Mutual grafting affects the selenium uptake of two *Solanum photeinocarpum* ecotypes

Renyan Liao¹*, Zhiwei Yang¹, Jiayan Pang¹, Kewen Huang²

¹Ya’an Polytechnic College, Ya’an, Sichuan 625000, China
²College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China

*Corresponding author’s e-mail: 51699474@qq.com

Abstract. The aim of this pot experiment was to determine the grafting affecting the selenium (Se) absorption of two *Solanum photeinocarpum* ecotypes. Two *S. photeinocarpum* ecotypes (farmland and mining) were subjected to mutual grafting, and then their Se accumulation 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 root biomass, while the opposite grafting combination showed decreased whole shoot biomass. Mutual grafting also increased the Se content in grafted *S. photeinocarpum*, and the combination of the scion (farmland ecotype) and the rootstock (mining ecotype) showed the highest values. The e scion (farmland ecotype) and the rootstock (mining ecotype) combination showed increased Se absorption and transportation, but the largest amount of extracted Se was in whole shoots of the ungrafted farmland ecotype. Thus, the ungrafted *S. photeinocarpum* farmland ecotype had the best nutritional value in terms of Se content.

1. Introduction
Grafting is a method of asexual propagation that combines a rootstock and a scion at an incision point, after which homogeneous tissue develops and a whole plant is formed [1]. It is widely used in the cultivation and breeding of horticultural plants, because it not only maintains the excellent traits of the female parent (scion), but also promotes ion absorption. For example, the contents of nitrogen, phosphorus, potassium (K), sodium (Na), and other trace elements in peach leaves and flowers were significantly increased after grafting scions onto different rootstocks [2]. The grafting combinations of watermelon/ *Citrullus lanatus* and watermelon/ *Citrullus moschata* were shown to improve the K uptake of grafted seedlings and improve the plant’s resistance to low-K stress [3]. Although many studies have shown that grafting has a positive effect on the absorption of macro or micronutrients by plants, a suitable grafting combination can effectively reduce the uptake of heavy metals and salt ions through ion exclusion or retention. For example, under salt stress, the transcript levels of *PMA* and *SOS1*, which encode plasma membrane transporters, in grafted pumpkin/cucumber seedlings were higher than that in cucumber ungrafted seedlings, resulting in greater Na⁺ pumping from the cytoplasm of root cells [4]. In soil containing cadmium (Cd), a grafted apple with *Malus baccata* as the rootstock showed reduced Cd contents in shoots because Cd influx was inhibited in the root system [5]. These studies show that the absorption and transportation of different ions is significantly affected by grafting. Compared with other elements, Se has a typical dose effect on plants, which is beneficial depending on its concentration [6]. However, few studies have explored the grafting effects on Se
uptake of plants.

*Solanum photeinocarpum* has high medicinal value [7-8]. In this study, we explored the mutual grafting affecting the Se uptake of two *S. photeinocarpum* ecotypes (farmland and mining), which could be useful for improving the medicinal plants production.

2. Materials and methods

2.1. Sample collection

The farmland ecotype seeds were picked from a mature *S. photeinocarpum* plant growing in the farmland of Yucheng County, China. The mining ecotype seeds were picked from a mature *S. photeinocarpum* plant growing at the Tangjiashan lead-zinc mine in Hanyuan County, 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, when *S. photeinocarpum* plants were in the full bloom stage, each plant of *S. photeinocarpum* was harvested. The plants were divided into roots, rootstock stems, scion stems, and scion leaves. Ungrafted *S. photeinocarpum* plants were also divided into roots, rootstock stems, scion stems, and scion leaves at the same height as grafted *S. photeinocarpum* plants. The samples were washed, and dried to constant weight for biomass determination. The Se content in dried plant materials was determined by atomic fluorescence spectrometry [15]. The translocation factor (TF) = Se content in shoots/ Se content in roots [16-18].

3. Results

3.1. Biomass of *S. photeinocarpum*

For ungrafted *S. photeinocarpum*, the biomasses of all parts of F-CK were higher than M-CK (Table 1). The M/F biomass was higher than F-CK, while no significant (p > 0.05) difference were in root biomass between F/M and M-CK. There were no significant (p > 0.05) differences in the biomass of scion stems, scion leaves, and whole shoots between M/F and M-CK, while the biomasses of scion stems, scion leaves, and whole shoots were 46.71% (p < 0.05), 36.15% (p < 0.05), and 37.89% (p < 0.05) lower, respectively, in F/M than in F-CK. No significant (p > 0.05) difference was in the rootstock stems biomass among M/F and F-CK, F/M, and M-CK.
Table 1 Biomass of *S. photeinocarpum*

| Treatments | Roots (g plant⁻¹) | Rootstock stems (g plant⁻¹) | Scion stems (g plant⁻¹) | Scion leaves (g plant⁻¹) | Whole shoots (g plant⁻¹) |
|------------|-------------------|----------------------------|------------------------|--------------------------|--------------------------|
| F-CK       | 1.043±0.048b      | 0.981±0.044a               | 2.535±0.054a           | 3.145±0.079a             | 6.661±0.177a             |
| M-CK       | 0.821±0.031c      | 0.723±0.017b               | 1.766±0.037b           | 2.065±0.081b             | 4.554±0.134bc            |
| M/F        | 1.633±0.074a      | 1.027±0.054a               | 1.667±0.045b           | 2.060±0.093b             | 4.754±0.192b             |
| F/M        | 0.858±0.018c      | 0.778±0.020b               | 1.351±0.048c           | 2.008±0.057b             | 4.137±0.124c             |

3.2. Se contents in *S. photeinocarpum*

For ungrafted *S. photeinocarpum*, the Se contents in different parts were higher in M-CK than in F-CK to varying degrees (Table 2). The Se contents in both roots and rootstock stems of M/F were higher than F-CK, and F/M higher than M-CK. The Se contents in scion stems, leaves, and whole shoots were 29.72% (*p* < 0.05), 26.55% (*p* < 0.05) and 28.84% (*p* < 0.05) higher, respectively, in F/M than in F-CK. The Se contents did not differ significantly (*p* > 0.05) between M/F and M-CK. The TF could be ranked as follows: M/F > M-CK > F/M > F-CK.

Table 2 Se contents in *S. photeinocarpum*

| Treatments | Roots (mg kg⁻¹) | Rootstock stems (mg kg⁻¹) | Scion stems (mg kg⁻¹) | leaves (mg kg⁻¹) | Whole shoots (mg kg⁻¹) | TF |
|------------|----------------|---------------------------|----------------------|-----------------|------------------------|----|
| F-CK       | 81.76±1.51c    | 15.83±0.54c               | 13.36±0.28c           | 21.24±0.91c     | 17.44±0.62c            | 0.213 |
| M-CK       | 83.28±1.81bc   | 17.83±0.61b               | 14.54±0.45bc          | 23.32±0.81bc    | 19.04±0.67bc           | 0.229 |
| M/F        | 88.44±2.21b    | 18.04±0.49b               | 15.32±0.85b           | 25.67±0.99ab    | 20.39±0.86ab           | 0.231 |
| F/M        | 101.4±2.98a    | 20.01±0.95a               | 17.33±0.46a           | 26.88±1.29a     | 22.47±0.94a            | 0.222 |

The translocation factor (TF) = Se content in shoots/Se content in roots.

3.3. Amount of extracted Se in *S. photeinocarpum*

Table 3 Amount of Se extracted by *S. photeinocarpum*

| Treatments | Roots (μg plant⁻¹) | Rootstock stems (μg plant⁻¹) | Scion stems (μg plant⁻¹) | Scion leaves (μg plant⁻¹) | Whole shoots (μg plant⁻¹) |
|------------|-------------------|-----------------------------|-------------------------|--------------------------|--------------------------|
| F-CK       | 85.28±5.508bc    | 15.53±1.216ab              | 33.87±1.435a            | 66.80±4.533a             | 116.20±7.184a            |
| M-CK       | 68.37±4.073c     | 12.89±0.742b               | 25.68±1.336b            | 48.16±3.543b             | 86.73±5.621b             |
| M/F        | 144.4±10.11a     | 18.53±1.478a               | 25.54±2.107b            | 52.88±4.434b             | 96.95±8.019ab            |
| F/M        | 87.04±4.426b     | 15.57±1.131ab              | 23.41±1.450b            | 53.98±4.101b             | 92.96±6.682b             |

In both roots and rootstock stems, there were no significant (*p* > 0.05) differences in Se extraction between F-CK and M-CK. In contrast, in scion stems, scion leaves, and whole shoots, the Se extraction was higher in F-CK than in M-CK (Table 3). The amount of extracted Se in roots was 69.32% (*p* < 0.05) higher in M/F than in F-CK, and 27.31% (*p* < 0.05) higher in F/M than in M-CK. In scion stems, scion leaves, and whole shoots, the amount of extracted Se was 30.88% (*p* < 0.05), 19.19% (*p* < 0.05), and 20.00% (*p* < 0.05) lower, respectively, in F/M than in F-CK, but did not differ significantly (*p* > 0.05) between M/F and M-CK.
4. Discussion

Complex interactions between the rootstock and scion have significant regulatory effects on the biomass allocation and growth vigour of grafted plants [19-20]. Several hypotheses have been proposed for the regulation of plant growth by grafting; for example, trophic exchange, plant growth regulators, and RNA trafficking between the rootstock and scion [21]. In the present study, M/F increased the root biomass of *S. photeinocarpum* compared with M-CK, but did not significantly differ on whole shoot biomass. However, compared with F-CK, F/M had a lower whole shoot biomass, but its root biomass was not significantly different. So, both grafting affected the growth of grafted plants, even if the differences between rootstock and scion originated from the different ecotypes.

Sulphur and Se have similar biochemical properties, and consequently Se is mainly absorbed by plants through the sulphate transport pathway [22-23]. Therefore, the genetic background of the rootstock and scion is an important factor affecting the Se-enrichment ability of plants. The results of these experiments show that, compared with ungrafted *S. photeinocarpum*, mutually grafted plants showed increased Se contents in all organs, with the maximum Se contents and TF in F/M plants. These results suggest that the rootstock and the scion not only facilitate Se absorption by *S. photeinocarpum*, but also promote long-distance Se transport within the plant. In terms of Se extraction by *S. photeinocarpum*, mutual grafting increased the extracted Se amount in roots. The largest amount of extracted Se was in whole shoots in F-CK, mainly because the shoot biomass was higher in F-CK than in the other treatments.

5. Conclusions

The mutual grafting of two *S. photeinocarpum* ecotypes (farmland and mining) had different effects on the allocation of biomass of grafted *S. photeinocarpum* plants under Se stress. Although both grafting treatments increased the Se contents in grafted *S. photeinocarpum*, this effect was strongest in the combination of the scion (farmland ecotype) and the rootstock (mining ecotype). Whole shoots of ungrafted farmland *S. photeinocarpum* had the highest biomass, and the largest amount of extracted Se. In summary, grafting of the scion (farmland ecotype) onto the rootstock (mining ecotype) not only facilitated Se absorption by *S. photeinocarpum*, but also promoted long-distance Se transport within the plant. Ungrafted farmland *S. photeinocarpum* had the best nutritional value in terms of Se content in edible parts.

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