Mutual grafting affects the physiology of two *Solanum photeinocarpum* ecotypes under selenium stress

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**Abstract.** The aim of this pot experiment was to determine the grafting affecting the physiology of two *Solanum photeinocarpum* ecotypes under selenium (Se) stress. Two *S. photeinocarpum* ecotypes (farmland and mining) were subjected to mutual grafting, and then their physiological characteristics were determined after growth in soil containing Se at 10 mg kg\(^{-1}\). Compared with ungrafted *S. photeinocarpum*, the combination of the scion (farmland ecotype) and the rootstock (mining ecotype) showed increased photosynthetic pigment contents, while the opposite grafting combination showed decreased photosynthetic pigment contents. Mutual grafting also increased the antioxidant enzyme activity grafted *S. photeinocarpum*, and these parameters showed the highest values in the combination of the scion (farmland ecotype) and the rootstock (mining ecotype).

1. Introduction
Selenium (Se) is the indispensable element for plants and is involved in diverse metabolic pathways [1]. Although Se resources are abundant around the world, the per capita Se intake in many regions is seriously insufficient due to its uneven distribution [2]. The food chain Se is mainly derived from the system of soil–plant, and is affected by the Se concentration in soil and how much is accumulated by plants [3]. Therefore, screening for Se-accumulating plant species and strengthening their Se-enrichment ability are feasible methods to increase Se content in the food chain.

*Solanum photeinocarpum* is the medicinal material in the Solanaceae family that is widely distributed. Its young leaves have a high nutritional value and are often consumed as a vegetable [4]. *S. photeinocarpum* also has high medicinal value [5-6]. A previous study showed that the Se content in *S. photeinocarpum* was increased with increasing soil Se concentrations, and its dry matter accumulation was significantly inhibited in soil containing Se at a high concentration [7]. Our previous study found that whether the farmland or mining ecotype of *S. photeinocarpum* was used as the rootstock, mutual grafting could increase the resistance of grafted *S. photeinocarpum* under Cd-contaminated soil stress [8]. However, it remained unknown whether grafting could improve the stress resistance of *S. photeinocarpum*. Therefore, in this study, we explored the mutual grafting affecting the physiological characteristics of farmland and mining ecotypes of *S. photeinocarpum* grown in soil containing Se. The results of this study could be benefit for improving the Se-accumulation ability of plants.
2. Materials and methods

2.1. Sample collection
The farmland ecotype seeds were picketed from a mature *S. photeinocarpum* plant growing in the farmland of Yucheng County, Sichuan Province, China. The mining ecotype seeds were picketed from a mature *S. photeinocarpum* plant growing at the Tangjiashan lead-zinc mine in Hanyuan County, Sichuan Province, China [9-10].

Soil samples were collected from farmland in April 2019. After air-drying and crushing, each polyethylene pot (15 cm × 18 cm, high × diameter) was put into 3.0 kg soil. Then, Na₂SeO₃ solution was added into soil, and made the final Se concentration of 10 mg kg⁻¹ [11]. The soil was left to equilibrate for 60 days and then thoroughly mixed before use in further experiments.

2.2. Grafting
In June, 2019, two *S. photeinocarpum* ecotypes seeds were sown and cultivated in a greenhouse under in the pot placed in greenhouse of the conditions of reference [12]. Grafting was conducted when *S. photeinocarpum* plants were about 10 cm high. The following grafted and ungrafted plant materials were evaluated in this experiment: the mining *S. photeinocarpum* ecotype as the scion, grafted onto rootstock of the farmland *S. photeinocarpum* ecotype (grafted seedlings were designated as M/F); the farmland *S. photeinocarpum* ecotype as the scion, grafted onto rootstock of the mining *S. photeinocarpum* ecotype (grafted seedlings were designated as F/M); the ungrafted farmland ecotype *S. photeinocarpum* (designated as F-CK); the ungrafted mining ecotype *S. photeinocarpum* (designated as M-CK). The split grafting method was used, and the joined part of the rootstock and scion was firmly bound with grafting tape. The tape was gently removed from surviving seedlings [13-14].

2.3. Experiment design
In August, 2019, *S. photeinocarpum* seedlings of M/F, F/M, F-CK, and M-CK with comparable growth were transplanted into Se-containing soil and cultivated under an outdoor shelter. Four *S. photeinocarpum* seedlings were transplanted in pot. Each treatment had three replicates. During cultivation, soil was watered as necessary to keep it moist and weeds were removed as necessary. After 40 days of growth, the upper young leaves of *S. photeinocarpum* were collected for analysis. The superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium photoreduction method, the peroxidase (POD) activity was determined by the guaiacol colorimetric method, catalase (CAT) activity was determined by the potassium permanganate titration method, and the soluble protein content were determined by the Coomassie brilliant blue staining method, respectively [14]. The mature leaves of *S. photeinocarpum* plants were collected and used for determining the photosynthetic pigment contents after extraction with acetone-ethanol [14].

3. Results

3.1. Photosynthetic pigments content of *S. photeinocarpum*

| Treatments | Chlorophyll *a* content (mg g⁻¹) | Chlorophyll *b* content (mg g⁻¹) | Total Chlorophyll content (mg g⁻¹) | Carotenoid content (mg g⁻¹) |
|------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------|
| F-CK       | 0.94±0.012c                       | 0.45±0.010c                       | 1.39±0.022c                       | 0.38±0.003b                  |
| M-CK       | 1.09±0.007a                       | 0.55±0.012a                       | 1.65±0.019a                       | 0.45±0.013a                  |
| M/F        | 1.04±0.002b                       | 0.525±0.009ab                     | 1.569±0.011b                      | 0.431±0.012a                 |
| F/M        | 1.038±0.006b                      | 0.518±0.011b                      | 1.556±0.016b                      | 0.419±0.016a                 |

The chlorophyll *a*, chlorophyll *b*, and carotenoid contents of M-CK were higher than F-CK (Table 1).
Compared with F-CK, F/M showed increasing contents of chlorophyll a, chlorophyll b, total chlorophylls, and carotenoids (by 10.31%, 14.86%, 11.78%, and 10.26%, respectively). The photosynthetic pigment contents in M/F were lower than M-CK; compared with M-CK, M/F showed 4.57% ($p < 0.05$) lower chlorophyll a content and 4.62% ($p < 0.05$) lower total chlorophyll content.

3.2. Antioxidant enzyme activity in S. proteinocarpum

The SOD activity, CAT activity, and soluble protein content of F-CK were higher than M-CK (Table 2). The activities of SOD, POD and CAT were 13.28% ($p < 0.05$), 136.49% ($p < 0.05$), and 11.33% ($p < 0.05$) higher, respectively, of F/M than F-CK. The POD activity and CAT activity were 116.57% ($p < 0.05$) and 25.34% ($p < 0.05$) higher, respectively, in M/F than in M-CK. The soluble protein content was 12.05% ($p > 0.05$) higher in F/M than in F-CK, and 47.92% ($p < 0.05$) higher in M/F than in M-CK.

Table 2 Antioxidant enzyme activity in S. proteinocarpum

| Treatments | SOD activity (U g$^{-1}$) | POD activity (U g$^{-1}$ min$^{-1}$) | CAT activity (mg g$^{-1}$ min$^{-1}$) | Soluble protein content (mg g$^{-1}$) |
|------------|---------------------------|----------------------------------|-----------------------------------|----------------------------------|
| F-CK       | 515.02±11.425b            | 8978.35±200.937d                 | 5.711±0.024b                      | 45.98±2.461bc                   |
| M-CK       | 513.53±17.323b            | 12241.16±258.915c                | 5.023±0.046c                      | 37.63±0.765c                   |
| M/F        | 552.31±27.946ab           | 26510.90±132.169a                | 6.296±0.028a                      | 55.69±1.888a                   |
| F/M        | 583.41±14.198a            | 21233.18±158.544b                | 6.358±0.043a                      | 51.52±5.306ab                  |

4. Discussion

Plants convert solar energy into stable chemical energy through photosynthesis. Thus, photosynthetic pigment contents can reflect plant growth to a certain extent. Under biotic or abiotic stress, grafting can enhance plants’ resistance by increasing the photosynthetic pigment contents [15-17]. Interestingly, our results show that the photosynthetic pigment contents in plants growing in Se-containing soil were significantly affected by grafting; i.e., increased in F/M but decreased in M/F, compared with their ungrafted controls. This result differs from those reported in another study [8], and may be related to the differences in soil Se concentrations between previous studies and our study.

Plants have evolved various anti-stress mechanisms to withstand unfavourable environmental conditions. Plants can effectively remove excess reactive oxygen radicals (ROS) from cells through the antioxidant enzyme system, thereby maintaining the dynamic balance of ROS [18]. They can also maintain turgor pressure and stabilize the structure and function of proteins and membranes through the regulation of soluble sugars, soluble proteins, and amino acids [19]. In the early stage after grafting, a large amount of ROS accumulates because the membrane structure of plant cells has been disrupted by cutting. This effectively activates the antioxidant system in grafted plants [20-21]. In this study, we found that mutual grafting increased the SOD, POD, and CAT activities, and also increased the soluble protein contents, compared with those in the ungrafted S. proteinocarpum controls, which is consistent with the results of other studies [22-23]. In addition, antioxidant enzyme activity was higher in F/M than in M/F, which may have been related to the higher Se content in F/M than in M/F. When plants absorb excess Se, lipid peroxidation increases rapidly, leading to an up-regulation of antioxidant enzyme activity [24].

5. Conclusions

The mutual grafting of two S. proteinocarpum ecotypes (farmland and mining) had different effects on the photosynthetic pigment content, and antioxidant enzyme activity of grafted S. proteinocarpum under Se stress. Compared with ungrafted S. proteinocarpum, the combination of the scion (farmland ecotype) and the rootstock (mining ecotype) showed increased photosynthetic pigment contents, while the opposite grafting combination showed decreased photosynthetic pigment contents. Mutual grafting also increased the antioxidant enzyme activity grafted S. proteinocarpum, and these parameters showed the highest values in the combination of the scion (farmland ecotype) and the rootstock...
(mining ecotype).

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