Effects of Grafting on Photosynthetic Characteristics of Potential Cd-Hyperaccumulator *Solanum photeinocarpum* under Cd Stress

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Abstract: A pot experiment was conducted to study the effects of grafting on photosynthetic characteristics of potential Cd-hyperaccumulator *Solanum photeinocarpum* under Cd stress. Four treatments were used in the experiment: ungrafted (UG), self-rooted grafting by the same *S. photeinocarpum* seedling (SG), self-rooted grafting by two different sizes of *S. photeinocarpum* seedlings (DG) and grafting on the rootstock of wild potato (PG). The results showed that SG and DG enhanced net photosynthetic rate (Pn) of *S. photeinocarpum* (Pn of DG was higher than SG), but PG decreased that. The light use efficiency (LUE) was the same as Pn. The grafting treatment decreased transpiration rate (Tr), stomatal conductance (Gs) and CO_2 concentration of intercellular (Ci) of *S. photeinocarpum*, and increased water use efficiency (WUE) of *S. photeinocarpum*. Therefore, grafting could use to enhance the photosynthetic ability of Cd-hyperaccumulator *S. photeinocarpum* for the phytoremediation.

Introduction

Grafting is a method which has used widely in plant propagation of horticulture, and plays a significant role in improving the yield and quality of fruit and vegetables [1]. Under heavy metal stress, the photosynthetic enzymes activity and chlorophyll synthesis are inhibited, resulting in reduced plant photosynthesis [2]. Some studies show that grafting can enhance the photosynthetic ability of plant, especially under stress condition [3-4]. So, if we use grafting method on the heavy metal hyperaccumulator plant, the photosynthetic ability of hyperaccumulator plant could enhance, and the phytoremediation ability of hyperaccumulator could improve too. *Solanum photeinocarpum* is a potential Cd-hyperaccumulator that grows rapidly and has a strong propagative ability [5]. To further enhancing the phytoremediation of *S. photeinocarpum*, a pot experiment was conducted to study the effects of grafting on photosynthetic characteristics of potential Cd-hyperaccumulator *Solanum photeinocarpum* under Cd stress. The objectives of this study were to determine whether grafting could enhance photosynthetic ability of *S. photeinocarpum* after Cd exposure.

Materials and Method

Materials. The seeds of *S. photeinocarpum* were collected from a *S. photeinocarpum* plant at the Ya'an campus farm of the Sichuan Agricultural University (29° 59′ N, 102° 59′ E), China in July 2012, air-dried and stored at 4 °C. The inceptisol soil samples (purple soil in the Genetic Soil Classification of China) were collected from the Ya'an campus farm. The basic properties of the soil were the same as reference [6], and the total Cd content was 0.101 mg·kg^{-1}.

Grafting. The seeds of *S. photeinocarpum* were sown in the farmland of the Ya'an campus farm. 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 grafting treatments in the experiment. (1) ungrafted (UG): *S. photeinocarpum* seedlings remained untreated. (2) Self-rooted grafting by the same *S. photeinocarpum* seedling (SG): The scion and rootstock were

from the same *S. photeinocarpum* seedling. The lower parts of the *S. photeinocarpum* seedlings, with heights of 6 cm, were the rootstocks, and the upper parts of the same *S. photeinocarpum* seedlings, with heights of 4 cm, were the scions. The scions and rootstocks were consistent in physiology. (3) Self-rooted grafting by two different sizes of *S. photeinocarpum* seedlings (DG): The scion and rootstock were from two different growth stages of *S. photeinocarpum* seedlings. The rootstocks of the *S. photeinocarpum* seedlings were the same as in the SG, and the lower parts, with heights of 6 cm (10 cm for the whole plant), were the rootstocks. The scions were the upper parts (4 cm) of *S. photeinocarpum* seedlings with 5 cm of whole plant (four expanded euphyllas). The scions and rootstocks were not consistent in physiology. (4) Grafting on rootstock of wild potato (PG): Wild potato seedlings, with heights of 10 cm, were collected from the Ya'an campus farm. The lower part of the wild potato seedlings, with heights of 6 cm, were the rootstocks, and the upper parts of the *S. photeinocarpum* seedlings, with heights of 4 cm, were the scions. The scions of *S. photeinocarpum* seedlings were the same as in the SG. The scions and rootstocks were not consistent in physiology. 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, and all the germinating buds of rootstocks were also removed.

Experimental Design. The experiment was conducted at the Ya'an campus farm from April to June 2013. The soil samples were air-dried and passed through a 5-mm mesh in April 2013, 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 60 $mg \cdot kg^{-1}$ with a saturated heavy metal solution in the form of $CdCl_2 \cdot 2.5H_2O$. 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 May 2013. 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 CO_2 concentration 400 $\mu mol \cdot CO_2$ mol^{-1}, temperature 25□, light intensity 1200 μmol $m^{-2} \cdot s^{-1}$. The determination of photosynthetic parameters were net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and 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 [7].

Results and Discussion

Net Photosynthetic Rate (Pn). Compared with UG, SG and DG enhanced Pn of *S. photeinocarpum* under Cd stress, but PG decreased that (Fig. 1). The Pn of *S. photeinocarpum* was ranked as: DG > SG > UG > PG. SD and DG enhanced Pn of *S. photeinocarpum* by 15.23% ($p < 0.05$) and 25.82% ($p < 0.05$) respectively, compared with UG, and PG decreased by 4.64% ($p > 0.05$) compared with UG. Because of the physiological resistance generated by the graft healing process could continue during the growth and development of the scion [8-9], the Pn of SD and DG enhanced under Cd stress, and the resistance of SG to Cd stress was lower than that of DG because of the differences in physiology when grafting. For PG, because wild potato and *S. photeinocarpum* were two different species, and the affinity of wild potato was lower than that of *S. photeinocarpum* as the rootstock of *S. photeinocarpum*. So, the Pn of PG was the minimum of treatments.

Transpiration Rate (Tr). Compared with UG, SG, DG and PG decreased the Tr of *S. photeinocarpum* (Fig. 2). The Tr of *S. photeinocarpum* was ranked as: UG > SG > DG > PG. This result was in contrast to Pn, which was consistent with other studies [3-4]. SG, DG and PG decreased the Tr of *S. photeinocarpum* by 39.34% ($p < 0.05$), 44.85% ($p < 0.05$) and 62.13% ($p < 0.05$) respectively, compared with UG.

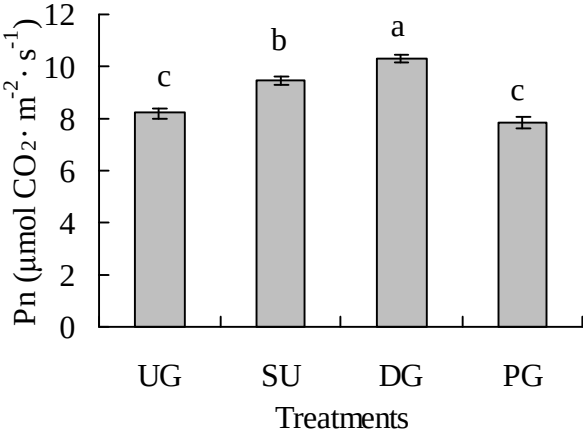

Fig. 1 Pn of grafted *S. photeinocarpum*

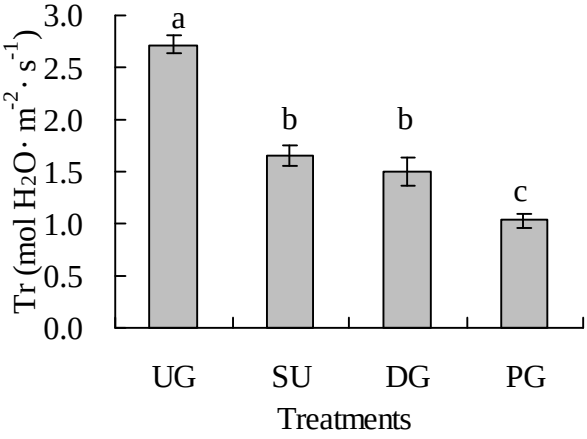

Fig. 2 Tr of grafted *S. photeinocarpum*

Water Use Efficiency (WUE). The Fig. 3 shows that WUE of *S. photeinocarpum* increased by grafting treatment under Cd stress. Compared with UG, SG, DG and PG increased WUE of *S. photeinocarpum* by 90.06% ($p < 0.05$), 127.48% ($p < 0.05$) and 150.66% ($p < 0.05$) respectively, indicating that grafting could effectively improve the utilization of water, which was consistent with other studies [3-4].

Light Use Efficiency (LUE). The same as Pn, SG and DG increased LUE of *S. photeinocarpum* under Cd stress, but PG decreased that (Fig. 4). The LUE of *S. photeinocarpum* was ranked as: DG > SG > UG > PG. SG, DG and PG increased LUE of *S. photeinocarpum* by 15.17% ($p < 0.05$) and 25.96% ($p < 0.05$) respectively, compared with UG, and PG decreased by 4.57% ($p > 0.05$) compared with UG.

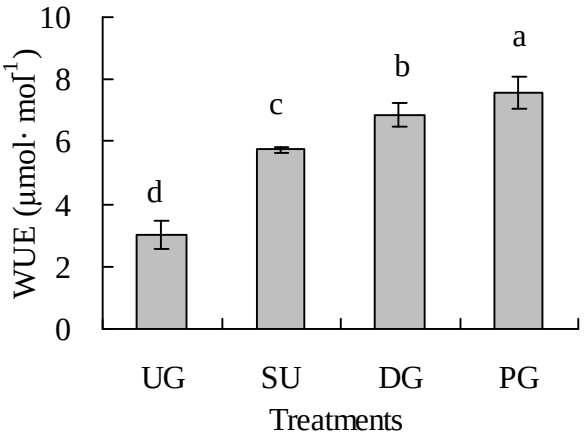

Fig. 3 WUE of grafted *S. photeinocarpum*

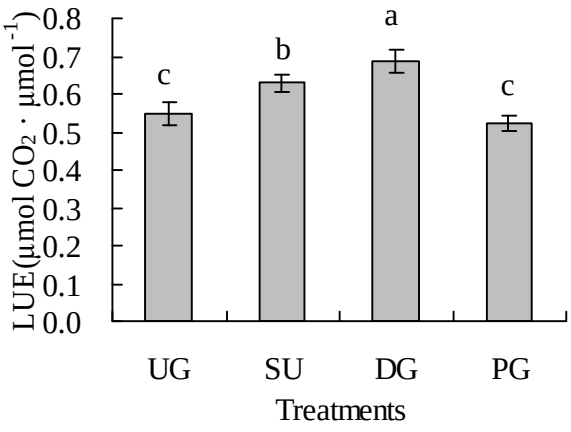

Fig. 4 LUE of grafted *S. photeinocarpum*

Stomatal Conductance (Gs). After grafting, Gs of *S. photeinocarpum* decreased (Fig. 5), which was benefit to Pn. The Gs of *S. photeinocarpum* was ranked as: UG > SG > DG > PG. Compared with UG, SG, DG and PG decreased Gs of *S. photeinocarpum* by 7.57% ($p > 0.05$), 29.73% ($p < 0.05$) and 50.27% ($p < 0.05$) respectively.

CO_2 Concentration of Intercellular (Ci). The same as Gs, after grafting, Ci of *S. photeinocarpum* decreased (Fig. 6). The Ci of *S. photeinocarpum* was ranked as: UG > SG > PG > DG. Compared with UG, SG, DG and PG decreased Ci of *S. photeinocarpum* by 0.73% ($p > 0.05$), 15.87% ($p < 0.05$) and 15.45% ($p < 0.05$) respectively.

Conclusions

Using the same species to graft could enhance Pn of *S. photeinocarpum*, and Pn of DG was higher than SG under Cd stress. The wild potato as rootstock decreased Pn of *S. photeinocarpum*. The result of

LUE was the same as Pn. Grafting decreased Tr, Gs and Ci of *S. photeinocarpum*, and increased WUE of *S. photeinocarpum*. Therefore, grafting could use to enhance the photosynthetic ability of Cd-Hyperaccumulator *S. photeinocarpum*, which would help to improve the phytoremediation ability of *S. photeinocarpum*.

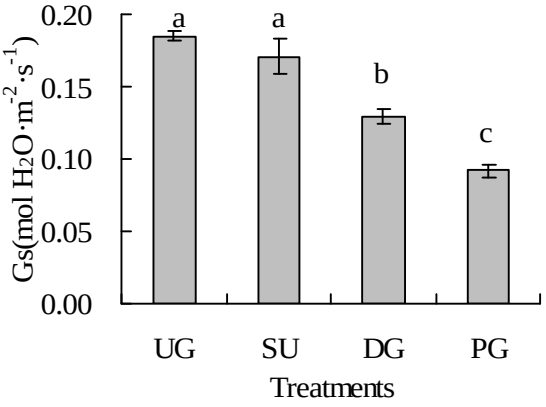

Fig. 5 Gs of grafted *S. photeinocarpum*

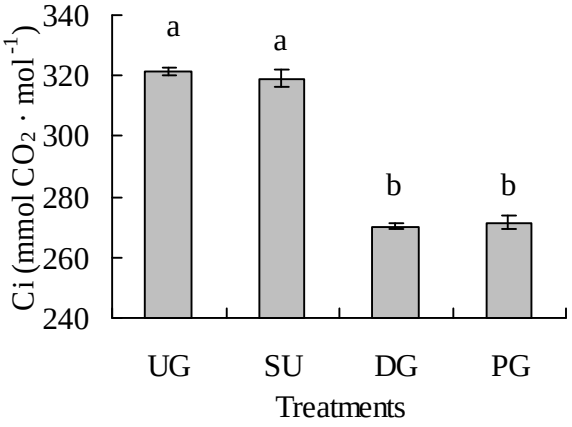

Fig. 6 Ci of grafted *S. photeinocarpum*

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