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Research article

Selenium enhancement strategy under precise fertilization in foxtail millet rhizosphere

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A R T I C L E I N F O

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Root application position
Millet
Selenium content

A B S T R A C T

Enhancing selenium content in millet is a crucial strategy to address malnutrition due to selenium deficiency. Jingu 21 was used as the experimental material in this study. The effects of selenium fertilizer application amount, vertical position of fertilization, and horizontal position of fertilization on the selenium content in various millet organs were assessed using a three-factor, five-level quadratic rotation combination design. The results indicate that selenium fertilizer application amount, vertical fertilization position, and horizontal fertilization position significantly affected the selenium content in various millet organs. Analysis of the selenium accumulation for different millet organs show that the recommended optimal agronomic strategy for producing selenium-enriched millet comprises a selenium fertilizer application amount ranging from 100.65 to 120.15 kg/hm^2, a vertical fertilization position of 10.28–11.76 cm, and a horizontal fertilization position of 6.74–7.29 cm. This study elucidates the patterns of selenium content accumulation under precise fertilization measures of millet and provides valuable insights for implementing selenium enhancement techniques in the production of selenium-enriched millet.

1. Introduction

Selenium is an essential trace mineral element for human health. Several studies report that selenium contributes to overall well-being by enhancing and restoring endurance and slowing the aging process [1–3]. Selenium deficiency can lead to diseases such as Keshan disease, Kashin-Beck disease, and endemic fluorosis [4,5]. Global statistics indicate that approximately one billion people suffer from malnutrition due to inadequate selenium intake [6,7], with over 105 million people in China experiencing adverse effects from selenium deficiency [8]. Currently, the average selenium intake among Chinese residents is 26.63 μg/d, significantly lower than

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the recommended daily selenium intake (60 μg/d) for optimal human health [9]. The dietary primary source of selenium intake is from plant-based foods. Studies report that approximately 70 % of selenium intake in China is derived from grains, emphasizing the significance of replenishment selenium through grain-based dietary approaches to tackle malnutrition [10].

Millet, an important plant-based cereal, is cultivated extensively in many countries [11,12]. Millet has high nutritional and health value compared to other grains. Millet is rich in eight essential amino acids, dietary fiber, vitamins, minerals, polyphenols, and flavonoids. Notably, polyphenols have potent antioxidant properties that can alleviate fatigue, enhance the immune system, and inhibit the proliferation of cancer cells. Additionally, millet possesses high levels of dietary fiber and satiety-inducing characteristics, making it a valuable component of "Nutraceuticals" [13–16]. However, millet typically exhibits lower selenium content compared to other grains, for instance, wheat in the main producing areas of China [17]. Therefore, it is imperative to enhance the selenium content in millet through agronomic practices or biofortification methods.

Crops absorb and assimilate selenium mainly from the soil. However, the level of selenium content in arable soil in China is relatively low, with 72 % of the national land area classified as selenium-deficient regions [18]. Soils in regions renowned for significant millet production, such as Shanxi, Inner Mongolia, and Shaanxi, are characterized by severe selenium deficiency. In these areas, relying solely on natural agricultural production to enhance the selenium content in millet is challenging. Therefore, biofortification methods involving adding exogenous selenium are essential for producing selenium-enriched millet, which mainly include root application of selenium fertilizer, foliar spray of selenium, and coating seeds with selenium, but the primary method is root application of selenium fertilizer [19–26].

Root application of selenium fertilizer enhances the abundance and diversity of bacterial and fungal communities in the rhizosphere soil [27]. In contrast to other selenium application methods, root application of selenium fertilizer enriches the available selenium content in the soil and improves the distribution ratio of soil nutrients. Consequently, this positively affects various physiological processes in crops such as rice and wheat, ultimately enhancing overall crop productivity [28–36]. Fertilizer application amount and location are crucial components of the 4R (Right source, Right rate, Right time, Right place) root zone fertilization technique and play a significant role in determining fertilizer efficiency [37]. Optimal fertilizer application amount and position are essential prerequisites for ensuring high quality and yield of crops. Excessive or insufficient (too close or too far) application of fertilizers can lead to wastage and potential reduction in crop yields [38–40]. Current research on the effect of fertilizer application amount or root application location mainly focuses on elements such as nitrogen, phosphorous, and potassium and major grain crops like wheat and maize. However, there is limited comprehensive research on the effect of application amount and root application position on selenium in millet. This study aims to explore the impact of selenium fertilizer application amount and application location on the selenium content in various organs of millet under root zone fertilization practices.

2. Materials and methods

2.1. Experimental site description

The experiment was conducted from May 2022 to October 2023 at the Functional Millet Experimental Base in Taoyuanbao Village, Taigu District, Jinzhong City, Shanxi Province. The study site is situated on the northern periphery of the Loess Plateau and is characterized by typical saline-alkali soil and a temperate semi-arid continental monsoon climate. The soil at the experimental site had an organic matter content of 13.15 g/kg, a total nitrogen content of 0.79 g/kg, an available phosphorus content of 17.52 mg/kg, an available potassium content of 209.46 mg/kg, a pH of 8.03, and a total selenium content of 0.49 mg/kg.

2.2. Experimental materials and design

The Jingu 21 millet variety was used as the experimental material. This variety is registered under the code GPD millet (2017) 140009 and was provided by Shanxi Luyu Seed Industry Co., Ltd, China. Selenium-enriched fertilizer (SETEK-19BF-001) obtained from Suzhou SETEK Co. Ltd, China, containing a total Selenium content of 1000.0 mg/kg, was used in the study. The selenium-enriched fertilizer primarily comprised nano-selenium (particle size≤500 nm), with an organic matter content of≥45 %, total nutrient content (nitrogen + phosphorus pentoxide + potassium oxide) of≥5 %, and moisture (free water) content of ≤30 %. The selenium fertilizer application amount (X_1), vertical position of fertilization (X_2), and horizontal position of fertilization (X_3) were considered in this study, with each factor set at five levels (Table 1). A three-factor, five-level quadratic regression orthogonal rotation combination design was used, resulting in a total of 23 experimental treatment combinations (Fig. 1). Each experimental plot had an area of 1.1 m ×

Table 1
Experimental factor levels and encoding.

Code	Selenium fertilizer application amount (kg/hm^2)	Vertical position of fertilization (cm)	Horizontal position of fertilization (cm)
−1.682	37.5	4.0	0.0
−1	75.0	8.0	4.0
0	112.5	12.0	8.0
1	150.0	16.0	12.0
1.682	187.5	20.0	16.0
△j	37.5	4.0	4.0

8 m and was replicated three times, arranged in a randomized block design. A protective row of 1 m was established around the perimeter of the experimental site, with an additional 0.5 m protective row set between plots. On May 12, 2022, irrigation was conducted once following local standards. Subsequently, deep tillage and soil leveling were performed on May 18 to ensure uniform distribution of soil nutrition and consistent soil properties. On May 20, manual furrows were established, and selenium-enriched fertilizer was applied on one side. Sowing was carried out concurrently, with a row spacing of 50 cm and a seeding rate of 7.5 kg/ hm^2. Other management procedures were consistent with local conventional field management practices and kept the production conditions uniform. A validation experiment was conducted in 2023 following the same experimental protocol used in 2022 to verify the accuracy of the mathematical model for millet grain established in 2022 and to assess the optimal root application strategy for selenium content.

2.3. Sample collection and analysis

After the millet plants reached maturity, three representative plants were randomly selected from each experimental plot. The selected plants were washed three times with tap water to eliminate surface soil and other impurities. Subsequently, the plants were washed thrice with distilled water and dried. The entire plant was divided into five parts: root, stem, leaf, millet grain (after dehusked), and millet bran. Each part was then placed in parchment bags, blanched at 105 °C for 30 min and dried at 55 °C. The samples were ground into powder and sieved through a 100-mesh for selenium content analysis. A multi-element X-ray rapid analyzer was used to determine selenium content, following the procedure outlined in LS/T6115-2016. The specific steps for selenium content analysis were as follows: known concentration samples were placed in sample bottles and analyzed to construct a standard curve. The standard curve was validated using GBW10189 a standard substance for millet flour component analysis. Subsequently, the samples were analyzed in quantitative measurement mode, with the instrument automatically calculating the component levels and displaying the results.

2.4. Data processing and statistical analysis

The selenium content values in each organ of millet were shown in Table 2. Data were organized using Microsoft Office Excel 2016, and data analysis was performed using SPSS 25.0 (Inc., Chicago, IL, USA) Statistics software. The regression equations linking the three factors and the selenium content in various organs of millet plants were established using an iterative stepwise approximation method to find the maximum value. The significance level was set at 0.10. Contour plots of single factors and interaction effects were generated based on the results of response surface regression analysis. These plots were used to evaluate the effect of individual factors and the interaction of these factors on the selenium content in various plant organs. The selenium content model was subjected to a combination of simulations for optimization. Frequency analysis and statistical optimization techniques were utilized to identify the combination strategy within a 95 % confidence interval that approximated the highest selenium content.

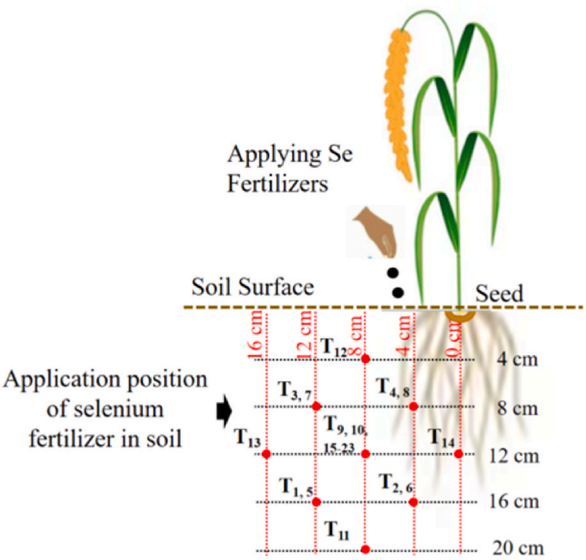

Fig. 1. The position of selenium fertilizer application in the soil. Note: The red dots indicate the locations of the respective treatments in this experiment. The treatments were positioned to the upper left of the red dots. The red dot represents multiple treatments, each comprising a different amount of selenium fertilizer.

Table 2
Experimental design and selenium content in each organ of millet.

Treatment	X_1	X_2	X_3	Selenium Content (mg/kg)				
				Root	Stem	Leaf	Grain	Bran
1	1	1	1	0.527 ± 0.01	0.166 ± 0.01	0.222 ± 0.02	0.403 ± 0.08	0.141 ± 0.08
2	1	1	−1	0.562 ± 0.02	0.199 ± 0.01	0.211 ± 0.02	0.395 ± 0.07	0.157 ± 0.04
3	1	−1	1	0.701 ± 0.02	0.206 ± 0.02	0.246 ± 0.02	0.501 ± 0.05	0.192 ± 0.03
4	1	−1	−1	0.568 ± 0.02	0.162 ± 0.02	0.226 ± 0.02	0.411 ± 0.03	0.225 ± 0.07
5	−1	1	1	0.569 ± 0.01	0.178 ± 0.02	0.178 ± 0.02	0.381 ± 0.09	0.214 ± 0.01
6	−1	1	−1	0.674 ± 0.01	0.171 ± 0.02	0.191 ± 0.02	0.518 ± 0.01	0.221 ± 0.02
7	−1	−1	1	0.612 ± 0.02	0.161 ± 0.02	0.151 ± 0.01	0.381 ± 0.08	0.176 ± 0.01
8	−1	−1	−1	0.566 ± 0.08	0.115 ± 0.02	0.191 ± 0.02	0.420 ± 0.07	0.165 ± 0.05
9	1.682	0	0	0.580 ± 0.05	0.146 ± 0.02	0.212 ± 0.02	0.364 ± 0.06	0.100 ± 0.08
10	−1.682	0	0	0.558 ± 0.04	0.122 ± 0.01	0.157 ± 0.02	0.326 ± 0.01	0.172 ± 0.10
11	0	1.682	0	0.680 ± 0.01	0.239 ± 0.02	0.328 ± 0.02	0.606 ± 0.16	0.273 ± 0.07
12	0	−1.682	0	0.800 ± 0.12	0.223 ± 0.02	0.276 ± 0.02	0.450 ± 0.22	0.230 ± 0.02
13	0	0	1.682	0.617 ± 0.01	0.151 ± 0.02	0.183 ± 0.02	0.494 ± 0.07	0.163 ± 0.01
14	0	0	−1.682	0.510 ± 0.03	0.140 ± 0.02	0.178 ± 0.02	0.370 ± 0.08	0.150 ± 0.01
15	0	0	0	0.590 ± 0.03	0.191 ± 0.02	0.200 ± 0.02	0.400 ± 0.04	0.206 ± 0.04
16	0	0	0	0.611 ± 0.02	0.171 ± 0.02	0.280 ± 0.02	0.417 ± 0.02	0.216 ± 0.04
17	0	0	0	0.593 ± 0.01	0.211 ± 0.02	0.194 ± 0.02	0.450 ± 0.05	0.196 ± 0.07
18	0	0	0	0.627 ± 0.02	0.156 ± 0.02	0.226 ± 0.00	0.415 ± 0.05	0.211 ± 0.01
19	0	0	0	0.625 ± 0.05	0.197 ± 0.02	0.263 ± 0.02	0.455 ± 0.08	0.204 ± 0.04
20	0	0	0	0.625 ± 0.09	0.176 ± 0.02	0.220 ± 0.02	0.444 ± 0.04	0.174 ± 0.03
21	0	0	0	0.652 ± 0.16	0.192 ± 0.02	0.233 ± 0.04	0.383 ± 0.07	0.195 ± 0.02
22	0	0	0	0.729 ± 0.02	0.177 ± 0.02	0.219 ± 0.08	0.371 ± 0.07	0.177 ± 0.01
23	0	0	0	0.611 ± 0.07	0.191 ± 0.02	0.223 ± 0.02	0.428 ± 0.06	0.151 ± 0.04

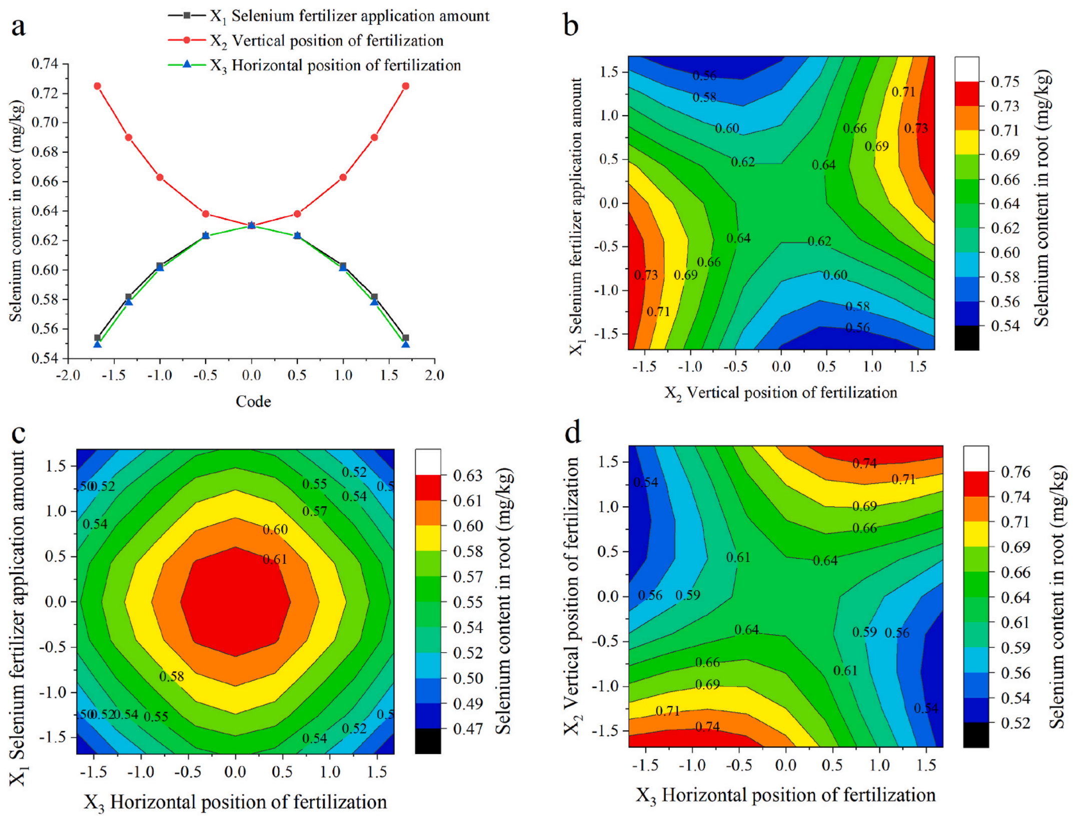

Fig. 2. Effects of selenium fertilizer application amount and position on selenium content in millet root. Note: Effect of individual factors (a), synergistic effect of selenium fertilizer application amount and vertical fertilization position (b), synergistic effect of selenium fertilizer application amount and horizontal fertilization position (c), synergistic effect of vertical fertilization position and horizontal fertilization position (d).

3. Results

3.1. Effects of selenium fertilizer application amount and position on the selenium content in millet root

A regression equation for the selenium content in millet root under the three factors is established as follows: $Y = 0.62979–0.02678X_1^2+0.03367X_2^2-0.02873X_3^2-0.03063X_1X_2-0.03988 \times 2 \times {}_3$. The regression equation accurately models the relationship between the variables ($F_{regression} = 3.419$, $P = 0.0356$), whereas the lack-of-fit term shows that the model accurately fits the data ($F_{lack\text{-}of\text{-}fit} = 1.716$, $P = 0.2003$). These findings indicate that the regression equation effectively models the relationship between root selenium content and the three factors. The optimized agronomic scheme for maximizing millet root selenium content is determined through calculation as $X_1 = -1$, $X_2 = 1.682$, $X_3 = -1$, corresponding to a selenium fertilizer application amount of 75.0 kg/hm^2, a vertical fertilization position of 20 cm, and a horizontal fertilization position of 4 cm. Under these conditions, the millet root selenium content is 0.788 mg/kg. To achieve millet root selenium content greater than 0.620 mg/kg (based on the lower bound of the 95 % confidence interval), the optimized selenium fertilizer application amount ranges from 99.60 to 125.40 kg/hm^2, with a vertical fertilization position between 10.20 and 13.80 cm, and a horizontal fertilization position from 6.62 to 9.38 cm.

Millet root selenium content increases with selenium fertilizer application amount (Fig. 2a). However, beyond a certain amount, the content begins to decrease. The content initially decreases with an increase in vertical fertilization position but starts to increase after surpassing a certain depth. The root selenium content increases with an increase in horizontal fertilization position but begins to decline after surpassing a certain distance. Notably, analysis of the individual factors shows that selenium fertilizer application amount ($P = 0.3274$), vertical fertilization position ($P = 0.6277$), and horizontal fertilization position ($P = 0.4345$) do not significantly influence millet root selenium content. Millet root selenium content initially decreases and then increases with an increase in selenium fertilizer application amount and vertical fertilization position, as well as with increasing vertical fertilization position and horizontal fertilization position (Fig. 2b and d). Millet root selenium content initially increases and then decreases with an increasing selenium fertilizer application amount and horizontal fertilization position (Fig. 2c). The interaction between selenium fertilizer application amount and vertical fertilization position ($P = 0.0900$), as well as vertical fertilization position and horizontal fertilization position ($P = 0.0330$) significantly affects millet root selenium content, whereas the effect of the interaction between selenium fertilizer application amount and horizontal fertilization position is insignificant ($P = 0.2616$).

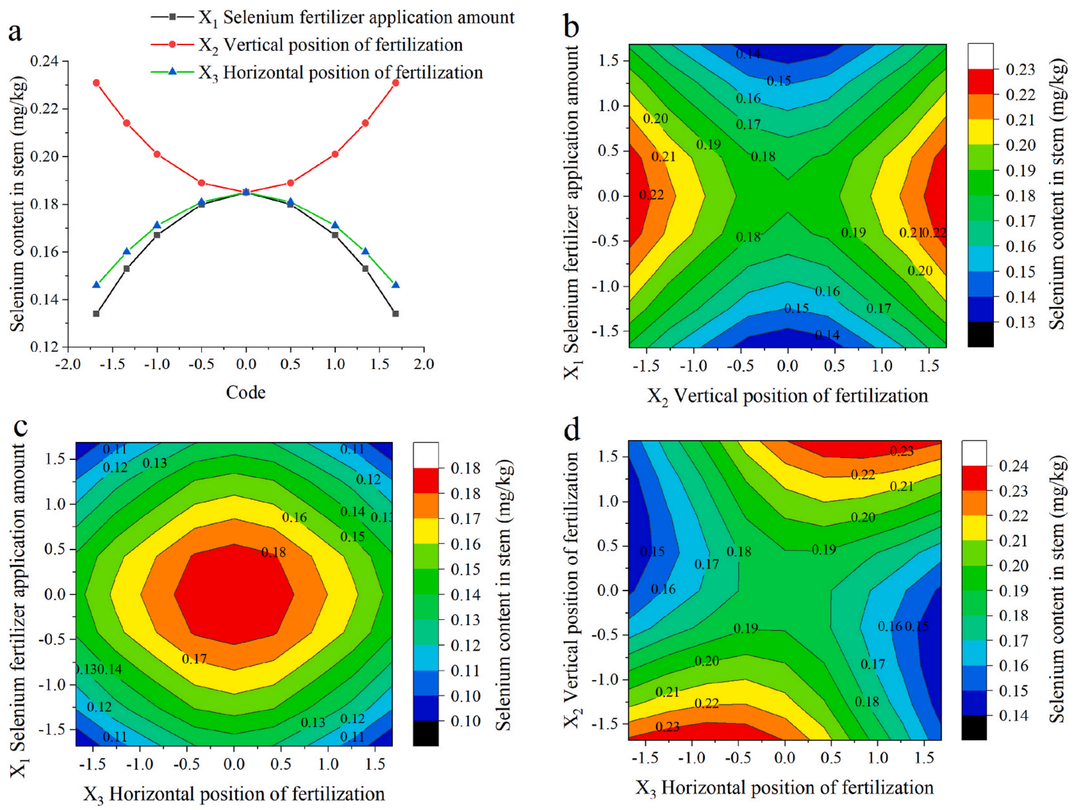

Fig. 3. Effects of selenium fertilizer application amount and position on selenium content in millet stem. Note: Effect of individual factors (a), synergistic effect of selenium fertilizer application amount and vertical fertilization position (b), synergistic effect of selenium fertilizer application amount and horizontal fertilization position (c), synergistic effect of vertical fertilization position and horizontal fertilization position (d).

3.2. Effects of selenium fertilizer application amount and positions on the selenium content in millet stem

A regression equation is established for millet stem selenium content under the three factors as follows: Y = 0.18466–0.01782X_1^2+0.01648X_2^2-0.01375X_3^2-0.01450 × 2 × 3. The regression equation accurately exhibits the relationship between stem selenium content and the three factors ($F_{regression}$ = 4.776, P = 0.0121), whereas the lack-of-fit term shows that the model accurately fits the data ($F_{lack\text{-}of\text{-}fit}$ = 1.974, P = 0.1500). These results show that the regression model effectively models the relationship between stem selenium content and the three factors. The optimal agronomic practices for maximizing selenium content in millet stem are identified as $X_1 = 0$, $X_2 = -1.682$, and $X_3 = 1$, corresponding to a selenium fertilizer concentration of 112.5 kg/hm^2, a vertical fertilization position of 4 cm, and a horizontal fertilization position of 12 cm. The selenium content in the millet stem is 0.242 mg/kg under these conditions. The optimized selenium fertilizer application to achieve millet stem selenium content greater than 0.180 mg/kg (based on the lower bound of the 95 % confidence interval) ranges from 99.45 to 125.55 kg/hm^2, with a vertical fertilization position between 10.06 and 13.94 cm, and a horizontal fertilization position from 6.63 to 9.37 cm.

The effect of selenium fertilizer application amount or horizontal fertilization position on millet stem selenium content exhibits a downward-opening parabolic shape, whereas the impact of the vertical fertilization position shows an upward-opening parabolic shape (Fig. 3a). However, the effect of individual factors including selenium fertilizer application amount (P = 0.3553), vertical fertilization position (P = 0.5519), and horizontal fertilization position (P = 0.5303) do not exhibit a significant effect on stem selenium content. Millet stem selenium content remains unchanged with the increased selenium fertilizer application amount and vertical fertilization position (Fig. 3b). Millet stem selenium content initially increases and then decreases with the increased selenium fertilizer application amount and horizontal fertilization position (Fig. 3c). Conversely, millet stem selenium content initially decreases and then increases with increasing vertical fertilization position and horizontal fertilization position (Fig. 3d). Vertical fertilization position and horizontal fertilization position (P = 0.0512) exhibit a significant synergistic effect on millet stem selenium content, whereas the interactions between selenium fertilizer application amount and vertical fertilization position (P = 0.1828), and selenium fertilizer application amount and horizontal fertilization position (P = 0.4507) exhibit an insignificant effect on millet stem selenium content.

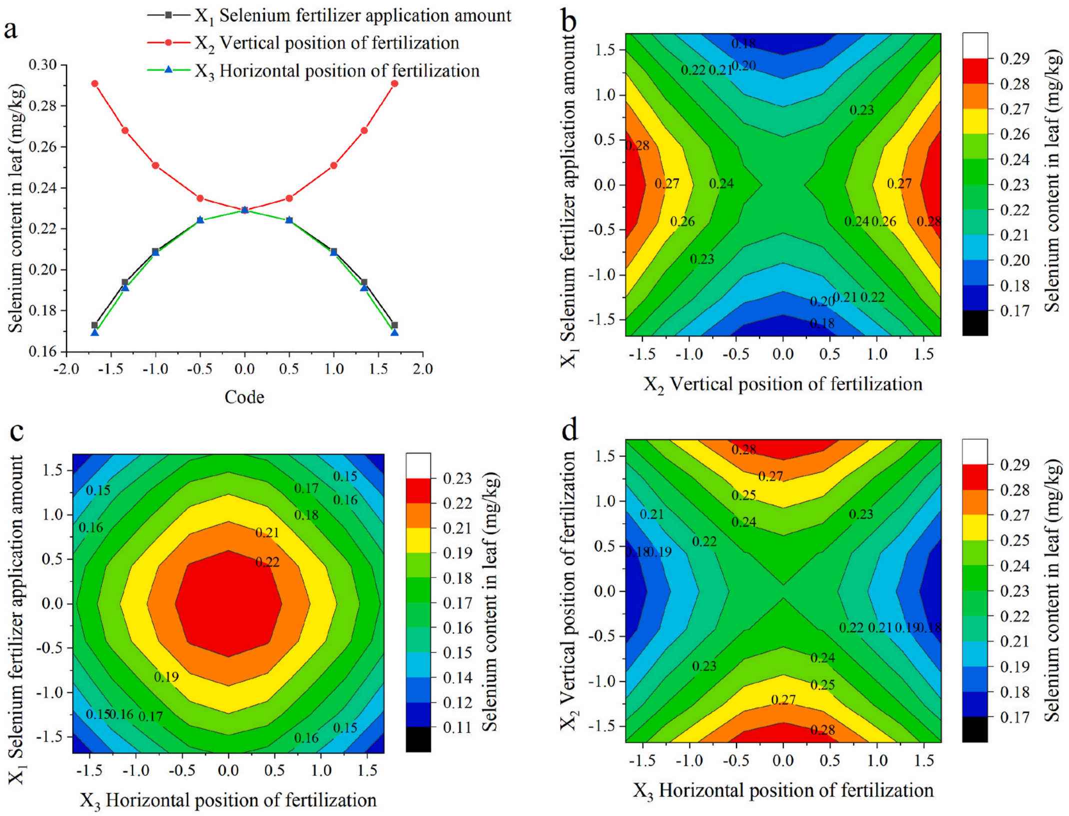

Fig. 4. Effects of selenium fertilizer application amount and position on millet leaf selenium content. Note: Effect of individual factors (a), synergistic effect of selenium fertilizer application amount and vertical fertilization position (b), synergistic effect of selenium fertilizer application amount and horizontal fertilization position (c), synergistic effect of vertical fertilization position and horizontal fertilization position (d).

3.3. Effects of selenium fertilizer application amount and position on the selenium content in millet leaf

The regression equation established for the selenium content in millet leaf under the three factors is: $Y = 0.22910–0.01975X_1^2+0.02179X_2^2-0.02117X_3^2$. The regression equation accurately models the relationship between leaf selenium content and the three factors ($F_{regression} = 2.545$, $P = 0.0825$), and the lack-of-fit term shows that the regression model accurately fits the data ($F_{lack\text{-}of\text{-}fit} = 1.991$, $P = 0.1471$), implying that the regression equation is accurate in modeling the effect of these factors on leaf selenium content. The optimal agronomic solution for maximizing the selenium content in millet leaf is determined as $X_1 = 0$, $X_2 = -1.682$, and $X_3 = 0$, corresponding to a selenium fertilizer application amount of 112.5 kg/hm^2, a vertical fertilization position of 4 cm, and a horizontal fertilization position of 8 cm. Under these conditions, the selenium content in millet leaf is 0.291 mg/kg. The optimized selenium fertilizer application amount to achieve millet leaf selenium content greater than 0.220 mg/kg (based on the lower bound of the 95 % confidence interval) ranges from 100.65 to 124.35 kg/hm^2, with a vertical fertilization position between 9.79 and 14.21 cm, and a horizontal fertilization position from 6.74 to 9.26 cm.

The effects of selenium fertilizer application amount or horizontal fertilization position on millet leaf selenium content exhibit a downward-opening parabolic shape, whereas the effect of the vertical fertilization position demonstrated an upward-opening parabolic shape (Fig. 4a). Single-factor analyses show that selenium fertilizer application amount ($P = 0.4113$), vertical fertilization position ($P = 0.4206$), and horizontal fertilization position ($P = 0.8032$) do not significantly affect millet leaf selenium content. Increasing selenium fertilizer application amount and vertical fertilization position, as well as vertical fertilization position and horizontal fertilization position, do not significantly affect millet leaf selenium content (Fig. 4b and d). Millet leaf selenium content initially increases and then decreases with increasing selenium fertilizer application amount and horizontal fertilization position (Fig. 4c). The interaction effects of selenium fertilizer application amount and vertical fertilization position ($P = 0.4836$), selenium fertilizer application amount and horizontal fertilization position ($P = 0.3754$), and vertical fertilization position and horizontal fertilization position ($P = 0.8471$) exhibit insignificant effects on millet leaf selenium content.

3.4. Effects of selenium fertilizer application amount and position on millet grain selenium content

A regression equation is established to explore the relationship among selenium content in millet grains and the three factors as follows: $Y = 0.41832–0.02038X_2-0.02098X_3-0.02788X_1^2+0.03682X_2^2-0.02650 \times 1 \times {}_2+0.03425X_1X_3-0.02250 \times 2 \times {}_3$. The results

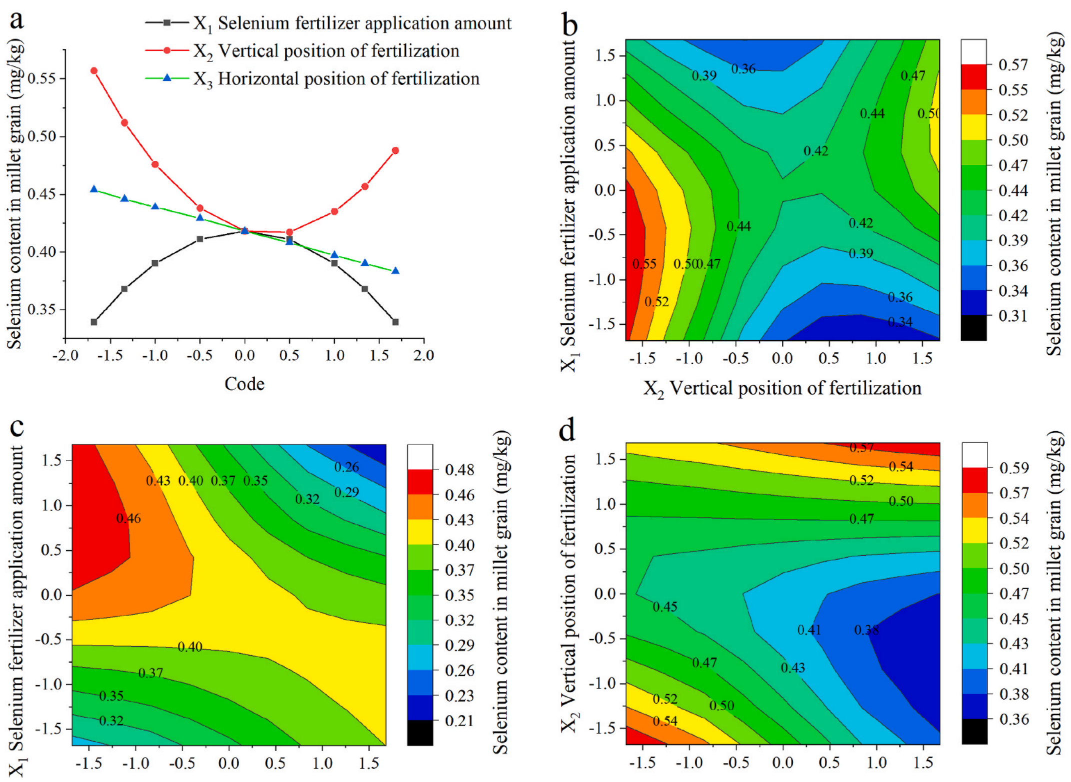

Fig. 5. Effects of selenium fertilizer application amount and position on selenium content in millet grains. Note: Effect of individual factors (a), synergistic effect of selenium fertilizer application amount and vertical fertilization position (b), synergistic effect of selenium fertilizer application amount and horizontal fertilization position (c), synergistic effect of vertical fertilization position and horizontal fertilization position (d).

show that the regression equation accurately models the relationship between millet grain selenium content and the three factors ($F_{regression} = 5.620$, $P = 0.0069$). The lack-of-fit term shows that the regression model effectively fits the data on selenium content and the three factors ($F_{lack\text{-}of\text{-}fit} = 2.265$, $P = 0.1091$). The optimal agronomic solution for maximizing the selenium content in millet grains is $X_1 = -1.682$, $X_2 = 1.682$, and $X_3 = -1.682$, corresponding to a selenium fertilizer application amount of 37.5 kg/hm^2, a vertical fertilization position of 20 cm, and a horizontal fertilization position of 0 cm, resulting in a millet grain selenium content of 0.680 mg/kg. The optimized application of selenium fertilizer required to achieve millet grain selenium content exceeding 0.430 mg/kg (based on the lower bound of the 95 % confidence interval) ranges from 97.05 to 120.15 kg/hm^2, with a vertical fertilization position between 8.74 and 11.76 cm, and a horizontal fertilization position from 4.66 to 7.29 cm.

The selenium content in millet grains increases with an increase in selenium fertilizer application amount. However, after reaching a certain threshold, the selenium content starts to decline, indicating that an optimal selenium concentration promotes crop absorption, whereas excessively high or low concentrations negatively affect selenium uptake. An increase in depth of the vertical fertilization position leads to a decrease in selenium content in millet grains, followed by a gradual increase after surpassing a certain depth. An increase in horizontal fertilization position results in a gradual decrease in selenium content in millet grains. Single-factor analysis shows that the effect of selenium fertilizer application amount ($P = 0.6911$) on the selenium content in millet grains is not significant, whereas vertical fertilization position ($P = 0.056$) and horizontal fertilization position ($P = 0.050$) exhibit significant effects on the selenium content in millet grains. The selenium content in millet grains initially decreases and then increases with an increase in both selenium fertilizer application amount and vertical fertilization position, as well as under increasing vertical fertilization position and horizontal fertilization position (Fig. 5b and d). Conversely, the selenium content in millet grains initially increases and then decreases with an increase in both selenium fertilizer application amount and horizontal fertilization position (Fig. 5c). The interaction effects of selenium fertilizer application amount and vertical fertilization position ($P = 0.0571$), selenium fertilizer application amount and horizontal fertilization position ($P = 0.0183$), and vertical fertilization position and horizontal fertilization position ($P = 0.0997$) significantly affect the selenium content in millet grains.

3.5. Effects of selenium fertilizer application amount and position on the selenium content of millet bran

The regression equation for the selenium content of millet bran under the three factors is established as follows: $Y = 0.19207 - 0.01869X_1^2 + 0.02219X_2^2 - 0.01145X_3^2 - 0.02663 \times 1 \times {}_2$. The results show that the regression equation accurately models the

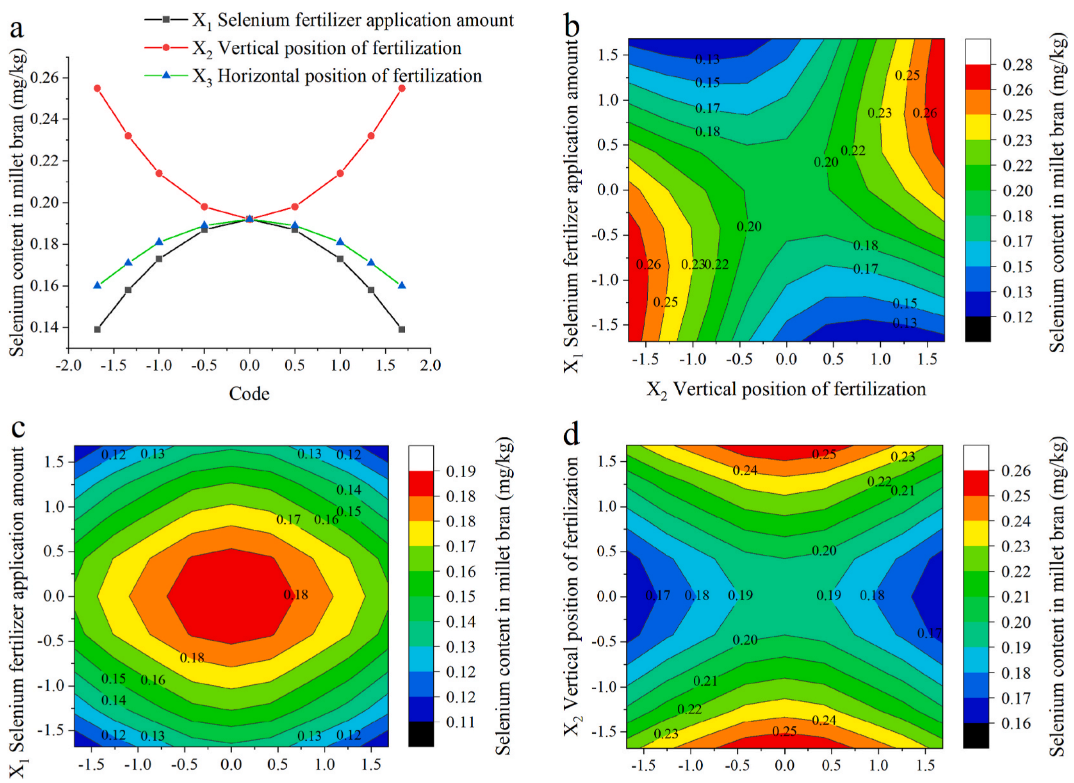

Fig. 6. Effects of selenium fertilizer application amount and position on selenium content in millet bran. Note: Effect of individual factors (a), synergistic effect of selenium fertilizer application amount and vertical fertilization position (b), synergistic effect of selenium fertilizer application amount and horizontal fertilization position (c), synergistic effect of vertical fertilization position and horizontal fertilization position (d).

relationship among the three factors ($F_{regression} = 4.782$, $P = 0.0121$), and the lack-of-fit term shows that the regression model exhibits an accurate fit for the data ($F_{lack\text{-}of\text{-}fit} = 1.530$, $P = 0.2476$). This finding indicates that the regression model effectively predicts the relationship among the three factors. Analysis shows that the optimal agronomic scheme for maximizing the selenium content in millet bran is $X_1 = -1$, $X_2 = 1.682$, and $X_3 = 0$, representing a selenium fertilizer concentration of 75 kg/hm^2, a vertical fertilization position of 20 cm, and a horizontal fertilization position of 8 cm. Under these conditions, the selenium content in millet bran is 0.281 mg/kg. The optimized selenium fertilizer application scheme to achieve millet bran selenium content greater than 0.190 mg/kg (based on the lower bound of the 95 % confidence interval) ranges from 100.05 to 124.95 kg/hm^2, with a vertical fertilization position between 10.28 and 13.72 cm, and a horizontal fertilization position from 6.65 to 9.35 cm.

The influence of selenium fertilizer application amount or horizontal fertilization position on the selenium content on millet bran shows a downward-opening parabolic trend, whereas the vertical fertilization position exhibits an upward-opening parabolic trend (Fig. 6a). Single-factor analysis indicates that selenium fertilizer application amount ($P = 0.4972$), vertical fertilization position ($P = 0.2734$), and horizontal fertilization position ($P = 0.4505$) exhibit insignificant effects on the content of selenium in millet bran. The selenium content of millet bran initially decreases and then increases with an increase in selenium fertilizer application amount and vertical fertilization position, as well as under an increase in vertical fertilization position and horizontal fertilization position (Fig. 6b and d). On the contrary, the selenium content initially increases and then decreases with an increase in selenium fertilizer application amount and horizontal fertilization position (Fig. 6c). The interaction between selenium fertilizer application amount and vertical fertilization position ($P = 0.0061$) significantly influences the selenium content of millet bran, whereas the interactions between selenium fertilizer application amount and horizontal fertilization position ($P = 0.4309$) and between vertical fertilization position and horizontal fertilization position ($P = 0.9880$) exhibit insignificant effects on the selenium content of millet bran.

3.6. Verification of the selenium enrichment model on the optimal millet rhizosphere fertilization strategy

Validation experiments are conducted in 2023 based on the optimal solution, which comprises a selenium fertilizer application amount of 37.5 kg/hm^2, a vertical fertilization position of 20 cm, and a horizontal fertilization position of 0 cm. The actual selenium content in root, stem, leaf, millet grain, and millet bran obtained in the validation experiment conducted in 2023 varies from the selenium contents obtained from the model established in 2022 by 0.23 %, 4.79 %, 0.10 %, 1.47 %, and 0.93 %, respectively (Table 3). These values indicate that the results are relatively consistent between the two periods. Based on the determined agronomic schemes for each organ, the final recommended agronomic scheme for selenium-enriched millet production is a selenium fertilizer application amount of 100.65–120.15 kg/hm^2, a vertical fertilization position of 10.28–11.76 cm, and a horizontal fertilization position of 6.74–7.29 cm.

4. Discussion

Selenium is a beneficial element for plants, which significantly promotes plant growth and development. However, its effectiveness is intricately associated with the concentration of applied selenium, exhibiting an overall trend of stimulation at low concentrations and inhibition at high concentrations [41]. The results of this study indicate that the selenium content in various parts of millet increased with an increase in selenium fertilizer application amount, reaching a maximum before decreasing. These results are consistent with the findings reported by Li et al. [42], demonstrating a clear trend of stimulation at low concentrations and inhibition at high concentrations. This observation may be attributed to the energy-dependent nature of selenium absorption by plant roots, where excessive selenium can damage plant cell membranes, disrupt intracellular water balance, and inhibit plant respiration and photosynthesis [43,44]. A previous study on the impact of root-applied selenium-enriched organic fertilizer on millet demonstrated that the optimal effect occurred at an application amount of 150 kg/hm^2 [45]. This amount was different from the recommended selenium fertilizer application rate of 100.65–120.15 kg/hm^2 obtained in our study. This disparity could be attributed to the varying methods of selenium fertilizer application, whereby conventional basal application was used in the previous study, whereas precision root application based on specific positions was used in our study. This variation in the approach to applying root selenium fertilizer could be a significant factor influencing the observed results.

Root fertilization methods include surface broadcasting, seepage, co-application with seedbed, and deep application in vertical and horizontal positions [46]. Although surface broadcasting is the most predominant method, advances in fertilization technology and the use of mechanized equipment are gradually phased this method out due to its low fertilizer utilization rate (9%–20 %) [47]. The appropriate fertilization position is a critical factor in determining fertilizer utilization efficiency. Li et al. (2019) recommended an optimal agronomic scheme for Zhangzagu 10 comprising a horizontal fertilization position ranging from 16.80 to 18.75 cm and a

Table 3
Results on the validation of the rhizosphere fertilization model for the determination of optimal selenium enrichment strategy.

Organ	The selenium content from the model (mg/kg)	The selenium content in the year 2023 (mg/kg)	Coefficient of variation (%)
Roots	0.863	0.861 ± 0.09	0.10
Stems	0.188	0.179 ± 0.04	1.82
Leaves	0.201	0.199 ± 0.07	0.50
Millet Grains	0.680	0.671 ± 0.05	1.76
Millet Bran	0.215	0.213 ± 0.02	0.39

fertilization depth between 20.80 and 23.75 cm [48,49]. The recommended vertical fertilization position of 8.74–11.76 cm and horizontal fertilization position of 4.66–7.29 cm in our study differ from the previous recommendation. The difference may be attributed to variations in fertilizer types and crop varieties. Therefore, further research should be conducted to elucidate the specific reasons. However, the recommended fertilization depth in our study is consistent with the findings reported by Bryant-Schlobohm et al. and Guo et al. [50,51]. Hou et al. (2023) explored the effect of the position of selenium fertilizer application on wheat physiological characteristics and selenium accumulation and observed that the optimal position for selenium-enriched wheat production was a horizontal fertilization position of 5 cm and a vertical depth of 5 cm from the soil surface [52]. The horizontal fertilization position is consistent with the recommended horizontal fertilization position in our study but the vertical fertilization position is different, possibly due to variations in the maximum depth of root penetration between millet and wheat [53]. In our study, the selenium content in various organs initially decreases and then increases with increased fertilization depth. This result is consistent with the findings reported by Wu (2019) [45], who observed that top-dressing selenium fertilizer during the jointing stage was more effective than root application. This observation implied a correlation between the optimal fertilization depth for selenium-enriched millet production and the growth stage characterized by the strongest root development of the millet. Further research should be conducted to explore the specific reasons underlying this correlation.

5. Conclusions

Millet is an important coarse grain crop characterized by significantly high nutritional content. The precise root application of selenium fertilizer is essential in producing selenium-enriched millet along with high nutritional quality. The effects of selenium fertilizer application amount and position on the selenium content in different millet organs under rhizosphere fertilization practices were explored in this study. A selenium content regression model was used to simulate the optimal combination of the three factors, combined with frequency analysis and statistical optimization. These methods were used to determine the optimal combination measures to achieve the highest selenium content within a 95 % confidence interval. The results revealed that the selenium fertilizer application quantity and position exerted varying effects on the selenium levels in different millet organs. Selenium content in various millet organs initially increased and then decreased with an increase in selenium fertilizer application amount or horizontal fertilization position. Conversely, selenium levels initially reduced and then increased with an increase in vertical fertilization position. The recommended optimal agronomic approach for cultivating selenium-enriched millet to achieve the maximum selenium content in all millet organs is a selenium fertilizer application amount of 100.65–120.15 kg/hm^2, a vertical fertilization position of 10.28–11.76 cm, and a horizontal fertilization position of 6.74–7.29 cm. These findings offer valuable insights for implementing selenium enhancement techniques in producing selenium-enriched millet.

Data availability statement

Data will be made available upon reasonable request.

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CRediT authorship contribution statement

Xiaohu Wang: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Gege Wu:** Investigation. **Yuanqi Wang:** Investigation. **Mengdi Lu:** Investigation. **Yukun Guo:** Investigation. **Wei Yin:** Investigation. **Chenlu Sun:** Investigation. **Youtao Chen:** Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Data curation. **Xuebin Yin:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization.

Declaration of competing interest

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

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