Advances in Engineering Research (AER), volume 143
6th International Conference on Energy and Environmental Protection (ICEEP 2017)

Two Ecotypes Mutual Grafting Improves Photosynthesis of Post Generations of *Solanum photeinocarpum* under Cadmium Stress

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Keywords: *Solanum photeinocarpum*; Mutual grafting; Photosynthesis; Post generation

Abstract: The effects of mutual grafting on the photosynthesis of two ecotypes of post generation *Solanum photeinocarpum* under cadmium (Cd) stress were studied by the pot experiment, and the photosynthetic pigment contents, photosynthetic characteristics and soluble sugar contents in post generations of *S. photeinocarpum* were determined. Under Cd stress, two ecotypes of *S. photeinocarpum* mutual grafting improves the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of their post generations. The mutual grafting increased the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) of post generations of *S. photeinocarpum*, and decreased the value of pressure deficit leaf (Vpdl) of that compared with the un-grafted control, respectively. Compared with the un-grafted control, the grafting increased the soluble sugar contents in roots, stems, leaves and shoots of post generations of *S. photeinocarpum*. Therefore, two ecotypes mutual grafting could improve the photosynthesis of post generations of *S. photeinocarpum* under cadmium stress.

Introduction

Solanum photeinocarpum is a potential cadmium (Cd) hyperaccumulator plant [1]. Compared with the farmland ecotype, the mining ecotype of *S. photeinocarpum* was short and lower biomass, but had more Cd contents in plant [2]. Compared with the other Cd-hyperaccumulator plants [3-4], the phytoremediation ability of *S. photeinocarpum* is low. So, to further enhancing the phytoremediation ability of *S. photeinocarpum*, a pot experiment was conducted to study the effects of mutual grafting on the photosynthesis of two ecotypes of post generations *S. photeinocarpum* under Cd stress. The objectives of this study were to determine whether mutual grafting could enhance photosynthetic ability of two ecotypes of post generations *S. photeinocarpum* under Cd stress, and provide a reference for other hyperaccumulator plants.

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) Un-grafted of farmland ecotype (FCK). (2) Un-grafted of mining ecotype (MCK). (3) The farmland ecotype as scion grafted on the rootstocks of mining ecotype (FSC). (4) The mining ecotype as scion grafted on the rootstocks of farmland ecotype (MSC). 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. At maturity (50 d after grafting), fruits of *S. photeinocarpum* from CK, scion and rootstock were collected, and the seeds were taken out from fruits, air-dried and stored separately at 4 °C, which were recorded as un-grafted of farmland ecotype (FCK1), rootstock of farmland ecotype (FRT1), scion of farmland ecotype (FSC1), un-grafted of mining ecotype (MCK1), rootstock of mining ecotype (MRT1) and scion of mining ecotype (MSC1).

Experimental Design. The experiment was conducted at the Chengdu campus from April to July 2017. The soil samples were air-dried and passed through a 5-mm mesh in April 2017, 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 [5] with a saturated heavy metal solution in the form of $CdCl_2 \cdot 2.5H_2O$. The soils were mixed immediately and again after 8 weeks, during which soil moisture was kept at 80%. The seeds of *S. photeinocarpum* were sown in the un-contaminated soil in May 2017. Four uniform *S. photeinocarpum* seedlings with four expanded true leaves of each treatment were transplanted into each pot in June 2017. Each treatment was repeated three times with the 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 μmol/mol, temperature 30 °C, light intensity 1000 μmol/m^2/s. The determination of photosynthetic parameters were net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and value of pressure deficit leaf (Vpdl), and each treatment was repeated three times. After that, the upper mature leaves of plants were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) contents [6]. Then, the whole plants were then gently removed, the roots, stems and leaves were washed with tap water followed by deionized water, and dried at 80°C to constant weight. The soluble sugar contents in shoots of *S. media* were determined by anthrone colorimetry with dry weight plant samples [6].

Statistical Analyses. Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference (LSD) at the p = 0.05 confidence level.

Results and Discussion

Photosynthetic Pigment Content in Post Generations of *S. photeinocarpum*. The chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of farmland ecotype of *S. photeinocarpum* were higher than the mining ecotype (Table 1). Compared with FCK1, the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of FRT1 and FSC1 increased. FRT1 and FSC1 increased the total chlorophyll content by 13.54% ($p < 0.05$) and 34.06% ($p < 0.05$), respectively, compared with FCK1, and increased carotenoid content by 22.64% ($p < 0.05$) and 42.77% ($p < 0.05$), respectively. Compared with MCK1, the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of MRT1 and MSC1 increased. MRT1 and MSC1 increased the total chlorophyll content by 24.68% ($p < 0.05$) and 16.00% ($p < 0.05$), respectively, compared with MCK1, and increased carotenoid content by 33.55% ($p < 0.05$) and 18.60% ($p < 0.05$), respectively. The order of chlorophyll a/b was FCK1 > FRT1 > MCK1 > MSC1 > FSC1 > MRT1.

Table 1 Photosynthetic pigment content in post generations of *S. photeinocarpum*

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
FCK1	0.851±0.036c	0.294±0.005c	1.145±0.041c	2.895	0.318±0.015d
FRT1	0.963±0.044b	0.337±0.023bc	1.300±0.066b	2.858	0.390±0.017bc
FSC1	1.069±0.037a	0.466±0.017a	1.535±0.054a	2.294	0.454±0.018a
MCK1	0.699±0.029d	0.245±0.016d	0.944±0.013d	2.853	0.301±0.014d
MRT1	0.809±0.039c	0.368±0.031b	1.177±0.070bc	2.198	0.402±0.012b
MSC1	0.791±0.055cd	0.304±0.017c	1.095±0.038c	2.602	0.357±0.017c

Values are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

Photosynthetic Characteristics of Post Generations of *S. photeinocarpum*. Compared with the un-grafted control, the grafting improved the photosynthesis of post generations of *S. photeinocarpum* under Cd stress (Table 2). The grafting increased the Pn, Gs, Ci, Tr of post generations of *S. photeinocarpum*, and decreased the Vpdl of that compared with the un-grafted control, respectively. Compared with FCK1, FRT1 and FSC1 increased Pn by 4.66% ($p > 0.05$) and 41.83% ($p < 0.05$), respectively, increased Gs by 22.85% ($p < 0.05$) and 46.33% ($p < 0.05$), respectively, increased Ci by 5.86% ($p > 0.05$) and 15.62% ($p < 0.05$), respectively, and increased Tr by 7.42% ($p > 0.05$) and 32.54% ($p < 0.05$), respectively. Compared with MCK1, MRT1 and MSC1 increased Pn by 35.97% ($p < 0.05$) and 10.36% ($p < 0.05$), respectively, increased Gs by 58.92% ($p < 0.05$) and 19.57% ($p < 0.05$), respectively, increased Ci by 15.37% ($p < 0.05$) and 8.18% ($p < 0.05$), respectively, and increased Tr by 56.74% ($p < 0.05$) and 23.64% ($p < 0.05$), respectively. FRT1 and FSC1 decreased Vpdl by 4.61% ($p > 0.05$) and 31.80% ($p < 0.05$), respectively, compared with FCK1, and MRT1 and MSC1 decreased Vpdl by 35.64% ($p < 0.05$) and 25.96% ($p < 0.05$), respectively, compared with MCK1.

Table 2 Photosynthetic characteristics of post generations of *S. photeinocarpum*

Treatments	Pn (μmol $CO_2/m^2/s$)	Gs (mol $H_2O/m^2/s$)	Ci (μmol CO_2/mol)	Tr (mmol $H_2O/m^2/s$)	Vpdl (kPa)
FCK1	29.81±1.01cd	0.477±0.016d	233.78±7.32d	4.18±0.23c	2.17±0.10a
FRT1	31.20±2.00cd	0.586±0.014c	247.48±4.26cd	4.49±0.10c	2.07±0.07a
FSC1	42.28±2.34a	0.698±0.019b	270.29±8.80ab	5.54±0.39b	1.48±0.02b
MCK1	29.16±0.60d	0.465±0.018d	238.09±7.21d	4.23±0.29c	2.08±0.05a
MRT1	39.65±1.18b	0.739±0.026a	274.68±7.42a	6.63±0.32a	1.32±0.04c
MSC1	32.18±0.56c	0.556±0.030c	257.56±9.59bc	5.23±0.13b	1.54±0.06b

Values are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

Soluble Sugar Content in Post Generations of *S. photeinocarpum*. Compared with the un-grafted control, the grafting increased the soluble sugar contents in roots, stems, leaves and shoots of post generations of *S. photeinocarpum* under Cd stress (Table 3). Compared with FCK1, FRT1 and FSC1 increased the soluble sugar content in roots by 17.13% ($p < 0.05$) and 26.73% ($p < 0.05$), respectively, increased the soluble sugar content in stems by 23.40% ($p < 0.05$) and 39.72% ($p < 0.05$), respectively, increased the soluble sugar content in leaves by 11.18% ($p < 0.05$) and 20.66% ($p < 0.05$), respectively, and increased the soluble sugar content in shoots by 16.54% ($p < 0.05$) and 28.75% ($p < 0.05$), respectively. Compared with MCK1, MRT1 and MSC1 increased the soluble sugar content in roots by 51.50% ($p < 0.05$) and 26.53% ($p < 0.05$), respectively, increased the soluble sugar content in stems by 48.99% ($p < 0.05$) and 14.69% ($p > 0.05$), respectively, increased the soluble sugar content in leaves by 33.09% ($p < 0.05$) and 14.27% ($p < 0.05$), respectively, and increased the soluble sugar content in shoots by 39.04% ($p < 0.05$) and 13.47% ($p < 0.05$), respectively.

Table 3 Soluble sugar content in post generations of *S. photeinocarpum*

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)	Shoots (mg/g)
FCK1	58.03±3.15d	46.07±3.15c	69.66±2.91c	58.75±2.96c
FRT1	67.97±3.82bc	56.85±3.68b	77.45±1.13b	68.47±2.24b
FSC1	73.54±1.87ab	64.37±1.70a	84.05±2.38a	75.64±2.04a
MCK1	50.99±1.71e	44.58±3.46c	60.84±1.01d	53.23±1.08d
MRT1	77.25±2.78a	66.42±0.62a	80.97±3.70ab	74.01±2.22a
MSC1	64.52±2.91cd	51.13±1.26bc	69.52±1.80c	60.40±1.51c

Values are means ± standard errors. Means with the same letter within each column are not significantly different at $p < 0.05$.

Conclusions

Under Cd stress, two ecotypes of *S. photeinocarpum* mutual grafting improves the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of their post generations. The mutual grafting increased the Pn, Gs, Ci, Tr of post generations of *S. photeinocarpum*, and decreased the Vpdl of that compared with the un-grafted control, respectively. Compared with the un-grafted control, the grafting increased the soluble sugar contents in roots, stems, leaves and shoots of post generations of *S. photeinocarpum*. Therefore, two ecotypes mutual grafting could improve the photosynthesis of post generations of *S. photeinocarpum* under cadmium stress.

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).

References

[1] X.F. Zhang, H.P. Xia, Z.A. Li, P. Zhuang and B. Gao: Journal of Hazardous Materials Vol. 189 (2011), p. 414.

[2] J. Wang, L. Jin, L. Luo, M. Liao, X. Lv, Z. Wang, D. Liang and H. Xia: Environmental Monitoring and Assessment Vol. 188 (2016), p. 82.

[3] X.X. Long, Y.H. Wang and H.Y. Liu: Journal of Plant Ecology Vol. 32 (2008), p. 168.

[4] S.H. Wei, G. Clark, A.I. Doronila, J. Jin and A.C. Monsant: International Journal of Phytoremediation Vol. 15 (2013), p. 199.

[5] L. Lin, M. Liao, L. Mei, J. Cheng, J. Liu, L. Luo and Y. Liu: Environmental Progress & Sustainable Energy Vol. 33 (2014), p. 1251.

[6] Z.B. Hao, J. Chang and Z. Xu: *Plant Physiology Experiment* (Harbin Institute of Technology Press, China 2004).