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CHARACTERISTICS OF SOIL SELENIUM ENRICHMENT AND ITS EFFECTS ON THE ABOVEGROUND PARTS OF *ABELMOSCHUS MANIHOT* (L.) MEDIC

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Abstract

Pot experiments were carried out to study characteristics of soil selenium (Se) enrichment and its influence on Chinese medicinal plant *Abelmoschus manihot,* and selenium contents in aboveground parts of *A. manihot* under conditions of natural Se-rich, low soils and exogenous Se addition were analyzed. Results indicated that contents of total Se and various Se fractions of natural Se-rich soil were significantly higher than that of Se-low soil ($P < 0.05$), and Se content in aboveground parts of *A. manihot* grown in natural Se-rich soil was significantly higher than that in natural Se-low soil ($P < 0.05$). The Se content in aboveground parts of *A. manihot* increased with the increase of soil total Se content, soil pH and contents of various Se fractions, showing significant tolerance to high Se. Moreover, it was initially found that *A. manihot* could effectively absorb soil Se even in relatively low Se soil conditions (soil Se content of 0.11~0.12 mg/kg) and thus reaching the standard of Se-rich crops.

Introduction

Selenium (Se) is an essential nutrient element for humans and animals and has the effect of removing peroxides, preventing cell damage and delaying cell senescence. Subsequent studies have further shown that Se has important functions in anti-cancer, anti-Keshan disease, anti-Kashin-Beck disease, anti-aging, and enhancing immune function (Peng and Chen 2007, Yu *et al.* 2017). However, China is in the low Se belt of the earth, and 72% of the country's land area is Se deficient to varying degrees, resulting in a general lack of Se intake in the country (Tan *et al.* 1982). Therefore, Se supplementation has become a trend in the pursuit of health. As inorganic Se is not easily absorbed and utilized by the human body and is easy to cause poisoning, therefore, Se supplementation by eating Se-enriched agricultural products is one of the safest and most effective Se supplementation methods (Ellis and Salt 2003, Finley and Davis 2010). The study of Se-enriched plants has expanded from the earliest food crops such as rice and corn to fruits, vegetables, tea, tobacco and horticultural plants, and the factors affect the ability of plants to enrich Se have been studied (Liu *et al.* 2018, Wang *et al.* 2015).

Abelmoschus manihot (L.) Medic is a nearly endangered Chinese medicinal plant discovered by the Chinese Academy of Agricultural Sciences in Xingtai, Hebei in August 2003. It has the most edible, medicinal and health care value among more than 200 okra plants, and has become a research hotspot (Lu and Jia 2015). These plants are rich in nutrients such as polysaccharides, flavonoids, vitamin E, organic acids, unsaturated fatty acids, and has anti-cancer, enhancing the body's antioxidant capacity, lowering blood sugar and blood lipids and other functions (Lu and Jia 2015, Liu 2018). Studying the ability of *A. manihot* to enrich Se will be of great significance to

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further improve its medicinal value. The area of Se-enriched soil (Se content greater than 0.2 mg/kg) has been found to be as high as 8245.12 km^2 in Guanzhong area of Shaanxi Province, China, which provides a geochemical basis for characteristic planting, scientific planting and development and utilization of Se-enriched agricultural products. Therefore, it is of great significance to study the characteristics of Se content in Se-enriched soil and its effect on Se content of *A. manihot*. Moreover, with unique geological environment, landform, soil and climatic conditions, the southern Shaanxi (Qinba Mountains) has become an important "biological gene bank" and a Chinese medicine bank in China. Moreover, there are more than 2,000 kinds of Chinese herbal medicines, and it is known as a "natural medicine bank". Therefore, the introduction of *A. manihot* from southern Shaanxi for cultivation in Guanzhong area is of great significance for the demonstration of Se-enriched planting, the promotion of planting of Se-enriched crops, and the selection of superior Se-enriched crops in Guanzhong.

Thus in the present study, the Se-rich soil and Se-low soil in Shaanxi Guanzhong were taken as research objects, the characteristics of Se and other element content in aboveground parts of *A. manihot* under the conditions of natural Se-rich and Se-low soil and exogenous Se addition were studied through pot experiment, and the planting suitability of Se-enriched *A. manihot* in Guanzhong area was further analyzed.

Materials and Methods

The Se-rich soil (average selenium content 0.73 mg/kg) and Se-low soil (average selenium content 0.12 mg/he) were collected from the corresponding fields in Zhangba Village (109°23′03″ E, 34°38′24″ N) and Xiangqiao Village (109°21′42″ E, 34°36′42″ N) in Lintong District, Shaanxi, China. Then, the Se content in Se-low soil was regulated by adding Sodium selenite (Na_2SeO_3, analytically pure), and was set to 5 levels of Se concentration treatment. And each treatment added exogenous Se concentration (converted from Na_2SeO_3 to Se content) were: T0 (0 mg/kg), T1 (1 mg/kg), T2 (3 mg/kg), T3 (4 mg/kg), T4 (5 mg/kg). The pot experiment of *A. manihot* was carried out in the greenhouse of the Shaanxi Se-enriched crop planting demonstration base, and each treatment in the pot experiment was set as follows: natural Se-rich soil and Se-low soil pot experiment were performed with 3 replicates each, and exogenous Se addition experiments were performed with 3 replicates for each treatment, with a total of 18 potted plants. The growth period of *A. manihot* is about 2 months.

Soil samples were naturally air-dried, crushed agglomerates with wooden sticks, passed through a 20-mesh nylon sieve, and sent to the laboratory for analysis. The *A. manihot* was uprooted at the flowering stage, rinsed with tap water, and then rinsed with deionized water for three times, and then divided into roots and shoots (aboveground parts). All aboveground samples were dried, weighed, crushed, sieved, and stored in sealed bags for later testing.

The analysis methods of soil alkali-hydrolyzed nitrogen, soil available phosphorus, and soil available potassium were alkali-hydrolyzed diffusion method, 0.5 mol/l $NaHCO_3$ extraction-molybdenum antimony anti-spectrophotometry, and NH_4OAc extraction-flame photometry, respectively. The analysis methods of soil pH and soil organic matter were pH meter electrode method and redox capacity method, respectively. The analysis method of the soil Se speciation indicators was plasma emission spectrometry. The method for analyzing soil total Se and Se in the aboveground parts of *A. manihot* was atomic fluorescence spectrometry. The method for analyzing nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg) , iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), boron (B) and molybdenum (Mo) in soil and in the aboveground parts was inductively coupled plasma mass spectrometry.

The sample analysis and testing were undertaken by the Xi'an Mineral Resources Supervision and Testing Center of the Ministry of Land and Resources. During the test, national first-level standards were randomly added to control the analysis quality. The reporting rate of all samples was 100%, and the qualification rate of accuracy and precision monitoring sample was over 98%. Date statistics and analyses were processed by Excel 2013, SPSS18.0 and SigmaPlot 10.0 software. All data were expressed as mean ± standard error (SE).

Results and Discussion

Selenium is a thiophilic element and a biophilic element, and is prone to secondary enrichment or depletion in the process of epigenetic geochemical cycles. Se-rich soil is the soil rich in Se. Tan (1989) proposed the threshold value of Se ecological landscape in China based on endemic diseases and low Se environment in China, and divided the Se content in soil into Se deficiency ($< 0.125\times10^{-6}$), potential Se deficiency [$(0.125\sim0.175)\times10^{-6}$], sufficient Se [$(0.175\sim0.40)\times10^{-6}$], Se enrichment [$(0.40\sim3.0)\times10^{-6}$] and Se poisoning ($> 3.0\times10^{-6}$). Secondly, referring to the classification standard of Se in soil proposed by Li *et al.* (2000), and further referring to the delineation of Se-rich soil in "Delimitation and Labeling of Natural Se-rich Land" (Trial) (DD 2019-10). Soils separately collected from Zhangba Village and Xiangqiao Village in this study was natural Se-rich soil (selenium content of 0.73 mg/kg) and Se-low soil (selenium content of 0.12 mg/kg). It can be seen from Fig. 1 that the Se content in the natural Se-rich soil was significantly higher than that in the Se-low soil ($P < 0.05$), and the former was more than 6 times that of the latter. Moreover, the content of organic matter and most mineral elements in the natural Se-rich soil was significantly higher than that in the Se-low soil in this study (Table 1), and this might be one of the reasons for the higher Se content in the natural Se-rich soil (Xu *et al.* 2020).

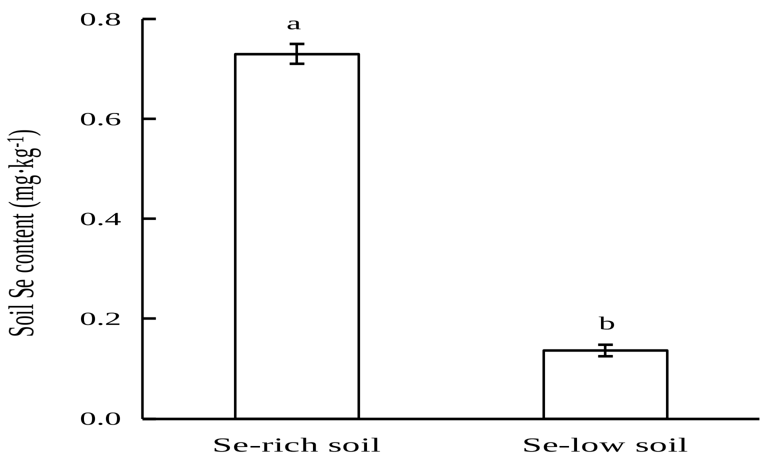

Fig. 1. Total Se content of natural Se-rich and Se-low soils. Different lowercase letters indicate significant differences between the two groups at the 0.05 level.

The differences in the content of different forms of Se in natural Se-rich soil and Se-low soil are consistent with previous research results, showing that the content of soluble and exchangeable selenium is generally low, and Se exists mainly in organically bound and residual state (Fig. 2) (Zhang *et al*. 2002, Wang 2012). Moreover, the Se content of all forms in Se-rich soil is higher than that in Se-low soil (Fig. 2). This might be due to the complex effect of soil organic matter on

Se form and availability. That is to say, the high content of organic matter will increase the content of organic Se, and along with the mineralization of organic Se, the easily soluble small molecules organic Se and inorganic Se in the soil will increase, and thus the bioavailability of Se will be improved (Li *et al.* 2017, Dinh *et al.* 2017).

Table 1. General chemical and physical properties of natural Se-rich and Se-low soils.

Properties	Se-rich soil	Se-low soil	Properties	Se-rich soil	Se-low soil
Organic matter (%)	1.96 ± 0.04 a	0.98 ± 0.05 b	Ca (%)	4.31 ± 0.03 a	4.53 ± 0.05 b
pH value	8.82 ± 0.01 a	8.63 ± 0.03 b	Mg (%)	1.49 ± 0.01 a	1.26 ± 0.02 b
Alkali-hydrolyzable N (mg/kg)	64.90 ± 2.27 a	49.20 ± 1.10 b	Fe (%)	2.97 ± 0.00 a	2.72 ± 0.03 b
Available P (mg/kg)	13.67 ± 1.72 a	14.10 ± 0.90 a	Mn (mg/kg)	713.00 ± 4.51 a	674.33 ± 9.39 b
Available K (mg/kg)	175.00 ± 2.11 a	135.00 ± 0.97 b	Cu (mg/kg)	24.40 ± 0.30 a	19.97 ± 0.38 b
N (mg/kg)	1197.00 ± 11.01 a	652.00 ± 9.01 b	Zn (mg/kg)	70.30 ± 0.47 a	55.30 ± 1.15 b
P (mg/kg)	1224.33 ± 13.97 a	856.33 ± 29.76 b	Na (%)	1.33 ± 0.02 a	1.43 ± 0.01 b
K (g/kg)	20.50 ± 2.93 a	19.20 ± 1.45 a	B (mg/kg)	58.40 ± 3.50 a	47.37 ± 1.19 b
S (mg/kg)	258.67 ± 1.67 a	155.67 ± 4.18 b	Mo (mg/kg)	0.95 ± 0.00 a	0.87 ± 0.04 a

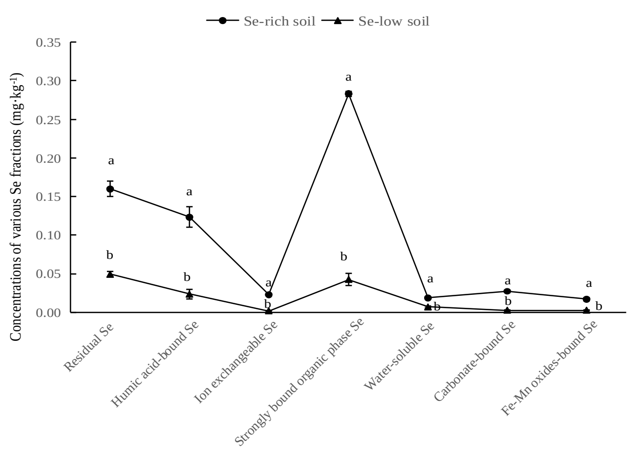

Fig. 2. Concentrations of various Se fractions in natural Se-rich and Se-low soils. Different lowercase letters indicate significant differences in the concentrations of various Se fractions between Se-rich and Se-low soils at the 0.05 level.

It was found that the contents of only Na and Se in aboveground parts of *A. manihot* grown in natural Se-rich soil were significantly higher than that in Se-low soil ($P < 0.05$) (Fig. 3), and the significantly higher Na and Se content in the aboveground parts of *A. manihot* in natural Se-rich soil might be due to that plant uptake of Se in the form of sodium selenate or sodium selenite and thereby increasing plant Na content (Sun *et al.* 2020).

It was also believed that low availability of Se in soil would lead to low Se content in plants, and thus lead to Se deficiency in humans or animals (Winkel *et al.* 2012). Moreover, it was generally believed that: the water-soluble Se, ion-exchangeable Se and carbonate-bound Se were the readily available Se that was easily bioavailable, and that was the soil-available Se; the humic acid-bound and Fe-Mn oxides-bound Se were moderately available Se; the strongly bound organic phase Se and residues Se were inert state Se (Zhou *et al.* 2016, Liang *et al.* 2017). Therefore, the available Se content of natural Se-rich soils in this study was significantly higher than that of Se-low soils (Fig. 2), and this would be the reason why the Se content of *A. manihot* grown in natural Se-rich soils was significantly higher than that of *A. manihot* grown in Se-low soils.

Table 2. Element content characteristics in aboveground parts of *A. manihot* under treatments of different Se concentrations.

Elements	T0	T1	T2	T3	T4
N (%)	1.48 ± 0.41 a	1.35 ± 0.18 a	1.68 ± 0.01 a	1.51 ± 0.16 a	1.63 ± 0.35 a
P (%)	0.26 ± 0.01 a	0.29 ± 0.03 a	0.30 ± 0.02 a	0.24 ± 0.00 a	0.27 ± 0.03 a
S (%)	0.36 ± 0.05 a	0.39 ± 0.03 a	0.37 ± 0.01 a	0.34 ± 0.04 a	0.40 ± 0.04 a
K (%)	2.43 ± 0.07 a	2.30 ± 0.16 a	2.41 ± 0.13 a	2.33 ± 0.09 a	2.23 ± 0.10 a
Ca (%)	1.73 ± 0.33 a	2.04 ± 0.21 a	1.83 ± 0.12 a	1.76 ± 0.23 a	2.17 ± 0.36 a
Mg (%)	1.42 ± 0.21 a	1.37 ± 0.12 a	1.39 ± 0.04 a	1.33 ± 0.09 a	1.37 ± 0.15 a
Fe (mg/kg)	179.33 ± 4.37 a	209.33 ± 32.42 a	177.00 ± 5.51 a	165.33 ± 11.85 a	202.00 ± 41.62 a
Mn (mg/kg)	62.70 ± 45.18ab	51.13 ± 14.27 b	62.43 ± 27.58ab	111.27 ± 74.66ab	121.67 ± 69.17ab
Cu (mg/kg)	7.95 ± 0.59 ab	9.13 ± 1.17 ab	9.57 ± 0.40 a	6.70 ± 0.71 b	8.05 ± 0.75 ab
Zn (mg/kg)	19.70 ± 2.50 a	19.80 ± 2.80 a	18.13 ± 1.66 a	14.27 ± 1.01 a	20.37 ± 3.24 a
B (mg/kg)	60.40 ± 14.45 a	77.10 ± 14.73 a	68.17 ± 5.25 a	65.93 ± 9.05 a	64.57 ± 8.03 a
Mo (mg/kg)	1.42 ± 0.19 a	1.44 ± 0.19 a	1.33 ± 0.05 ab	0.93 ± 0.02 b	1.07 ± 0.08 ab
Na (%)	0.20 ± 0.05 a	0.29 ± 0.05 a	0.25 ± 0.13 a	0.53 ± 0.23 a	0.55 ± 0.21 a
Se (mg/kg)	0.04 ± 0.00 d	0.63 ± 0.09 c	1.58 ± 0.14 b	2.54 ± 0.12 a	2.62 ± 0.13 a

Different lowercase letters indicate significant differences among treatments at the 0.05 level. T0, T1, T2, T3 and T4 represented for each treatment added exogenous Se concentration of 0, 1, 3, 4 and 5 mg/kg, respectively.

Lu *et al.* (2018) found that Se content of wheat grains grown on Se-deficient soil (soil Se content of 0.205 mg/kg) did not meet the Se-enriched standard. Whereas the present study showed that the Se content in the aboveground parts of *A. manihot* grown in the natural Se-rich soil and in the natural Se-low soil all met the Se content standard of Se-enriched edible agricultural products (medicinal plants) in Shaanxi Province (DB61/T 556-2018) (> 0.02 mg/kg). The present study indicated that the *A. manihot*, a traditional Chinese medicine, still had a strong Se enrichment ability in low Se soil conditions and both the natural Se-rich and Se-low soils (soil Se content of 0.11 mg/kg ~0.12 mg/kg) in Guanzhong area in this study were suitable for the production of Se-enriched *A. manihot*.

It can be seen from Table 2, Mn, Cu and Mo contents showed some differences among soils treated with different concentrations of Se ($P < 0.05$), and especially, the Se content in the aboveground parts of *A. manihot* showed a significant difference among soils treated with different concentrations of Se ($P < 0.001$). In addition, except between T4 and T5 treatments, there were significant differences in Se content in the aboveground parts of *A. manihot* between any other two treatments (P < 0.05). Se content in the aboveground parts of *A. manihot* showed an obvious trend of increasing with the increase of soil Se content, and the Se content level was much higher than that of the *A. manihot* growing on the natural Se-rich soil (0.16 mg/kg) (Table 2). This indicated that the soil Se content had a significant effect on the Se enrichment of *A. manihot*. However, it can also be found that when the amount of Se added in the soil increased from 4.0 mg/it to 5.0 mg/it, the average Se content in the aboveground parts of *A. manihot* tended to be relatively stable, indicating that there might be an extreme value of Se absorption in *A. manihot* (Yang *et al*. 2022).

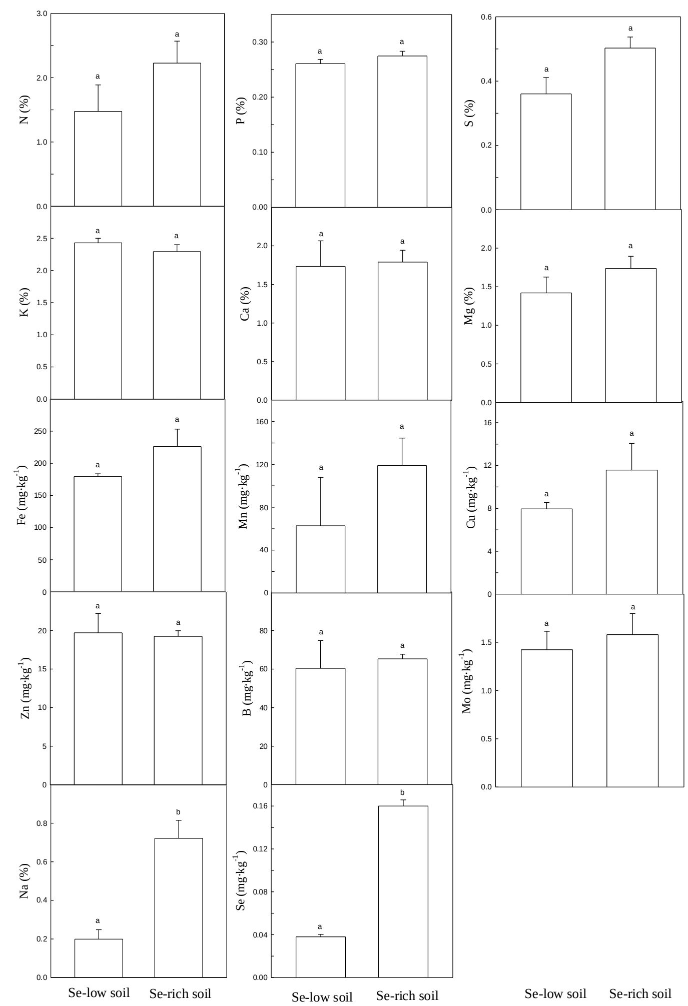

Fig. 3. Element content characteristics in aboveground parts of *A. manihot* in natural Se-rich and Se-low soils. Different lowercase letters indicate significant differences between the two groups at the 0.05 level.

The biological characteristics of plants themselves also affect the bioavailability of Se to a large extent, that is, different plants have different enrichment abilities for Se. It was observed that *Cruciferae* (such as mustard, cabbage) had a strong ability to enrich Se (Duan *et al.* 2011a, 2011b), while *A. manihot* was an annual herb belonging to the family Malvaceae and there were few studies on its absorption efficiency of Se in soil. Compared with the Se content (0.424 mg/re) of *A. manihot*, obtained as a high Se-enriched crop from the Se-enriched cultivation test carried by the project of selenium-enriched crop planting demonstration base in Shaanxi Province, the Se accumulated significantly in the *A. manihot* with the increase of Se in the soil in this study. It was further obversed that *A. manihot* had a strong Se enrichment ability and is a high-Se-tolerant crop, and is suitable for promotion and cultivation in Guanzhong as a Se-enriched functional health food.

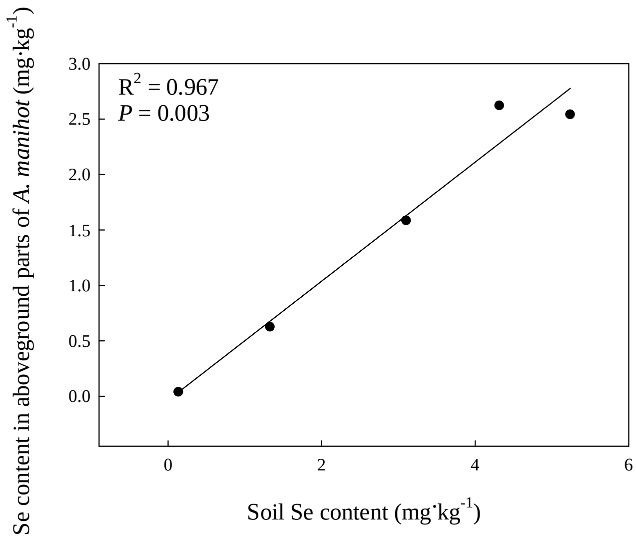

Fig. 4. Correlations between soil Se content and Se content in aboveground parts of *A. manihot* under treatments of different Se concentrations.

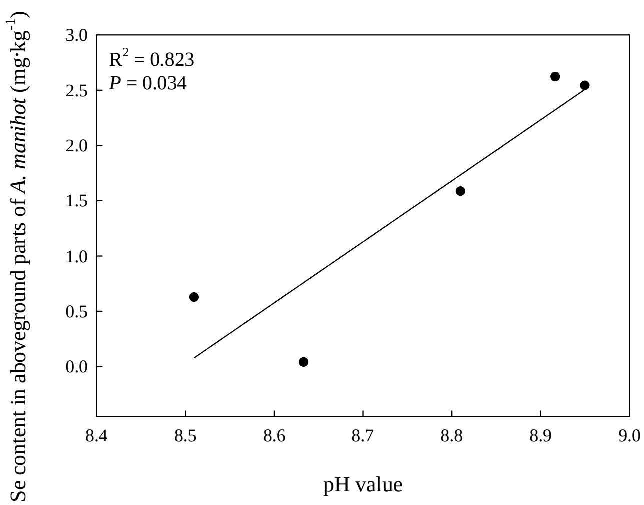

Fig. 5. Correlations between soil pH and Se content in aboveground parts of *A. manihot* under treatments of different Se concentrations.

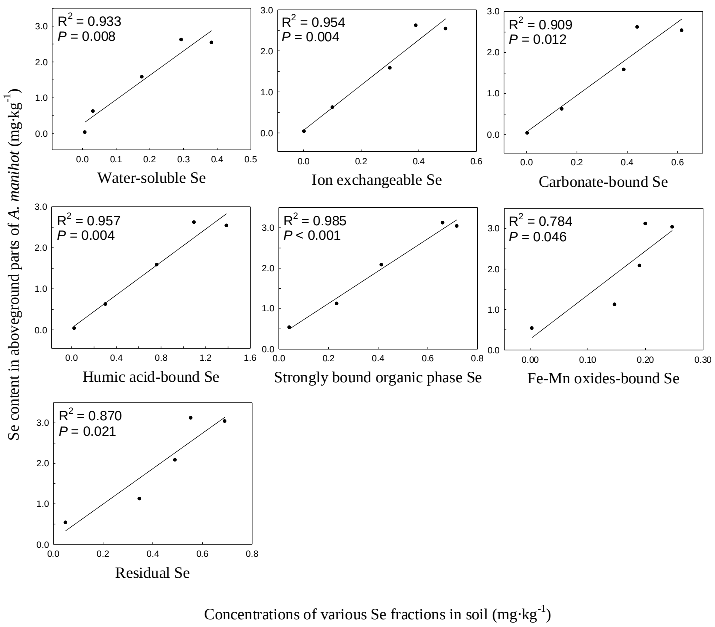

Fig. 6. Correlations between various Se fraction content in soil and Se content in aboveground parts of *A. manihot* under treatments of different Se concentrations.

Table 3. Pearson's correlation between soil total Se content and soil chemical factors.

Soil physical and chemical factors	Soil total Se content	
	Pearson's r	*P* value
pH value	0.900	0.037
Water-soluble Se	0.985	0.002
Ion exchangeable Se	0.998	0.000
Carbonate-bound Se	0.991	0.001
Humic acid-bound Se	0.999	0.000
Strongly bound organic phase Se	0.993	0.001
Fe-Mn oxides-bound Se	0.919	0.027
Residual Se	0.965	0.008

The soil properties that affect the absorption of Se by crops include soil Se content, pH, organic matter and nutrient elements (Zhou *et al.* 2014). In the present study, it was found that under different concentrations of exogenous Se addition, the Se content in the aboveground parts of *A. manihot* increased significantly with the increase of soil total Se content, the soil pH and the content of Se in various forms in soil ($P < 0.05$) (Figs. 4, 5, and 6). These results were consistent with the results of other studies (Duan *et al.* 2011a, Jiang *et al.* 2015, Dai and Tu, 2018) and soil pH may be affected by different concentrations of exogenous Se additions and showed a significant correlation with plant Se content (Table 3) (Khanna *et al.* 2022).

Data showed that the Se content in the aboveground part of *A. manihot* was significantly related to the content of seven Se forms in soil (Fig. 6). The change of Se content of various forms in soil should be caused by the addition of different concentrations of exogenous Se (Table 3), and the variation of Se content in *A. manihot* might be caused by the change of soil available Se content (Khanna *et al.* 2022).

The present study firstly clarified the characteristics of soil selenium enrichment and its influence on the Chinese medicinal plant *Abelmoschus manihot.* The following conclusions may be drawn from the present study: Firstly, the total selenium content and the content of various forms of selenium in the natural selenium-rich soil in Guanzhong, Shaanxi Province were significantly higher than those in the low-selenium soil, and the selenium content in the aboveground part of *A. manihot* grown in natural selenium-rich soil was significantly higher than that in the aboveground part of *A. manihot* grown in low-selenium soil; Secondly, under different concentrations of exogenous selenium, Se content in the aboveground part of *A. manihot* was significantly correlated with the total selenium content, pH value and the content of selenium in various forms in the soil; Thirdly, *A. manihot* showed significant high selenium tolerance, and it was initially found that it could effectively absorb soil selenium in natural selenium-rich soil, artificial selenium application and even relatively low selenium soil conditions, reaching the standard of selenium-rich crops. In summary, the Guanzhong area is suitable for planting the selenium-enriched traditional Chinese medicine *A. manihot*, and as a kind of Chinese medicine that is on the verge of extinction, *A. manihot* has strong selenium-enriching ability, good development prospects, and is suitable for large-scale planting. The research will be of great significance to provide theoretical basis and data support for the in-depth development and marketization of Se-enriched *A. manihot* and increase the added value of Se-rich land.

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