

## Effects of Mutual Grafting on Photosynthetic Characteristics of two ecotypes of *Solanum photeinocarpum* under Cadmium Stress

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**Keywords:** Mutual grafting; Photosynthetic characteristic; *Solanum photeinocarpum*; Cd stress

**Abstract:** A pot experiment was conducted to study the effects of mutual grafting on photosynthetic characteristics of two ecotypes of *Solanum photeinocarpum* under cadmium (Cd) stress. Four treatments were used in the experiment: ungrafted of farmland ecotype (F-CK), ungrafted of mining ecotype (F-CK), the farmland ecotype as scion grafted on the rootstocks of mining ecotype (F-Scion), the mining ecotype as scion grafted on the rootstocks of farmland ecotype (M-Scion). The results showed that mutual grafting of two ecotypes of *S. photeinocarpum* could enhance the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and CO<sub>2</sub> concentration of intercellular (Ci) of scion of *S. photeinocarpum*. The result of light use efficiency (LUE) was the same as Pn, but the mutual grafting decreased the vapor pressure deficit of leaf (VpdL) of *S. photeinocarpum*. F-scion decreased water use efficiency (WUE) compared with M-CK, but M-scion increased WUE compared with M-CK. Therefore, mutual grafting could use to enhance the photosynthetic ability of two ecotypes of *S. photeinocarpum* under Cd stress, which would help to improve the phytoremediation ability of two ecotypes of *S. photeinocarpum*.

### Introduction

In the nature, due to differences in the ecological environment, the different ecotypes of plants format, and their morphology and physiology are completely different [1-2]. For hyperaccumulator plants, the cadmium (Cd) accumulation in mining ecotype of *Sedum alfredii* Hance is much higher than that in non-mining ecotype [3]. The Cd contents in different areas of *Solanum nigrum* L. is also very different [4], and other scholars' studies have yielded similar results [5]. *Solanum photeinocarpum* is one of the wild vegetables, and also a potential Cd-hyperaccumulator plant [6]. Using the grafting can enhance the photosynthesis ability of *S. photeinocarpum* [7]. In the previous studies, the morphology and Cd accumulation of different ecotypes of *S. photeinocarpum* were quite difference. Compared with the farmland ecotype, the mining ecotype of *S. photeinocarpum* was short and lower biomass, but had more Cd contents in plant [8]. So, to further enhancing the phytoremediation of *S. photeinocarpum*, a pot experiment was conducted to study the effects of mutual grafting on photosynthetic characteristics of two ecotypes of *S. photeinocarpum* under Cd stress. The objectives of this study were to determine whether mutual grafting could enhance photosynthetic ability of two ecotypes of *S. photeinocarpum* under Cd stress.

### Materials and Methods

**Materials.** The seeds of two ecotypes (mining ecotype and farmland ecotype) of *S. photeinocarpum* were collected from Tangjiashan lead-zinc mine and farmland of Ya'an campus farm of Sichuan Agricultural University in May, 2016, air-dried and stored at 4 °C respectively. The Tangjiashan

lead-zinc mine (29° 24' N, 102° 38' E) locates in Hanyuan County, Sichuan Province, China, with an typical dry-hot valley climate. The farm of Sichuan Agricultural University (29° 59' N, 102° 59' E) locates in Yucheng County, Sichuan Province, China, with an humid subtropical monsoon climate.

**Grafting.** The seeds of two ecotypes of *S. photeinocarpum* were sown in the farmland of the Chengdu campus in June, 2016. When the *S. photeinocarpum* seedlings reached a height of ~10 cm (eight expanded euphyllas, rapid growth stage), the grafting was conducted. The grafting method was cleft grafting bound with 1-cm-wide plastic film. All of the leaves of the rootstocks remained. There were four treatments in the experiment. (1) Ungrafted of farmland ecotype (F-CK). (2) Ungrafted of mining ecotype (F-CK). (3) The farmland ecotype as scion grafted on the rootstocks of mining ecotype (F-Scion). (4) The mining ecotype as scion grafted on the rootstocks of farmland ecotype (M-Scion). When the grafting was completed, the soil moisture content was maintained at 80% of field capacity, and all of the seedlings were covered with transparent plastic film and a shade net. After 10 d, the transparent plastic film, the shade net and the plastic binding films were removed.

**Experimental Design.** The experiment was conducted at the Chengdu campus from June to August 2016. The soil samples were air-dried and passed through a 5-mm mesh in June 2016, and then 3.0 kg of soil was weighed into each polyethylene pot (15 cm tall, 18 cm diameter). Cd was added to make a final soil Cd concentration of  $10 \text{ mg} \cdot \text{kg}^{-1}$  with a saturated heavy metal solution in the form of  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ . The soils were mixed immediately and again after 4 weeks, during which soil moisture was kept at 80%. Four uniformly prepared *S. photeinocarpum* seedlings of each treatment were transplanted into each pot in July 2016. Each treatment was repeated three times with a 10-cm spacing between pots. The soil moisture content was maintained at 80% of field capacity until the plants were harvested. After *S. photeinocarpum* matured (30 d of cultivation at the fully blooming stage), the photosynthesis of each plant was determined by using LI-6400 portable photosynthesis meter (LI-COR Inc., USA). The photosynthetic parameters of the photosynthesis meter were manual control  $\text{CO}_2$  concentration  $400 \mu\text{mol} \cdot \text{mol}^{-1}$ , temperature  $25^\circ\text{C}$ , light intensity  $1000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . The determination of photosynthetic parameters were net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and  $\text{CO}_2$  concentration of intercellular (Ci), and each treatment was repeated three times. Water use efficiency (WUE) = net photosynthetic rate (Pn) / transpiration rate (Tr), Light use efficiency (LUE) = net photosynthetic rate (Pn) / light intensity [9].

## Results and Discussion

**Net Photosynthetic Rate (Pn).** Compared with F-CK, the Pn of M-CK was lower than F-CK (Table 1), indicating that farmland ecotype of *S. photeinocarpum* had higher photosynthesis than mining ecotype. The grafting enhanced the Pn of F-scion by 14.91% ( $p < 0.05$ ) compared with F-CK under Cd stress, and enhanced the Pn of M-scion by 17.89% ( $p < 0.05$ ) compared with M-CK. So, the grafting could enhance the photosynthesis ability of *S. photeinocarpum* under Cd stress, and the enhancement degree of farmland ecotype was higher than mining ecotype.

**Transpiration Rate (Tr).** The same as the Pn, the Tr of farmland ecotype of *S. photeinocarpum* was also higher than mining ecotype (Table 1). The grafting increased the Tr of two ecotype of *S. photeinocarpum* compared with their control respectively. The grafting increased the Tr of F-scion by 73.76% ( $p < 0.05$ ) compared with F-CK, and increased the Tr of M-scion by 13.04% ( $p < 0.05$ ) compared with M-CK. So, the Tr enhancement degree of farmland ecotype was higher than mining ecotype.

**Stomatal Conductance (Gs).** After grafting, the Gs of two ecotypes of *S. photeinocarpum* increased (Table 1), which was benefit to the Pn. The Gs of *S. photeinocarpum* was ranked as: F-scion > F-CK > M-scion > M-CK. Compared with F-CK, F-scion decreased the Gs of *S. photeinocarpum* by 85.82% ( $p < 0.05$ ). Compared with M-CK, M-scion decreased the Gs of *S. photeinocarpum* by 15.04% ( $p < 0.05$ ). So, the Gs enhancement degree of farmland ecotype was higher than mining ecotype.

**CO<sub>2</sub> Concentration of Intercellular (Ci).** The same as Gs, after grafting, Ci of two ecotype of *S. photeinocarpum* increased (Table 1). The Ci of *S. photeinocarpum* was ranked as: F-scion > F-CK > M-scion > M-CK. Compared with F-CK, F-scion decreased the Ci of *S. photeinocarpum* by 11.09% ( $p < 0.05$ ), and M-scion decreased the Ci of *S. photeinocarpum* by 2.48% ( $p < 0.05$ ) compared with M-CK.

Table 1 Pn, Tr, Gs and Ci of mutual grafted *S. photeinocarpum*

Treatments	Pn ( $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	Tr ( $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	Gs ( $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	Ci ( $\text{mmol CO}_2 \cdot \text{mol}^{-1}$ )
F-CK	5.03±0.21b	0.972±0.023b	0.0677±0.0031b	268.65±5.56b
F-scion	5.78±0.26a	1.689±0.069a	0.1258±0.0053a	298.44±3.15a
M-CK	2.85±0.20d	0.629±0.016d	0.0552±0.0033c	265.15±7.52b
M-scion	3.36±0.02c	0.711±0.013c	0.0635±0.0016b	271.73±5.89b

Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ( $p < 0.05$ ). F-CK = ungrafted of farmland ecotype, F-CK = ungrafted of mining ecotype, F-Scion = the farmland ecotype as scion grafted on the rootstocks of mining ecotype, M-Scion = the mining ecotype as scion grafted on the rootstocks of farmland ecotype.

**Water Use Efficiency (WUE).** The Fig. 1 shows that WUE of two ecotypes of *S. photeinocarpum* effected by grafting treatment under Cd stress. Compared with M-CK, F-scion decreased WUE by 33.87% ( $p < 0.05$ ), but M-scion increased WUE by 33.87% ( $p < 0.05$ ) compared with M-CK.

**Light Use Efficiency (LUE).** The same as the Pn, the grafting increased LUE of two ecotypes of *S. photeinocarpum* under Cd stress (Fig. 2). The LUE of *S. photeinocarpum* was ranked as: F-scion > F-CK > M-scion > M-CK. F-scion increased LUE of *S. photeinocarpum* by 14.91% ( $p < 0.05$ ) compared with F-CK, and M-scion increased LUE by 17.89% ( $p < 0.05$ ) compared with M-CK.

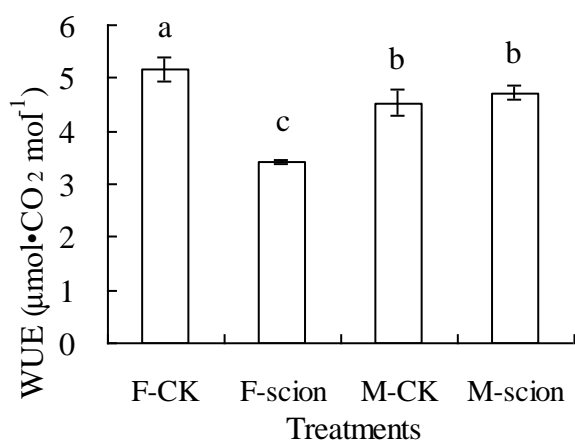


Fig. 1 WUE of mutual grafted *S. photeinocarpum*. Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ( $p < 0.05$ ). F-CK = ungrafted of farmland ecotype, F-CK = ungrafted of mining ecotype, F-Scion = the farmland ecotype as scion grafted on the rootstocks of mining ecotype, M-Scion = the mining ecotype as scion grafted on the rootstocks of farmland ecotype.

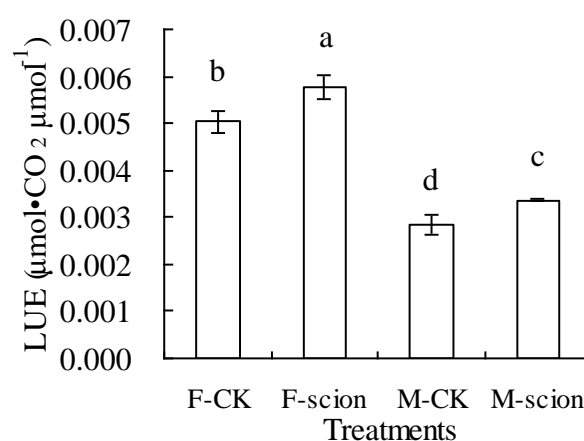


Fig. 2 LUE of mutual grafted *S. photeinocarpum*. Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ( $p < 0.05$ ). F-CK = ungrafted of farmland ecotype, F-CK = ungrafted of mining ecotype, F-Scion = the farmland ecotype as scion grafted on the rootstocks of mining ecotype, M-Scion = the mining ecotype as scion grafted on the rootstocks of farmland ecotype.

### Vapor Pressure Deficit of Leaf (VpdL). The

Fig. 3 shows that the VpdL of two ecotypes of *S. photeinocarpum* effected by grafting treatment under Cd stress. The VpdL of farmland ecotype of *S. photeinocarpum* had lower VpdL than mining ecotype. Compared with M-CK, F-scion decreased the VpdL by 4.30% ( $p < 0.05$ ), and M-scion increased the VpdL by 12.53% ( $p < 0.05$ ) compared with M-CK.

### Conclusions

Mutual grafting of two ecotypes of *S. photeinocarpum* could enhance the Pn, Tr, Gs and Ci of scion of *S. photeinocarpum*. The result of LUE was the same as Pn, but the mutual grafting decreased the VpdL of *S. photeinocarpum*. F-scion decreased WUE compared with M-CK, but M-scion increased WUE compared with M-CK. Therefore, mutual grafting could use to enhance the photosynthetic ability of two ecotypes of *S. photeinocarpum* under Cd stress, which would help to improve the phytoremediation ability of two ecotypes of *S. photeinocarpum*.

### Acknowledgements

This work was financially supported by the 2016 Innovation Training Program of University Student (201610626032) and the Application Infrastructure Project of Science and Technology Department of Sichuan Province (2016JY0258).

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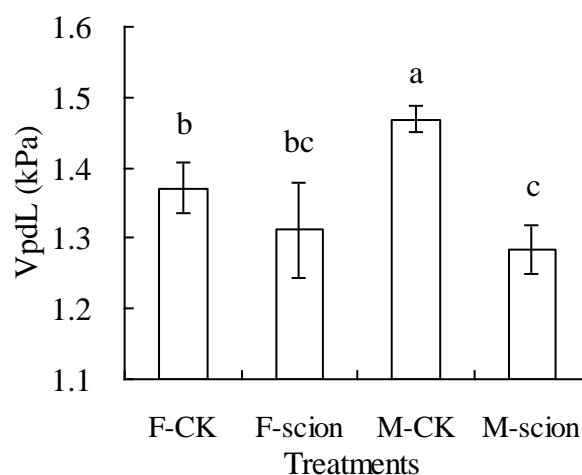


Fig. 3 VpdL of mutual grafted *S. photeinocarpum*. Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ( $p < 0.05$ ). F-CK = ungrafted of farmland ecotype, F-CK = ungrafted of mining ecotype, F-Scion = the farmland ecotype as scion grafted on the rootstocks of mining ecotype, M-Scion = the mining ecotype as scion grafted on the rootstocks of farmland ecotype.

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