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Effects of selenomethionine on the growth and physiological characteristics of *Scrophularia ningpoensis* seedlings

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Abstract: In order to provide a theoretical basis for the application of organic selenium (Se) in the production and cultivation of *Scrophularia ningpoensis* Hemsl. We investigated the effects of selenomethionine (SeMet) on the growth and physiological characteristics of *S. ningpoensis* seedlings. The results showed that SeMet significantly improved the antioxidant capacity by enhancing the activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) in the leaves of *S. ningpoensis*, which significantly reduced the content of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), as compared to the control. SeMet also significantly improved the water metabolism by increasing the transpiration rate, stomatal conductance, water use efficiency (WUE), relative water content, and water saturation deficit of *S. ningpoensis* leaves. Moreover, SeMet significantly enhanced photosynthetic performance by decreasing non-photochemical quenching (NPQ) and increasing the soil and plant analyser development (SPAD) value, net photosynthetic rate, PSII actual photochemical efficiency Y(II), photochemical quenching (qP), PSII photochemical effective quantum yield (F_v'/F_m') and apparent electron transport rate (ETR). Meanwhile, SeMet significantly improved the plant's height, basal diameter, root/shoot ratio and dry weight of shoots and roots in *S. ningpoensis*. Various SeMet 30 and 60 mg/L SeMet concentrations demonstrated better effects on the growth and physiological characteristics of *S. ningpoensis.* The above results indicate that appropriate concentrations of SeMet can enhance the growth of *S. ningpoensis* and can be improved by increasing its antioxidant capacity, water metabolism, and photosynthetic performance. This provides a theoretical foundation for using organic selenium in growing and producing *S. ningpoensis.*

Keywords: trace element; medicinal plant; water physiology; photosynthetic physiology; plant growth

Scrophularia ningpoensis Hemsl. is a plant belonging to the family Scrophulariaceae Juss. Its dry roots are used as medicine. The medicinal ingredients of *S. ningpoensis* mainly include iridoids, polysaccharides, phenylpropanes, organic acids and other compounds (Liu et al. 2021, 2022). According to previous reports from modern pharmacology, *S. ningpoensis* plays an important role in protecting the cardiovascular system, enhancing antioxidation activity and reducing blood sugar levels (Zhang et al. 2021, Zhu et al. 2023). Therefore, *S. ningpoensis* is an important medicinal plant in China. As the growth is the basis for the formation and accumulation of medicinal ingredients, it is important to improve the growth of *S. ningpoensis* through cultivation measures.

The application of exogenous substances is an important cultivation measure for improving crop growth. Exogenous substances commonly applied to plants include potassium dihydrogen phosphate (KH_2PO_4), phenolic acids, polyamines, organic se-

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lenium (Se), etc. KH_2PO_4 concentration of 5 g/L is favourable for *Acer pseudosieboldianum* (Pax) Kom growth. seedling (Yang et al. 2014). 1 000 mg/L phenolic acids can significantly promote the growth and photosynthetic characteristics of *Santalum album* L. seedlings. Among them, 1 000 mg/L meta phenol has a significant impact on seedlings height, aboveground biomass, underground biomass, total biomass and photosynthetic of *S. album* seedlings, significantly higher than 1 000 mg/L clove acid, ferulic acid and control (Li et al. 2022). The growth of seedlings was promoted by exogenous polyamines (Hu and Zuo 2004). Spraying 2.0 kg and 3.0 kg of high-efficiency organic selenium biological fertiliser per 667 m^2 of leaf surface promoted the vegetative growth of cv. Yanfu 3 apple trees and leaf photosynthesis (Yang et al. 2023). The single-element enriched selenium nutrient solution concentration of 50 mg/L performed the best, significantly improving watermelons' growth (Song et al. 2023).

As a component of several major metabolic pathways, Se plays an important role in human health (Rayman 2006, Winkel et al. 2012). It is also considered an essential trace element for humans, animals and certain species of microorganisms (Stroud et al. 2010). Plant-based foods are the primary source of Se supplementation for humans. The concentration of Se in food, as well as the amount of food consumed, significantly affects the dietary intake of Se (Tao et al. 2023). Therefore, studying how to increase Se content in plants is significant. Foliar spraying with exogenous agents increases Se content in plants (Deng et al. 2019) and significantly increases photosynthetic indices, chlorophyll fluorescence parameters and antioxidant enzyme activities. Appropriate concentrations of sodium selenite can promote root length, stem diameter, root volume and chlorophyll content in loofah plants, positively impacting their growth and photosynthesis (Li et al. 2022). Applying selenomethionine (SeMet) also significantly increased the net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r) and water use efficiency (WUE) of strawberry leaves and had significant effects on chlorophyll fluorescence parameters (Gao et al. 2020). The superoxide dismutase (SOD) and peroxidase (POD) activities of peanut seedlings were increased. At the same time, malondialdehyde (MDA) content was decreased when an appropriate amount of Se fertiliser was applied (Shi et al. 2022). Exogenous spraying sodium selenite solution could significantly improve the activities of key enzymes in the biosynthetic pathway of ascorbic acid in strawberry fruits, thereby significantly increasing the content of ascorbic acid in strawberry fruits (Cai et al. 2023).

SeMet is a type of organic selenium that offers both safety and high efficiency compared to inorganic selenium. SeMet has been used to improve the growth of strawberries, peanuts and luffas (Li et al. 2022, Shi et al. 2022, Cai et al. 2023). 25 mg/L ethephon significantly promoted plant growth and increases the biological yield of *S. ningpoensis* (Xie and He 2013). However, applying SeMet can improve the growth of *S. ningpoensis*, which has not been reported. Therefore, it is of great significance to investigate the effects of SeMet on the growth and physiological characteristics of *S. ningpoensis* seedlings.

In conclusion, the effects of SeMet on the growth physiology of different plants demonstrate that its application in the production practices of various plant species is feasible. In this study, we hypothesised that applying SeMet would improve plant growth in *S. ningpoensis* seedlings were changed to increase their antioxidant capacity, water physiology and photosynthetic physiology of *S. ningpoensis* seedlings. To test the above hypothesis, we investigated the effects of SeMet on antioxidant capacity, water physiology, photosynthetic physiology and growth physiology of *S. ningpoensis* seedlings. This study aimed to investigate the regulatory effects of SeMet on the growth of *S. ningpoensis* seedlings, which will provide a theoretical basis for the application of *S. ningpoensis* seedlings grown using selenium-enhanced cultivation methods.

MATERIAL AND METHODS

Plant material and treatments. An experiment was conducted at Henan Institute of Science and Technology, an urban area of Xinxiang (35°16'43"N, 113°56'24"E) in the North China Plain. The sprouts of *Scrophularia ningpoensis* Hemsl. they were selected and planted in plastic gallon barrels with similar shapes and sizes. Three sprouts were planted in each barrel. The size of the gallon barrel is 36.5 cm × 28.8 cm × 37.0 cm (upper diameter × lower diameter × height). The barrel was filled with 35.0 kg of soil, which was prepared by mixing the common soil with the nutrient soil (Xian Xintiantu Garden Co, Ltd., Shanxi, China) in a weight ratio of 7:3. The soil texture belongs to the brown soil, which was defined as Eutric Cambisols according to the FAO-UNESCO Soil Map of the World

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soil taxonomy system. The basic properties of the mixed soil were as follows: the cation exchange capacity (CEC) of 23 $mmol_+$/100 g, the pH value of 6.52, soil organic carbon (SOC) of 9.69 g/kg, electrical conductivity (321.3 uS/cm), available nitrogen (21.0 mg/kg), available potassium (73.3 mg/kg) with available phosphorus (29.0 mg/kg). After *S. ningpoensis*, there were two pairs of leaves. The water content of the nutrition soil was controlled at 60% of its field capacity. To prevent water loss from each barrel, a weighing method was used to maintain the water content of the soil at 60% of its field capacity daily. The experimental materials were planted on February 28, 2022. In this study, there were five treatments in this study, namely CK (0 mg/L), T1 (10 mg/L), T2 (30 mg/L), T3 (60 mg/L) and T4 (120 mg/L). Each treatment had three replicates for a total of 15-gallon pots. Each pot was sprayed with 100 mL of leaves. The first application of SeMet occurred in mid-April. In early May 2022, data on growth and photosynthetic indices of *S. ningpoensis* were recorded.

Photosynthetic physiology. We determined P_n, WUE, T_r and g_s using a Li-cor6400 photosynthetic instrument (Lincoln, USA). The SPAD value of leaves was determined using a SPAD-502 chlorophyll meter between 9:30 and 11:00. Each treatment was performed with three replicates.

Chlorophyll fluorescence. Optimal/maximal photochemical efficiency of PSII in the dark (F_v/F_m), photochemical efficiency of PSII in the light (F_v'/F_m'), non-photochemical quenching coefficient (NPQ), photochemical quenching (qP), PSII actual photochemical efficiency (Y(II)) and photosynthetic electron transport rate (ETR) were measured using a PAM-2500 portable modulated chlorophyll fluorometer from 9:30 to 11:00. The leaves were dark treatment for 20 min beforehand. Each treatment was performed with three replicates.

Assays of antioxidant enzymes. The activities of SOD (EC 1.15.1.1), POD (EC 1.11.1.7) and catalase (CAT) (EC 1.11.1.6) were analysed according to Ma et al. (2022), Zhang et al. (2021) and Sun et al. (2022), respectively. Ascorbate peroxidase (APX, EC 1.11.1.11) was analysed as described in the method of Shan and Liang (2010). The enzymes' specific activities were expressed as U/g fresh weight (FW). Each treatment was performed three with replicates.

Assays of MDA and hydrogen peroxide (H_2O_2) contents. MDA content was measured using the method described by Heath and Packer (1968). The H_2O_2 content was measured according to Brennan and Frenkel (1977). It was calculated from a standardised curve of H_2O_2. Each treatment was performed three with replicates.

Assays of glutathione, vitamin C and total phenol contents. Glutathione (GSH) content was determined according to Griffith (1980). The vitamin C (Vc) content was measured according to Farajzadeh and Nagizadeh (2003). The content of total phenols was determined according to Wang et al. (2020). Each treatment was performed with three replicates.

Assays of plant growth indicators. The specific indicators included plant height, basal diameter, dry weight of roots and shoots and the root/shoot ratio. We measured plant height using a tape measure. The base diameter was measured using a vernier calliper. The shoots were dried at 80 °C for 36 h and weighed using an electronic balance (Ningbo Jinnuo Balance Instrument Co., Ltd, Zhejiang, China). The underground part (root) was dried at 60 °C for 12 h and then perspired for another 12 h. This process was repeated more than three times until the root turned black. After complete drying, the root was weighed on an electronic balance. The root/shoot ratio is the ratio of root dry weight to shoot dry weight. Each treatment was performed with three replicates.

Relative water content. Relative water content (RWC) was measured according to Hou et al. (2018). RWC was calculated as follows: RWC = [((FW – dry weight (DW))/(saturated weight (TW) – (DW))] × 100. The harvested leaves were weighed as fresh weight. After being soaked for 24 h, they were weighed again as saturated weight. Their dry weight was then calculated by drying weight of the leaves dried at 80 °C for 6 h.

Membership function method. The membership function method and the inverse membership function method were used to comprehensively evaluate the effects of SeMet on the growth and physiological characteristics of *S. ningpoensis* seedlings were used. And the calculation method was as follows. The membership function was used for the positive correlation index, while the inverse membership function method was employed for the negative correlation index. The calculation formula for the membership function method is: $R(X_i) = (X_i - X_{min})/(X_{max} - X_{min})$. The calculation formula for the inverse membership function method is $R(X_i) = 1 - (X_i - X_{min})/(X_{max} - X_{min})$. In the given formula, X_i represents the measured value of a specific indicator. X_{max} and X_{min} denote the maximum and minimum values of

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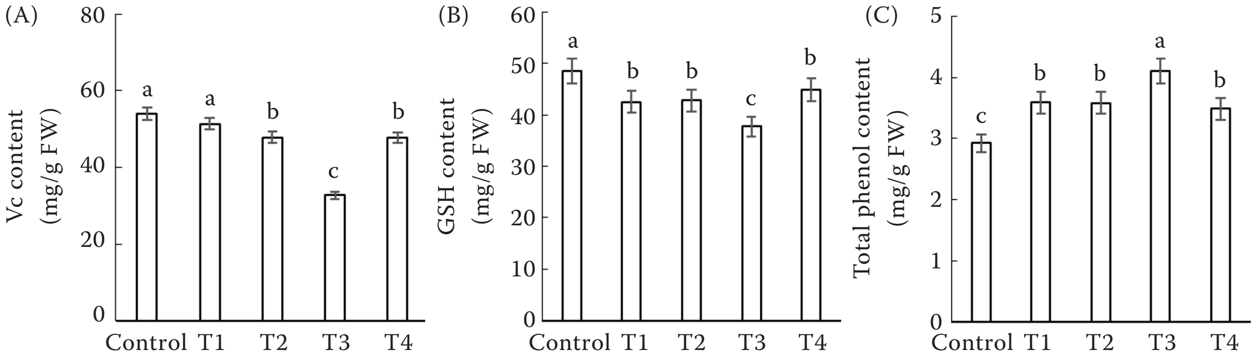

Figure 1. Effects of selenomethionine (SeMet) at different concentrations on (A) vitamin C (Vc); (B) glutathione (GSH) and (C) total phenol contents in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at $P < 0.05$. FW – fresh weight; Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

that same indicator, respectively. The higher the average value of the membership function, the better the growth conditions for *S. ningpoensis* seedlings.

Data analysis. The data were analysed using SAS (Statistical Analysis System, Cary, USA). The plots were created using Origin 2017 (Northampton, USA). The treatments were compared using one-way analysis of variance (ANOVA) and Fisher's least significant difference (*LSD*) tests. The differences were considered to be significant at $P < 0.05$.

RESULTS

Effects of SeMet on the antioxidant capacity of *S. ningpoensis* leaves. Compared to the control group, four Se treatments significantly increased the content of antioxidant substances, such as total phenols and the activity of antioxidant enzymes, including SOD, POD, CAT and APX in *S. ningpoensis* (Figures 1C and 2). However, upon exposure to SeMet, a few decreases in non-enzymatic antioxidants such as vitamin C and glutathione were observed in *S. ningpoensis* leaves (Figures 1A, B). Meanwhile, SeMet significantly decreased the content of MDA and H_2O_2 in *S. ningpoensis* leaves (Figure 3). With the increase of organic selenium concentration, the promoting effect of SOD, POD, CAT, APX and total phenol in leaves of *S. ningpoensis* first increased and then decreased. However, the effect of Vc, GSH, MDA and H_2O_2 was the opposite. Among various

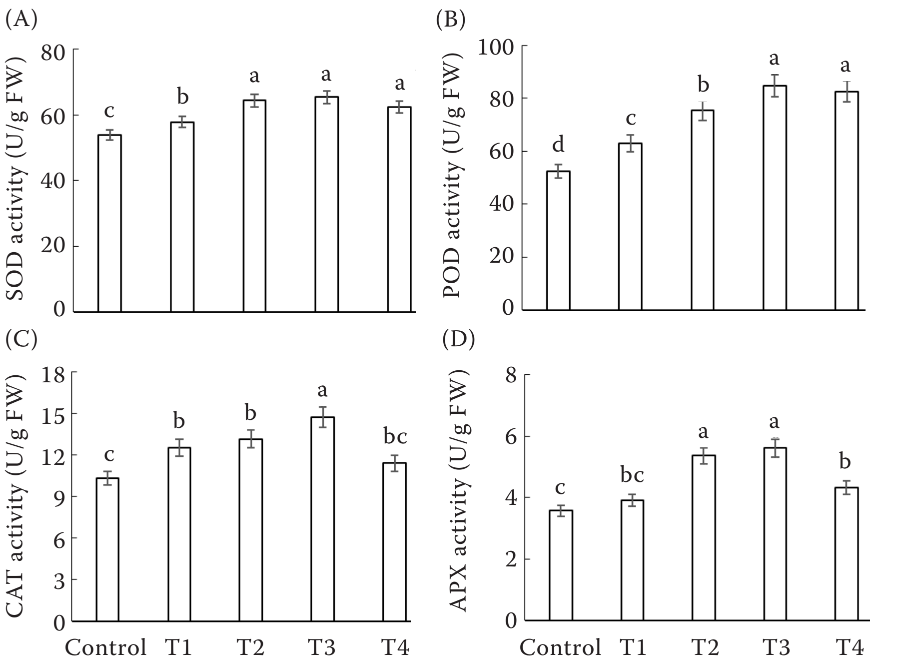

Figure 2. Effects of seleno methionine (SeMet) at different concentrations on (A) superoxide dismutase (SOD); (B) peroxidase (POD); (C) catalase (CAT) and (D) ascorbate peroxidase (APX) activities in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at $P < 0.05$. FW – fresh weight; Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

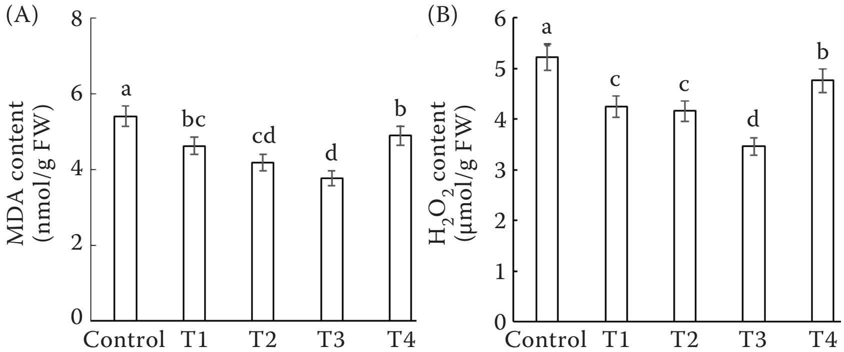

Figure 3. Effects of selenomethionine (SeMet) at different concentrations on (A) malondialdehyde (MDA) and (B) hydrogen peroxide (H_2O_2) activities in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at P < 0.05. FW – fresh weight; Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

concentrations, 60 mg/L of SeMet exhibited the most favourable results for all metrics except Vc and GSH. Compared to the control, *S. ningpoensis* leaves exposed to 60 mg/L Se increased total phenol content and the activities of antioxidant enzymes SOD, POD, CAT and APX by 39.9, 21.5, 61.4, 42.7 and 57.1%, respectively. Meanwhile, 60 mg/L SeMet decreased the contents of Vc, MDA and H_2O_2 by 39.51, 30.3 and 33.7%, respectively. These results suggested that SeMet could improve the antioxidant capacity of *S. ningpoensis* by increasing total phenol content and the activities of antioxidant enzymes SOD, POD, CAT and APX in its leaves.

Effects of SeMet on water physiology of *S. ningpoensis* leaves. Compared to the control, SeMet significantly increased g_s, WUE and leaf RWC in *S. ningpoensis* (Figures 4B, C, D). However, most SeMet treatments have no significant impact on T_r except 120 mg/L SeMet (Figure 4A). With the increase of organic Se concentration, the promoting impacts on g_s, WUE and leaf RWC in *S. ningpoensis* leaves showed a convex parabolic trend, reaching its peak at 60 mg/L SeMet. Compared to the control, 60 mg/L SeMet increased stomatal conductance, water use efficiency and leaf relative water content by 20.3, 18.4 and 19.2%, respectively. These results suggested that

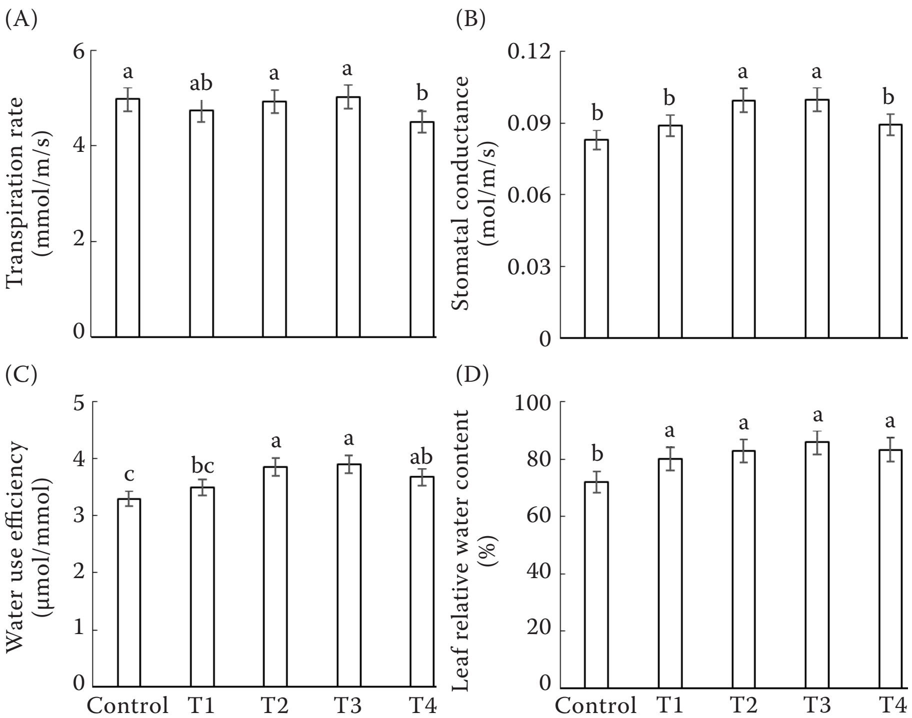

Figure 4. Effects of selenomethionine (SeMet) at different concentrations on (A) transpiration rate; (B) stomatal conductance; (C) water use efficiency and (D)leaf relative water content in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at P < 0.05. Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

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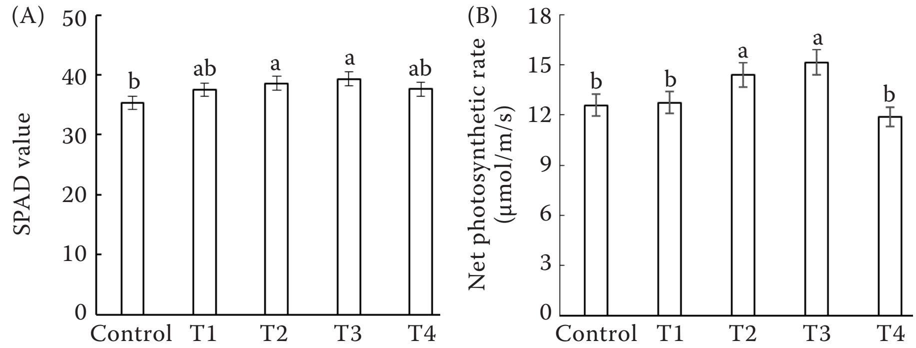

Figure 5. Effects of selenomethionine (SeMet) at different concentrations on (A) soil and plant analyser development (SPAD) value and (B) net photosynthetic rate in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at P < 0.05. Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

SeMet can improve water use efficiency and enhance the capacity of water metabolism of *S. ningpoensis.*

Effects of SeMet on photosynthetic performance of *S. ningpoensis*. Compared to the control, SeMet significantly increased SPAD value, P_n, F_v'/F_m', qP, Y(II) and ETR of *S. ningpoensis* (Figures 5 and 6). However, SeMet significantly decreased NPQ and had no significant effect on F_v/F_m and qP. Among

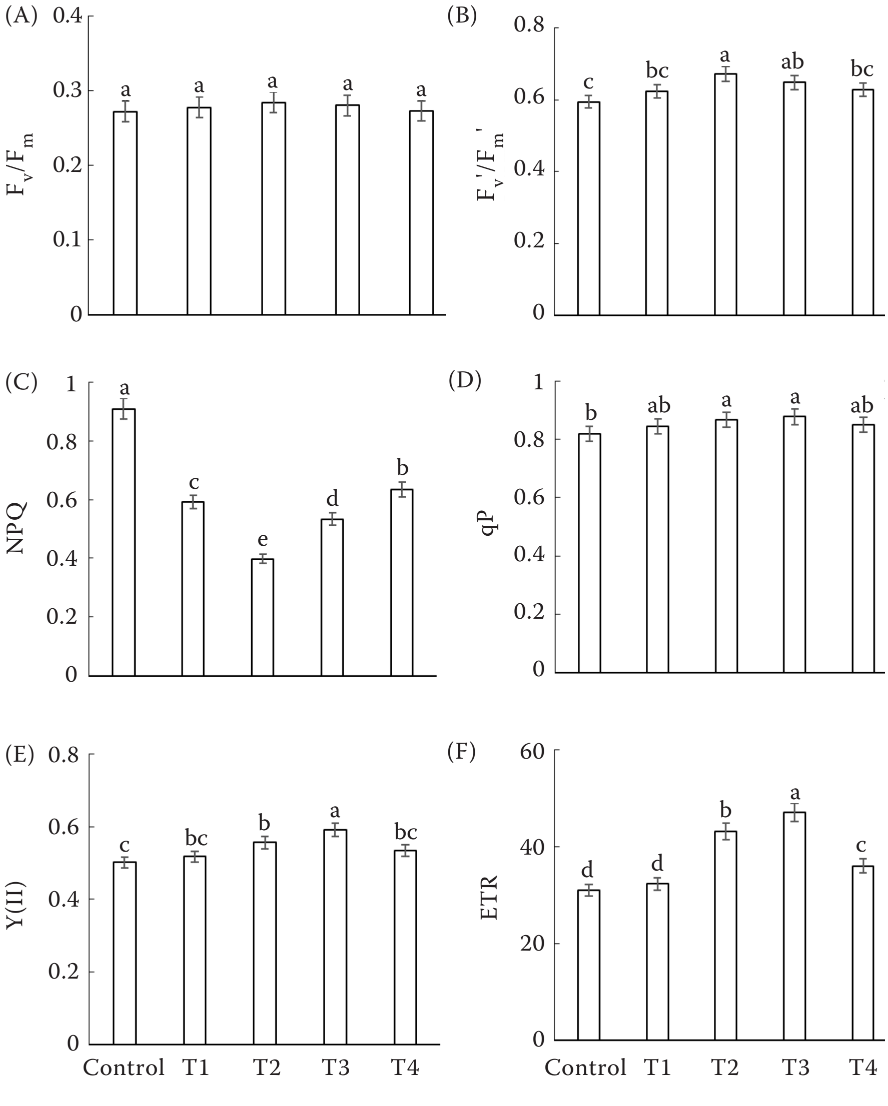

Figure 6. Effects of seleno methionine (SeMet) at different concentrations on (A) optimal/maximal photochemical efficiency of PSII in the dark F_v/F_m; (B) photochemical efficiency of PSII in the light (F_v'/F_m'); (C) non-photochemical quenching (NPQ); (D) photochemical quenching (qP); (E) PSII actual photochemical efficiency (Y(II)) and (F) electron transport rate (ETR) in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at P < 0.05. CK – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

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Table 1. The comprehensive evaluation for the effects of different concentrations of selenomethionine (SeMet) on the growth and physiological characteristics of *Scrophularia ningpoensis* at seedling stage

Indicator	Control	T1	T2	T3	T4
Superoxide dismutase activity	0.20	0.45	0.86	0.93	0.73
Peroxidase activity	0.01	0.29	0.64	0.89	0.84
Catalase activity	0.06	0.51	0.63	0.96	0.28
Ascorbate peroxidase activity	0.03	0.17	0.82	0.93	0.36
Vitamin C content	0.06	0.18	0.33	0.97	0.33
Glutathione content	0.97	0.45	0.48	0.04	0.66
Total phenol content	0.06	0.53	0.52	0.89	0.46
Hydrogen peroxide content	0.11	0.58	0.62	0.96	0.33
Malondialdehyde content	0.10	0.50	0.72	0.93	0.36
Soil and plant analyser development (SPAD) value	0.13	0.49	0.67	0.78	0.50
Net photosynthetic rate	0.27	0.31	0.72	0.90	0.09
Optimal/maximal photochemical efficiency of PSII in the dark	0.28	0.43	0.62	0.50	0.30
Photochemical efficiency of PSII in the light	0.25	0.50	0.91	0.71	0.54
Non-photochemical quenching coefficient	0.01	0.61	0.98	0.72	0.53
Photochemical quenching	0.14	0.44	0.68	0.79	0.50
PSII actual photochemical efficiency	0.11	0.26	0.62	0.95	0.41
Electron transport rate	0.05	0.12	0.67	0.88	0.31
Transpiration rate	0.78	0.49	0.72	0.85	0.19
Stomatal conductance	0.07	0.30	0.71	0.72	0.32
Water use efficiency	0.19	0.43	0.86	0.92	0.65
Leaf relative water content	0.46	0.72	0.81	0.91	0.83
Plant height	0.13	0.52	0.69	0.87	0.43
Basal diameter	0.30	0.74	0.67	0.91	0.13
Dry weight of root	0.01	0.25	0.85	0.99	0.57
Dry weight of the shoot	0.01	0.28	0.90	0.96	0.65
Root/shoot ratio	0.02	0.28	0.70	0.96	0.44
The average membership function value	0.19	0.42	0.71	0.84	0.45
Evaluation order	5	4	2	1	3

Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

different concentrations, 30 mg/L of SeMet displayed the most favourable results for F_v'/F_m' and NPQ. In contrast, 60 mg/L of SeMet demonstrated excellent outcomes regarding SPAD values, net photosynthetic rates, Y(II) and ETR. With increased organic selenium concentration, the promoting effect on SPAD, P_n, Y (II), and ETR of *S. ningpoensis* leaves changed from weak to strong and then weakened. According to Table 1, in different concentrations of SeMet, the maximum effect on the photosynthetic capacity of leaves was 60 mg/L SeMet, followed by 30 mg/L SeMet. Compared to the control, a concentration of 30 mg/L SeMet increased F_v'/F_m' by 13.0%. The amount NPQ was reduced by 56.3%, and 60 mg/L of SeMet increased SPAD value, net photosynthetic rate, Y(II) and ETR by 11.3, 20.3, 17.9 and 52.0%, respectively. These results suggest that SeMet can improve the photosynthetic performance of *S. ningpoensis*, increase its chlorophyll content, and enhance the functionality of photosynthetic system II (PSII).

Effects of SeMet on the growth and biomass of *S. ningpoensis*. Compared to the control, SeMet significantly increased plant height, basal diameter, root/shoot ratio and dry weight of shoots and roots in *S. ningpoensis* (Figures 7 and 8). Among different concentrations, 60 mg/L of SeMet demonstrated the most favourable results for the abovementioned indicators. Compared to the control, a concentration of

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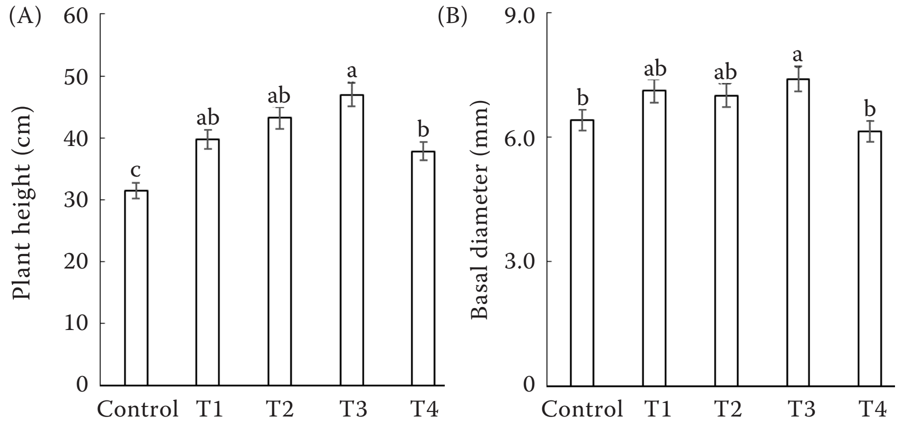

Figure 7. Effects of selenomethionine (SeMet) at different concentrations on (A) plant height and (B) basal diameter in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at $P < 0.05$. Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

60 mg/L SeMet increased plant height, basal diameter, root/shoot ratio and dry weight of shoots and roots by 49.2, 15.4, 26.8, 91.3 and 145.2%, respectively. These results suggest that SeMet could improve the growth and biomass of *S. ningpoensis*, especially by facilitating biomass distribution to the roots. The promoting effect increased and decreased with the increase in organic selenium concentration. And 60 mg/L SeMet was the peak, providing the best treatment effect.

The comprehensive evaluation of the effects of different concentrations of SeMet on the growth and physiological characteristics of ***S. ningpoensis*** **at the seedling stage.** Table 1 shows a comprehensive evaluation of the effects of various concentrations of SeMet on the growth and physiological characteristics of *S. ningpoensis*, which was identified at the seedling stage using the membership function method. It can be seen from Table 1 that different concentrations of SeMet exhibited improved outcomes for the growth and physiological traits of *S. ningpoensis* at the seedling stage compared to the control. Among different concentrations of SeMet, the evaluation order is from large to small regarding their effects on the growth and physiological characteristics of *S. ningpoensis*, which had a concentration of 60 > 30 > 120 > 10 mg/L SeMet. Under the treatment of 60 mg/L SeMet, the indexes of *S. ningpoensis* were arranged in the following order: biomass > growth > photosynthetic performance > water physiology > antioxidant capacity. In conclusion, organic Se can

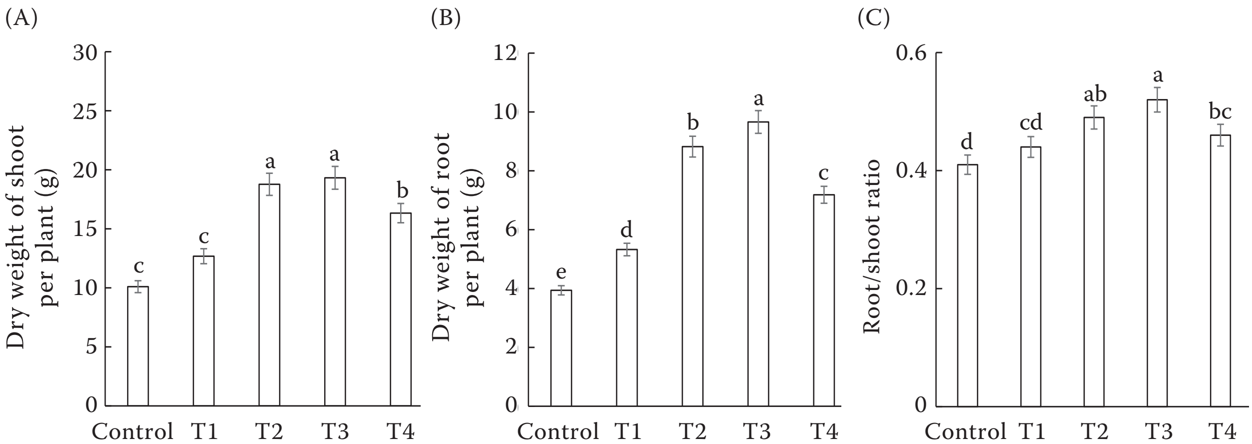

Figure 8. Effects of selenomethionine (SeMet) at different concentrations on (A, B) dry weight of shoot and root and (C) root/shoot ratio in *Scrophularia ningpoensis* seedlings. Different small letters in this table stand for significant difference among different treatments at $P < 0.05$. Control – 0 mg/L; T1 – 10 mg/L; T2 – 30 mg/L; T3 – 60 mg/L; T4 – 120 mg/L of SeMet

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effectively improve the antioxidant capacity, water metabolism and photosynthetic performance of *S. ningpoensis.* Seedings are promoted to grow and accumulate dry matter, which accumulates. These results indicate that 60 mg/L SeMet shows the best effect on the growth and physiological characteristics of *S. ningpoensis*, which can be used to produce and cultivate *S. ningpoensis.*

DISCUSSION

Se is an essential trace element for the human body. A deficiency of Se can lead to various human diseases. Appropriate selenium supplementation can not only prevent Keshan disease, cardiovascular issues and cancer but also help enhance human immunity (Deng et al. 2019). Over the past few decades, Se-enriched plants have been developed to demote deficiency problems for those living in low Se regions who cannot maintain the recommended intake level (Navarro-Alarcon et al. 2008). Applying Se fertilisers is one of the most simple and robust techniques to increase Se content in plants (Tangjaidee et al. 2023). Therefore, by improving the cultivation measures of Se-rich plants, the quality of Se-rich plants can be improved, their yield can be increased, and the development of Se-rich plants can be promoted. To the best of our knowledge, the use of organic Se has been used for biological fortification purposes in *S. ningpoensis* seedlings has not been evaluated.

The large accumulation of reactive oxygen species (ROS) in medicinal plants can lead to membrane lipid peroxidation, which affects the normal metabolism of cells, inhibits their photosynthesis and causes cell death in severe cases. Plants have two antioxidant mechanisms; one is the non-enzymatic pathway, where metabolites such as GSH directly scavenge ROS. And the other antioxidant mechanism is to regulate oxidative stress through enzymatic reduction of ROS, involving a series of antioxidant enzymes, including POD, SOD and APX (Jiménez et al. 1998, Rizwan et al. 2017). Phenolic compounds play a beneficial role in reducing oxidative stress, as they are involved in the detoxification of ROS (Wang et al. 2011). Total phenol content showed the highest value after 60 mg/L SeMet treatment, so they seemed to have a greater ability to scavenge ROS (Figure 1C). Foliar spraying with organic Se significantly increased the antioxidant enzymes SOD, POD, CAT and APX of *S. ningpoensis* seedlings by 21.5–57.1% while decreasing the content of Vc, GSH, MDA and H_2O_2 (Figures 1 and 3). Appropriate spraying amount of organic Se can enhance the activity of CAT, POD, SOD and APX (Figure 2), increase the concentration of total phenols, decrease the levels of H_2O_2 and MDA, and minimise the accumulation of ROS in *S. ningpoensis.* The results of this study were the same as those of the macrotyloma uniflorum and vigna radiate, broccoli, wheat seedlings and foxtail millet (Liang et al. 2014, Sharma and Uttam 2020, Wu et al. 2022).

Leaf water status affects the photosynthetic rate, growth and development, light utilisation rate, and yield of medicinal plants, reflecting their environmental adaptability and resource use efficiency. Studies have shown that the RWC of tomato leaves has been significantly increased by 10% after applying an appropriate Se fertiliser (Haghighi et al. 2014). This indicates that applying Se fertiliser to plants can effectively increase the water content of plant leaves. Lei et al. (2023) found that the Se application could improve the WUE of common buckwheat. A low concentration of Se solution could also delay the senescence of the cut chrysanthemum and maintain the RWC of leaves (Yu et al. 2019). A similar observation in tea showed that nano-Se could improve g_s (Wu et al. 2021). The results of this study showed that compared to the control group, the stomatal conductance, water utilisation rate and relative water content of leaves in the organic selenium treatment group increased by 20.3, 18.4 and 19.2% (Figure 4). However, there was no significant difference in the transpiration rate between the organic selenium treatment and control groups. Since leaf RWC depends on the water absorption and T_r of plants. After applying SeMet, RWC, water utilisation rate and g_s of the leaves of *S. ningpoensis* were measured. Seedlings growth was significantly improved, showing that organic Se can enhance the water utilisation efficiency of *S. ningpoensis* seedlings and provide ideas for water-saving irrigation. Future field experiments can further verify the optimal application concentration of organic Se.

SeMet can enhance photosynthesis and respiration by increasing the photosynthetic capacity and light utilisation rate of plant leaves and enhancing mitochondrial activity. Luo et al. (2021) showed that applying 5.0 mg/kg Se fertiliser could not only significantly increase the carotenoid content of rice but also increase the intercellular carbon dioxide concentration, T_r and P_n of leaves. They found that an appropriate amount of Se could improve rice

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chloroplasts' ETR, respiration rate, and oxidative phosphorylation efficiency and significantly enhance mitochondrial activity. The results of this study showed that the SPAD, P_n, F_v'/F_m', Y(II) and ETR of the organic Se treatment group were higher than those of the control group (Figures 5 and 6). The NPQ was lower than that of the control group, while F_v/F_m and qP of the organic Se treatment group were not significantly different from the control group (Figures 5A, C, D). Among them, the 30 mg/L organic Se treatment group and the 60 mg/L organic Se treatment group showed the best results. Our previous research has demonstrated that SeMet could improve Y(II), F_v/F_m, F_v'/F_m', ETR and qP but reduce NPQ during each strawberry period (Gao et al. 2022). This is the same as for luffa (Li et al. 2022). Therefore, P_n and the utilisation rate of light energy for *S. ningpoensis* could be increased by applying the proper amount of organic Se to the leaf surface. In this experiment, we measured the partial photosynthetic indices for *S. ningpoensis* seedlings, which can be combined with photosynthetic pigments to explore further the effects of organic Se on the photosynthesis of *S. ningpoensis*.

She has a dual effect on plant growth. The proper amount of Se fertiliser promotes plant growth and development and improves their quality. However, excessive Se fertiliser has toxic effects on plants and inhibits their growth. Singh et al. (2023) showed that in a hydroponic test of halotolerant microalga *Dunaliella salina*, the low concentration of Se fertiliser promoted the growth and development of halotolerant microalga *Dunaliella salina*. However, the high concentration of Se fertiliser inhibited its growth and subsequently reduced the yield. With an increase in Se fertiliser concentration, the rate at which mung bean sprout colours fade has been accelerated, and leaf shedding has become more severe (Cheng et al. 2023). The results of this experiment showed that the growth indices and biomass of the *S. ningpoensis* seedlings in the organic Se treatment group had better results than those in the control group. More information is needed to determine the duality of Se and the growth development in *S. ningpoensis* seedlings.

At present, there are different studies on the relationship between Se fertiliser and crop yield. One is that the application of Se has no effect on crop yield and dry matter weight. The application of Se fertiliser cannot directly improve crop yield and dry matter weight, but it indirectly affects crop growth due to environmental factors. Zhang et al. (2015) showed that applying Se foliar fertiliser did not significantly affect the biomass of naked oat grains. These findings were in line with previous studies (Zheng et al. 2013, Liu et al. 2016, Tian et al. 2022). The other is that the application of Se can enhance crop stress resistance, promote crop growth and development, and thus increase crop yield and dry matter weight. Studies have shown that the application of two exogenous Se fertilisers in soil increased the biomass of tomatoes (Fan et al. 2024). Guo et al. (2023) observed that 0.5 and 1.0 mg/L Se^{6+} treatment showed relatively remarkable effects on improving the edible yield, quality and antioxidant capacity of sprouts. In the present study, the exogenous Se addition greatly improved plant growth, including plant height, basal diameter, dry weight of shoot per plant, dry weight of root per plant and root/shoot ratio (Figures 7 and 8). Similar evidence has also been found that Se played a beneficial role in plant growth (Huang and Wu 1997, Wu et al. 1998, Li et al. 2003, Kan et al. 2021). Therefore, the net photosynthetic rate and light energy utilisation rate of *S. ningpoensis* could be effectively increased after the leaves were treated with 30 mg/L and 60 mg/L SeMet, thereby increasing the yield of *S. ningpoensis*. This experiment primarily focused on the seedling stage of *S. ningpoensis*. The experiment can be combined with the entire growth period of *S. ningpoensis* in the future. Its potential use needs to be evaluated under field conditions in Se-deficient soils.

There was a significant positive correlation between the Se content and the exogenous Se application concentration in medicinal and edible homologous plants. Therefore, the goal of producing safe and Se-rich Chinese medicinal materials can be achieved by adjusting the application concentration of exogenous Se. In this study, organic Se effectively improved the antioxidant capacity, water metabolism, and photosynthetic performance of *S. ningpoensis* seedlings, thereby promoting seedling growth and dry matter accumulation. Among them, the present study found that a 60 mg/L concentration of organic Se treatment was the most effective, followed by a 30 mg/L concentration of organic Se treatment. This was the same as for peaches, strawberries and luffa (Wang et al. 2013, Gao et al. 2022, Li et al. 2022). The above research aims to clarify the physiological mechanism by which SeMet increases the growth of *S. ningpoensis* seedlings and provides a theoretical basis for applying organic Se in *S. ningpoensis* production.

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