



Amino acid promotes selenium uptake in medicinal plant *Plantago asiatica*

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Abstract The medicinal plant, *Plantago asiatica* have high selenium (Se) accumulation ability but is considered lower compared to other Se-hyperaccumulators. In this experiment, we evaluated the effects of different amino acid concentrations (600, 900, 1200, and 1500-fold dilutions) on the growth and Se uptake in *P. asiatica* for possible improvement of Se accumulation ability and medicinal value of *P. asiatica*. The 600, 900, and 1200-fold amino acid dilutions increased the root and shoot biomass of *P. asiatica*. Additionally, the photosynthetic pigments contents (chlorophyll *a*, chlorophyll *b*, and total chlorophyll) and antioxidant enzymes activities (superoxide dismutase, peroxidase, and catalase) of *P. asiatica* were increased by the different amino acid concentrations. However, these amino acid concentrations reduced the soluble protein content of *P. asiatica* to some extent. The Se content and extraction from *P. asiatica* were also enhanced and had a quadratic polynomial regression relationship with the Se extraction tissues and their Se contents. In addition, there were significant correlations between the biomass of Se extraction tissues and their Se contents. Our findings indicate that various amino acid concentrations promote growth and Se uptake in *P. asiatica*, but 900-fold amino acid dilution is the best concentration for enhancing Se accumulation ability in *P. asiatica* shoots.

Keywords Amino acid · Growth · *Plantago asiatica* · Selenium

Introduction

Selenium (Se) is one of the essential trace elements in the human body, with multiple functions such as anti-oxidation, immunity strengthening, anti-cancer activities, protein synthesis regulation, and enhancing reproductive functions (Rayman 2000; Chauhan et al. 2019). However, Se intake varies worldwide compared with many other micronutrients due to the difference in its amount in food and the availability in the food chain (Rayman 2012; Chauhan et al. 2019). Low Se concentrations (lower than 1 mg/kg) can enhance plant stress tolerance, stimulate plant growth, improve photosynthesis, and inhibit the absorption and accumulation of toxic metals. Such concentrations can also improve plant antioxidant and reactive oxygen species (ROS) system activities (Pilon-Smits et al. 2009; Chauhan et al. 2017; Feng et al. 2013). Conversely, Se promotes oxidation and can increase ROS and lipid peroxidation in plants at higher concentrations (Terry et al. 2000). Some Se-hyperaccumulator plants are weeds (Yuan et al. 2013; Shao et al. 2007); therefore, it is important to increase Se uptake by food or medicinal plants useful for human health. However, there are few reports on promoting Se accumulation in plants.

There are some agronomic measures such as intercropping, straw returning, application of plant hormones and biostimulants that can promote plant growth and nutrient absorption (Bao et al. 2009; Liang et al. 2021; van Asten et al. 2005; Xie et al. 2019). Biostimulants are the hotspots for study and application in recent years, which include the

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amino acids, humic acid, seaweed extract, chitosan, etc. (Xie et al. 2019). Amino acids are organic compounds containing basic amino and acidic carboxyl groups, used to synthesize proteins and many low-molecular-weight compounds of great physiological importance (Hou et al. 2015). Amino acids are very essential for cell structures, enhancing protein nutrition and human immune function, and maintaining body growth, development, and health (Hou et al. 2015; Li et al. 2007). In plants, amino acids are involved in many important processes including biosynthesis of important substances, resistance to stress, signal transduction, and stress responses (Rai 2002; Hildebrandt et al. 2015). Studies have found that spraying certain amino acids on the leaves can increase biomass, yield components and effectively promote growth in onions (Khalil et al. 2008). Seed treatment and foliar application of amino acids can improve soybean water shortage tolerance and increase plant dry biomass and yield (Teixeira et al. 2020). Moreover, exogenous application of proline promotes the absorption of K^+ , Ca^{2+} , nitrogen, and phosphorus in two maize varieties during water stress (Ali et al. 2008). Under heavy metal stress, applying glycine on plants successfully improves growth, photosynthesis, antioxidant enzyme activity, nutrient absorption, and reduces heavy metal absorption and oxidative stress (Hawkesford and Zhao 2007). Another study found that amino acid and protein levels may be related to the accumulation and transport of Se in crops since the selenate and selenite absorbed by plants need to be transported by the sulfate transporter family (Ali et al. 2020). These studies show that applying amino acids may effectively promote plant growth and nutrient (including Se) absorption and transportation, but there are few reports on it.

Plantago asiatica, a perennial herbaceous plant, is a folk medicine with many edible and medicinal values (Ma 2006; Lin and Kan 1990). It contains various biologically active substances, which can treat various diseases and be used as antipyretics, diuretics, antivirals, and wound healing agents (Chiang et al. 2003; Lithander 1992). A previous study showed that *P. asiatica* has a high accumulation ability of Se (Liao et al. 2018); however, the accumulation rate is lower compared with other Se hyperaccumulators such as *Cardamine hupingshanensis* (Yuan et al. 2013) and *Thlaspi arvenst* (Shao et al. 2007). If amino acids were applied on *P. asiatica*, the Se uptake and medicinal value of *P. asiatica* could be further improved. So, in this experiment, the effects of amino acid on the growth and Se accumulation of *P. asiatica* were studied. This study aimed to determine the most effective amino acid concentrations for promoting growth and Se uptake in *P. asiatica*, to provide a reference for *P. asiatica* production.

Materials and Methods

Materials

Plantago asiatica seeds were collected from fields around Ya'an Polytechnic College in Yucheng District, China, in June 2020. The seeds were air-dried and stored at 4 °C.

Inceptisol soil samples were collected from the farmland fields around Ya'an Polytechnic College in August 2021. Basic properties of the soil samples were: pH value 7.71, organic matter concentration 13.45 g/kg, alkaline nitrogen concentration 102.35 mg/kg, available phosphorus concentration 68.45 mg/kg, available potassium 48.49 concentration mg/kg, and total Se concentration 0.02 mg/kg.

The amino acid used in the experiment was the amino acid water soluble fertilizer, which was produced by Shanxi Kingshine Bio-technology Co., Ltd. (in China). It was an aqueous solution with the total amino acid concentration ≥ 100 g/L and copper + iron + manganese + zinc + boron concentration ≥ 20 g/L.

Experimental design

The experiment was conducted at Ya'an Polytechnic College from August 2021 to November 2021. The soil samples were treated as described by Lin et al. (2020a, b) after placing 3.0 kg of the soil samples in plastic pots measuring 15 cm \times 18 cm (height \times diameter). A final Se concentration (5 mg/kg) of the soil samples was achieved by mixing them with pure analytical sodium selenite (Liao et al. 2018). *P. asiatica* seeds were germinated in the incubator, and four uniform seedlings with five expanded true leaves were transplanted into each pot. The seedlings were watered daily to maintain the soil moisture content at 80% of the field capacity until the plants were harvested. A week after the transplantation, gradient concentrations of the amino acid solution (0, 600-, 900-, 1200-, and 1500-fold dilutions) were sprayed on the leaves and stems of the plants. The spraying criterion ensured that a layer of small water droplets uniformly attached to the leaves during each phase of spraying (about 20 mL solution for each pot). The amino acid solution (0, 600-, 900-, 1200-, and 1500-fold dilutions) was again sprayed a week later after the first spraying phase. Each treatment was conducted in triplicates.

Mature leaves (the fourth pair of leaves from the top) were collected from each plant after 2 months (November 2021) following the first application of amino acid. These leaf samples were used to determine the contents of soluble proteins and photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) and the activities of antioxidant enzymes [superoxide dismutase

(SOD), peroxidase (POD), and catalase (CAT)]. Photosynthetic pigments content was determined using ethanol and acetone extraction methods (Liu et al. 2021; Hao et al. 2004). Meanwhile, SOD, POD, and CAT activities were determined through nitroblue tetrazolium photoreduction, guaiacol colorimetric, and UV spectrophotometry methods, respectively. The Coomassie-brilliant blue staining method was used to determine the soluble protein content described previously (Lin et al. 2020a, b; Liu et al. 2021; Hao et al. 2004). Thereafter, the whole plant was harvested and treated following a method described previously (Lin et al. 2020a, b). The root and shoot biomass (dry weight) were determined using an electronic balance, and dried plant tissues were ground for Se content evaluation. Briefly, plant samples (0.5 g) were digested with nitric and perchloric acids ($\text{HNO}_3/\text{HClO}_4$; (4:1, v/v)) and reduced with hydrochloric acid, upon which Se content was determined using inductively coupled plasma (ICP) spectrometer (iCAP 6300, Thermo Scientific, Waltham, MA, USA). Other parameters were determined as follows: Se translocation factor (TF) = Se content in shoots/Se content in roots (Rastmanesh et al. 2010); Extracted Se = Se content in plant \times plant biomass (Zhang et al. 2010).

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 20.0. Data were analyzed using one-way analysis of variance (ANOVA) with the least significant difference (LSD) post hoc test at P value < 0.05 . Pearson correlation and the quadratic polynomial functions were used for correlation and regression analyses, respectively. The correlation results were visualized using Cytoscape v. 3.5.1 (The Cytoscape Consortium, New York, USA).

Results

Biomass of *P. asiatica*

The amino acid concentrations of 600, 900, and 1200-fold dilutions significantly increased the root and shoot biomass of *P. asiatica* (P value < 0.05) compared with the control. The root biomass increased by 8.42%, 9.82%, and 14.04%, while the shoot biomass increased by 8.75%, 13.31%, and 15.59% for the three amino acid concentrations, respectively. However, the 1500-fold dilution of the amino acid had no significant (P value > 0.05) effect (Fig. 1A, B). Regression analysis showed that the amino acid concentration had a quadratic polynomial regression relationship with the root and shoot biomass (Fig. 1A, B).

Photosynthetic pigment contents of *P. asiatica*

The amino acid concentrations of 600, 900, 1200, and 1500-fold dilutions significantly increased chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents of *P. asiatica* (P value < 0.05) compared with the control (Table 1). The total chlorophyll content was increased by 9.20%, 17.21%, 18.91%, and 10.90%, at 600, 900, 1200, and 1500-fold dilutions, respectively, compared with the control. Moreover, the 1200-fold amino acid dilution significantly increased the carotenoid content of *P. asiatica* (P value < 0.05), while the 600, 900, and 1500-fold amino acid dilutions had no significant effect (P value > 0.05) compared with the control.

Antioxidant enzymes activities and soluble protein contents of *P. asiatica*

The 600, 900, 1200, and 1500-fold amino acid dilutions significantly increased the antioxidant enzymes (SOD, POD, and CAT) activities of *P. asiatica* (P value < 0.05) compared with the control (Table 2). The 600, 900, 1200, and 1500-fold amino acid dilutions increased the SOD activity by 4.37%, 6.10%, 8.24%, and 4.04%, respectively. Similarly, the POD activity increased by 6.49%, 10.23%, 13.37%, and 7.62%, while the CAT activity increased by 14.07%, 83.47%, 86.10%, and 72.93%, at the four amino acid concentrations, respectively. Conversely, the 900, 1200, and 1500-fold amino acid dilutions significantly decreased the soluble protein contents of *P. asiatica* (P value < 0.05), while the 600-fold amino acid dilution had no significant effect (P value > 0.05) compared with the control.

Selenium content of *P. asiatica*

The Se root content of *P. asiatica* significantly increased (P value < 0.05) by 103.46%, 92.06%, 83.27%, and 67.94% at 600, 900, 1200, and 1500-fold amino acid dilutions respectively, compared with the control (Fig. 2A). Similarly, the Se shoot content of *P. asiatica* significantly increased (P value < 0.05) by 25.47%, 45.67%, 28.36%, and 22.29% at 600, 900, 1200, and 1500-fold amino acid dilutions, respectively, compared with the control (Fig. 2B). Regression analysis showed a quadratic polynomial regression relationship between the amino acid concentrations and the Se contents in roots and shoots (Fig. 2A, B). The four amino acid concentrations (600, 900, 1200, and 1500-fold dilutions) significantly reduced the TF in *P. asiatica* (P value < 0.05) compared with the control (Fig. 3). However, there were no significant differences (P values > 0.05) in the TF contents

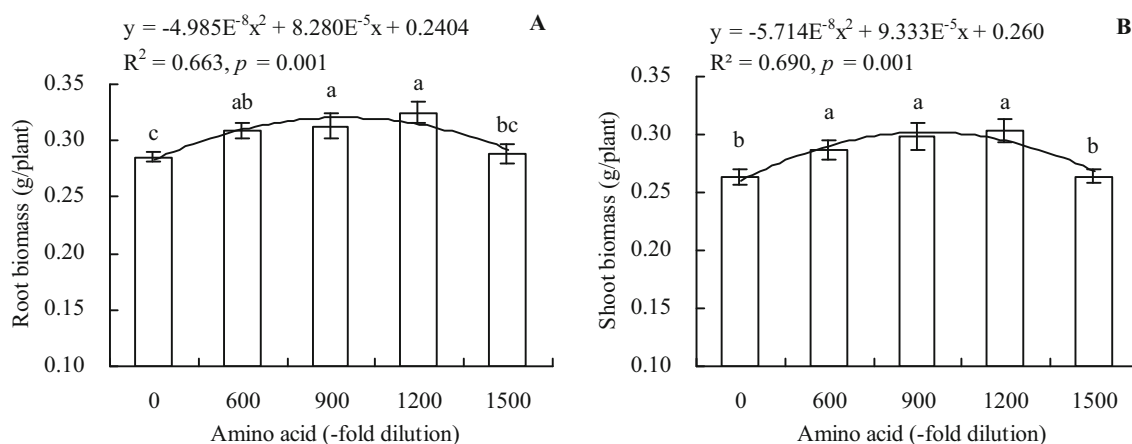


Fig. 1 Effects of amino acid on biomass of *P. asiatica*. **A** Root biomass; **B** shoot biomass. Different lowercase letters indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference (P value < 0.05)

Table 1 Effects of amino acid on photosynthetic pigment contents in *P. asiatica*

Amino acid (fold dilution)	Chlorophyll <i>a</i> (mg/g)	Chlorophyll <i>b</i> (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
0	0.439 ± 0.003c	0.148 ± 0.003b	0.587 ± 0.006c	0.094 ± 0.002b
600	0.480 ± 0.015b	0.161 ± 0.005a	0.641 ± 0.020b	0.099 ± 0.005b
900	0.518 ± 0.011a	0.170 ± 0.004a	0.688 ± 0.015a	0.104 ± 0.004ab
1200	0.526 ± 0.014a	0.171 ± 0.003a	0.698 ± 0.011a	0.110 ± 0.006a
1500	0.489 ± 0.006b	0.161 ± 0.007a	0.651 ± 0.012b	0.099 ± 0.004b

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference (P value < 0.05)

Table 2 Effects of amino acid on antioxidant enzyme activities and soluble protein content of *P. asiatica*

Amino acid (-fold dilution)	POD activity (U/g/min)	SOD activity (U/g)	CAT activity (mg/g/min)	Soluble protein content (mg/g)
0	1214 ± 5.66d	203.4 ± 0.30d	3.007 ± 0.032d	37.33 ± 0.95a
600	1267 ± 4.24c	216.6 ± 1.38c	3.430 ± 0.112c	36.47 ± 0.85a
900	1288 ± 11.31b	224.2 ± 1.02b	5.517 ± 0.060a	29.43 ± 1.01b
1200	1314 ± 2.83a	230.6 ± 1.21a	5.596 ± 0.104a	29.24 ± 1.20b
1500	1263 ± 9.90c	218.9 ± 1.14c	5.200 ± 0.101b	29.86 ± 0.46b

Different lowercase letters within a column indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference (P value < 0.05)

among the different concentrations of amino acid treatments.

Selenium extraction from *P. asiatica*

The 600, 900, 1200, and 1500-fold amino acid dilutions significantly increased ($p < 0.05$) Se extraction from the *P. asiatica* roots by 120.66%, 110.82%, 108.85%, and 69.84%, respectively (Fig. 4A). The Se extraction from the *P. asiatica* shoots also increased significantly ($p < 0.05$) by 35.85%, 64.53%, 47.92%, and 22.26%, at 600, 900, 1200,

and 1500-fold amino acid dilutions, respectively (Fig. 4B). The regression analysis demonstrated a quadratic polynomial regression relationship between the amino acid concentrations and the Se extraction from roots and shoots (Fig. 4A, B).

Correlations between the biomass of Se extraction tissues and their Se content

The root biomass had a significant correlation ($0.01 < P$ value < 0.05) with the root and shoot Se contents and an

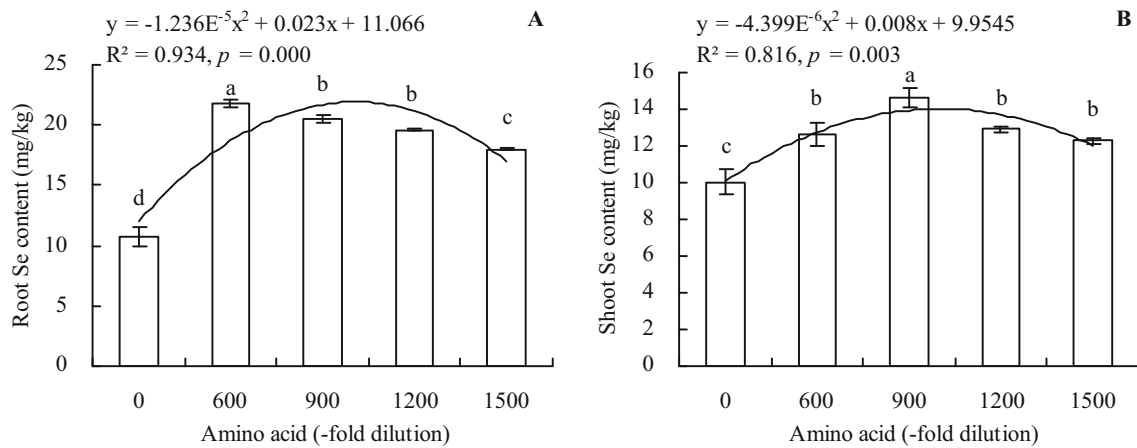


Fig. 2 Effects of amino acid on Se contents in *P. asiatica*. **A** Root Se content; **B** shoot Se content. Different lowercase letters indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference (P value < 0.05)

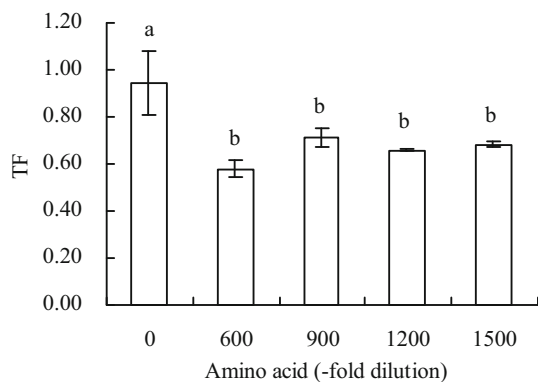


Fig. 3 Effects of amino acid on translocation factor (TF) of *P. asiatica*. Different lowercase letters indicate significant differences based on one-way analysis of variance (ANOVA) with the least significant difference (P value < 0.05)

extremely significant correlation (P value < 0.01) with the root and shoot Se extractions (Fig. 5). Conversely, the

shoot biomass exhibited a significant correlation ($0.01 < P$ value < 0.05) with the root and shoot Se contents and root Se extraction. An extremely significant correlation (P value < 0.01) existed between the shoot biomass and the shoot Se extraction. The root and shoot Se contents also had extremely significant correlations (P value < 0.01) with the root and shoot Se extractions, respectively.

Discussion

Amino acids are the basic units of plant proteins and could be homogenous nutrients for plants, increasing chlorophyll content, enhancing photosynthetic capacity, and promoting rapid growth in plants (Yan et al. 2002). The application of amino acids can increase the growth of new shoots and the number of branches in *Syzygium grijsii*, but to a limited

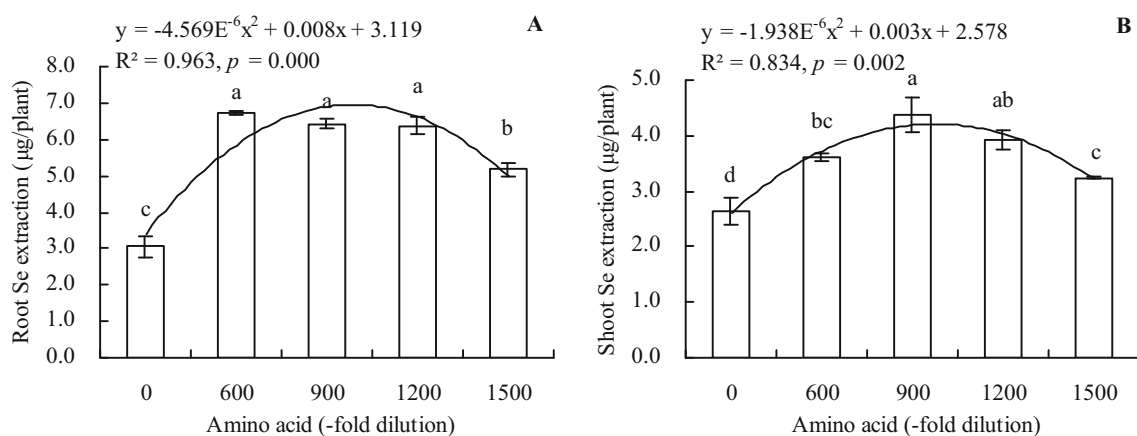


Fig. 4 Effects of amino acid on Se extraction from *P. asiatica*. **A** Root Se extraction; **B** shoot Se extraction. Different lowercase letters indicate significant differences based on one-way analysis of

variance (ANOVA) with the least significant difference (P value < 0.05). Se extraction by plants = Se content in plants × plant biomass

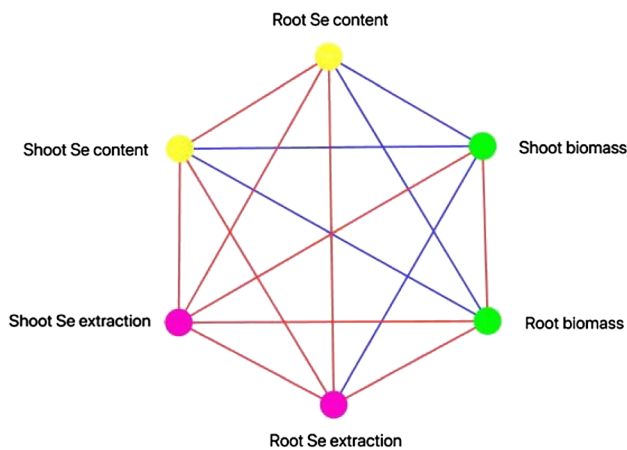


Fig. 5 Correlations between the biomass of Se extraction tissues and their Se content. The correlation was based on the Pearson correlation analysis function of SPSS 20.0.0. The blue solid line indicates the significance level ($0.01 < P \text{ value} < 0.05$), and the red solid line indicates the extreme significance level ($P \text{ value} < 0.01$)

extent (Liu et al. 2011). Moreover, different concentrations of glycine and glutamine can also increase the dry and fresh weights in lettuce (Noroozlo et al. 2019). In this experiment, the 600, 900, and 1200-fold amino acid dilutions increased the biomass of *P. asiatica*, while the 1500-fold amino acid dilution had no significant effect, which is consistent with the results of previous studies (Liu et al. 2011; Noroozlo et al. 2019). In addition, the amino acid concentrations had a quadratic polynomial regression relationship with the root and shoot biomass of *P. asiatica*. This may be because amino acids contain the most important nutrient element, nitrogen, which promotes plant metabolism and morphogenesis, thus directly or indirectly affecting plant physiological activities (Abd El-Aal et al. 2010). Therefore, amino acids could promote growth in *P. asiatica*.

Photosynthesis is essential for the growth and development of plants, and amino acids promote the synthesis of photosynthetic pigments, thereby enhancing plant photosynthesis and growth (Li et al. 2008). Application of exogenous amino acid increases the chlorophyll content and affects photosynthetic characteristics in lettuce, with the L-methionine concentration of 0.2 mg/L exhibiting the best effect (Khan et al. 2019). Under salt stress, the total chlorophyll content of tomatoes is increased by applying amino acids, and the chlorophyll content increases with the increase of amino acid concentration (Tantawy et al. 2009). In this study, the four amino acid concentrations increased the contents of chlorophyll *a*, chlorophyll *b*, and total chlorophyll in *P. asiatica*. Only the 1200-fold amino acid dilution increased the carotenoid content of *P. asiatica*, similar to the results of previous studies (Khan et al. 2019; Tantawy et al. 2009). These findings may be attributed to

the stimulatory effect of amino acids on chlorophyll biosynthesis and reduced chlorophyll degradation in plants, which leads to increased photosynthetic pigment contents in plants (Causin 1996; Sourı et al. 2017).

Plants have high-efficiency and complex enzyme and non-enzymatic antioxidant defense systems, which play important roles in removing active oxygen and maintaining normal plant oxidative metabolism (Gapper and Dolan 2006). Amino acids are widely involved in enzymatic reactions as enzyme components and various physiological processes of plants (Alfosea-Simón et al. 2021). The application of amino acids on the leaves and seeds of soybeans can affect the antioxidant enzyme activities; for example, glycine increases POD and SOD activities, while cysteine increases the CAT activity (Teixeira et al. 2017). Additionally, amino acid treatment increases the CAT activity of wheat but has no significant effect on the POD activity under salinity stress (Bahari et al. 2013). In this study, we found that the various amino acid concentrations increased the SOD, POD, and CAT activities of *P. asiatica*. The soluble protein content of *P. asiatica* decreased at 900, 1200, and 1500-fold amino acid dilutions but showed no change at 600-fold amino acid dilution. These results are similar to previous studies (Bahari et al. 2013; Teixeira et al. 2017) and may be due to the protective role of amino acids to the cell components against stress and oxidation (Sourı and Hatamian 2019). In addition, amino acids play a key role in plant stress responses and metabolism signaling, and osmotic protection, thereby enhancing the antioxidant capacity of the system (Hildebrandt et al. 2015).

Selenium is an essential trace element for Se-accumulating plants, and its content in crops is closely related to the soil Se concentration (An 2017; Chauhan et al. 2017; Feng et al. 2013). Moreover, an appropriate Se concentration can promote plant growth and stress resistance by enhancing the antioxidant enzyme activities (Astaneh et al. 2018). Different plant species have varying Se accumulation abilities (Lin et al. 2020a, b; Liao et al. 2018), which are affected by the exogenous Se concentrations and types (Slekovec and Goessler 2005; Cerdán et al. 2008). Amino acids are widely involved in enzymatic reactions as enzyme components and various physiological processes, such as absorbing mineral elements in plants (Alfosea-Simón et al. 2021). In this experiment, the various amino acid concentrations enhanced the Se extraction and its content in *P. asiatica*. This may be due to the involvement of amino acids in plant signal metabolism since the activation of amino acid receptors can unlock a series of signal mechanisms related to nutrient absorption and transportation (Häusler et al. 2014). Additionally, amino acids can combine with Se forming organic Se-amino acids, thereby affecting the accumulation of Se in plants. However, whether plants also directly absorb organic Se from the soil

remains to be studied (Mayland et al. 1991). In this study, the amino acid reduced the TF of *P. asiatica*, indicating that the amino acid weakened Se translocation from the roots to the shoots in *P. asiatica*. The amino acid also had a quadratic polynomial regression relationship with Se content and extraction from *P. asiatica*. There were significant correlations between the biomass of Se extraction tissues and their Se content. So, amino acid could increase the resistance of *P. asiatica* to Se (Rai 2002; Hildebrandt et al. 2015) and promote the nutrient absorption and growth of *P. asiatica* (Ali et al. 2008), resulting in the improvement of Se accumulation ability of *P. asiatica*.

Conclusions

Amino acid promoted growth in *P. asiatica* by increasing its biomass and the photosynthetic pigments contents. The amino acid also improved the stress resistance in *P. asiatica* by enhancing the antioxidant enzyme activities. In addition, amino acid increased the Se content and enhanced Se extraction from *P. asiatica*, and exhibited a quadratic polynomial regression relationship with Se content and extraction. There were significant correlations between the biomass of Se extraction tissues and their Se content. The findings indicate that amino acids can promote growth and Se accumulation in *P. asiatica*. However, there is a need to investigate the mechanisms underlying the effects of amino acids on Se accumulation in plants.

Declarations

Conflict of interest The authors declare no conflict and competing interest.

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