Journal of Food Composition and Analysis 136 (2024) 106822

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Bio-fortification with selenium (Se) improves quality and nutrient profile in citrus fruit

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ARTICLE INFO

Keywords:
Biofortification
Selenium
Citrus
Application stage
Fruit weight
Fruit quality
Human health

ABSTRACT

Selenium (Se) is an essential nutrient for the human body. Humans can enhance Se absorption by consuming horticultural plants, particularly fruits. Therefore, there is need for Se biofortification in fruits to meet the human demand for Se. The objective of this study was to determine the optimal Se application rate, application stage and method, and to assess their impacts on improving both the quality and nutrient profile of a sweet orange cultivar 'Hongjiangcheng' (*Citrus sinensis* (L.) Osbeck). 'Hongjiangcheng' is a notable variety of mandarin orange, acclaimed for its large size, thin and smooth skin, orange-red flesh, tender and juicy texture, balanced sweetness and acidityand distinctive flavor. A field experiment was conducted at the Qiaotou Town Test Base in Chengmai County, Hainan Province, China. Biofortification of Se was done through foliar and soil application methods. Treatments were: Control (C) with no application of Se, foliar application of 25 (SeF1), 50 (SeF2), 100 (SeF3) and 200 (SeF4) mg/L and soil application of 100 mg/L (SeS1) as well as a combination of 100 mg/L Se in soil along with 50 mg/L Se on leaves (SeS1F2). At start of the experiment, 189 healthy, four-year-old citrus trees of similar size and normal growth were selected. The trees were divided into three groups of 63 each. Each group received the aforementioned treatments at the young fruit, expanding fruit and premature fruit stages. The Se was applied once during each stage before 9:00 am on sunny days. Treatments were arranged in a randomized complete block design with 9 biological replicates (7 treatments × 9 replicates = 63 citrus trees/ application stage). Foliar application of Se at rate of 200 mg/L (SeF4) enhanced total Se content in leaves by 105, 34 and 69 % at young fruit, expanding fruit and premature fruit stages compared to control ($p\leq0.05$). Respective increments in fruits were 264, 22 and 21 %. The total Se content in leaves were 32 and 40 % higher in the SeF4 (foliar) compared to SeS1 and SeS1F2 (soil) treatments across all development stages ($p\leq0.05$), respectively. The respective increments in fruits total Se content were 16 and 52 %. Only at the young fruit stage, the organic Se content in fruits was significantly higher in the SeF4 compared to soil application treatments ($p\leq0.05$). Single fruit weight was enhanced by 16.61, 13.69 and 4.36 g by foliar than soil application at young fruit, expanding fruit and premature fruit stages, respectively. It was significantly higher (155.53 vs. 113.13 g) after application of SeF4 treatment at young fruit stage relative to all other treatments ($p\leq0.05$). The application of SeF4 at the young fruit stage resulted in an increased seed rate (97 %), total soluble solids (28 %) and solid-to-acid ratio (75 %), while titratable acid decreased by 26 % compared to the control. Interestingly, Se application had non-significant effects on the fruit shape index, peel rate and residue rate across all stages ($p>0.05$). Additionally, a positive correlation was observed between fruit Se content and several quality indices like total soluble solids, solid acid ratio, fruit shape index and fruit weight at young fruit stage ($p\leq0.05$). It is concluded that foliar application of Se at 200 mg/L (SeF4) during young fruit stage improved citrus Se content, its fruit weight and quality indices establishing these fruits as a valuable source of Se-rich food for human consumption.

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https://doi.org/10.1016/j.jfca.2024.106822
Received 16 April 2024; Received in revised form 25 August 2024; Accepted 2 October 2024
Available online 5 October 2024

1. Introduction

Selenium (Se) is an important trace element contributing to human health through its pivotal roles in various physiological processes (Banuelos et al., 2023). It is a crucial component of antioxidant enzymes such as glutathione peroxidases that play a vital role in alleviating cellular damage caused by free radicals hence mitigating carcinogenic factors. Established guidelines of FAO and WHO recommend a daily Se intake of 60 μg per day for women and 70 μg per day for men, with the United Kingdom specifying a daily recommendation of 50 μg per day (Hao et al., 2022; Schöne et al., 2023). The Chinese Society of Nutrition has outlined a daily Se intake spanning from 78 to 400 μg per day (Yang et al., 2021). However, the recent daily intake of Se is falling below the recommended threshold of 60–70 μg per day, presenting a significant health concern, especially in developing Asian countries (Dijck-Brouwer et al., 2022; Qian et al., 2023). Similarly, half of China's agricultural land facing Se deficiency and around 60 % of the population consuming 40 % less Se, there is a potential increase in the risk of Keshan and Keshin–Beck diseases, an endemic heart condition with high case-fatality (Liu et al., 2021a). One of the options to increase human Se intake in foods is through biofortification which aims to enhance bioactivity and Se levels in the edible parts of plants.

Se biofortification is achieved through methods like soil application and foliar sprays, enhancing its content in edible parts of crops. Other techniques may include seed priming, genetic engineering and hydroponic systems for tailored Se enrichment. All methods of Se biofortification have shown positive results in increasing its content in edible parts of plants. However, among these methods, foliar application has proven to be the most effective (Izydorczyk et al., 2021). Foliar application, which involves spraying Se-containing solutions directly onto plant leaves, has demonstrated efficiency, especially in situations where Se uptake from the soil is challenging. Moreover, providing plants with Se through foliar application involves minimal use of Se salts, making it a convenient, cost-effective and safe method to enhance its content in edible crops (Djujić et al., 2000). Foliar spray of Se is proved to be effective for agronomic biofortification in cereals (wheat, rice, maize) (Lara et al., 2019, Lidon et al., 2019, Ngigi et al., 2019), vegetables (spinach, carrot, broccolini) (Moteshare et al., 2020, Rakoczy-Lelek et al., 2021, Poblaciones and Broadley, 2022) as well as in some fruits (apple, pear, strawberry) (Budke et al., 2021, Huang et al., 2023). Extensive research has been done on most of herbaceous crops on Se biofortification, however, research demonstrating the feasibility of enhancing Se intake in citrus through the consumption of selenized fruits lacks a comprehensive understanding (D'Amato et al., 2020; Danso et al., 2023).

Citrus, commonly known as sour fruit, is a major global fruit crop, cultivated in over 140 countries across tropical to subtropical regions, making it widely available and a valuable addition to the human diet (Russo et al., 2021; Volk et al., 2023). As citrus fruits have become one of the most extensively consumed fruit varieties globally, they are distinguished by their abundant provision of essential micronutrients and bioactive compounds, effectively meeting dietary nutritional needs (Foong et al., 2020; Suri et al., 2022). Considering the vital role of Se in human health, the biofortification of citrus fruits is crucial, especially considering their widespread availability and consumption (Sardar et al., 2022; Chen et al., 2023). Biofortified citrus enriched with Se can mitigate its deficiencies in humans, thereby elevating dietary Se levels and eliminating the potential risks associated with excessive Se intake from mineral supplements. There have been few studies investigating the influence of Se application on citrus fruits and detailed information regarding its effects on the nutrient profile is not thoroughly understood (Xieping et al., 2018; Wen et al., 2021).

The objective of this study was to determine the optimal Se application rate, application stage and method, and to assess their impacts on improving both the quality and nutrient profile of a sweet orange cultivar 'Hongjiangcheng' (*C. sinensis* (L.) Osbeck). It was hypothesized that foliar application of Se at young fruit stage to 'Hongjiangcheng' would increase fruit quality and plant Se content. 'Hongjiangcheng' is a distinguished variety of mandarin orange named after its place of origin in Hongjiangcheng Township, Guangdong Nongken Hongjiang Farm. In 1971, production technicians at Team 19 of Hongjiang Farm selected a mutant plant from the orange garden, which turned out to be a graft chimera mutation. This variety is recognized for its large size, thin and smooth skin, orange-red flesh, tender and juicy texture, balanced sweetness and acidity, and unique flavor. It is known as the "China Orange King" and the "State Banquet Fruit," making it a renowned new variety of mandarin orange in China. The present research is designed to establish an intellectual and scientific framework for the secure and efficient supplementation of Se rich content in citrus cultivation, establishing the groundwork for the growth of Se-rich citrus in Se-deficient areas.

2. Materials and methods

2.1. Chemicals and reagents

Sodium selenite (Na_2SeO_3) diluted in a 0.5 % surfactant solution (Assist®, BASF) was used to prepare various doses of Se. Selenite was used as their uptake is reported to be 1.5 and 5 times higher in canola and wheat than selenate (Kikkert and Berkelaar, 2013). Furthermore, Se exists as selenite in acidic soils (Gupta and Gupta, 2016). Given that our soil pH was 5.8, we used Na_2SeO_3.

2.2. Experiment location and treatments

A field experiment was carried out at the Qiaotou Town Test Base in Chengmai County, located in Hainan Province, China, at latitude 19.92°N and longitude 109.91°E. The study area has a tropical monsoon climate where mean monthly temperature ranged from 18.0 to 33.5°C and cumulative rainfall fluctuated between 20.6 and 315.6 mm during the experimental period (Fig. 1). The study was conducted on Hongjiangcheng citrus trees (*C. sinensis* (L.) Osbeck), planted with an individual tree spacing of 3.0 m and row spacing of 4.5 m, leading to a tree density of 750 plants/ha. The soil in the citrus orchard is classified as latosol and it is derived from granite. The soil has a pH of 5.8, EC of 52 mS/m organic matter content of 2.34 %, total nitrogen content of 0.11 %, available phosphorus content of 20.9 mg/kg, available potassium content of 400.0 mg/kg and a selenium (Se) content of 0.35 mg/kg in the 0–20 cm soil layer.

Biofortification of Se was done using both foliar and soil application

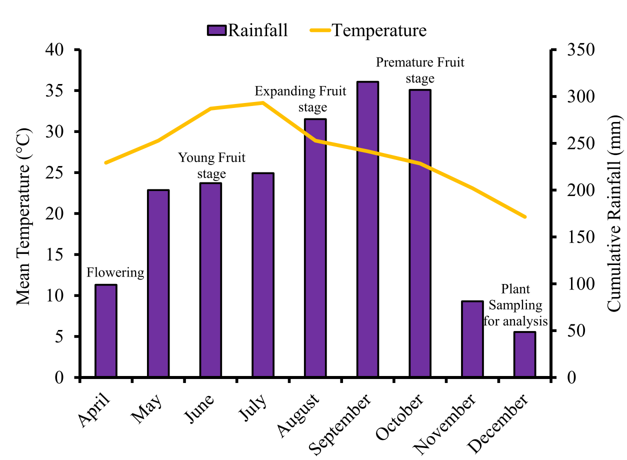

Fig. 1. Mean monthly temperature (solid line) and cumulative rainfall (bars) during the experimental period. The text above the bars indicates the months of onset of flowering, Se application stages and plant sampling phase.

methods. The treatments included: Control (C) with no Se application, foliar applications of 25 (SeF1), 50 (SeF2), 100 (SeF3) and 200 (SeF4) mg/L, soil application of 100 mg/L (SeS1) and a combination of 100 mg/L Se in soil along with 50 mg/L Se on leaves (SeS1F2). During foliar application, the Se solution was applied at a rate of 2 L per plant. In the soil application for the SeS1 treatment, the Se solution was irrigated at a rate of 2 L per plant. For the SeS1F2 treatment, 1 L per plant was applied to the soil, while 1 L per plant was applied as a foliar spray. Treatments were applied only once during each of the following stages to prevent Se toxicity: young fruit, expanding fruit, and premature fruit before 9:00 am on sunny and windless days. The various stages of treatment applications were measured from the time flowering began on April 25th, 2022. These stages included young fruit at 1 month and 23 days after flowering (AF) on June 17th, 2022, expanding fruit at 3 months and 26 days AF on August 20th, 2022, and premature fruit at 5 months and 24 days AF on October 19th, 2022. At the start of the experiment, 189 healthy, four-year-old citrus trees of similar size and normal growth were selected. These trees were divided into three groups of 63 trees each. One group received the treatments (foliar and soil applications including control) at the young fruit stage, the second group at the expanding fruit stage, and the third group at the premature fruit stage. Each treatment consisted of 9 biological replicates arranged in a randomized complete block design. There were 7 treatments with 9 replicates each, totaling 63 citrus trees per growth stage. In summary, there were 63 citrus trees for the young fruit stage, 63 for the expanding fruit stage, and 63 for the premature fruit stage, making a total of 189 citrus trees. Relative soil moisture contents were 76, 75 and 77 % during treatment application at young fruit, expanding fruit, and premature fruit stages.

2.3. Pretreatment and sampling

Mature fruits and leaves were sampled once on the same day following the treatment application. However, due to the different timings of treatment application, sampling was conducted 176 days after treatment (DAT) for the young fruit stage, 112 DAT for the expanding fruit stage and 52 DAT for the premature fruit stage. During sampling, leaves were selected from the upper portion of the middle spring shoot and fruits from the central tree crown. For each treatment direction (east, west, north and south), thirty leaves and twenty fruits were collected. These samples were temporarily stored in an ice incubator and subsequently transported to the laboratory. The leaves underwent washing, drying, grinding into powder and thereafter storage, whereas the fruits were preserved in a −70°C cold storage until further analysis.

2.4. Measurement of Se content

Citrus leaves and fruit were thoroughly rinsed with ultrapure deionized water and then dried in an oven at 60°C until they reached a constant weight (Hong et al., 2019). After drying, they were ground to a uniform particle size using a blender (MR 350 CA, Braun, Spain). The total Se content in fruits and leaves was determined using a method developed by Liu et al. (2021b) with slight modifications. The sample digestion involved a mixture of perchloric acid and nitric acid (HNO_3-$HClO_4$) in a ratio of 1:4 (v/v) at 180°C until the solution became colorless. The resulting digestive solution was then restored to 2 mL with 6 mol/L HCl, followed by a process of cooling and filtration. Afterwards, the hydride generation-atomic fluorescence spectroscopy (HG-AFS-9230) (Jitian Instruments Co., Beijing, China) was used to measure total Se concentration in each sample. The inorganic Se content of each sample was measured by atomic absorption spectrometry (AAS) as described in Zhao et al. (2019). For this purpose, 1 g of grounded citrus leaves or 2 g of fruit sample was taken into Erlenmeyer glass flask and mixed with 30 mL of ultra-pure water. After 30 minutes of sonication, the supernatant was obtained by centrifugation at 1006.2 g for 15 minutes and transferred to a separating funnel. To this supernatant, 2.5 mL of 12 mol/L HCl and 10 % (w/w) potassium ferricyanide were added. After 20 minutes, the aqueous phase was collected into a beaker. This solution containing inorganic Se was heated for 3 minutes after adding 2.5 mL of 6 mol/L HCl and then diluted to 10 mL with ultra-pure water before being subjected to AAS analysis. Organic Se content was calculated by subtracting inorganic Se content from total Se content (Yuan et al., 2023) as given below:

$$\text{Proportion of inorganic Se}(\%) = \frac{\text{Inorganic Se content}}{\text{Total Se content}} \times 100 \quad (1)$$

$$\text{Proportion of organic Se}(\%) = \textit{Total Se content}(\%) - \text{Inorganic Se content}(\%) \quad (2)$$

2.5. Fruit quality and nutritional profile

Thirty fruit samples were randomly selected for longitudinal and transverse diameters using a vernier caliper to assess aesthetic quality. The fruit shape index was calculated using following equation:

$$\text{Fruit shape index} = \frac{\text{Fruit longitudinal diameter}}{\text{Fruit transverse diameter}} \quad (3)$$

The mass of single fruit, its peel, seeds and residue were measured using an electronic balance. Citrus fruit residue rate is the percentage of the fruit that remains as discarded waste after processing and is not utilized for commercial purposes (Zema et al., 2018). Following formulas were used to calculate the peel rate, seed rate and residual rate:

$$\text{Peel rate}(\%) = \frac{\text{Peel mass}}{\text{Single fruit mass}} \times 100 \quad (4)$$

$$\text{Seed rate}(\%) = \frac{\text{Seed mass}}{\text{Single fruit mass}} \times 100 \quad (5)$$

$$\text{Residue rate}(\%) = \frac{\text{Residue mass}}{\text{Single fruit mass}} \times 100 \quad (6)$$

To evaluate internal quality of fruit, juice was manually extracted from fruits, measured fruit weight after peeling, weighed the peel, seed and juice. Subsequently, the edible rate (%) and juice rate (%) were determined using formulas below:

$$\text{Juice rate}(\%) = \frac{\text{Juice weight}}{\text{Single fruit weight}} \times 100 \quad (7)$$

$$\text{Edible rate}(\%) = \frac{\text{Single fruit weight} - \text{Peel weight} - \text{Seed weight}}{\text{Single fruit weight}} \times 100 \quad (8)$$

The citrus fruit juice was filtered through four layers of gauze and analyzed for total soluble solids (TSS), titratable acid (TA) and vitamin C content by using procedure as described in Sdiri et al. (2012). Citrus juice TSS-to-TA ratio was calculated using formula below:

$$\text{Solid : acid ratio} = \frac{\text{TSS}}{\text{TA}} \tag{9}$$

2.6. Statistical analyses

The effects of Se biofortification treatments on relevant citrus traits was statistically analyzed through univariate analysis by using SPSS statistics software 20, IBM, USA (IBM Corp., Armonk, NY, USA). The treatment effect was tested through analysis of variance (ANOVA) at 5 % probably level. If the effect of treatments was significant then their means were analyzed through LSD test. Pearson correlation coefficient was used to estimate the strength and direction of the relation between fruit total Se content and its quality attributes at young fruit, expanding fruit and premature fruit stages. The large language models (Chat GPT) were used to correct the language errors in this manuscript.

3. Results

3.1. Total selenium (Se) content of citrus leaves and fruits

Total Se content of citrus leaves and fruits after biofortification are shown in Table 1. At young fruit stage, Se-biofortification improved Se content for SeF4 by 2.1 and 3.6 folds in leaves and fruits relative to control, respectively ($p \leq 0.05$). At the expanding fruit stage, the total Se content increased 1.8 folds in leaves for SeF3, while in fruits, it increased 2.0 folds with SeS1 treatments ($p \leq 0.05$). At premature fruit stage, only leaves Se content were enhanced by 1.7 times after application of SeF3 and SeF4 as compared to control ($p \leq 0.05$). However, no significant differences were observed in Se content of fruits among all treatments at premature fruit stage ($p > 0.05$). The total Se content in leaves were notably higher in the SeF4 treatment (foliar) compared to SeS1 and SeS1F2 (soil) treatments ($p \leq 0.05$) across all stages. Although Se content in fruits did not show significant differences at young fruit and premature fruit stages, it was on average 33 % higher in the SeF4 treatment ($p > 0.05$). Interestingly, at the expanding fruit stage, SeF4 has 38 % and 27 % lower Se content in fruits compared to SeS1 and SeS1F2, respectively ($p \leq 0.05$). On average, foliar application of Se across all treatments increased leaf content by 52, 65 and 50 % compared to the control during the young fruit, expanding fruit and premature fruit stages, respectively. Similarly, the corresponding increases in Se content of fruits were 214 %, 9 % and 16 %. The average soil application of Se for both treatments enhanced Se content of leaves by 63, 6.5 and 36 % compared to the control at young fruit, expanding fruit and premature fruit stages, respectively. The respective increases in Se content of fruits were 175 %, 98 % and 20 % (Table 1). Total Se contents of both leaves and fruits were reduced at premature fruit stage relative to young fruit stage.

Table 1
Mean (±SE[a]) total selenium (Se) content (μg/kg) of citrus leaves and fruits at various stages after biofortification.

Treatments	Young Fruit Stage		Expanding Fruit Stage		Premature Fruit Stage	
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
[c]C	56.59 $\pm 1.19^{bb}$	3.37 $\pm 0.31^{a}$	54.46 $\pm 0.38^{a}$	1.27 $\pm 0.01^{a}$	50.42 $\pm 1.26^{a}$	1.26 $\pm 0.02^{a}$
SeF1	55.69 $\pm 1.21^{a}$	6.55 $\pm 2.59^{ab}$	87.49 $\pm 0.38^{b}$	1.28 $\pm 0.17^{a}$	50.64 $\pm 1.84^{a}$	1.26 $\pm 0.01^{a}$
SeF2	79.35 $\pm 0.40^{c}$	11.73 $\pm 1.02^{c}$	89.14 $\pm 1.35^{b}$	1.39 $\pm 0.09^{a}$	80.28 $\pm 3.41^{c}$	1.58 $\pm 0.01^{a}$
SeF3	94.06 $\pm 2.98^{d}$	11.83 $\pm 1.55^{c}$	95.29 $\pm 1.30^{c}$	1.30 $\pm 0.12^{a}$	86.48 $\pm 5.14^{c}$	1.50 $\pm 0.08^{a}$
SeF4	115.92 $\pm 0.33^{e}$	12.26 $\pm 1.17^{c}$	87.39 $\pm 0.22^{b}$	1.55 $\pm 0.28^{a}$	85.40 $\pm 2.51^{c}$	1.52 $\pm 0.04^{a}$
SeS1	92.14 $\pm 3.02^{d}$	9.25 $\pm 0.09^{bc}$	58.00 $\pm 2.68^{a}$	2.51 $\pm 0.24^{b}$	68.44 $\pm 2.40^{b}$	1.51 $\pm 0.38^{a}$
SeS1F2	89.49 $\pm 1.73^{d}$	6.68 $\pm 0.67^{ab}$	55.87 $\pm 2.23^{a}$	2.13 $\pm 0.21^{b}$	60.33 $\pm 2.55^{b}$	1.27 $\pm 0.26^{a}$

(a)SE; Standard error, (b) Different small letters as superscripts within a column represent significant differences at 5 % probability level after LSD test, (C); Control with no application of Se, SeF1; foliar application of 25, SeF2; 50, SeF3; 100 and SeF4; 200 mg/L and SeS1; soil application of 100 mg/L as well as SeS1F2; combination of 100 mg/L Se in soil along with 50 mg/L Se on leaves.

Generally, foliar application increased the total Se content by 56 % in leaves and 79 % in fruits, whereas soil application (SeS1) led to a 35 % increase in leaves and a 98 % increase in fruits compared to the control. Also, a combination between soil and foliar application (SeS1F2) increased the total Se content in leaves and fruits by 35 % and 56 % than control, respectively. Mostly, the highest increase in total Se content in leaves and fruits was at young fruit stage.

3.2. Organic and inorganic Se content of citrus leaves

The inorganic Se content in citrus leaves showed no significant difference following applications of SeF3 and SeF4, as well as SeS1F2, compared to the control at the young fruit stage ($p > 0.05$; Fig. 2A). However, these concentrations were significantly lower than the control at the expanding fruit and premature fruit stages ($p \leq 0.05$; Fig. 2B & C). On the other hand, the organic Se content in leaves exhibited a significant increase following Se application at all stages of fruit development ($p \leq 0.05$; Fig. 2), except for SeF1 at the young fruit stage, where there was no significant difference ($p > 0.05$). Increasing the foliar application rate of Se from 0 to 200 mg/L led to a rise in the organic Se content of the leaves at the young fruit stage, with the highest content observed at SeF4 (Fig. 2A). Soil application of 100 mg/L (SeS1) and a combination of 100 mg/L Se in soil with 50 mg/L Se on leaves (SeS1F2) resulted in decreased organic Se content in leaves compared to foliar application at expanding fruit and premature stages (Fig. 2B & C). Furthermore, the young fruit stage exhibited the highest levels of organic Se in leaves compared to other stages (Fig. 2). Foliar application of 200 mg/L Se (SeF4) to citrus leaves at the young fruit stage resulted in the most significant increase in the proportion of organic Se in the leaves compared to the control ($p \leq 0.05$; Fig. 3). However, no significant differences in the percentage of organic Se were observed among the foliar and soil application treatments at this stage ($p > 0.05$). At the expanding fruit stage, the highest organic Se content was measured following the SeF1 treatment compared to other treatments ($p \leq 0.05$; Fig. 3). However, at the premature fruit stage, the highest organic Se content was found in the SeS1F2 treatment ($p \leq 0.05$; Fig. 3).

3.3. Organic and inorganic Se content of citrus fruits

At young fruit stage, the inorganic Se content in citrus fruit were increased under the SeF2 treatment, whereas no significant differences were observed among all other foliar applied treatments (Fig. 4A). At expanding fruit stage, soil and combined applications (SeS1 and SeS1F2) resulted in higher levels of inorganic Se content in the fruits compared to foliar applied Se ($p \leq 0.05$; Fig. 4). Interestingly, at the premature fruit development stage, significantly lower inorganic Se was observed in SeF4 and SeS1 relative to all other treatments ($p \leq 0.05$; Fig. 4C). The foliar application of Se at young fruit stage significantly elevated the organic Se content in fruits than control ($p \leq 0.05$; Fig. 4A). The highest dose of Se (SeF4) resulted in an enhancement of organic Se content in citrus fruits, reaching values of 11.96 μg/kg for young fruit and 1.35 μg/kg for premature fruit stages compared to control. Additionally, during the expanding fruit stage, the soil applied treatment SeS1 produced the highest organic Se content in fruits, with a value of 1.62 μg/kg. At the young fruit stage, the organic Se content in fruits was significantly higher in the SeF4 treatment compared to soil application alone ($p \leq 0.05$; Fig. 4A). Remarkable organic Se contents of 97.53 %, 83.28 %, and 88.73 % of the total Se were observed in SeF4 at the young fruit, expanding fruit, and premature stages, respectively. The highest dose of

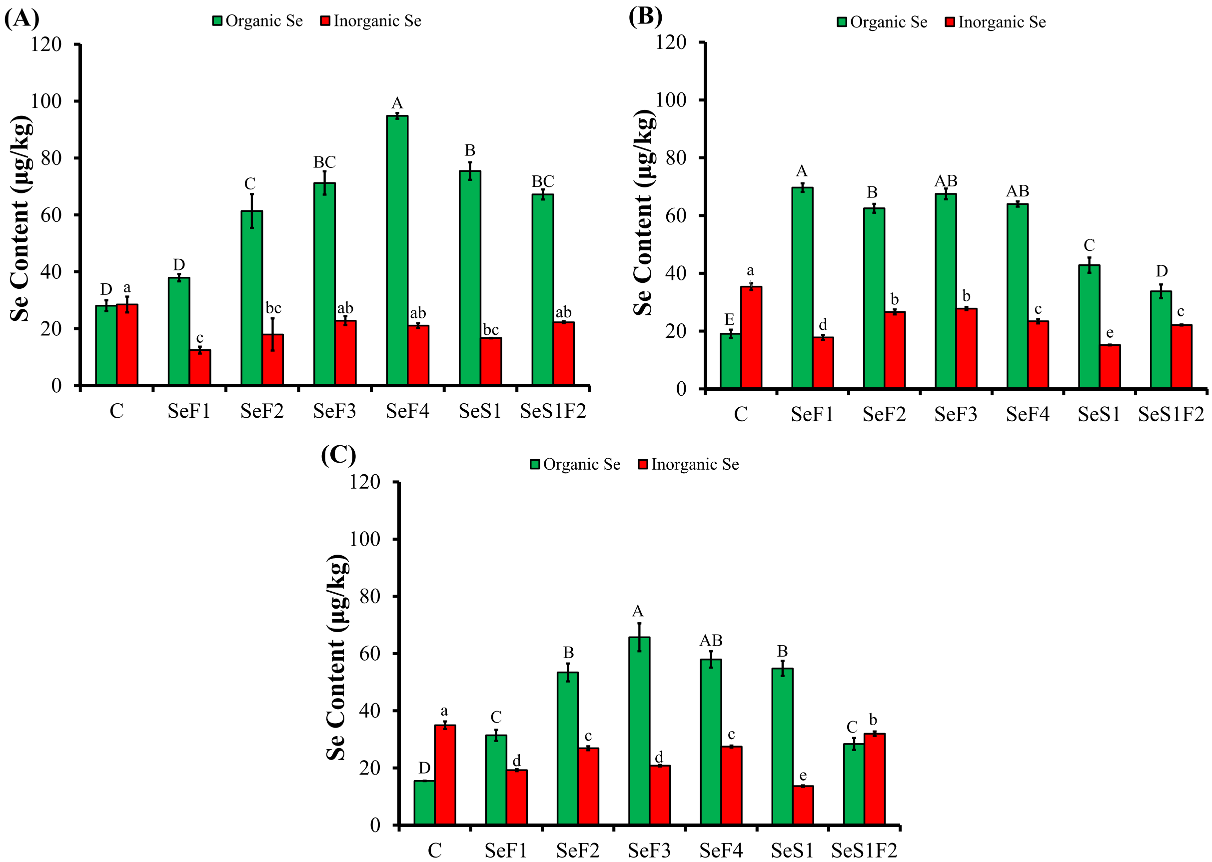

Fig. 2. Selenium (Se) composition of citrus leaves at (A) young fruit, (B) Expanding fruit and (C) premature fruit stages as affected by foliar and soil application of Se. Error bar represents standard error of the mean. Different uppercase letters indicate significant differences among organic Se treatments, while lowercase letters indicate significant differences within inorganic Se at 5 % probability level after LSD test.

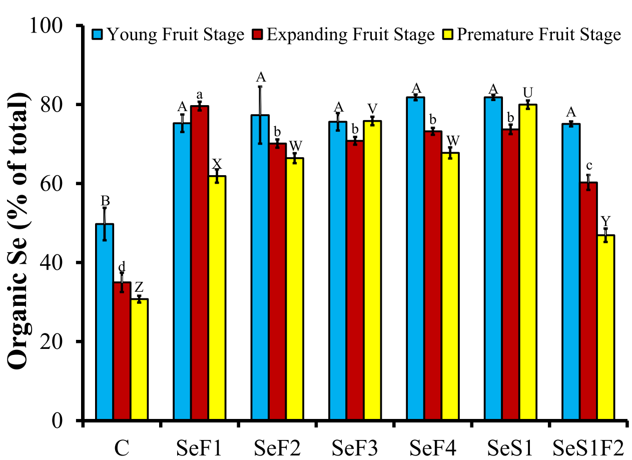

Fig. 3. Organic Se (% of total) of leaves at various application stages as affected by foliar and soil application of Se. Error bar represents standard error of the mean. Different uppercase letters, such as A-B, indicate significant differences among treatments at the young fruit stage, while other uppercase letters, such as U-Z, represent significant differences at premature fruit stage. Lowercase letters, such as a-d, denote significant differences among treatments at expanding fruit stage, all at a 5 % probability level after LSD test.

200 mg/L Se (SeF4) was shown to be the most effective treatment for increasing the proportion of organic Se in citrus fruits at the young fruit stage, with the exception of the SeF3 treatment (Fig. 5).

3.4. External quality of citrus fruits

The application of Se through both foliar and soil methods did not affect external fruit quality parameters such as fruit shape index, peel rate and residue rate at any stage of fruit development ($p>0.05$; Table 2). During the young fruit stage, the foliar application of Se significantly enhanced the seed rate by 102 % and 97 % with SeF3 and SeF4 treatments, respectively, compared to the control ($p\leq0.05$; Table 2). However, there were no significant differences observed after soil application of Se compared to the control in terms of seed rate during the young fruit stage ($p>0.05$). During the expanding fruit stage, the seed rate was statistically enhanced by 34 % with SeF4 and by 64 % with SeS1 relative to the control, while no differences were found among all other treatments. However, during the premature fruit development stage, no significant differences in seed rate were observed between the treated and untreated groups ($p>0.05$). Single fruit weight increased by 56 %, 39 %and 13 % with the SeF4 treatment as compared to control during the young fruit, expanding fruit and premature fruit stages, respectively ($p\leq0.05$; Table 2). Soil application of Se did not affect single fruit size during the young fruit and expanding fruit stages, while only a 13 % increment was observed at the premature fruit stage, which was significant compared to the control. Overall, foliar application of Se improved single fruit weight by 28 %, 25 %and 12 % during the young fruit, expanding fruit and premature fruit stages, respectively. However, the respective increments were only 16 %, 17 %and 13 % after soil application of Se during the young fruit, expanding fruit and premature fruit stages, respectively. Therefore, foliar application outperformed soil application in terms of increasing single fruit weight. Among the foliar applied treatments, SeF4 was found to be the most effective.

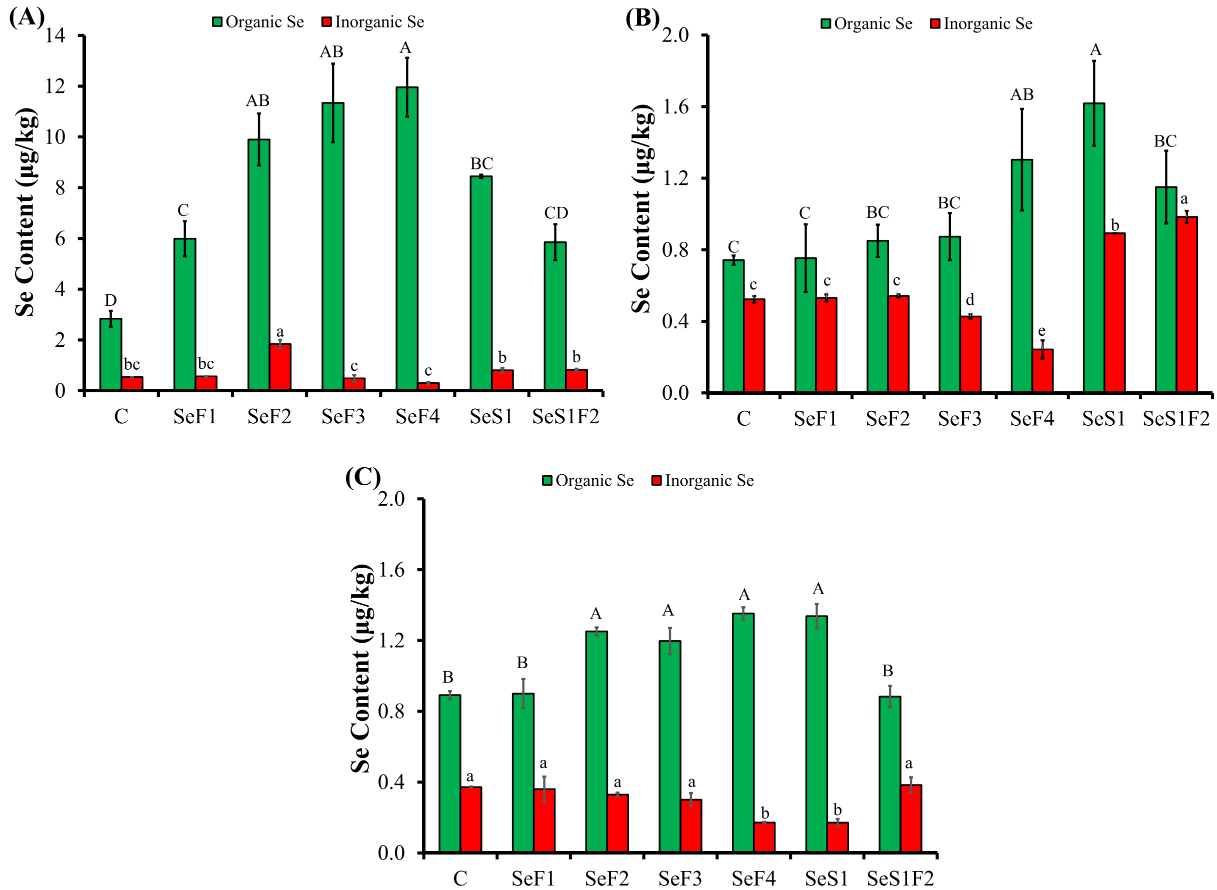

Fig. 4. Selenium (Se) composition of citrus fruits at (A) young fruit, (B) Expanding fruit and (C) premature fruit stages as affected by foliar and soil application of Se. Error bar represents standard error of the mean. Different uppercase letters indicate significant differences among organic Se treatments, while lowercase letters indicate significant differences within inorganic Se at 5 % probability level after LSD test.

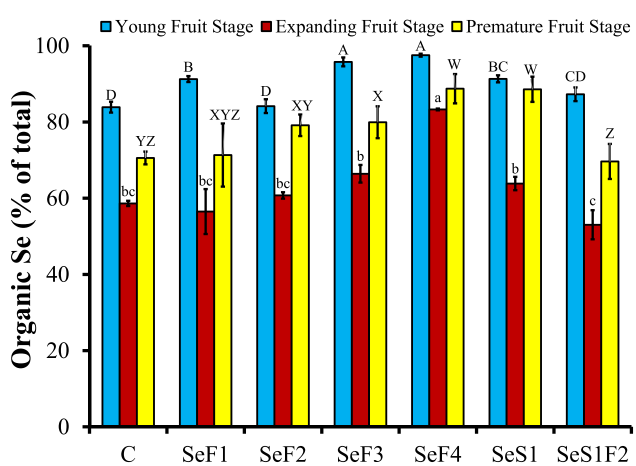

Fig. 5. Organic Se (% of total) of fruits at various application stages as affected by foliar and soil application of Se. Error bar represents standard error of the mean. Different uppercase letters, such as A-D, indicate significant differences among treatments at the young fruit stage, while other uppercase letters, such as W-Z, represent significant differences at premature fruit stage. Lowercase letters, such as a-c, denote significant differences among treatments at expanding fruit stage, all at a 5 % probability level after LSD test.

3.5. *Internal quality of citrus Fruits*

Foliar application of 200 mg/L Se (SeF4) during the young fruit and expanding fruit stages resulted in notable increases in total soluble solids (TSS) by 28 and 21 % accompanied by increments in the solid-acid ratio (TSS/TA) by 75 and 72 % as compared to the control, respectively ($p \leq 0.05$; Table 3). However, titratable acid (TA) was decreased by 26, 29 and 26 % during young fruit, expanding fruit and premature fruit stages in SeF4 relative to control ($p \leq 0.05$). Notably, there was no significant differences among treatments in vitamin C content during the young fruit stage. However, fruit vitamin C was improved by 8 % after application of SeF4 during expanding fruit stage, but there was no significant increase during premature fruit stage. Interestingly, soil application of Se did not affect the fruit's vitamin C content, except for a 15 % reduction following SeS1 application at the premature stage compared to the control. Soil application did not improved TSS across all fruit development stages ($p > 0.05$). The increments in solid-acid ratio due to combined foliar and soil application (SeS1F2) was 56 % than control ($p \leq 0.05$). However, a 31 % reduction in titratable acid was observed at the young fruit stage following the application of SeS1F2 compared to the control ($p \leq 0.05$). This reduction increased to 39 % with SeS1 at the expanding fruit stage, while no differences were noted between soil applied treatments and the control at the premature fruit stage.

The fruit juice rate remained unaffected by both foliar and soil Se application methods at all stages of fruit development, except for a 6 %

Table 2
Mean (±SE[a]) external quality parameters of citrus fruits at various stages after biofortification.

Application stage	Treatment	Fruit shape index	Peel rate (%)	Residue rate (%)	Seed rate (%)	Single fruit weight (g)
Young Fruit	[c]C	0.90±0.03[ab]	25.18±1.15[a]	22.02±0.76[a]	1.21±0.18[a]	99.75±6.40[a]
	SeF1	0.93±0.02[a]	26.87±0.65[a]	21.26±0.75[a]	1.24±0.07[a]	114.71±2.51[a]
	SeF2	0.94±0.02[a]	27.70±1.27[a]	21.14±0.31[a]	1.16±0.19[a]	116.94±1.91[a]
	SeF3	0.95±0.02[a]	26.54±1.18[a]	20.11±0.95[a]	2.44±0.68[b]	124.65±3.61[a]
	SeF4	0.96±0.04[a]	27.07±0.51[a]	19.97±0.59[a]	2.38±0.15[b]	155.53±6.36[b]
	SeS1	0.96±0.01[a]	27.08±1.14[a]	20.90±0.42[a]	1.14±0.17[a]	115.94±9.70[a]
	SeS1F2	0.93±0.01[a]	27.70±0.26[a]	21.69±0.70[a]	1.83±0.36[ab]	106.76±4.14[a]
Expanding Fruit	C	0.90±0.01[a]	26.24±0.23[a]	23.06±2.23[a]	1.46±0.04[ab]	92.80±7.43[a]
	SeF1	0.93±0.02[a]	25.91±0.66[a]	23.05±0.42[a]	1.33±0.07[ab]	108.09±3.69[ab]
	SeF2	0.90±0.01[a]	26.51±2.13[a]	21.97±1.73[a]	1.44±0.09[ab]	109.73±4.67[ab]
	SeF3	0.91±0.01[a]	26.86±2.26[a]	21.31±0.08[a]	1.78±0.25[abc]	116.81±3.52[bc]
	SeF4	0.91±0.02[a]	27.30±0.55[a]	21.02±1.54[a]	1.96±0.41[bc]	129.19±2.15[c]
	SeS1	0.90±0.03[a]	26.68±0.74[a]	20.06±0.13[a]	2.40±0.30[c]	108.53±1.66[ab]
	SeS1F2	0.94±0.01[a]	26.55±2.26[a]	22.23±0.97[a]	1.01±0.33[a]	96.01±8.94[a]
Premature Fruit	C	0.93±0.03[a]	22.96±1.09[a]	21.96±0.82[a]	1.33±0.10[a]	95.45±6.14[a]
	SeF1	0.95±0.01[a]	22.24±0.67[a]	21.68±0.58[a]	0.98±0.06[a]	106.76±2.49[ab]
	SeF2	0.95±0.01[a]	22.75±0.29[a]	21.40±1.12[a]	1.17±0.26[a]	106.30±0.91[ab]
	SeF3	0.95±0.01[a]	22.96±0.75[a]	21.38±1.72[a]	0.97±0.03[a]	108.39±0.88[b]
	SeF4	0.95±0.01[a]	22.26±3.85[a]	21.00±0.44[a]	1.50±0.28[a]	108.13±5.70[b]
	SeS1	0.92±0.02[a]	22.63±0.20[a]	21.43±0.70[a]	1.09±0.31[a]	108.13±3.72[b]
	SeS1F2	0.93±0.02[a]	21.69±0.52[a]	20.90±1.10[a]	0.99±0.15[a]	97.92±0.81[ab]

(a)SE; Standard error, (b) Different small letters as superscripts within a column represent significant differences at 5 % probability level after LSD test, (C) Control with no application of Se, SeF1; foliar application of 25, SeF2; 50, SeF3; 100 and SeF4; 200 mg/L and SeS1; soil application of 100 mg/L as well as SeS1F2; combination of 100 mg/L Se in soil along with 50 mg/L Se on leaves.

Table 3
Mean (±SE[a]) internal quality parameters of citrus fruits at various stages after biofortification.

Application stage	Treatment	Total soluble solids (TSS %)	Titratable acid (TA %)	Vitamin C (mg/100 g)	Solid-acid ratio (TSS/TA)	Juice rate (%)	Edible rate (%)
Young Fruit	[c]C	6.67±0.33[ab]	3.49±0.34[b]	37.12±0.52[a]	1.96±0.27[a]	51.97±0.66[b]	72.28±0.30[a]
	SeF1	7.50±0.29[abc]	2.98±0.07[ab]	37.35±0.40[a]	2.52±0.06[ab]	51.83±1.06[ab]	72.42±0.10[a]
	SeF2	7.83±0.33[abc]	2.95±0.08[ab]	37.92±0.63[a]	2.65±0.05[abc]	50.67±0.97[ab]	72.14±1.12[a]
	SeF3	8.17±0.33[bc]	2.83±0.06[a]	37.68±0.23[a]	2.88±0.14[bc]	51.35±1.21[ab]	72.13±2.40[a]
	SeF4	8.54±0.04[c]	2.59±0.33[a]	38.79±0.22[a]	3.43±0.49[c]	50.64±0.49[ab]	72.66±0.53[a]
	SeS1	7.83±0.60[abc]	2.92±0.10[ab]	38.69±0.67[a]	2.70±0.27[abc]	50.88±1.21[ab]	71.78±1.31[a]
	SeS1F2	7.33±0.33[ab]	2.42±0.10[a]	38.14±0.68[a]	3.05±0.27[bc]	48.78±0.49[a]	70.47±0.22[a]
Expanding Fruit	C	7.60±0.06[a]	3.80±0.03[d]	38.48±0.20[a]	2.00±0.01[a]	47.85±2.06[a]	70.90±0.84[a]
	SeF1	8.17±0.33[abc]	3.28±0.07[c]	40.85±0.52[b]	2.49±0.09[ab]	49.71±1.05[a]	72.43±0.44[a]
	SeF2	8.51±0.01[abc]	3.28±0.09[c]	41.28±0.29[b]	2.60±0.07[bc]	50.08±2.70[a]	72.05±2.10[a]
	SeF3	8.83±0.33[bc]	2.92±0.10[b]	41.41±0.93[b]	3.04±0.19 [cd]	49.23±1.89[a]	72.03±1.57[a]
	SeF4	9.17±0.33[c]	2.69±0.09[b]	41.75±0.69[b]	3.43±0.22[d]	49.06±3.09[a]	71.08±0.57[a]
	SeS1	7.50±0.58[a]	2.32±0.10[a]	40.62±0.56[b]	3.24±0.29[d]	50.73±0.29[a]	70.93±0.73[a]
	SeS1F2	8.03±0.03[ab]	3.61±0.10[d]	37.92±0.59[a]	2.23±0.06[ab]	47.21±0.47[a]	69.44±0.66[a]
Premature Fruit	C	6.93±0.23[a]	3.57±0.14[b]	36.00±1.99[b]	1.95±0.14[a]	49.45±2.45[a]	70.74±0.10[a]
	SeF1	7.00±0.29[a]	2.95±0.43[ab]	36.67±0.74[b]	2.48±0.40[a]	49.37±1.76[a]	70.30±0.61[a]
	SeF2	7.17±0.17[a]	2.73±0.30[ab]	35.88±0.99[b]	2.68±0.28[a]	50.36±0.47[a]	71.09±0.15[a]
	SeF3	7.00±0.50[a]	2.69±0.23[ab]	36.63±0.88[b]	2.66±0.38[a]	49.63±0.56[a]	70.74±0.77[a]
	SeF4	7.50±0.29[a]	2.63±0.33[a]	36.67±1.77[b]	2.93±0.35[a]	49.58±0.59[a]	70.91±1.30[a]
	SeS1	7.00±0.29[a]	2.72±0.10[ab]	30.68±1.09[a]	2.59±0.20[a]	50.52±0.49[a]	71.28±0.71[a]
	SeS1F2	7.33±0.17[a]	2.82±0.17[ab]	38.25±0.90[b]	2.62±0.18[a]	47.75±0.77[a]	69.65±0.21[a]

(a)SE; Standard error, (b)Different small letters as superscripts within a column represent significant differences at 5 % probability level after LSD test, (C); Control with no application of Se, SeF1; foliar application of 25, SeF2; 50, SeF3; 100 and SeF4; 200 mg/L and SeS1; soil application of 100 mg/L as well as SeS1F2; combination of 100 mg/L Se in soil along with 50 mg/L Se on leaves.

reduction caused by SeS1F2 during the young fruit stage. Additionally, Se application did not impact the quality of the fruit's edible component ($p>0.05$).

3.6. Correlation of selenium with internal and external fruit quality parameters

Pearson's correlation coefficients between fruit total Se content and its quality attributes at young fruit, expanding fruit and premature fruit stages are presented in Table 4. A positive correlation was observed between total Se content in fruits and various quality parameters, including total soluble solids (TSS; $r=0.769$; $p<0.001$), solid-acid ratio ($r=0.457$; $p=0.037$), fruit shape index ($r=0.435$; $p=0.049$ and single fruit weight ($r=0.566$; $p=0.008$) at the young fruit stage. However, all other quality attributes showed no significant relationship with fruit Se content at this stage ($p>0.05$). Notably, no internal or external fruit quality parameters exhibited any significant relationship with fruit Se content at the expanding fruit and premature fruit development stages ($p>0.05$; Table 4). There was only a tendency for negative relationships between fruit Se content and titratable acid and positive relationships with seed rate across all fruit developmental stages ($p>0.05$).

Table 4
Pearson's correlation coefficients between Se content in citrus fruits and both internal as well as external fruit quality parameters at various stages after biofortification.

Parameter	Application stage	TSS[1] (%)	TA[2] (%)	Vit. C[3] (mg/100 g)	Solid-acid ratio	Fruit shape index	Juice rate (%)	Edible rate (%)	Peel rate (%)	Residue rate (%)	Seed rate (%)	Single fruit weight (g)
Fruit total Se content (μg/kg)	Young fruit	0.769** (<0.001)¶	−0.247 (0.281)	0.311 (0.170)	0.457* (0.037)	0.435* (0.049)	−0.197 (0.392)	−0.122 (0.597)	0.243 (0.288)	−0.364 (0.105)	0.415 (0.061)	0.566** (0.008)
	Expanding Fruit	0.249 (0.277)	−0.389 (0.081)	−0.180 (0.434)	0.256 (0.264)	−0.036 (0.878)	0.043 (0.853)	−0.297 (0.192)	−0.063 (0.785)	−0.316 (0.162)	0.304 (0.180)	−0.077 (0.740)
	Premature Fruit	−0.281 (0.217)	−0.144 (0.532)	−0.013 (0.957)	−0.001 (0.996)	0.385 (0.085)	−0.018 (0.938)	0.339 (0.133)	−0.010 (0.967)	0.187 (0.416)	0.392 (0.079)	0.396 (0.076)

* Significant correlation at the 0.05 level, ¶ Values in parenthesis are p-value,
** Significant correlation at the 0.01 level,
1 TSS; Total soluble solids;
2 TA; Titratable acid,
3 Vit. C; Vitamin C.

4. Discussion

In general, foliar application of selenium (Se) resulted in increased total Se content in both leaves and fruits of citrus (Table 1). Previous studies have shown comparable increases in Se content in the seeds of rice, wheat, soybean and maize, as well as in pear and apple fruits, following foliar application of Se (Radawiec et al., 2021; Zahedi et al., 2020; Joy et al., 2022; Wójcik, 2023). We found that foliar application of Se at a concentration of 200 mg/L (SeF4) resulted in 28 and 54 % greater Se in citrus leaves and fruits as compared to soil treatment at young fruit stage, respectively (Table 1). In rice, foliar application of selenite to leaves increased Se absorption by 5–6 times compared to soil application (Lidon et al., 2019). Kao et al. (2023) and Sheikhalipour et al. (2021) observed that more than 80 % of the selenite and selenate applied to the soil were either leached out by irrigation water or potentially absorbed by soil particles. This resulted in reduced availability of Se for plant uptake. Unlike soil application, foliar application of Se improves its uptake and recovery efficiency by reducing its immobilization in the soil and shortening the transport distance of Se from plant roots to shoots (Silva et al., 2023). Furthermore, it allows Se to be delivered precisely to specific plant parts, such as leaves or fruits, optimizing its utilization by the plant. Therefore, foliar application of Se is biologically preferable than soil treatment (de Lima et al., 2023).

In our study, treatments were applied at various stages of fruit development to determine the optimal stage for Se biofortification, it was observed that applying treatments during the young fruit stage generally increased Se content, particularly with SeF4 treatment (Table 1). This finding aligns with previous research demonstrating Se absorption at higher foliar Se concentrations (100 and 150 mg/L Na2SeO3) during fruit expansion in sweet persimmons and the young fruit stage in apples (Yan et al., 2021; Wójcik et al., 2024). The foliar application of Se in jujube also exhibited varied effects on Se assimilation capacities across different developmental stages (Wang et al., 2020). The young fruit stage is characterized by active fruit growth, necessitating ample nutrient uptake and substantial fertilizer application. In contrast, fertilizer application decreases during later stages of maturity (Ma et al., 2022). We analyzed the time span from treatment application to fruit harvesting and found it was 176 days for young fruit, 112 days for expanding fruit, and only 52 days for the premature fruit stage. We believe this duration has influenced Se content in both fruit and leaves. Additionally, heavy rainfall in October (307 mm; Fig. 1), immediately following the application of treatments, reduced Se content in both leaves and fruit at premature fruit stage. This might be due to the washing off of applied Se from leaf surfaces and the dilution of Se concentrations after foliar application, and Se leaching following soil applications (Dhillon et al., 2008; Fernández et al., 2013).

Interestingly, the highest dose of foliar application (SeF4) did not affect the inorganic Se content in leaves and fruits at the young fruit stage, but it reduced the content at the expanding and premature fruit stages compared to the control (Figs. 2 and 4). Conversely, high levels of organic Se were observed, demonstrating a positive correlation between Se assimilation and increasing Se concentration, with the peak observed when 200 mg/L of Se (SeF4) was applied through foliar application, particularly at the young fruit stage. Our results are in line with prior research indicating that organic Se accounts for approximately 80 % of the total Se in edible fruit portions (Sarwar et al., 2020; Arı et al., 2022). The application of sodium selenite in a surfactant solution significantly enhanced the effectiveness of Se biofortification in citrus. After uptake, selenite undergoes reduction to selenide, facilitated by enzymes like sulfite reductase, and possibly assisted by glutaredoxins or glutathione (Moulick et al., 2024). The resulting selenide then reacts with O-acetylserine (OAS) in the presence of cysteine synthase, forming Se cysteine (SeCys). This SeCys can take two main paths for further transformation: it can be methylated by selenocysteine methyltransferase to become methyl-selenium cysteine (methyl-SeCys), or it can be converted into Se methionine (SeMet) through processes involving enzymes like selenocysteine lyase (Adebayo et al., 2020). These organic Se compounds, including SeCys, methyl-SeCys, and SeMet, are essential for citrus metabolism and contribute to its nutritional value for human consumption (Chauhan et al., 2019). These organic Se compounds can replace sulfur-containing amino acids in plant proteins (Zagrodzki et al., 2023). Moreover, high total Se availability through foliar application can increase Se uptake and transport in citrus fruits, providing more substrate for the synthesis of organic Se compounds within plant tissues.

The foliar application of Se at a concentration of 200 mg/L, particularly during young fruit stage resulted in increased seed rate. Increasing the seed rate of citrus fruits following Se biofortification enhances their nutritional content by increasing the number of Se-biofortified seeds. However, an excessive number of seeds per fruit may be perceived as indicating lower quality, which could negatively influence consumer perception. House and Gao (2009) found that a seed rate below 10 % did not affect consumer willingness to purchase. In our case, the maximum seed rate after Se biofortification was 2.44 % (SeF3), which falls within the range of consumer preference. Single fruit weight was significantly higher (155.53 vs. 113.13 g) after application of SeF4 treatment at young fruit stage relative to all other treatments ($p \leq 0.05$; Table 2). This enhancement ultimately improved fruit quality by enhancing the activity of antioxidant enzymes and increasing the overall yield of the plant (Babalar et al., 2019; Zahedi et al., 2020). The activity of antioxidant enzymes is boosted by foliar application of Se, both through direct enhancement of their catalytic activity and indirectly via the regulation of gene expression associated with antioxidant defense pathways (Lanza and Dos Reis 2021). Consequently, the plant resilience against oxidative stress is enhanced, leading to an increase in its yield. The single fruit weight decreased from the young fruit stage to the expanding and premature stages, with mean values of 127.96, 115.96, and 107.39 g/fruit after foliar application, respectively. In comparison, the respective values were 111.35 g, 102.27 g, and 103.03 g per fruit after soil application of Se. This might be attributed to reduction in peel rate and juice rate of fruits as plants matures (Tables 2 and 3). Besides, gradual decrease in mean temperature from young fruit (35.5°C), expanding (30.0°C), premature stage (25.5°C) to harvesting (20.0°C) (Fig. 1) might lead to a reduction in metabolic activity, nutrient uptake and cell division, resulting in smaller fruit sizes. Besides, Se concentration was lower in leaves and fruits at premature stage (Table 1) might lead to smaller fruit sizes as the fruit matures. However, application of Se through both foliar and soil methods did not impact fruit shape index, peel rate and residue rate at any stage of fruit development (Table 2). In contrast, previous research has shown that foliar Se application improved various quality attributes in pomegranates, such as peel thickness, fruit diameter and length, as well as nutritional quality, while also reducing fruit cracking (Zahedi et al., 2019).

Foliar application of Se in citrus led to higher levels of total soluble solids (TSS) and an increased ratio of total soluble solids to titratable acidity (TSS/TA), particularly evident with the highest Se dose at young fruit and expanding fruit stages (SeF4; Table 3). Similar findings have been reported in pomegranates (Zahedi et al., 2019), sapotas (Lalithya et al., 2014), and potatoes (Ibrahim and Ibrahim, 2016). This enhancement in citrus fruit TSS following Se application is likely attributed to an elevation in total soluble sugars, possibly linked to increased activity of fructose 1,6-bisphosphatase, a key enzyme in carbohydrate metabolism known to positively influence citrus flavor (Owusu-Sekyere et al., 2013). Research also indicates that foliar application of boron and zinc has been effective in increasing total sugar content in mandarin (Citrus reticulata) fruits (Babu and Yadav, 2005). In contrast, Pezzarossa et al. (2012) found no increase in TSS for peach and pear following foliar Se application, which they attributed to the late ripening of these fruits. Interestingly, soil application did not increase TSS across all fruit development stages (Table 3) compared to the control. There was a decrease in TSS observed in apples following soil application of Se, potentially due to an inhibition of the starch conversion process into sugars, particularly noticeable in less mature apples (Wójcik et al., 2024). Interestingly, both foliar and soil applications of Se did not influence fruit vitamin C content during the young fruit stage (Table 3). This finding contrasts with studies by Ibrahim et al. (2014), Wen et al. (2021) and Zhan et al. (2021), which reported an increase in vitamin C content in citrus following Se application. The lack of effect on vitamin C content at the young fruit stage may be attributed to the early Se application timing, as vitamin C biosynthesis in citrus fruits is most active during ripening stages (Magwaza et al., 2017). This hypothesis is supported by an observed 8 % higher vitamin C content after SeF4 application during the expanding fruit stage (Table 3), indicating a stage-specific response to Se application in fruit vitamin C content. Citrus fruit juice rate and edible rate remained unaffected by both foliar and soil Se application methods at all stages of fruit development. The impact of Se on the actual volume of citrus juice as well as edible rate is generally minor compared to its effects on quality parameters and overall plant health (Maas, 1993). The application of Se significantly enhanced the quality of agricultural products such as strawberries, corn and tomatoes (Zahedi et al., 2020; Jalali et al., 2022). Besides, we used moderate levels of Se doses which do not cause negative impacts on these parameters.

The application of Se demonstrated superior effectiveness during the young fruit stage, showing a positive correlation between total Se content in fruits and various quality parameters such as total soluble solids, solid-acid ratio, fruit shape index, and single fruit weight (Table 4). In contrast, no significant relationships were found between fruit Se content and internal or external fruit quality parameters during the expanding fruit and premature fruit development stages. Similar positive effects of early Se application on quality parameters have been observed in lettuce (Ríos et al., 2010) and tea (Hu et al., 2003). Conversely, studies have shown no significant correlation between fruit Se content and its quality parameters after its application at late development stages (Barker and Pilbeam, 2015). These findings underscore the significance of enhancing fruit Se content through external treatments to improve both quality traits and fruit production.

5. Conclusions

The foliar application of Se significantly enhanced the Se content and quality of citrus fruits, as compared to its soil application. Particularly, foliar application of 200 mg/L Se during young fruit stage proved to be the most effective approach for enhancing Se bio-fortification, through increasing organic Se content in fruits, single fruit weight, total soluble solids (TSS), solid-acid ratio (TSS/TA) while reducing titratable acidity. However, vitamin C, juice rate and edible rate were unaffected. A positive correlation was observed between total Se content in fruits and various quality parameters, including total soluble solids, solid-acid ratio, fruit shape index and single fruit weight at this stage. These findings offer valuable insights for the development of strategies aimed at enhancing Se bio-fortification in citrus, potentially addressing Se deficiency in the regions where citrus is a dietary staple.

CRediT authorship contribution statement

Yongxia Liu: Investigation, Conceptualization. **Zheli Ding:** Supervision, Data curation. **Tianyan Yun:** Conceptualization. **Tao Jing:** Investigation, Formal analysis. **Ghulam Abbas Shah:** Methodology. **Lixia Wang:** Supervision, Funding acquisition, Data curation. **Yingdui He:** Funding acquisition, Data curation, Conceptualization. **rasha Afify:** Writing – review & editing, Data curation. **Sona El-Nwehy:** Writing – review & editing, Formal analysis, Data curation. **Mamdouh Eissa:** Formal analysis. **Xiaoping Zang:** Software, Methodology.

Declaration of Competing Interest

All the authors declare that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Data availability

Data will be made available on request.

Acknowledgements

Financial support for this study was provided by Hainan Natural Science Foundation Project (317229), China Agriculture Research System of MOF and MARA (CARS-31-25) and Central Public-interest Scientific Institution Basal Research Fund (1630092022001 & 1630052024011).

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