

Effect and mechanism of exogenous selenium on selenium content and quality of fresh tea leaves

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Abstract

To study the effect and mechanism of selenium sources on the selenium content and quality of fresh tea leaves, tea seedlings (*Camellia sinensis* (L.) O. Kuntze) were the research object. A solution of 100 mg/L sodium selenate (Na₂SeO₄), sodium selenite (Na₂SeO₃), and selenium yeast (selenium yeast) were applied on the leaves surface of 5-week-old tea plants, and the selenium levels in the leaves, roots, and stems were determined at 20 weeks of age. The effects of different selenium sources on the mineral nutrient content, antioxidant enzyme activity, and quality parameters content in leaves were analyzed. The mechanism was analyzed by detecting the expression levels of related genes. The results showed that the three selenium sources can increase the growth of tea seedlings and the selenium content in leaves and stems, and the selenium yeast treatment had the most significant effect. Selenium spraying promoted the absorption of mineral nutrients such as nitrogen, phosphorus, and potassium, but had no significant impact on the absorption of calcium and magnesium. Spraying the three selenium sources dramatically increased the activities of APX, POD, and SOD antioxidant enzymes, among which the selenium yeast treatment had the most significant effect. However, there was no significant impact on the MDA level in this study. Selenium sources markedly increased leaves total amino acid levels, accompanied by up-regulation the genes of amino acid synthetic enzymes (*CsGS*, *CsGOGAT*, and *CsGDH*). Leaves glucose, tea polyphenol, total soluble protein, catechin, flavonoid contents, and sucrose were higher in selenium sources treatments than in control treatment seedlings. Moreover, selenium sources up-regulated expression of *CsHMGR*, *CsAPX*, and *CsTCSI* genes. selenium yeast had the best comprehensive effect of the three selenium sources. These results confirmed that selenium sources play a positive role on the selenium content and quality of tea by increasing the antioxidant capacity of leaves, the absorption rate of mineral nutrients, and regulating expression of related genes in *Camellia sinensis*.

Keywords: antioxidase; mineral nutrient; selenium; tea; the leaves quality

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Introduction

As a necessary microelement for humans, selenium is vital for health (Smits *et al.*, 2019; Gui *et al.*, 2022). About 0.5-1 billion people face the risk of selenium deficiency worldwide because of low selenium levels in their diets (Gui *et al.*, 2022). Selenium can strengthen the ability of oxidation resistance in the human body and also prevent some diseases, including hepatopathy, tumor, cardiovascular, and Kaschin-Beck disease (Wang *et al.*, 2017; Vinceti *et al.*, 2018). As an antidote to heavy metals, selenium can detoxify arsenic, lead, cadmium, and mercury in the human body; it can be discharged from the body by combining these heavy metals into compounds (Farooq *et al.*, 2019). As selenium is a mineral element, the human body cannot synthesize it and relies on food to obtain it (Gui *et al.*, 2022). Therefore, how to safely and effectively supplement selenium has become an urgent problem. Many studies showed that converting inorganic selenium into organic selenium for human absorption and utilization is an essential way for humans to safely supplement selenium (Zhou *et al.*, 2020; Gui *et al.*, 2022). In selenium transformation, plants have significant advantages, for they have a high selenium conversion rate and rich organic selenium content (Zhou *et al.*, 2020; Gong and Zhang, 2022). Especially, cultivated plants (such as broccoli) as a human food source can be used as simple and effective selenium carriers (Gui *et al.*, 2022; Zhang and Mao, 2022). Selenium-fertilizer can effectively increase the total selenium level in plants, and inorganic selenium can be bio-transformed into organic selenium, thereby satisfying the selenium absorption for humans (Bañuelos *et al.*, 2015). Na_2SeO_4 , Na_2SeO_3 , and selenium yeast are rapidly transformed into organic forms following absorption by roots and thus are extensively used as selenium fertilizer for plants (Gui *et al.*, 2022). Sodium selenite and selenium yeast raise the selenium level and nutritional quality in broccoli florets (Zhou *et al.*, 2018; Gui *et al.*, 2022). Sodium selenate and sodium selenite were applied on leaves of carrots, and the selenium level in carrots was significantly increased (Kápolna *et al.*, 2009). Exogenously added sodium selenate resulted in selenium-biofortified tomato fruits, with higher levels of kaempferol and chalcone (Schiavon *et al.*, 2013).

As a beverage made from its leaves after being processed, tea (*Camellia sinensis* (L.) O. Kuntze) is a large commercial crop (Shao *et al.*, 2019; Qiu *et al.*, 2020). With the improvement of consumption levels, healthy tea has attracted more and more attention in the market (Eliseev *et al.*, 2020). The production of selenium-rich tea supplements selenium from daily tea, which can become an essential means to improve the selenium level of the human body (Di *et al.*, 2021). Selenium-enriched tea cultivation is of great practical significance to further enhance its food quality (leaves glucose, total soluble protein, sucrose, catechin, tea polyphenol, total amino acid, and flavonoid contents), improve its health functions such as selenium-rich, and develop and produce selenium-enriched tea (Li *et al.*, 2021). Li *et al.* (2021) reported that nano-selenium dramatically enhanced the defense response of tea, the nutrients, and secondary metabolites protein (catechins contents, soluble sugar, carotenoid, and tea polyphenols). Hu *et al.* (2003) reported that selenite increased the vitamin C and amino acid levels in tea plants.

The food quality of tea is associated with enzymes in leaves, including glutamate dehydrogenase (*GDH*), glutamate synthase (*GOGAT*), and glutamine synthetase (*GS*) (Lin *et al.*, 2012). These enzymes participate in the pathway of amino acid biosynthesis (Lin *et al.*, 2012). Expressions of *GDH*, *GOGAT*, and *GS* play a vital role in the quality of fresh tea leaves (Qiu *et al.*, 2020). Li *et al.* (2021) reported that the synthesis and metabolism of proline, glutamic acid, arginine, and theanine can be regulated by the cycle of *GS-GOGAT* in tea plants. Exogenously added selenium can increase the secondary metabolism in tea leaves, thereby increasing the levels of flavonoids (quercetin, rutin, kaempferol, myricetin, and apigenin) and total phenols (Li *et al.*, 2021). However, it is not clear whether selenium sources regulate the relative gene expressions involved in antioxidant protection systems (such as ascorbate peroxidase biosynthesis), absorption of nutrients, and the food quality in tea plants.

We hypothesized that selenium sources could play a role in improving the selenium level in tea seedlings, in connection with the heightened absorption of mineral nutrients, the quality parameters content in leaves,

and antioxidant enzyme protection system. To confirm the hypothesis, 100 mg/L Na_2SeO_4 , Na_2SeO_3 , or selenium yeast in solution were applied on the leaf's surfaces of tea seedlings (*Camellia sinensis* cv. 'Xinyang 10'), and the selenium levels in leaves, roots, and stems were determined. The effects of the three selenium sources on mineral nutrient content, antioxidant enzyme activity, and quality parameters content in leaves were analyzed. The mechanism was analyzed by detecting the related gene expressions. This could be a new strategy and provide a theoretical foundation for future about selenium-fertilizer application in tea cultivation.

Materials and Methods

Experimental materials

Cloned seedlings of 'Xinyang 10' were provided by the Xinyang Agriculture and Forestry University. The seedlings were placed in sand (< 4 mm) on March 01, 2021, which had been sterilized in an autoclave (0.12 MPa, 121 °C, 1.5 h), and grown for 20 days (26/20 °C day/night temperature conditions) in the glasshouse of Xinyang Agriculture and Forestry University. Then the seedlings were transplanted into plastic pots (4.0 L) of a uniform size on March 21, 2021. Each pot contained 4.0 kg of autoclaved sand (0.12 MPa, 121 °C, 1.5 h).

Experimental design

A solution of purified water and Na_2SeO_4 (100 mg/L), Na_2SeO_3 (100 mg/L), and selenium yeast (100 mg/L) were applied on the leaves surface of tea plants on April 01, 2021. Each treatment was arranged in a completely randomized block design and replicated 6 times, which produced a total of 24 pots. They were sprayed once a week for 4 times, with each pot receiving 3 L. Each pot was irrigated with 150 mL Hoagland solutions (pH 5.2) every other day in soil. The basic culture Hoagland solution refers to the strategy of Zhang *et al.* (2018) (4.00 mmol/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 6.00 mmol/L KNO_3 , 2.00 mmol/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.00 mmol/L $\text{NH}_4\text{H}_2\text{PO}_4$, 45.00 $\mu\text{mol/L}$ H_3BO_3 , 9.00 $\mu\text{mol/L}$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.80 $\mu\text{mol/L}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.30 $\mu\text{mol/L}$ $\text{CuSO}_4 \cdot 3\text{H}_2\text{O}$, 0.14 $\mu\text{mol/L}$ H_2MoO_4 , and 52 mmol/L EDTA-Fe, pH 5.95-6.15). Na_2SeO_4 , Na_2SeO_3 , and selenium yeast were bought from Angle Yeast Co., Ltd. (Yichang, China).

Variable measurements

The samples were taken on April 29, 2021. The selenium content in leaves, roots, and stems of tea seedlings were detected by HG-AFS (hydride-generation atomic fluorescence spectrometry) according to (Gui *et al.*, 2022).

The levels of leaves phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined by inductively coupled plasma-atomic emission spectrometry according to Shao *et al.* (2021). The nitrogen (N) content in the leaves was determined by the Smartchem 200 (Yang *et al.*, 2018). The leaves of tea were immediately frozen at -80 °C when washed with deionized water for analysis of the concentration of MDA and the activities of POD, SOD, and APX, according to Gui *et al.* (2022). The quality of fresh tea leaves parameters contents (total amino acid, glucose, tea polyphenol, fructose, catechin, sucrose, total soluble protein, and flavonoid) were determined as described by Chen *et al.* (2009).

The relative expressions of glutamate dehydrogenase (*CsGDH*), glutamine synthetase (*CsGS*), glutamate synthase (*CsGOGAT*), tea caffeine synthase 1 (*CsTCSI*), 3-hydroxy-3-methylglutaryl coenzyme (*CsHMGR*), and ascorbate peroxidase (*CsAPX*) in leaves were analyzed by real-time quantitative PCR (qRT-PCR). The specific primers of these genes were designed using Primer Premier 5 based on the Tea Plant Information Archive (<http://tpdb.shengxin.ren/>) and are shown in Table 1. The extraction of RNA, reverse transcription of RNA, and the reactions of qRT-PCR were obtained as described by Qiu *et al.* (2020). The relative expressions of genes were calculated by the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001).

Statistical analysis

All the data were statistically analyzed with one way ANOVA by SAS (9.1.3) software to determine the significance between treatments (Duncan's multiple range tests, $P < 0.05$). Graphs were plotted by Sigmaplot software (14.0).

Table 1. The specific primers of relevant genes designed for real time quantitative PCR amplification

Gene name	Accession	Sequence (5'-3') - forward	Sequence (5'-3') - reverse
<i>CsHMGR</i>	KJ946250	CTCTTCCTCCTCCTCCTC	CTTTGTGCCCTTGGATAG
<i>CsAPX</i>	EU547804	TTCTATCAGTTGGCTGGAGT	ATGGTCACATCCCTTATCG
<i>CsGS</i>	JN602372	CTCAGAAGCAAAGCAAGGAC	ACATCAGGGTGGCTGAAAAT
<i>CsGDH</i>	JN602371	AGCGGCAAATCATCCTACT	TCGTCCCACATGAAACCTT
<i>CsGOGAT</i>	JN602373	GCTTCAGGACGTTTTGGTG	CATGATGTGGAGGTGGGGAT
<i>CsTCSI</i>	AB031280	TCCGTGTTATGTGATGGGAG	ATGATGTGGAGGTGGGGATA
<i>CsGADPH</i>	TEA003029	TGGCATCGTTGAGGGTC	CAGTGGGAACACGAAAAG

Results*The selenium content in tea leaves, roots, and stems*

As shown in Figure 1, the three selenium sources improved the growth of tea seedlings to varying degrees. The selenium content in leaves, roots, and stems were all increased with treatment by the three selenium sources. As shown in Table 2, compared with the control group, the selenium yeast treatment significantly increased the selenium content 1788.34% and 124.14% in leaves and stems, respectively. Na_2SeO_4 and Na_2SeO_3 treatments increased the selenium content in leaves 1391.09% and 1413.22%, and 105.81% and 110.16% in stems, respectively. Statistical analysis showed no effects of these three selenium sources application on root selenium contents.



Figure 1. Whole plants of *Camellia sinensis* 'Xinyang 10' seedlings treated by different selenium sources

Table 2. Effects of different selenium sources on selenium content in leaves, roots, and stems of *Camellia sinensis* 'Xinyang 10' seedlings ($\mu\text{g}/\text{Kg}$)

Treatments	Leaves	Root	Stem
CK	108.9 \pm 30.2 ^c	10.8 \pm 0.9 ^a	55.1 \pm 4.4 ^c
Na ₂ SeO ₄	1623.8 \pm 110.2 ^b	10.4 \pm 0.8 ^a	113.4 \pm 10.6 ^b
Na ₂ SeO ₃	1647.9 \pm 102.2 ^b	11.1 \pm 0.7 ^a	115.8 \pm 10.8 ^b
Selenium yeast	2056.4 \pm 109.7 ^a	11.6 \pm 1.0 ^a	123.5 \pm 11.4 ^a

Data (means \pm SD, n = 4) followed by different letters in the same column are significantly different at P < 0.05.

The mineral nutrient contents in tea leaves

The leaves were harvested to measure the levels of N, P, K, Ca, and Mg. The statistical analysis in Table 3 shows that compared with the non-treated plants, the contents of Ca and Mg had no significant changes with three selenium treatment sources. In contrast, selenium yeast observably increased the levels of N, P, and K by 11.72%, 32.81%, and 39.56%, respectively. Na₂SeO₃ had the same effect on N as selenium yeast with a slightly increase in the contents in P and K. The Na₂SeO₄ treatment only slightly increased the contents of P and K.

Table 3. Effects of different selenium sources on mineral nutrient contents in the leaves of *Camellia sinensis* 'Xinyang 10' seedlings (mg/plant dry weight)

Treatments	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)
CK	12.8 \pm 0.91 ^b	0.64 \pm 0.03 ^c	5.41 \pm 0.45 ^c	2.09 \pm 0.02 ^a	0.81 \pm 0.07 ^a
Na ₂ SeO ₄	13.5 \pm 0.89 ^{ab}	0.77 \pm 0.04 ^b	6.57 \pm 0.52 ^b	2.18 \pm 0.01 ^a	0.81 \pm 0.06 ^a
Na ₂ SeO ₃	14.1 \pm 0.58 ^a	0.76 \pm 0.03 ^b	6.61 \pm 0.55 ^b	2.17 \pm 0.01 ^a	0.82 \pm 0.08 ^a
Selenium yeast	14.3 \pm 0.92 ^a	0.85 \pm 0.05 ^a	7.55 \pm 0.69 ^a	2.18 \pm 0.01 ^a	0.83 \pm 0.07 ^a

Data (means \pm SD, n = 4) followed by different letters in the same column are significantly different at P < 0.05.

The concatenation of MDA and the activities of POD, SOD, and APX in tea leaves

Exogenous selenium modulated the concatenation of MDA and the activities of the main antioxidant enzymes (POD, SOD, and APX) in the leaves. As shown in Table 4, the three selenium sources had no significant effects on MDA. However, the exogenous selenium treatments, Na₂SeO₄, Na₂SeO₃, and selenium yeast notably increased the activities of SOD, POD, and APX by 33.33%, 33.97%, 58.33%, and 21.78%, 86.14%, 90.10%, and 7.19%, 8.10%, 19.98%, respectively, compared to the control. Results showed that these three exogenous selenium applications effectively increased antioxidant enzyme activity and strengthened the antioxidant capacity of tea, particularly the selenium yeast treatment.

Table 4. Effects of different selenium sources on activities of SOD, POD, MDA, and APX in the leaves of *Camellia sinensis* 'Xinyang 10' seedlings

Treatments	SOD (U/g)	POD (U/min.g)	MDA (nmol/g)	APX (U/g)
CK	1.56 \pm 0.09 ^c	0.101 \pm 0.007 ^c	25.35 \pm 2.11 ^a	601.55 \pm 52.56 ^c
Na ₂ SeO ₄	2.08 \pm 0.15 ^b	0.123 \pm 0.011 ^b	25.02 \pm 1.92 ^a	644.79 \pm 51.22 ^b
Na ₂ SeO ₃	2.09 \pm 0.13 ^b	0.188 \pm 0.014 ^a	24.97 \pm 2.21 ^a	650.29 \pm 60.58 ^b
Selenium yeast	2.47 \pm 0.15 ^a	0.192 \pm 0.015 ^a	25.42 \pm 2.04 ^a	721.72 \pm 62.23 ^a

Data (means \pm SD, n = 4) followed by different letters in the same column are significantly different at P < 0.05.

The leaves quality parameters content of tea

As shown in Table 5, the three selenium sources exhibited significantly higher contents of leaves glucose, fructose, sucrose, tea polyphenol, catechin, total soluble protein, and flavonoid than the control, and the selenium yeast treatment gave the best result, increased 233.07%, 364.26%, 104.96%, 35.14%, 134.03%,

109.91%, 111.57%, respectively. Except for total amino acids, only the selenium yeast treatment performed significantly differently with a 15.25% increase compared to the control.

The relative expressions of fresh leaves quality genes

As shown in Figure 2, the three selenium sources significantly up-regulated the relative gene expressions (*CsHMGR*, *CsAPX*, *CsGS*, *CsGDH*, and *CsTCSI*) in leaves, compared to the non-treatment seedlings, particularly the selenium yeast treatment increased them by 1, 1.7, 1.4, 0.8, and 1.6, respectively. The transcript levels of *CsGOGAT*, by Na_2SeO_4 , Na_2SeO_3 , and selenium yeast treatments were significantly up-regulated it by 1.1, 1.15, and 1.21 compared to the control group, respectively (Figure 2).

Table 5. Effects of different selenium sources on the fresh leaves' quality parameters content (mg/plant DW) of *Camellia sinensis* 'Xinyang 10' seedlings

Treatments	Sucrose	Fructose	Glucose	Tea polyphenol	Total amino Acids	Catechins	Flavonoid	Total soluble proteins
CK	50.8±4.6 ^c	23.5±1.9 ^c	82.6±7.7 ^c	3011±205 ^c	1639±152 ^b	52.6±4.4 ^c	6.66±0.4 ^c	22.13±1.9 ^c
Na_2SeO_4	111.2±10.1 ^b	81.1±6.8 ^b	105.3±8.7 ^b	3541±236 ^b	1771±155 ^{ab}	102.1±8.7 ^b	9.18±0.7 ^b	31.02±2.2 ^b
Na_2SeO_3	109.2±9.2 ^b	79.1±6.2 ^b	109.6±8.8 ^b	3555±245 ^b	1780±157 ^{ab}	103.5±9.8 ^b	9.24±0.8 ^b	31.57±2.4 ^b
Selenium yeast	169.2±14.1 ^a	109.1±8.7 ^a	169.3±14.1 ^a	4069±308 ^a	1889±166 ^a	123.1±10.2 ^a	13.98±1.1 ^a	46.82±4.1 ^a

Data (means ± SD, n = 4) followed by different letters in the same column are significantly different at P < 0.05.

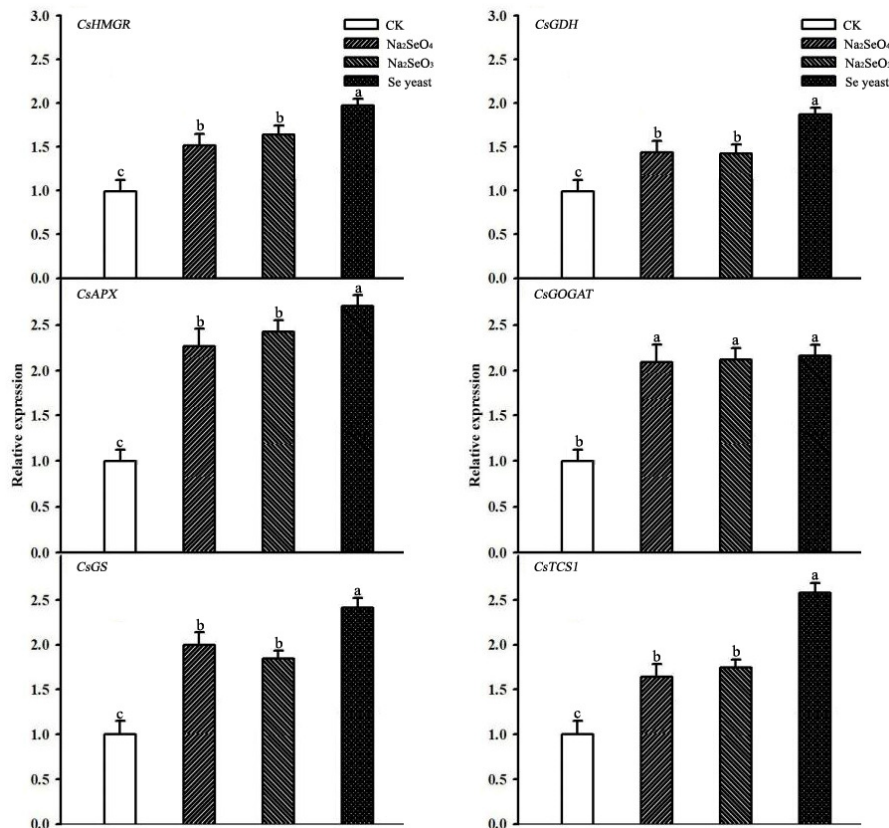


Figure 2. Effects of different selenium sources on relative expressions of leaves *CsHMGR*, *CsAPX*, *CsGS*, *CsGDH*, *CsGOGAT*, and *CsTCSI* in *Camellia sinensis* 'Xinyang 10' seedlings

Discussion

In horticultural crop cultivation, compared with soil application of selenium fertilizer, foliar spraying of selenium can reduce selenium loss and effectively increase selenium content in plants, which is a standard method to increase the selenium content in fruits or leaves (Babalar *et al.*, 2019; Meucci *et al.*, 2021). There are differences in the suitable foliar spraying of selenium for different horticultural crops. The spraying of lettuce with sodium selenite solution increased the selenium level in lettuce leaves, and also increased the net photosynthetic rate of leaves, transpiration rate, and stomatal conductivity, improved photosynthesis and antioxidant capacity, and promoted the growth and development of lettuce (Hawrylak-Nowak, 2015). Studies on cotton showed that selenium mineral fertilizer can advance the spitting period of cotton, improve the nutritional level, and promote the growth and development of its bolls (Zour *et al.*, 2009; Saleem *et al.*, 2020). The application of bio-selenium in the selenium-enriched tomato cultivation process can obtain higher selenium content in the fruit, and the fruit quality is also significantly improved (Zhu and Hai-Jun, 2018). Sodium selenite or sodium selenate can dramatically increase the selenium content in carrots by spraying leaves (Kápolna *et al.*, 2009). The level of organic selenium was significantly increased in tomato fruits when the supply of sodium selenate was via foliar spray (Schiavon *et al.*, 2013). Our results showed that foliar spraying of the three selenium sources (Na_2SeO_4 , Na_2SeO_3 , and selenium yeast) can significantly increase the selenium content in both tea leaves and stems, which is consistent with the results of (Liu *et al.*, 2021). Among them, the yeast selenium treatment has the best effect, which is consistent with the results of (Gui *et al.*, 2022). By contrast, the three selenium sources had no significant effect on the root selenium content of tea. So, selenium yeast can be widely used in the cultivation and production of selenium-rich tea.

Regarding the effect of selenium on the uptake of mineral nutrients in plants, our results showed that foliar selenium spraying promoted the absorption of N, P, and K by tea leaves, but had no significant effect on the content of Ca and Mg. Wen *et al.* (2022) showed that selenium spraying promoted the absorption of N and K in citrus leaves, but had no effect on Mg absorption, which is similar to the results of our study. However, Du *et al.* (2020) studied watermelons and showed that spraying selenium at a lower concentration can promote the absorption of Ca and Mg, while a higher concentration can inhibit the absorption of Ca and Mg. In addition, spraying selenium can promote the absorption of N and K. Why the results of this study are inconsistent with our results may be related to the concentration of selenium sprayed on the leaves surface. The relationship between selenium and mineral nutrient absorption in tea trees needs further study and discussion.

POD, SOD, and APX are scavengers of ROS in plants. They can remove excess ROS from plant cells during stress or aging, maintain the cellular metabolic balance, and improve plant resilience, which is related to the antioxidant capacity of plants. Malondialdehyde (MDA) is one of the essential products of membrane lipid peroxidation. The higher the content of MDA, the stronger the peroxidation degree (Lin *et al.*, 2019). Li *et al.* (2021) reported that nano-selenium dramatically enhanced the antioxidant system of tea. In the present experiment, the activities of SOD, POD, and APX in tea leaves sprayed with the three selenium sources were significantly higher than those in the water treatment. At the same time, the content of MDA was not very different, indicating that selenium can improve the antioxidant capacity of plants and protect the integrity of the cell membrane by removing MDA, a product of membrane lipid peroxidation. This is similar to the test results in strawberries: selenium in strawberries cannot only protect the integrity of the cell membrane, but can reduce the content of heavy metal ions, and effectively inhibit the absorption of heavy metals cadmium and lead in strawberry leaves and fruits (Zhang *et al.*, 2013). Furthermore, in our research, yeast selenium had the best effect on improving the activities of APX, POD, and SOD, and can be applied to strengthen the antioxidant capacity in the production and cultivation of tea.

Exogenous application of selenium fertilizer not only increases the selenium level in plants, but also regulates the growth and development of plants and affects the quality of fruits or leaves. Studies have shown that foliar spraying of sodium selenite can dramatically increase grape fruit quality, such as improving grape

single-grain weight, fruit horizontal and vertical meridians, Vc content, and selenium content, and can delay the senescence of fruits and leaves (Zhu *et al.*, 2017; Zhu and Hai-Jun, 2018). The selenium-enriched liquid fertilizer significantly increased the soluble solid content and single fruit weight of peach fruits (Zhang *et al.*, 2010). It made the peach peel less diseased, with good gloss, and increased the maturity period for 7 days (Zhang *et al.*, 2010). Ahmed *et al.* (2018) and Liao *et al.* (2021) showed that the use of foliar spraying of biological nano-selenium on citrus increased the soluble solid content of the fruit, and the fruit preservation period and shelf life can be extended by 15 days. Selenium-enriched tea showed better food quality in leaves, such as higher contents of glucose, sucrose, tea polyphenol, total soluble protein, flavonoid contents, total amino acids, and catechin (Li *et al.*, 2021). The vitamin C and amino acid levels of leaves can be markedly increased by selenite in tea (Hu *et al.*, 2003). These results are consistent with previous studies that the three selenium sources improved the quality of tea leaves to varying degrees, such as increasing of leaves sucrose, tea polyphenol, glucose, fructose, catechin, total soluble proteins, and flavonoid. Among the three selenium source treatments in this study, yeast selenium treatment had the best effect on improving tea food quality in leaves, which can be considered for application to tea plant production practice to improve the quality of fresh tea leaves.

In this study, selenium sources dramatically up-regulated the gene expression levels (*CsGOGAT*, *CsGS*, and *CsGDH*) in leaves. This is identical to the result reported by Qiu *et al.* (2020). However, Lin *et al.* (2012) showed that the expression of *GOGAT* was negatively associated with the level of theanine, the expression of *GS* was negatively related to the levels of lactamine, lysine, and theanine, and the expression of *GDH* was positively related to the level of theanine in tea. So, the change of amino acid components deserves further study. However, gene expressions (*GOGAT*, *GS*, and *GDH*) are related to the amino acid level (Lin *et al.*, 2012). As reported in this study, higher gene expressions (*CsGDH*, *CsGS*, and *CsGOGAT*) potentially promoted amino acid biosynthesis in tea. Therefore, it was concluded that selenium sources up-regulated *CsGOGAT*, *CsGS*, and *CsGDH* expression levels, to regulate the biosynthesis of amino acids. HMGR plays an essential role in the biosynthesis and metabolism of terpenes in plants, which is the first rate-limiting enzyme in the mevalonate pathway in plants (Li *et al.*, 2014). TCS1 catalyzes the N-3 and N-1 in methylation, and is the main enzyme in caffeine biosynthesis in tea (Jin *et al.*, 2016). Selenium sources up-regulated the relative gene expressions (*CsHMGR* and *CsTCS1*) in leaves, in the present study. It was concluded that selenium sources can up-regulate *CsTCS1* and *CsHMGR* to expedite the biosynthesis of caffeine and terpene, although caffeine and terpene were not tested in this study. APX, as the main enzyme removing reactive oxygen species, can improve the resistance in plants (Rakgotho *et al.*, 2022). Plants usually up-regulate the expression levels of one or more antioxidant enzyme genes, under adverse stress to strengthen the stress tolerance (Zhang *et al.*, 2017). The changes in leaves *CsAPX* expression of tea plants under selenium source treatments are in accord with the results of Xia *et al.* (2009) and Liu *et al.* (2021) in *Cucumis sativus* plants under low temperature stress. Hence, it can be concluded that selenium sources induce *CsAPX* expression to accommodate the stress resistance in plants.

Conclusions

The results showed that the three selenium sources can increase the growth of tea seedlings and the selenium content in leaves and stems. Selenium spraying promoted the absorption of mineral nutrients such as N, P, and K. The antioxidant capacity in leaves was improved, and the degree of peroxidation was reduced when sprayed with selenium sources. Furthermore, selenium source treatments significantly increased the food quality in leaves, accompanied by up-regulation of the related gene expressions (*CsGS*, *CsGOGAT*, *CsGDH*, *CsHMGR*, *CsAPX*, and *CsTCS1*). So, the selenium sources had positive effects on selenium content and the quality of fresh tea leaves through increasing the antioxidant capacity of leaves, the absorption rate of mineral

nutrients, and up-regulation of relevant gene expressions in *Camellia sinensis*. Finally, selenium yeast had the best comprehensive effect of the three selenium sources. In the future, the mechanism of selenium-rich in tea will be further studied from the perspectives of molecular biology, transcriptomics and metabolomics. Also, the botanists should pay more attention to the effect and mechanism of selenium on tea fresh leaves quality.

Authors' Contributions

Conceptualization; M.F.S. and Y.L.C., Data curation; J.J.W. and C.L.T., Formal analysis; M.F.S. and Y.L.C., Funding acquisition; P.Y. and M.F.S., Investigation; C.L.T., Project administration; J.J.W., Supervision; G.Y.G., Writing; M.F.S. and W.L. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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