

Effect of selenium fertilization on yield, quality, and organic selenium accumulation in pak choi (*Brassica rapa* L.)

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Abstract

This study aimed to evaluate the effects of different selenium (Se) fertilization methods on yield, quality, and organic Se accumulation in pak choi (*Brassica rapa* L.). A two-year small field experiment (2024–2025) was conducted with four treatments: control (CK), soil application (T1), foliar application (T2), and combined soil plus foliar application (T3). Results indicated that Se treatments significantly increased yield by 4.9–17.55%. Se concentration increased by 175.71–305.71% in root and by 202.81–314.08% in stem. Selenomethionine (SeMet) was the dominant organic form while selenocysteine (SeCys) also increased markedly. Crude protein, ash, and micronutrients (Fe, Zn, and Ca) were enhanced, especially under T3. Overall, the combined soil and foliar application (T3) proved most effective for improving yield, nutritional quality, and organic Se accumulation, offering a sustainable strategy for Se biofortification in leafy vegetables.

Keywords: Biofortification, *Brassica rapa* L., Nutritional quality, Selenium fertilization, Selenium speciation, Soil and foliar application

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Introduction

Pak choy (*Brassica rapa* L.) is one of the most widely consumed leafy vegetables in Asia due to its high nutritional value, short growth period, and stable yield. However, the micronutrient content of leafy vegetables, including pak choy, is often insufficient to meet daily dietary requirements among which selenium (Se) is considered an element playing an important role in human health. Historically, Se was once considered a toxic element until Schwarz and Foltz (1957) demonstrated its essentiality as a trace nutrient. Adequate Se intake has been reported to enhance immune responses and antiviral capacity (Arthur et al., 2003), whereas Se deficiency has been associated with more than 40 pathological conditions, including cancer, cardiovascular disorders, hepatic dysfunction, and impaired vision (Tapiero et al., 2003).

Se deficiency is particularly prevalent in vegetable growing areas in Asia due to low soil Se content, which increases the risk of malnutrition in the community. In certain regions of the world, such as China, Egypt, and Thailand (Tapiero et al., 2003), the intrinsic selenium (Se) concentration in soils is considerably low, leading to Se deficiency in human diets. In China, Se deficiency sometimes in severe forms has been reported in approximately 72% of counties, where the average daily Se intake among adults' ranges from 26 to 32 µg, which is markedly lower than the recommended daily intake of 50–200 µg (Gailer, 2009). However, upon exposure to suitable concentrations, selenium can be biotransformed into organic forms such as selenocysteine (SeCys) and selenomethionine (SeMet) (Zhu et al., 2009).

In Long Khanh City, Dong Nai Province, Vietnam, soil samples collected and analyzed in 2024 revealed Se concentrations ranging from 0.6 to 1.3 mg kg⁻¹ dry soil, which were below the WHO recommended threshold (Nguyen et al., 2025). Agronomic biofortification through Se supplementation in crops has been recognized as an effective and sustainable approach to improving dietary Se intake (Combs, 2001).

Several studies have shown that at low concentrations, Se not only can provide benefits to plants, especially under stress conditions (Hartikainen et al., 2000), but also help plants increase their resistance to adverse factors such as heavy metals, UV rays or drought (Proietti et al., 2013). The application of Se-containing fertilizers has been shown to enhance plant growth,

improve Se accumulation in the product, and limit adverse effects from fungal pathogens (Broadley et al., 2010). Recent investigations have indicated that foliar spraying of Se rapidly increases its concentration in edible tissues, whereas soil Se application provides a more stable Se source and contributes to yield improvement. However, studies evaluating the combined effects of soil and foliar Se application on leafy vegetables remain limited. Therefore, this study was conducted to assess the effectiveness of different Se fertilization methods on the growth performance and nutritional quality of pak choy. The results are expected to provide a scientific basis for developing Se biofortification strategies in leafy vegetables, thereby contributing to the alleviation of Se deficiency in Asian populations.

Material and Methods

Experimental site

Field experiments were conducted for two years (2/2024–2/2025) in Long Khanh City, Dong Nai Province, Vietnam (11.006234° N, 107.238112° E). The site is characterized by a tropical monsoon climate with an average temperature of 25–29 °C, annual rainfall ranging from 2,000 to 2,500 mm, and relative humidity 80–85%. The topsoil (0–20 cm) had pH 4.60 ± 0.58, available P₂O₅ 12.07 ± 5.49%, K₂O 14.84 ± 4.95%, organic matter 2.45 ± 0.69%, total N 0.13 ± 0.02%, and total Se 0.6–1.3 mg/kg dry soil.

Experimental design

Treatments were set up by single factor in a completely randomized design (CRD) with three replications. The factors were: CK (control), T1 (soil Se application), T2 (foliar Se application), and T3 (combined soil + foliar application). Seeds of *Brassica rapa* L. and sodium selenite (Na₂SeO₃ > 97%, Xinglu Chemical, Shanghai, China) were used.

Preparation of experimental soil

Fifteen days prior to sowing, the soil was plowed, cleared of debris, and divided into 20 plots (5 × 20 m² = 100 m²) to stabilize its physical and chemical properties. Because the soil at the experimental site exhibited a low pH (4.60 ± 0.58), lime (CaCO₃) was applied at a rate of 2.5 kg per 100 m² to adjust the pH to a favorable range (5.5–6.8) for pak choy (*Brassica rapa* L.) growth. Subsequently, each plot was fertilized with 300 kg of decomposed manure, 1.0 kg

of superphosphate (P_2O_5), 0.7 kg of urea (N), and 0.5 kg of potassium chloride (KCl) per 100 m².

Selenium application treatments

Treatment CK: control, no Se was supplemented. Treatment T1: 0.1 g of Se was accurately weighed, thoroughly mixed with 10 kg of dry sand, and uniformly spread over an area of 100 m². The mixture was lightly plowed into the top 0–15 cm of soil before sowing to ensure even Se distribution. Treatment T2: 0.1 g of Se was accurately weighed, dissolved in 10 L of clean water, and evenly sprayed over the foliage of seventeen days old plants across the 100 m² plot using a handheld sprayer (Cali16S). Treatment T3: (Combined soil and foliar application): The procedure was conducted in the same manner as Treatments T1 and T2; however, selenium was simultaneously applied to the soil and sprayed on the leaves at a rate of 0.05 g for each application. The Se application rates were selected based on safe levels that do not induce phytotoxicity or environmental contamination (Broadley et al., 2006; Zhou et al., 2023). During the experimental period, the plots were watered daily to maintain adequate soil moisture while avoiding waterlogging that could cause root rot. The entire experimental procedure was repeated in 2025.

Sampling and analysis

Pak choi plants were randomly selected at five locations (1 m²/location) in each experimental plot (100 m²) and recorded the following parameters: fresh weight (g), plant height (cm) to evaluate the yield of each treatment. Then, the samples were labeled according to the research treatment, and transferred to the laboratory. They were washed again with distilled water, oven-dried at 70 °C to constant weight (approximately three days), ground, and analyzed for Se content and nutritional composition.

Selenium determination and speciation

Weigh 0.20–0.25 (g) of sample into a 25 mL PTFE decomposition tube with 9 mL HNO₃ and 3 mL HClO₄, heat at 130 °C, one hour, followed by 5 mL HF addition until the solution becomes clear or light yellow, then the tube was allowed to cool to room temperature. Subsequently, 10 mL of 6 M HCl was added to reduce selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}). The solution was diluted to volume with double-distilled water in a 10 mL plastic tube. The total selenium concentration was determined using an atomic fluorescence spectrometer coupled with a

hydride generation system (AFS-8220, Titan Instruments, Beijing, China).

Determination of selenium species by enzymatic extraction and HPLC–ICP–MS

Selenium speciation in pak choi (*Brassica rapa*) samples was determined following enzymatic extraction coupled with high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC–ICP–MS).

Enzymatic extraction

Approximately 0.5 g of freeze-dried sample powder was weighed into a 50 mL polypropylene tube. The sample was suspended in 10 mL of 30 mM Tris–HCl buffer (pH 7.5) containing 0.1% β-mercaptoethanol to prevent oxidation of seleno-amino acids. Protease XIV (4 mg mL⁻¹) was added to the mixture, and enzymatic hydrolysis was carried out at 37°C for 16 h in a shaking water bath (100 rpm). After digestion, the extract was centrifuged at 10,000 × g for 15 min, and the supernatant was filtered through a 0.22 μm PTFE membrane prior to chromatographic analysis.

Chromatographic separation

Selenium species were separated on a Hamilton PRP-X100 anion-exchange column (250 × 4.6 mm, 10 μm). The mobile phase consisted of ammonium carbonate (NH₄)₂CO₃ buffer (pH 9.0) with a linear gradient from 12.5 mM to 60 mM over 10 min, followed by a 5 min re-equilibration. The flow rate was maintained at 1.0 mL min⁻¹, and the injection volume was 100 μL. The separated selenium species including Se(IV), Se(VI), selenocysteine (SeCys₂), selenomethionine (SeMet), and Se-methyl-selenocysteine (MeSeCys) were detected by ICP-MS under standard operating conditions.

Instrumental conditions

The ICP–MS was operated in He collision mode to minimize polyatomic interferences, with RF power of 1550 W, nebulizer gas flow of 1.0 L min⁻¹, and plasma gas flow of 15 L min⁻¹. Quantification was achieved using external calibration with certified selenium standards (Se(IV), Se(VI), SeMet, and SeCys₂). Data acquisition and chromatographic integration were performed using instrument software.

Nutritional composition analysis

Crude protein, fat, ash, and fiber contents were determined following AOAC (2016) methods.

Determination of amino acid content in pak choy

Weigh 0.1 g of sample into a 20 mL pressure-resistant flask, followed by the addition of 10 mL of 6 M HCl and three drops of phenolphthalein. The solution was blown with nitrogen gas for 3 minutes, then hydrolyzed under drying conditions at 110 °C for 22–24 hours. The mixture was cooled, and double-distilled water was used to transfer the entire hydrolysate into a 50 mL PTFE tube. A 2 mL aliquot of the filtrate was placed in a rotary evaporator and dried with steam at below 60 °C. Subsequently, 2 mL of buffer solution (pH 2.2) was added, and the solution was filtered through a membrane filter. The filtrate was then centrifuged and analyzed for amino acid composition using an amino acid analyzer (Biochrom, Cambridge, UK).

Determination of micromineral content (Fe, Zn, Ca, Mn and Cu) in pak choy

The concentrations of Fe, Zn, Ca, Mn, and Cu were determined following the same analytical procedure as described for Se.

Statistical analysis

Data were evaluated based on mean values, statistically significant differences between groups

were evaluated by analysis of variance (ANOVA) based on least square difference (LSD) with a confidence level of $p < 0.05$ (Duncan's test). Pearson correlations were calculated to test the relationships with 95% confidence intervals, using IBM SPSS 20 software.

Results

Effects of selenium application methods on the growth of pak choy

Compared with CK, the yield of pak choy increased from 4.90% to 17.54%, in which the T3 treatment achieved the highest value (Table 1). This increase may be due to the effect of Se on promoting plant height and leaf area, leading to improved photosynthetic efficiency and biomass accumulation of plants. The results showed that when Se is used at appropriate doses, Se is not only an essential micronutrient for plants but also plays a positive role in plant growth and development. In particular, T3 treatment had a superior yield compared to the other treatments, with an increase of 17.54% (in 2024) and 17.55% (in 2025) compared with CK. This confirmed that the combined treatment of Se application to soil and foliar spraying (T3) increased yield without adversely affecting plant growth.

Table-1. Effects of selenium application methods on the growth of *Brassica rapa* L.

Year	Treatment	Height (cm)	Weight (g)	Yield	
				(kg fresh weight/ ha)	Increase compared to CK (%)
2024	CK	25.80 ± 0.10d	425.60 ± 0.30d	106.400,00	-
	T1	30.23 ± 0.40b	480.33 ± 0.58b	120.083,33	12.86
	T2	29.17 ± 0.29c	446.47 ± 0.50c	111.616,67	4.90
	T3	35.47 ± 0.81a	500.23 ± 0.40a	125.058,33	17.54
2025	CK	25.70 ± 0.10d	425.67 ± 0.21d	106.416,67	-
	T1	30.40 ± 0.36b	480.43 ± 0.51b	120.108,33	12.87
	T2	29.30 ± 0.26c	446.80 ± 0.72c	111.700,00	4.96
	T3	35.57 ± 0.74a	500.30 ± 0.36a	125.075,00	17.55

Note: Letters in the same column (a, b, c, d) indicate statistically significant differences between Se fertilization treatments ($n = 3$); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²)

Effects of selenium application methods on total selenium content and speciation of pak choi

Results in Table 2 show that in 2024, compared to the control (CK), the Se content in roots increased by 230.00% (T1), 175.71% (T2), and 301.42% (T3), while in stems it increased by 214.08% (T1), 202.81% (T2), and 311.26% (T3), with similar trends observed in 2025 (234.28% (T1), 178.57% (T2), and 305.71%

(T3); 215.49% (T1), 204.22% (T2), and 314.08% (T3)).

These results demonstrated that Se supplementation significantly enhanced the Se content in pak choi, with treatment T3 showing the greatest effectiveness in both 2024 and 2025. Notably, when Se was applied to the soil (T1), the accumulated Se content in roots was higher than that in leaves, whereas foliar application (T2) resulted in greater Se accumulation in leaves than in roots.

Table-2. Selenium content (mg/kg dry weight) in roots and shoots of *Brassica rapa* L.

Year	Treatment	Selenium concentration (mg/kg dry weight) in pak choi			
		Root	Compared to CK (%)	Shoot	Compared to CK (%)
2024	CK	0.070 ± 0.01d	-	0.071 ± 0.01d	-
	T1	0.231 ± 0.02b	230.00	0.223 ± 0.01b	214.08
	T2	0.193 ± 0.01c	175.71	0.215 ± 0.01c	202.81
	T3	0.281 ± 0.01a	301.42	0.292 ± 0.01a	311.26
2025	CK	0.070 ± 0.01d	-	0.071 ± 0.01d	-
	T1	0.234 ± 0.01b	234.28	0.224 ± 0.01b	215.49
	T2	0.195 ± 0.01c	178.57	0.216 ± 0.01c	204.22
	T3	0.284 ± 0.02a	305.71	0.294 ± 0.01a	314.08

Note: Letters in the same column (a, b, c, d) indicate statistically significant differences between Se fertilization treatments (n= 3); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²).

As shown in Figure 1, the total selenium (Se) concentration in *Brassica rapa* L. ranged from 0.19 to 0.29 mg/kg dry weight. Compared with the control (CK), all Se-treated groups (T1, T2, and T3) exhibited significantly higher total Se content ($P < 0.05$). The main species of Se in plants were SeMet, Se⁴⁺ and SeCys. The results indicated that in the CK treatment, Se exists primarily in the inorganic form (Se⁴⁺). In

contrast, SeMet accounted for the largest proportion, followed by Se⁴⁺ and SeCys in all treatments. These findings demonstrate that Se supplementation promoted the transformation of exogenous Se⁴⁺ into organic forms, particularly SeMet, thereby enhancing the efficiency of Se biofortification and the nutritional value of pak choi.

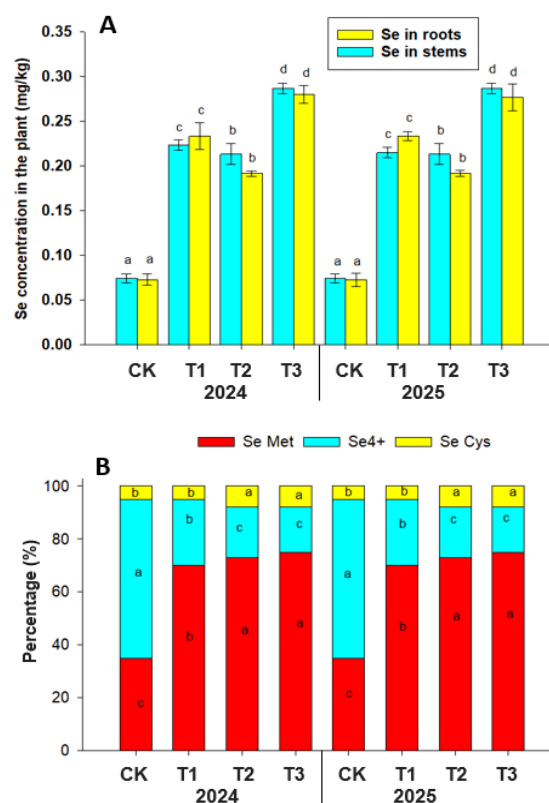


Figure-1. Total Se (A) content and Se (B) species in pak choi plants.

Note: Letters in the same column (a, b, c) indicate statistically significant differences between Se fertilization treatments ($n=3$); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²).

Effects of selenium application methods on nutritional value of pak choi

Crude ash and crude protein contents increased significantly ($P < 0.05$) in treatment T3 compared with CK. Specifically, crude protein and crude ash contents increased by 17.58% and 17.86% respectively in 2024, relative to CK, while they were 17.63% and 17.96% in 2025 (Table 3). These findings indicate that Se

supplementation enhanced the nutritional quality of pak choi, with the highest effectiveness observed in treatment T3. In contrast, crude fat and crude fiber contents showed no significant differences compared with CK. Furthermore, correlation analysis revealed that yield was significantly and positively correlated with crude protein, crude fat, and crude ash, but negatively correlated with crude fiber ($P < 0.01$).

Table-3. Nutritional composition of *Brassica rapa* L. under different selenium fertilization methods (g/kg dry weight).

Year	Treatment	Crude protein	Crude fiber	Crude ash	Crude fat
2024	CK	20.13 ± 0.15a	15.70 ± 0.30a	10.97 ± 0.06a	2.90 ± 0.17a
	T1	20.67 ± 1.00b	15.90 ± 0.46a	12.40 ± 0.36c	2.93 ± 0.12a
	T2	22.00 ± 1.15c	15.80 ± 0.35a	11.37 ± 0.51b	2.95 ± 0.05a
	T3	23.67 ± 0.58d	15.93 ± 0.65a	12.93 ± 0.12d	2.98 ± 0.03a
2025	CK	20.13 ± 0.10a	15.67 ± 0.31a	10.97 ± 0.15a	2.92 ± 0.12a
	T1	20.67 ± 0.90b	15.87 ± 0.42a	12.41 ± 0.44c	2.95 ± 0.08a
	T2	22.01 ± 0.91c	15.80 ± 0.35a	11.37 ± 0.55b	2.92 ± 0.13a
	T3	23.68 ± 0.64c	15.97 ± 0.60a	12.94 ± 0.06d	2.98 ± 0.03a

Note: Letters in the same column (a, b, c, d) indicate statistically significant differences between Se fertilization treatments (n= 3); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²).

Effects of selenium application methods on the amino acid composition of pak choi

Among the 19 amino acids analyzed, 14 amino acids including valine, isoleucine, methionine, phenylalanine, threonine, histidine, tyrosine, tryptophan, aspartic acid, serine, glycine, alanine, asparagine, and proline showed no statistically significant differences among treatments T1, T2, and T3 in both 2024 and 2025. In contrast, Se

supplementation in treatments T1, T2, and T3 led to significant increases ($P < 0.05$) in the levels of cysteine, leucine, lysine, glutamine, and glutamic acid. Among them, treatment T3 exhibited the highest increases, with values rising by 21.27%, 16.28%, 8.77%, 3.38%, and 3.94% in 2024, and by 23.40%, 18.56%, 8.77%, 3.38%, and 4.30% in 2025, respectively, compared with the control (CK) (Table 4).

Table-4. Amino acid composition of *Brassica rapa* under different selenium application methods (mg/g dry weight)

Year	Amino acid	CK	T1	T2	T3
2024	<i>Essential amino acid</i>				
	Valine	2.16±0.02 a	2.17±0.01 a	2.18±0.02 a	2.19±0.04 a
	Isoleucine	1.84±0.01 a	1.85±0.01 a	1.86±0.03 a	1.87±0.05 a
	Methionine	0.67±0.02 a	0.68±0.01 a	0.69±0.01 a	0.69±0.02 a
	Phenylalanine	1.94±0.01 a	1.96±0.02 a	1.96±0.03 a	1.96±0.03 a
	Threonine	1.74±0.01 a	1.75±0.02 a	1.75±0.03 a	1.76±0.04 a
	Histidine	0.49±0.02 a	0.50±0.02 a	0.51±0.05 a	0.52±0.06 a
	Cysteine	0.47±0.05 a	0.54±0.03 ab	0.52±0.03 ab	0.57±0.02 c
	Leucine	2.64±0.02 a	2.90±0.10 b	2.68±0.01 a	3.07±0.12 c
	Lysine	0.57±0.01 a	0.60±0.01 b	0.58±0.01 a	0.62±0.01 c
	Tyrosine	1.57±0.02 a	1.58±0.01 a	1.59±0.01 a	1.57±0.02 a
	Tryptophan	0.29±0.01 a	0.30±0.01 a	0.30±0.03 a	0.31±0.03 a
	Glutamine	0.59±0.02 a	0.60±0.01 b	0.61±0.05 ab	0.61±0.02 ab
	<i>Non-essential amino acid</i>				
	Aspartic acid	3.76±0.03 a	3.78±0.01 a	3.78±0.01 a	3.79±0.01 a
	Serine	2.07±0.02 a	2.08±0.02 a	2.08±0.02 a	2.09±0.01 a
	Glutamic acid	2.79±0.01 b	2.88±0.05 bc	2.82±0.05 ab	2.90±0.02 c
	Glycine	1.03±0.06 a	1.05±0.08 a	1.03±0.06 a	1.17±0.21 a
	Alanine	2.29±0.02 a	2.30±0.02 a	2.31±0.05 a	2.32±0.06 a
	Asparagine	0.72±0.03 a	0.73±0.03 a	0.74±0.03 a	0.75±0.05 a
	Proline	1.13±0.05 a	1.14±0.04 a	1.15±0.04 a	1.16±0.02 a
2025	<i>Essential amino acid</i>				
	Valine	2.17±0.01 a	2.18±0.01 a	2.18±0.01 a	2.20±0.03 a
	Isoleucine	1.84±0.01 a	1.85±0.01 a	1.86±0.03 a	1.87±0.05 a
	Methionine	0.67±0.02 a	0.68±0.02 a	0.69±0.01 a	0.70±0.03 a
	Phenylalanine	1.95±0.01 a	1.97±0.01 a	1.97±0.03 a	1.97±0.03 a
	Threonine	1.74±0.01 a	1.76±0.01 a	1.76±0.03 a	1.77±0.04 a
	Histidine	0.49±0.02 a	0.51±0.02 a	0.52±0.04 a	0.52±0.05 a
	Cysteine	0.47±0.05 a	0.54±0.04 ab	0.53±0.05 ab	0.58±0.01 c
	Leucine	2.64±0.02 a	2.90±0.10 b	2.69±0.01 a	3.13±0.12 c

Lysine	0.57±0.01 a	0.60±0.01 b	0.58±0.01 a	0.62±0.01 c
Tyrosine	1.57±0.02 a	1.58±0.02 a	1.59±0.01 a	1.60±0.02 a
Tryptophan	0.29±0.01 a	0.30±0.01 a	0.30±0.03 a	0.31±0.03 a
Glutamine	0.59±0.02 a	0.60±0.02 b	0.61±0.06 ab	0.61±0.02 ab
<i>Non-essential amino acid</i>				
Aspartic acid	3.76±0.03 a	3.78±0.03 a	3.78±0.01 a	3.79±0.02 a
Serine	2.07±0.02 a	2.09±0.02 a	2.09±0.01 a	2.10±0.02 a
Glutamic acid	2.79±0.01 b	2.88±0.05 bc	2.83±0.05 ab	2.91±0.04 c
Glycine	1.03±0.06 a	1.05±0.06 a	1.05±0.09 a	1.20±0.20 a
Alanine	2.29±0.02 a	2.30±0.02 a	2.31±0.05 a	2.32±0.06 a
Asparagine	0.72±0.03 a	0.74±0.03 a	0.74±0.04 a	0.75±0.05 a
Proline	1.13±0.05 a	1.16±0.05 a	1.16±0.05 a	1.18±0.01 a

Note: Letters in the same column (a, b, c) indicate statistically significant differences between Se fertilization treatments (n=3); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²).

Effects of selenium application on albumin and globulin content in pak choi

Table 5 shows that, compared with the control (CK), the globulin content in treatments T1, T2, and T3

increased significantly in both 2024 and 2025, with the highest effect observed in treatment T3 ($P < 0.05$). Between treatments T1 and T2, there were no statistically significant differences in albumin and globulin contents in either 2024 or 2025.

Table-5. Albumin and globulin contents of *Brassica rapa* L. under different selenium application methods (g/kg dry weight)

Year	Treatment	Albumin	Globulin
2024	CK	10.47±0.42a	4.67±0.58a
	T1	11.27±0.64a	5.33±0.70b
	T2	11.47±0.55a	5.37±1.01b
	T3	12.53±0.21a	6.10±0.56c
2025	CK	11.17±1.04a	4.80±0.35a
	T1	11.30±0.61a	5.37±0.71b
	T2	11.57±0.59a	5.57±0.67b
	T3	12.57±0.23a	6.13±0.61c

Note: Letters in the same column (a, b, c) indicate statistically significant differences between Se fertilization treatments (n=3); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²).

Effects of selenium application methods on mineral element composition in pak choi

The contents of Fe, Zn, and Ca in pak choi increased in all treatments compared with the control (CK), with the highest values recorded in treatment T3 ($P < 0.05$).

Specifically, in 2024, the increases in Fe, Zn, and Ca under T3 were 10.51%, 6.36%, and 10.28%, respectively, while in 2025, they were 11.33%, 7.36%, and 10.36%. In contrast, the contents of Mn and Cu in pak choi showed no significant differences among treatments compared with CK (Table 6).

Table-6. Concentrations of Ca, Mn, Fe, Zn, and Cu in *Brassica rapa* L. under different selenium fertilization methods (mg/kg dry weight).

Year	Treatment	Content (mg/kg)				
		Fe	Zn	Ca	Mn	Cu
2024	CK	80.20 ± 0.35 a	20.43 ± 0.51 a	10.70 ± 0.20 a	16.50 ± 0.50 a	3.36 ± 0.31 a
	T1	81.10 ± 0.17 b	21.13 ± 0.32 ab	11.40 ± 0.10 bc	16.60 ± 0.36 a	3.43 ± 0.20 a
	T2	80.73 ± 0.25 b	20.83 ± 0.21 ab	11.10 ± 0.36 ab	16.57 ± 0.40 a	3.39 ± 0.26 a
	T3	88.63 ± 0.28 c	21.73 ± 0.21 c	11.80 ± 0.10 c	16.77 ± 0.25 a	3.46 ± 0.14 a
2025	CK	80.21 ± 0.38 a	20.63 ± 0.85 a	10.71 ± 0.18 a	16.52 ± 0.50 a	3.37 ± 0.32 a
	T1	81.12 ± 0.14 b	21.27 ± 0.32 bc	11.41 ± 0.12 bc	16.62 ± 0.35 a	3.44 ± 0.21 a
	T2	80.75 ± 0.25 b	20.97 ± 0.40 ab	11.11 ± 0.38 ab	16.58 ± 0.40 a	3.41 ± 0.27 a
	T3	89.30 ± 0.56 c	22.15 ± 0.48 c	11.82 ± 0.07 c	16.78 ± 0.24 a	3.47 ± 0.15 a

Note: Letters in the same column (a, b, c) indicate statistically significant differences between Se fertilization treatments (n=3); CK (control); T1 (0.1g Se applied to soil/100m²); T2 (0.1g Se sprayed on leaves/100m²); T3 (0.05g Se applied to soil + 0.05g Se sprayed on leaves/100m²).

Discussion

The yield of pak choi increased significantly in treatment T3 compared with the control (CK) (Table 1). This effect was influenced by both the timing and method of Se application. When Se was applied to the soil (T1) at sowing, plants absorbed selenium continuously from the germination to maturity stages. In contrast, foliar spraying (T2) affected plants only from 15–17 days after sowing until harvest, thereby omitting several key developmental phases.

The combination of both methods (T3) maximized the advantages of T1 and T2, promoting greater plant height and leaf area, which enhanced photosynthetic efficiency, nutrient uptake, and biomass accumulation in pak choi.

The study by Li et al. (2021) on naked oats indicated that Se influences chlorophyll synthesis, photosynthetic regulation, and electron transfer during light absorption, thereby increasing chlorophyll content, enhancing photosynthetic capacity, and consequently improving yield. Similarly, Xue et al. (2001), Hartikainen et al. (2000) and Pedrero et al. (2008) reported that Se promotes plant growth by enhancing the activity of antioxidant enzymes, thereby reducing oxidative stress in plant cells, increasing leaf area and fresh weight, and ultimately contributing to higher crop yields. When selenium is absorbed at appropriate concentration, it can be biotransformed into organic forms such as selenocysteine (SeCys) and selenomethionine (SeMet) (Zhu et al., 2009). These compounds mitigate metal- or metalloid-induced oxidative stress by regulating reactive oxygen species

(ROS) metabolism including superoxide anion (O₂⁻), hydroxyl radicals (OH⁻), and hydrogen peroxide (H₂O₂)- through mechanisms such as scavenging excess reactive oxygen species, reducing lipid peroxidation, enhancing antioxidant enzyme activities, and preventing photosynthetic inhibition (Pandey and Gupta, 2015). Therefore, the appropriate application of Se is beneficial and can promote plant growth while enhancing resistance to various environmental stresses (Malik et al., 2012).

Malagoli et al. (2015) also noted that Se regulates gene expression related to plant growth and development, improving adaptability and nutrient uptake efficiency. According to Proietti et al. (2013), Se not only plays a key role in antioxidant defense and oxidative stress reduction, but also enhances plant tolerance to adverse conditions such as heavy metal toxicity, UV radiation, and drought. In addition, Broadley et al. (2010) demonstrated that the application of Se fertilizers can enhance plant growth, Se accumulation in plants, and resistance to fungal pathogens. In our study, Se application in pak choi provided essential nutrients to the soil, stimulated plant growth, and improved yield. Notably, treatment T3 showed higher productivity than T1 and T2, which may be attributed to synergistic effects between below-ground and above-ground plant parts following Se application. However, the specific mechanisms by which Se promotes growth and nutrient accumulation in plants require further investigation.

The T3 selenium biofortification treatment demonstrates strong potential for economic and environmental sustainability in smallholder farming systems. Applying Se at an optimal rate enhances crop

yield, nutritional quality, and antioxidant content while maintaining low input costs. Because Se is required only in trace amounts, the cost of supplementation remains negligible, making this approach highly affordable for resource-limited farmers. Furthermore, Se application can improve plant tolerance to abiotic stresses such as drought, salinity, and heavy metal exposure, thereby reducing potential yield losses and stabilizing income across growing seasons (Hawrylak-Nowak et al., 2015). Importantly, this treatment does not require major changes in cultivation practices or capital investment, allowing easy adoption within existing smallholder systems (Broadley et al., 2010). Overall, the T3 treatment combines agronomic efficiency with socio-economic feasibility, representing a sustainable biofortification strategy that can enhance smallholder productivity and contribute to food and nutritional security in developing regions (Schiavon and Pilon-Smits, 2017).

Results in Table 2 show that, in the foliar selenium application treatment (T2), the Se content in leaves was higher than that in roots ($0.215 > 0.193$). In contrast, when Se was applied to the soil (T1), Se accumulated more in the roots than in the leaves ($0.231 > 0.223$), with similar trends observed in 2025. These results can be explained as follows: When pak choi absorbs Se from the soil in the form of disodium selenite (Se^{4+}), Se is converted by the roots into selenate and transported to the shoots via sulfate transporters, before entering the chloroplasts in the leaves (Terry et al., 2000; Sors et al., 2005). Therefore, selenium tends to accumulate more in the roots under soil application. In contrast, when Se is applied directly to the leaves, it penetrates the vascular system without requiring root uptake or soil fixation, resulting in higher Se accumulation in the leaves than in the roots. According to Li et al. (2008), soil application of Se combined with fertilizers not only enables prolonged root uptake but also stimulates soil microbial activity, whereas foliar application allows Se to be absorbed directly through the leaf surface without translocation via roots. These findings are consistent with the results of our study.

Figure 1 shows that selenium (Se) supplementation in pak choi not only increases the total Se content but also promotes the transformation of Se into organic forms, with treatment T3 achieving the highest nutritional efficiency. Previously, the uptake of SeO_3^{2-} from soil was considered a passive process (Terry et al., 2000). However, recent studies have revealed that SeO_3^{2-} can

also be absorbed actively via phosphate transporters (Li et al., 2008). Once in the rhizosphere, SeO_3^{2-} reacts with glutathione (GSH) and is reduced to Se^{2-} , which then replaces sulfur (S) in essential sulfur-containing metabolites (including cysteine and methionine) due to their physicochemical similarity, leading to the rapid conversion of Se into highly mobile organic forms (SeCys and SeMet) that are subsequently transported to the aerial parts of the plant (Sors et al., 2005). Consequently, SeCys and SeMet are predominantly detected in the shoots of pak choi. SeCys and SeMet can be incorporated into selenoenzymes and selenoproteins, replacing Cys and Met after the reduction of SeO_3^{2-} (Navarro-Alarcon and Cabrera-Vique, 2008). SeCys is then further converted into other organic forms, namely SeMet and methyl selenocysteine (SeMeCys) and methyl selenomethionine (MeSeMet) by methylation (Eiche et al., 2015). Therefore, SeMet is thought to be more abundant than SeCys in pak choi shoots (Cubadda, 2010). However, Se has a very narrow biosafety range as its deficiency and toxicity thresholds are very close. Therefore, more studies are needed in the direction of precision agriculture to maintain Se content in plants at optimal levels, both ensuring the effective plant growth and avoiding the risk of soil pollution or adverse effects on human health.

The supplementation of Se significantly increased the crude protein and crude ash contents, thereby enhancing the nutritional value of pak choi (Table 3). This can be explained by the ability of Se to activate antioxidant enzyme systems and mitigate oxidative stress, which in turn promotes the synthesis of proteins and other organic compounds. These findings are consistent with those of Feng et al. (2013), who reported that Se can regulate the expression of genes related to nitrogen uptake and metabolism, contributing to protein accumulation in plant tissues and improving the overall nutritional quality.

In 2024, the crude protein content in the T1 treatment was lower than that in T3; however, by 2025, this difference was no longer significant. This may be due to the fact that a part of the Se applied to the soil was fixed in the solid phase, which reduced its uptake efficiency in the first year but maintained residual effects in subsequent years. The contents of crude fat and crude fiber showed no statistically significant differences among treatments, indicating that Se supplementation did not increase crude fiber levels, a beneficial factor for the sensory quality and edibility

of leafy vegetables, as excessive fiber can result in a tough and less palatable texture.

Se supplementation significantly increased the globulin content (Table 4), suggesting the role of Se in regulating protein synthesis and enhancing the activity of enzymes involved in essential amino acid biosynthesis, thereby improving the overall nutritional value of pak choi. The results demonstrated that Se fertilization, especially through the combined method of soil Se application and foliar spraying (T3), was considered an effective strategy to increase the content of high-biological-value protein in pak choi. This finding highlights the potential of Se biofortification as a promising agricultural approach to contribute to public health improvement.

As shown in Table 5, compared with the control (CK), Se application did not change the concentrations of 14 out of the 19 analyzed amino acids. This indicates that Se may not be directly involved in the biosynthesis of certain amino acids in pak choi. However, Se supplementation in treatment T3 significantly increased the levels of several important amino acids, including cystine, leucine, lysine, glutamine, and glutamic acid, with cystine showing the highest increase, reaching 21.27% in 2024 and 23.40% in 2025. The increase in Lysine and Leucine content is of practical significance, as these are two limiting amino acids in plant-based diets.

Selenium (Se) supplementation has been shown to regulate amino acid metabolism via the interaction between sulfur (S) and nitrogen (N) assimilation pathways. Because Se is chemically analogous to S, plants absorb selenate through sulfate transporters (SULTRs) and assimilate it along the sulfur pathway, forming selenocysteine (SeCys) that competes with cysteine in protein synthesis (Malagoli et al., 2015). At optimal levels, Se can stimulate nitrate reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT) activities, thereby enhancing the synthesis of glutamine and glutamate, two key amino acids in nitrogen metabolism (Zhou et al., 2024; Lyons et al., 2004). The increased levels of glutamine and glutamate provide substrates for the biosynthesis of other essential amino acids, including leucine and lysine, while Se's antioxidative role reduces oxidative stress and redirects carbon-nitrogen resources toward amino acid and protein synthesis. Therefore, the observed variations in cysteine, glutamine, glutamate, leucine, and lysine concentrations reflect Se's dual regulatory function in S assimilation and N

metabolism, highlighting its critical role in balancing redox homeostasis and primary metabolism in plants. Not all amino acids respond significantly to selenium (Se) supplementation, as the extent of metabolic alteration depends on each compound's position within the S-N-C metabolic network and the specificity of its biosynthetic enzymes. Amino acids such as valine, isoleucine, phenylalanine, threonine, tyrosine, histidine, and tryptophan are primarily synthesized through glycolytic and shikimate pathways, which are relatively independent of the sulfur (S) and nitrogen (N) assimilation routes directly influenced by Se (Malagoli et al., 2015; Wang et al., 2023; Zhou et al., 2024). Consequently, Se supplementation does not significantly alter the rate-limiting steps of their synthesis.

These results are consistent with the findings of Hasanuzzaman et al. (2020), who reported that the biochemical role of Se in mitigating oxidative stress and enhancing the activity of enzymes such as glutamine synthetase, nitrate reductase, and cystathionine- γ -synthase. These enzymes play central roles in nitrogen metabolism and the biosynthesis of sulfur-containing amino acids, particularly cystine. The observed increase in glutamic acid, an essential amino acid in the nitrogen metabolic network, also suggests that Se may promote amino acid biosynthesis by enhancing the availability of amino precursors. Therefore, Se supplementation not only improves the amino acid composition but also enhances the overall nutritional quality and protein value of pak choi, in which the T3 treatment achieved the highest efficiency.

The results of Table 6 show that, compared with CK, the T3 treatment significantly increased the Fe, Zn and Ca contents in pak choi ($P < 0.05$). This result indicates that Se supplementation through both soil application and foliar spraying represents the most effective approach to enhance mineral nutrient uptake in pak choi. In contrast, Se had no significant effect on Mn and Cu contents after two years of experimentation, suggesting that the influence of Se on mineral accumulation is selective and may be associated with ion transport mechanisms and element-specific interactions within plant tissues. These findings are consistent with those of Dinh et al. (2018), who demonstrated that Se not only improves agronomic performance but also enhances nutritional quality, particularly when combined with trace elements such as Zn and Fe, as well as beneficial soil microorganisms.

To ensure sustainable selenium (Se) enrichment in agricultural systems, continuous environmental monitoring is required. During soil Se supplementation, periodic soil testing should be conducted to quantify available and total Se levels, enabling appropriate adjustment of fertilization strategies. Maintaining Se within the optimal concentration range is critical to achieving biofortification goals without inducing phytotoxicity. Excessive Se accumulation can lead to soil contamination and potential toxicity for plants, livestock, and humans (Zhao and McGrath, 2009; Navarro-Alarcon and Cabrera-Vique, 2008). Therefore, Se application should be implemented under controlled supervision, integrating regular soil and crop analyses with good agricultural practices. Establishing threshold-based management protocols including rotational Se application, foliar over soil dosing when possible, and resting periods helps minimize environmental buildup while maintaining crop productivity. Such proactive monitoring frameworks are essential to support Se biofortification within a sustainable agriculture paradigm.

Conclusion

Among the three selenium (Se) fertilization strategies evaluated, the T3 treatment combining soil application with foliar spraying resulted in the greatest improvements in pak choi yield, as well as the highest total and organic Se accumulation and enhancement of key nutritional quality parameters.

For smallholder farmers, it is recommended to adopt low-dose foliar Se application at appropriate intervals rather than repeated soil supplementation. This approach, combined with routine soil monitoring after each growing season, can effectively prevent excessive Se buildup and maintain long-term soil health.

Future research should aim to: (1) Evaluate the spatial and temporal dynamics of Se accumulation and leaching under diverse soils and climatic conditions; (2) Investigate the long-term ecological impacts of Se on soil microbial diversity and trophic transfer within agricultural food chains; and (3) Develop integrated predictive models of Se–S–N cycling to refine application dosages and scheduling within sustainable cropping systems.

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Contribution of Authors

Thanh HN: Experimental design, data analysis and interpretation and manuscript writing.

Huong TM, Anh TTT, Pham T & Thanh HN: Experiment implementation and data collection.

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