (ii) the significant beneficial effect of the Se 75 treatment on stem weight. The aforementioned outcomes prove the practical importance of kohlrabi biofortification with Se, which provides both high yield and quality enhancement.

Other positive correlations arose between TP and AA, MS and TS, AA and both MS and TS content.

5. Conclusions

The results of the present investigation allowed to reveal important peculiarities of kohlrabi Se biofortification, such as the increase of yield, monosaccharides and ascorbic acid content, as well as the existence of great variations between the Brassicaceae species in the reaction to biofortification. These outcomes suggest great prospects of biofortified stems and leaves utilization as important functional food with high Se and ascorbic acid content, as well as increased values of carbohydrates and polyphenols.

6. Author contributions

Conceptualization, NG, GC; data curation and formal analysis, MA, AT; investigation, NG, MA; methodology, NG, AT, AS; draft manuscript writing, NG, MA, GC; manuscript revision and final editing, NG, AS and GC. All authors have read and agreed with the final version of the manuscript to be published.

7. Ethics approval and consent to participate

Not applicable.

8. Acknowledgment

The authors are grateful to L. Bondareva for providing kohlrabi seeds and to all the peer reviewers for their opinions and suggestions.

9. Funding

This research received no external funding.

10. Conflict of interest

The authors declare no conflict of interest.

11. References

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Abbreviations: AOA, total antioxidant activity; TP, total polyphenols; AA, ascorbic acid; DM, dry matter; dw, dry weight; fw, fresh weight; MS, monosaccharides; TS, total sugar; WSP, water soluble protein; NO₃, nitrates; BL, biofortification level.

Keywords: *Brassica oleracea* var. *gongylodes*; Selenium; Proteins; Sugars; Nitrates; Antioxidants

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