

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

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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 CO₂ 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 mg·kg⁻¹ with a saturated heavy metal solution in the form of CdCl₂·2.5H₂O. 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 CO₂ concentration 400 μmol·mol⁻¹, temperature 25 °C, light intensity 1000 μmol·m⁻² s⁻¹. The determination of photosynthetic parameters were net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and CO₂ 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.

CO₂ 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.

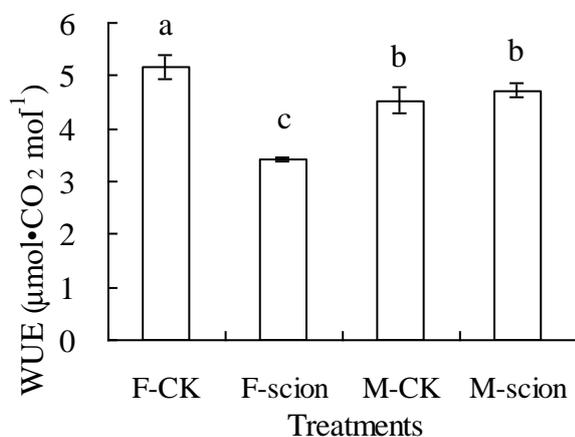


Fig. 1 WUE of mutual grafted *S. photeinocarpum*. Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). F-CK = ungrafted of farmland ecotype,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

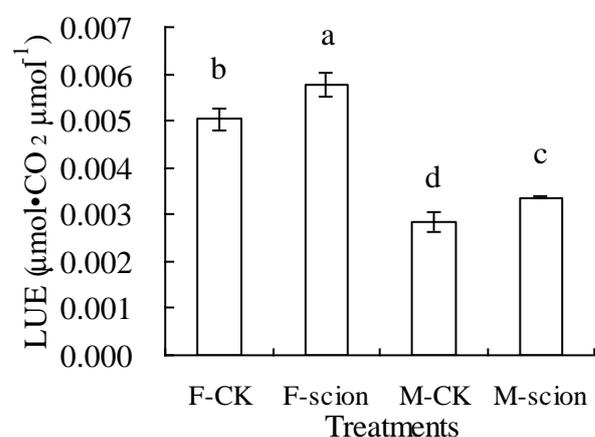


Fig. 2 LUE of mutual grafted *S. photeinocarpum*. Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). F-CK = ungrafted of farmland ecotype,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

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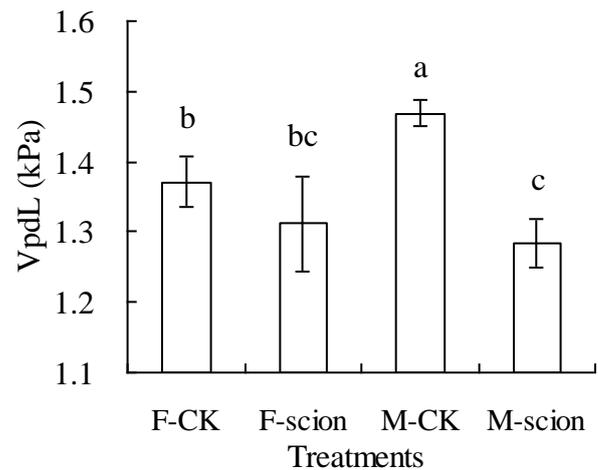


Fig. 3 VpdL of mutual grafted *S. photeinocarpum*. Values are means of three replicate pots. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). F-CK = ungrafted of farmland ecotype,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