EFFECTS OF SELENIUM THROUGH SOIL APPLICATION ON SOIL FERTILITY AND GRAIN QUALITY IN WINTER WHEAT

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Abstract. The field experiments were conducted at two location (Shenfen and Wujiazhuang) to evaluate Se enrichment through soil fertilization application on durum wheat. Our findings confirm soil Se application had different effects on soil fertility, which reduced the content of available P, but increased the content of available N and kept the content of available K, and soil sucrase, urease and alkaline phosphatase were activated. In addition, soil Se application not only significantly increased grain yield, but also due to most of the Se absorbed by the root system was transported to the aboveground, which significantly increased the concentration of total Se and organic Se in wheat grain. Then, through the regulation of Se metabolism, the absorption of Zn, Ca and Mg elements and the production of amino acids were promoted, and the bioavailability of Cu was reduced in wheat.
Keywords: *soil fertility, yield, selenium species, elements, amino acids*

Introduction

The metalloid selenium (Se) is an essential element in human nutrition. Crops play a critical physiological role in source of dietary Se for most people in the world (Li et al., 2018), which is a non-essential element for crops. However, the concentrations of Se in most crops produced in China are usually less than 60 μg kg^{-1} (William., 2009). According to the National Food Safety Standard in China, Se concentration in Se-enriched wheat was prescribed in the range of 150-300 ug kg^{-1} (GB/T 22499-2008 China). Increasing the production of Se-enriched crops is necessary, because Se supplementation from natural food sources is considered safer than directly ingesting inorganic Se (Liu et al., 2012).

Wheat is a Se sensitive plant, which accumulation capacity of Se was much higher than that of other cereal crops (Combs et al., 2022). It is common to improve the Se status of wheat grain by Se biofortification in the agricultural practice in China (Di et al., 2023; Wang et al., 2021). The background value of Se in soils in China is 0.29 μg g^{-1}, and 72% of the zones are classified as low and deficient levels (Hao et al., 2022). Although there are Se-enriched soils exist, the Se content in grains did not meet the standards of Se enrichment wheat in some slightly Se-enriched soils. Therefore, it is necessary to supplement exogenous Se to improve the Se content in grains according to the situation of soil Se, so as to meet the human demand for Se enrichment crops. Except the natural Se-enriched soil does not need to supply exogenous Se, the vast majority of areas need artificial application of exogenous Se fertilizer to increase the Se nutrient concentration in wheat, which is a short-term and effective method (Ali et al., 2017; Xia et al., 2019).

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The apply methods of Se fertilizer usually used soil or foliar application (Wang et al., 2017). So far, most of the studies found Se utilization efficiency was changed with the application method, dosage, varieties and climatic factors (Wan et al., 2018; Zhao et al., 2017). It is well known that interactions between nutrients should be considered during fertilization (Fernández et al., 2013). However, the effects of the interaction of soil-applied Se with available N, P, K or enzyme activity on soil under field conditions have not been investigated. Se not only affect crop nutrient absorption growth and increase crop organic Se concentration but also affect the intake of restricted amino acids, and exist the antagonistic and synergistic operations with some elements. It is generally considered that high dosages of Se may be toxic to plants and compete with some essential elements, such as Mn, Zn, Cu and Fe, however, in some cases, the stimulating effects of Se on Cu, Fe and Zn uptake in plants were also observed (Xia et al., 2019).

Therefore, it is of great significance to study the effects of soil Se applications on soil fertility, accumulation and transport and nutrient quality in the Se-enriched loess plateau rain-fed agricultural areas, so as to improve the nutritional quality in wheat, guide agricultural production and solve the health problems caused by Se deficiency in residents. In brief, the first aim of this study was to clarify the relationship between Se and Soil fertility. The second aim was to investigate the effects of soil Se applications on Se and its forms, mineral elements change and amino acids production in wheat grain.

Materials and methods

Field site description

Field experiments were conducted at two locations as follow: Shanxi Agricultural University Research Farm in Shenfen (37°25’ N, 112°36’ E, 774 m above sea level) and Wujiazhuang (37°26’ N, 112°31’ E, 802 m above sea level), Shanxi province, China. The experiments were carried out from September 2017 to June 2018. The Shannong129 (*Triticum aestivum L.*) was using in Shenfen and Wujiazhuang. The experimental fields were plain and water land, planting one crop a year. There were four distinctive seasons. Soil samples were collected from the surface layer with a depth of 0-20 cm before the sowing stage and selected physical and chemical properties of the soil are shown in *Table 1*.

Table1. *Selected physical and chemical properties of the soil*

Properties	Values		Methods
	Shenfen	Wujiazhuang	
Organic matter content (g kg^{-1})	17.27	19.96	Potassium dichromate volumetric
Total nitrogen (g kg^{-1})	1.38	0.99	Kjeldahl determination
Available phosphorus (mg kg^{-1})	5.21	4.51	Sodium bicarbonate extraction
Available kalium (mg kg^{-1})	89.54	195.86	Ammonium acetate flame photometric
Alkali-hydrolyzable nitrogen (mg kg^{-1})	49.24	59.51	Alkali-hydrolyzable proliferation
Total Se (mg kg^{-1})	0.50	0.28	ICP-MS

Experimental design

The randomized complete block design was used. Se was added in the form of Se enrichment solid fertilizer (Se concentration 50 mg·kg^{-1}) at rates of 0 g ha^{-1} (control), 37.50 g ha^{-1}. Our previous work showed that application Se concentration of 37.5 g ha^{-1},

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from the range of 0-112.5 g ha^{-1} in the form of Se^{4+} (Na_2SeO_3), could increase Se concentrations up to 290 μg kg^{-1} in the grains of wheat cultivars (Xia et al., 2019). Therefore, Se (Na_2SeO_3) of 37.5 g ha^{-1} was used for this study as well. Each treatment was done in triplicates, and each plot was 56 m^2 (7 m × 8 m) with an inter row spacing of 0.5 m. The Se enrichment solid fertilizer was applied as basal fertilizer and broadcast it in the soil at the sowing stage. The control no applied fertilizer. The seeds were sown in October 23, 2017 at a sowing density of 4500000 plant ha^{-1} and row spacing of 20 cm. The other fertilizer was not applied at wheat growing period. Other field management measures, such as irrigation were applied at greening stage, jointing stage and booting stage, 20 cubic meters each time and herbicides were applied in early April to control weeds. The harvest time was June 16, 2018.

Sample preparation

At the harvest, 0.66 m^2 was harvested in each plot, which was put into a mesh bag, drying, threshing, weighing and yield were calculation. Ten whole plants and its rhizosphere soil (0-20 cm) per plot were randomly sampled at mature stage. The plant was divided into four parts: grain, glume, stem + leaf, and root, which were carefully washed with tap water, followed by rinsing in deionized water. Non-rhizosphere soil was removed by shaking the roots gently, and soil remaining on the roots was collected in valve plastic bags as rhizosphere soil. The overground part were oven-dried at 65 °C for 24 h to constant weight, then the dried samples were ground into powder to pass through a sieve of 0.15 mm and sealed in ziplock bags before Se and its form, elements and amino acids analysis.

Soil nutrient analysis

The dried soil samples were screened for the analysis of soil nutrients. Available N, available P and available K indices were measured using the methods of alkali-hydrolyzable proliferation, sodium bicarbonate extraction and ammonium acetate flame photometric respectively.

Soil enzyme activity analysis

The dried soil samples were screened for the analysis of soil enzyme activity. Urease, alkaline phosphatase and sucrase indices were measured using the methods of indigo colorimetry, phenyldisodium phosphate colorimetric method and 3,5-dinitrosalicylic acid colorimetry respectively.

Se and elements analysis

The flour samples were used to investigate the concentrations of Se and elements. The flour samples were digested by using a HNO_3-H_2O_2 mixture in an alimentary furnace reaction system (LWY84B, China), and then the concentration of Se and minerals were measured by using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700x; Agilent Technologies, USA).

Se forms analysis

The flour samples was weighed. First, 5 mL Tris-HCl was added, which was extracted (30 min) by ultrasonic. Second, 50 mg cellulose enzyme, 0.4 mL proteinase K

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and 0.4 mL proteinase XIV were added, which was oscillated 48 h (50 °C, 250 r/min) by air bath thermostat. Finally, sample was centrifuged 30 min (1000 r/min, 4 °C), the supernatant was collected over 0.22 um drainage membrane. The Se forms in grains was measured by HPLC-ICP-MS.

Amino acids analysis

The hydrolyzed samples were analyzed according to the national food safety standard of the China (GB5009.124-2016). The flour samples was weighed. First, 10 mL HCl and phenol (3-4 drops) were added. Second, the hydrolysis tube was put into the refrigerant (3–5 min) and attached to the suction pipe of the vacuum pump and fill nitrogen (fill nitrogen three times). After that the hydrolysis tube was sealed and put in thermostat (110 °C, 22 h). The hydrolysate was filtered into a 50 mL volumetric flask and constanted to the scale. The filtrate was put into the test tube. In a test tube enrichment apparatus decompression drying in 48 °C heating environment. The dried residue was dissolved by water. Then the solution was vacuumed and dried again and until the top up. Finally, the buffer solution of pH 2.2 sodium citrate was added to the test tube. The solution is to be measured passing through 0.22 nm membrane. Amino acid samples were separated by ion exchange chromatography and measured using the automatic amino acid analyzer (model Biochrom 30+, England).

Calculation methods

Translocation Factor = The Se concentration of overground part (mg kg^{-1}) / The Se concentration of root (mg kg^{-1})

To quantitatively describe the inhibitory effect of Se at a given concentration, the relative inhibition rate of enzyme activity (η) was used.

$$\eta = (1 - Ase / Ack) \times 100\%$$

where Ase is the Soil enzyme activity of Se treatment, Ack is the Soil enzyme activity of CK treatment. The pollution degree was divided into three grades according to the decrease of soil enzyme activity. $\eta < 25\%$ (Slight pollution), $25\% < \eta < 45\%$ (middle level pollution), $\eta > 45\%$ (heavy pollution). If vertical is minus, indicated that it had activation effect on the enzyme activity.

Statistical analysis

For the initial experiment with the dosage of Se applications. The differences in the means were separated at $p < 0.05$ by the LSD (least significance difference) test. All computations were made by employing the statistical software (SAS, version 8.0).

Results

Soil available nutrient and enzyme activity

The soil available nutrient analysis carried out on two locations by Se application showed Se had affect the soil available nutrients at mature stage (*Fig. 1*). The contents of available N and P were changed significantly of Se treatment ($p < 0.05$). There was a

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negative correlation between the content of available P and Se. There was a positive correlation between the content of available N and Se. There was no significant correlation between the content of available K and Se. These results indicated that there might be synergistic effect the transport and absorption of Se and N, and antagonistic effect between Se and P, but not related to between Se and K in the soil by wheat root system.

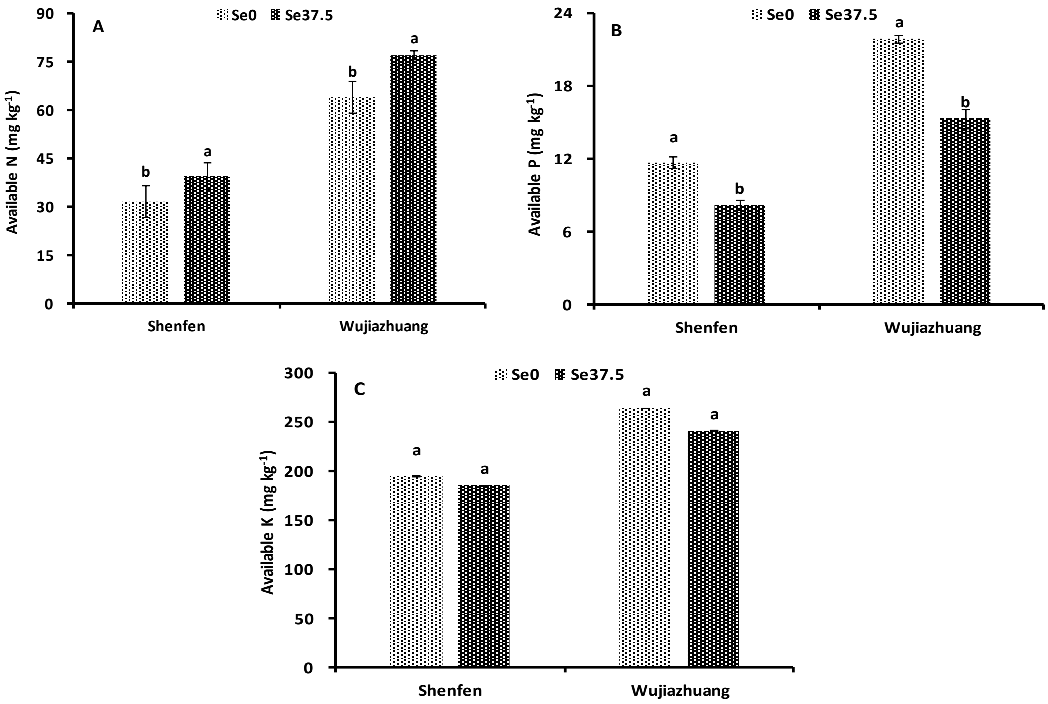

Figure 1. *Effects of soil Se application on soil available nutrient. Different lowercase letters (a, b) indicated significant differences among different treatment of the same location at p < 0.05. The same as below*

To clarify the relationship between Se application and soil enzyme activity, the relative inhibition rate of enzyme activity was calculated (*Fig. 2*). The enzyme activity was activated by Se application. The relative inhibitory rates of urease, alkaline phosphatase and sucrase was -17.3%, -30.0% and -25.6% in Shenfen, respectively. The relative inhibitory rates of urease, alkaline phosphatase and sucrase was -3.2%, -3.2% and -5.4% in Wujiazhuang, respectively. It could be seen that the inhibitory effect of soil enzyme activity in Wujiazhuang after Se fertilization was better than that in Shenfeng, indicated that the effect of exogenous Se on soil enzyme activity in Se-deficient soil was greater than that in mildly Se-enriched soil.

Yield and Se concentration

As presented in *Figure 3*, soil Se applications significantly affected grain yields and Se concentration at the Shenfen and Wujiazhuang ($p < 0.05$). At the Shenfen location, with relative high soil Se, applying Se to the soil increased the grain yield and Se concentration by 31.4%, 95.9%. Nevertheless, in the case of Wujiazhuang have relative low soil Se, applying Se to the soil only increased the grain yield and Se concentration by 9.8%, 300%. The results showed that the applying exogenous Se to the soil could increase the grain yield and Se concentration of wheat.

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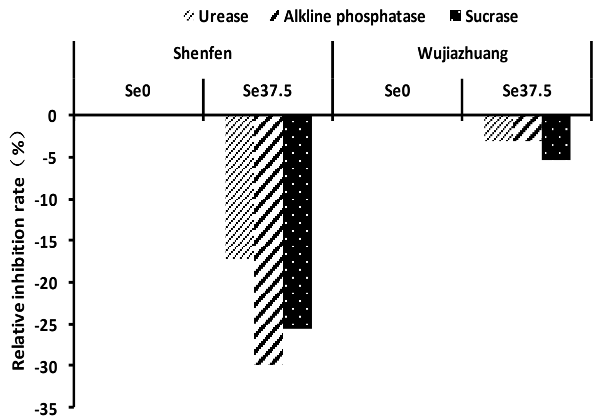

Figure 2. *Effects of soil Se application on soil relative inhibition rate*

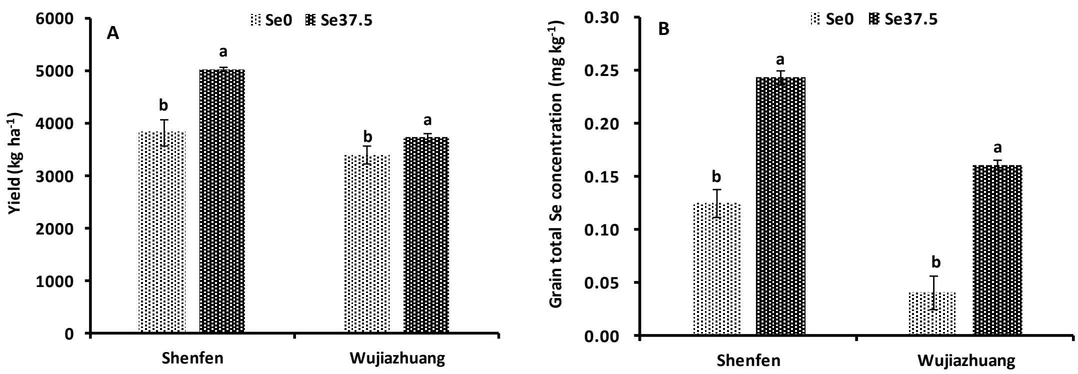

Figure 3. *Effects of soil Se application on yield and Se concentration. Different lowercase letters (a, b) indicated significant differences among different treatment of the same location at $p < 0.05$. The same as below*

As presented in *Table 2*, Se concentrations in root, stem + leaf, grain and glume decreased successively after soil Se application. In both sites, the total Se concentration in the overground part of wheat was higher than that in the corresponding root part. The transport factor were 1.437 and 1.396 in Shenfen and Wujiazhuang, respectively. This indicated that more Se transported to overground part by soil application. However, the two location differences were not significant.

Table 2. *Effects of soil Se application on distribution of every organ Se*

Location	Treatment	The Se concentration of different wheat organs (mg kg^{-1})				
		Root	Stalk + Leaf	Glume	Grain	Translocation factor
Shenfen	Se0	0.213b	0.103b	0.044b	0.104b	1.178
	Se37.5	0.504a	0.312a	0.161a	0.252a	1.437
Wujiazhuang	Se0	0.119b	0.043c	0.024b	0.040b	1.118
	Se37.5	0.445a	0.309b	0.151a	0.161a	1.396

Different lowercase letters (a, b) indicated significant differences among different treatment of the same location at $p < 0.05$. The same as below

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The retention time (tR) of each peak in the standard solution detection map was compared and confirmed, the five labeled peaks in the sample spectrogram from left to right were SeCys2, SeMeCys, Se^{4+}, SeMet, Se^{6+} and SeEt, respectively. However, tR = 32600 ms could not correspond to any Se morphological standard substance selected in this study, so it could not be identified (*Fig. 4*). Se species include organic Se and inorganic Se. Only organic Se was detected in grain with Se treatment (SeMet and SeCys2).

As shown in *Figure 5*, SeMet has the highest percentage in total Se concentration, which amplitude of variation was 55.63% ~ 92.83%. The concentration of SeCys2 was little, which amplitude of variation was 7.17% ~ 44.36%. In addition, after soil Se application, the Se species of grains were transformed. The concentration of SeCys2 decreased, more were converted to SeMet. After soil application of 0 g ha^{-1}, SeMet accounted for 55.63% ~ 55.71% of total Se. After soil application of 37.5 g ha^{-1}, the proportion of SeMet in total Se increased by 84.24%～92.83%.

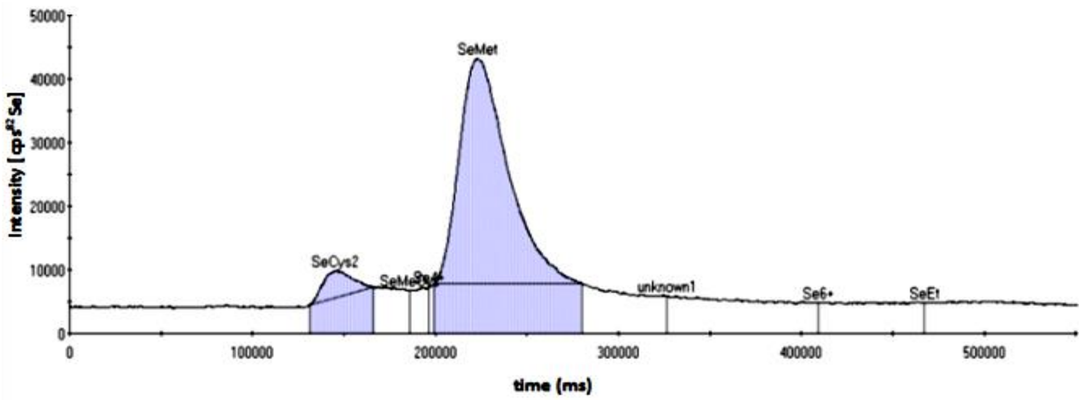

Figure 4. *Chromatograms of Se species in the grain*

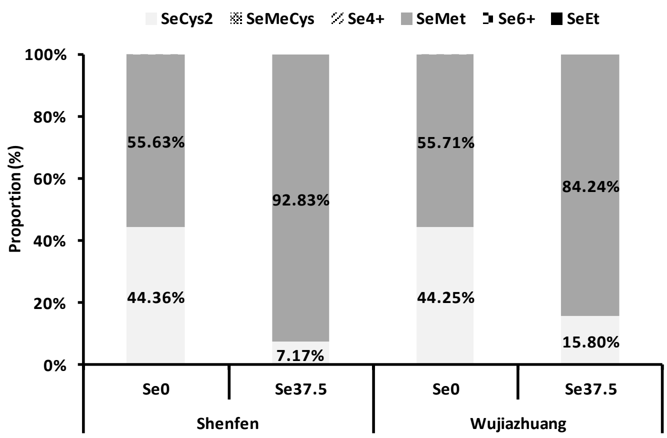

Figure 5. *Effects of soil Se application on proportion of Se forms in total Se*

Grain elements and amino acids concentration

The soil Se application significantly affect grain nutrient element concentration at Shenfen and Wujiazhuang (*Fig. 6*). After soil application of 37.5 g ha^{-1} significantly

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increased grain Zn, Ca and Mg concentrations at both locations, when compared to the control plots where wheat were not treated with soil Se ($p < 0.05$). The increase in grain Zn, Ca and Mg by soil Se applications were more pronounced at Shenfen (increase up to 15.5%, 49.8% and 30.3%, respectively). After soil application of 37.5 g ha^{-1} significantly decreased grain Cu concentration. However, soil Se application did not significantly affect grain Fe and Mn concentrations ($p > 0.05$). It indicated that Se may promote with Zn, Ca and Mg and inhibit with Cu.

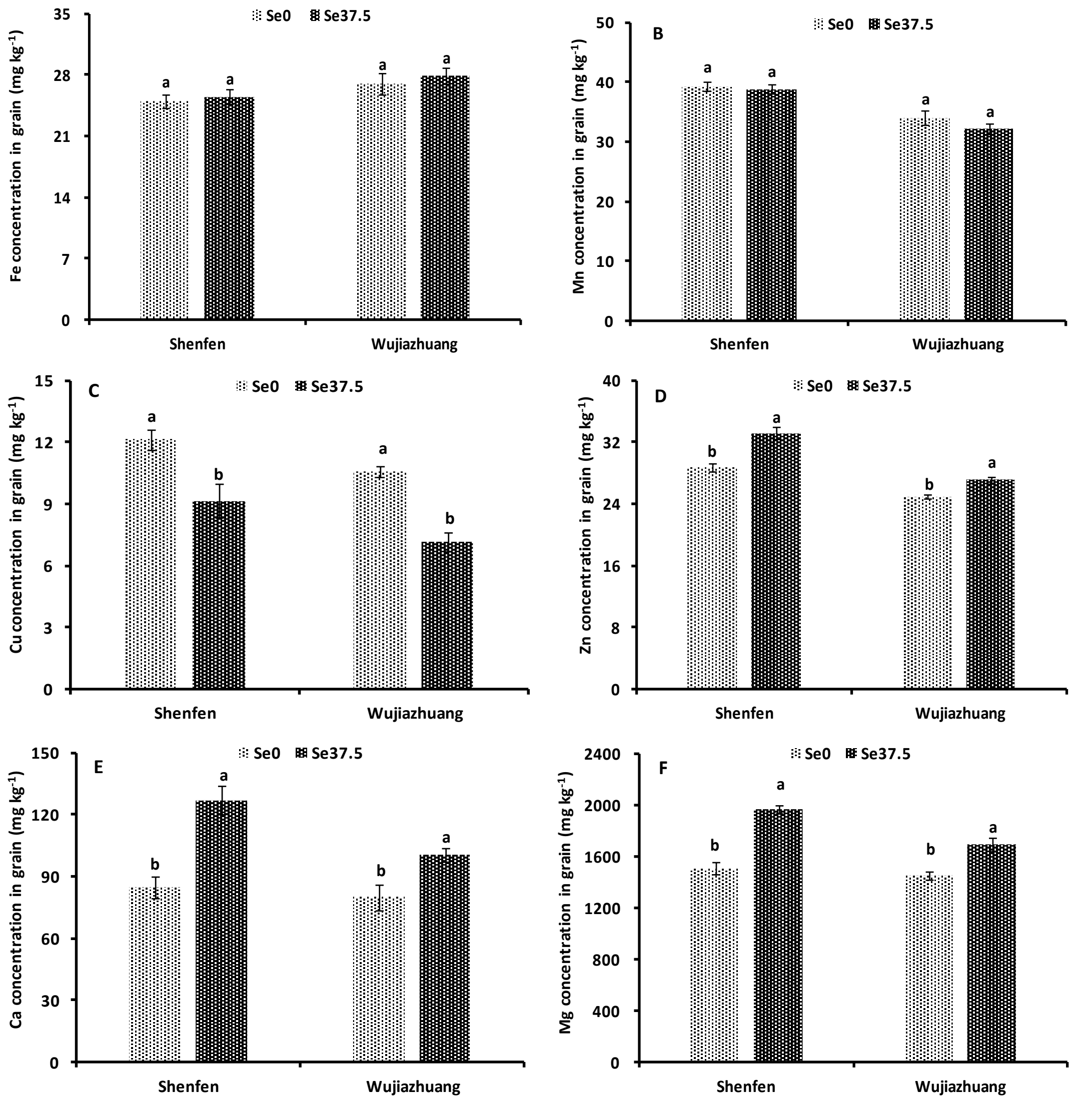

Figure 6. *Effects of soil Se application on concentration of nutrient element. Different lowercase letters (a, b) indicated significant differences among different treatment of the same location at p < 0.05. The same as below*

The amino acid composition (percent of dry weight) is illustrated in *Table 3*. There were sixteen kinds of amino acids were detected (including seven kinds of essential amino acids and nine kinds of essential amino acids). The essential amino acids including isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), valine (Val). The non-essential amino acids including alanine

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(Ala), arginine (Arg), aspartic acid (Asp), glutamate (Glu), proline (Pro), glycine (Gly), histidine (His), serine (Ser) and tyrosine (Tyr).

The total content of detected amino acids was higher in grain of Se-treated wheat than in the control. In the study presented here, soil application 37.5 g ha^{-1} Se had a positive effect on the grain total amino acid content, increasing by 8.3% in Shenfen and by 7.0% in Wujiazhuang compared with the control. We next investigated the amino acid composition in wheat with Se treatment. In terms of essential amino acids, levels Leu, Met, Phe and Val increased 28, 35 and 40%, respectively, because of the application of 37.5 g ha^{-1} Se. The one with a large increase after Se treatment at all concentrations was Phe. With regard to the essential amino acids, the content of Arg, Asp, Glu and Pro also underwent some noteworthy changes. The Arg, Asp, Glu and Pro level increased significantly after soil application of 37.5 g ha^{-1}, by 29.1% compared with the controls.

Table 3. *Effects of soil Se application on concentration of amino acids*

Amino acids		Shenfen		Wujiazhuang	
(% of dry weight)		**Se0**	**Se37.5**	**Se0**	**Se37.5**
EAA	Ile	1.03 ± 0.06	1.10 ± 0.03	1.32 ± 0.08	1.42 ± 0.05
	Leu	2.38 ± 0.05	2.50 ± 0.10*	2.49 ± 0.07	2.66 ± 0.15*
	Lys	0.85 ± 0.03	0.92 ± 0.05	0.92 ± 0.04	0.97 ± 0.01
	Met	0.35 ± 0.01	0.47 ± 0.01*	0.32 ± 0.02	0.45 ± 0.01*
	Phe	1.86 ± 0.05	2.11 ± 0.11*	2.18 ± 0.05	2.31 ± 0.13*
	Thr	0.72 ± 0.02	0.81 ± 0.04	0.86 ± 0.02	0.94 ± 0.03
	Val	1.25 ± 0.03	1.44 ± 0.07*	1.46 ± 0.02	1.56 ± 0.07*
NEAA	Ala	0.69 ± 0.02	0.71 ± 0.05	0.79 ± 0.01	0.85 ± 0.06
	Arg	2.11 ± 0.16	2.23 ± 0.13*	1.95 ± 0.16	2.19 ± 0.13*
	Asp	1.47 ± 0.04	1.63 ± 0.11*	1.65 ± 0.09	1.76 ± 0.07*
	Glu	14.29 ± 0.35	15.32 ± 0.72*	14.69 ± 0.38	15.66 ± 0.56*
	Pro	3.64 ± 0.08	4.11 ± 0.11*	4.11 ± 0.04	4.35 ± 0.03*
	Gly	0.71 ± 0.02	0.72 ± 0.02	0.73 ± 0.02	0.78 ± 0.02
	His	0.79 ± 0.03	0.83 ± 0.05	0.89 ± 0.03	0.93 ± 0.05
	Ser	1.17 ± 0.03	1.27 ± 0.09	1.37 ± 0.05	1.48 ± 0.06
	Tyr	1.40 ± 0.03	1.44 ± 0.11	1.06 ± 0.04	1.17 ± 0.08
∑EAA		8.44	9.32	9.65	10.31
∑NEAA		26.27	28.26	27.24	29.17
total AA		34.71	37.58	36.89	39.48

EAA, essential amino acid; NEAA, non-essential amino acid; AA, amino acid. Values represent the means ± the standard error (SE) of three replicates (N = 3). *indicated significance at the 0.05 levels of probability ($p < 0.05$)

Discussion

The absorption of Se were greatly influenced by soil physical and chemical properties in wheat. In this study, it was found that there may be synergistic effect of wheat root system on the absorption of Se and N in soil, and there may be antagonistic effect on the absorption of P, but not related to K. Se fertilizer could activate sucrase,

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urease and alkaline phosphatase in soil, which possible reason was that Se fertilizer was slowly decomposed and utilized by microorganisms, thus created favorable environmental conditions for the growth of microorganisms, and promoted the increased the number of microorganisms in the soil. It indicates that an appropriate amount of Se could activate the soil enzyme activity, which was conducive to promoting the material circulation and energy flow of the soil.

The effect of Se application on crop yield was related to the physicochemical properties of soil, fertilization method, fertilization period and other factors. Applying Se fertilizer to wheat soil in areas with severe Se deficiency can simultaneously improve yield and quality (Garousi, 2017). There were two views on the response of crop yield to exogenous Se, the one was that proper dose of Se can promote the growth and development of crops and increase the yield (Boldrin et al., 2016). The other one was that Se application does not affect crop yield (Rodrigo et al., 2013). However, it is generally agreed that Se application does not directly improve crop yield by its own nutritional function, but by changing the growing environment of crops. In this study, it was found that the application of proper Se fertilizer in soil could significantly increase the yield of wheat, and the increase of yield in areas with high basic Se content in soil was significantly greater than that in areas with low Se content. In addition, the root system of wheat absorbed a large amount of Se from soil or fertilizer, and the absorbed Se was more transported up to overground part, thus significantly increasing the content of organic Se and total Se in wheat grain. The U.S. food and nutrition commission reports that more than 90 percent of SeMet in crops is absorbed by the body. SeMet is an antioxidant (Panchuk et al., 2016), which had shown strong disease resistance in many diseases research, such as AIDS (Watanabe et al., 2016). SeCys2 is the constitutive part of the 21st amino acid, which played an important role in improving cognitive decline and alleviating Alzheimer’s disease (Zhang et al., 2016). In this study, it was found that the Se applied in seeds would transform between two forms, SeCys2 concentration decreased and SeMet concentration increased, which was consistent with the results of previous studies.

Mineral elements are the essential nutrient elements for crop growth and development, and also the material basis for crop yield and quality. This study found that Se fertilizer application not only could significantly improve the maturation of Se content in wheat grain, but also could improve the content of Zn, Ca and Mg, and decreased Cu content in grain. Se and Zn, Ca and Mg promoted each other, each other with Cu, which possible reason was that Se and Zn, Ca and Mg, combine to form soluble compounds, promote the absorption of Se, and combination between Cu into the undissolved compounds, the Se are precipitated, reduce the biological effectiveness of Se. This was inconsistent with the research results of Guerrero (Guerrero et al., 2014), which may be caused by a variety of factors such as differences in planting environment of selected crop types and soil types.

In addition, the concentration of amino acid significantly influence can be caused by nutrition and fertilization in plant. In this study detect the part of the essential amino acids increased due to supply Se. For example, in our study, the application of Se increased the Met content in wheat, the Jezek’s reported results was not consistent, this could be caused by many factors, such as the choice method of fertilization and crop types (Ježek et al., 2011). If people eat flour with SeMet and no Met, therefore, after people eat flour, SeMet will enter the protein synthesis pathway of human body and fail to play the role of Se, resulting in selenium deficiency (Adadi et al., 2019). However,

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our results showed that the content of Met increased after Se application. This indicated that although the content of SeMet was very high under the optimal fertilizer rate, it would not affect the protein synthesis pathway, thus making up for the lack of Se in our body. Phe content significantly increased after Se treatment. Munshi et al. found that Phe content increased from 20% to 53 with the increase of Na_2SeO_3 dosage in potato tubers (Smoleń et al., 2018). Moreover, Val had also undergone some changes under Se. The non-essential amino acids detected were also increased correspondingly. The application of selenium significantly affected the content of Asp, which could reduce the content of nitrate in plants and play a regulating role in the growth and development of crops. The free form of Pro is related to the resistance of plants to drought and salinization, and the content of Pro increases with the Se. Nevertheless, many researchers believe that the accumulation of Pro is only an indicator of several stresses (Ozturk et al., 2021; de la Torre et al., 2022), and it does not play a role in protecting plants from metal elements. Therefore, the mechanism of Se's influence on the content of amino acids in edible parts of crops needs to be further explored.

In general, the soil application Se could improve the level of Se nutrition, improved the grain amino acid content, improve the grain yield and Se, was the fundamental measures to solve the human Se deficiency, Furthermore, it provided theoretical basis and technical reference for solving the problems of inappropriate Se-enrich way. Since the absorption of Se in wheat caused changes in soil fertility index, so we should study from the perspective of microorganisms in the future to clarify the influence of Se absorption on the structure and diversity of microbial community.

Conclusion

Soil Se application had different effects on soil fertility, which reduced the content of available P, but increased the content of available N and kept the content of available K, and soil sucrase, urease and alkaline phosphatase were activated. Soil Se application not only significantly increased grain yield, but also most of the Se absorbed by the root system was transported to the overground part, which significantly increased the concentration of total Se and organic Se in wheat grain. Then, through the regulation of Se metabolism, the absorption of Zn, Ca and Mg elements and the production of amino acids were promoted, and the bioavailability of Cu was reduced in wheat.

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