

Effects of Two Ecotypes Mutual Grafting on Nutrient Absorption of Post Generations of *Solanum photeinocarpum* under Cadmium Stress

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Abstract: A pot experiment was carried out to study the effects of two ecotypes mutual grafting on nutrient absorption of post generations of *Solanum photeinocarpum* under cadmium stress. Two ecotypes mutual grafting increased the total N, total P contents in roots, stems and leaves of post generations of *S. photeinocarpum* under Cd stress, which were benefit to the growth of *S. photeinocarpum*. However, mutual grafting had little effects on total K content in roots of post generations of *S. photeinocarpum*, and had no significant effects on total K content in stems and leaves of that. Mutual grafting increased the available P and available K concentrations, but had little effects on soil alkali soluble N concentration. Therefore, two ecotypes mutual grafting could promote the nutrient uptake of post generations of *S. photeinocarpum* under Cd stress.

Introduction

Solanum photeinocarpum is a potential cadmium (Cd) hyperaccumulator plant [1]. In the wild, because of different climate environment, the different ecotypes of *S. photeinocarpum* formed, such as the farmland ecotype, mining ecotype [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 nutrient absorption of two ecotypes of post generation *S. photeinocarpum* under Cd stress. The objectives of this study were to determine whether mutual grafting could enhance nutrient absorption ability of two ecotypes of post generation *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 a 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 a 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 ephyllas, 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 $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$. 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 whole plants were dug up and divided into three parts of root, stem, leaf, then washed with tap water firstly, followed by deionized water. After that, the organs of all plants were dried at 80 °C until constant weight, weighed, ground to < 0.149 mm, and sealed into plastic bags for the determination of total nitrogen (N), total phosphorus (P) and total potassium (K) contents [6]. The soil sample was collected, air-dried and ground to < 1.0 mm for analysis of alkali soluble N, available P and available K concentrations [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

Total N Contents in Post Generations of *S. photeinocarpum*. The mutual grafting increased the total N contents in post generations of two ecotypes of *S. photeinocarpum* (Table 1). Compared with FCK1, FRT1 and FSC1 increased total N content in roots of *S. photeinocarpum* by 19.26% ($p < 0.05$) and 22.13% ($p < 0.05$), respectively, increased total N content in stems by 21.28% ($p < 0.05$) and 26.60% ($p < 0.05$), respectively, and increased total N content in leaves by 1.60% ($p > 0.05$) and 6.91% ($p < 0.05$), respectively. Compared with MCK1, MRT1 and MSC1 increased total N content in roots of *S. photeinocarpum* by 26.05% ($p < 0.05$) and 9.58% ($p < 0.05$), respectively, increased total N content in stems by 13.69% ($p < 0.05$) and 12.03% ($p < 0.05$), respectively, and increased total N content in leaves by 29.66% ($p < 0.05$) and 11.02% ($p < 0.05$), respectively.

Total P Contents in Post Generations of *S. photeinocarpum*. The mutual grafting increased the total P contents in post generations of two ecotypes of *S. photeinocarpum* (Table 2). Compared with FCK1, FRT1 and FSC1 increased total P content in roots of *S. photeinocarpum* by 21.32% ($p < 0.05$) and 35.74% ($p < 0.05$), respectively, increased total P content in stems by 13.33% ($p < 0.05$) and 40.89% ($p < 0.05$), respectively, and increased total P content in leaves by 3.73% ($p > 0.05$) and 44.10% ($p < 0.05$), respectively. Compared with MCK1, MRT1 and MSC1 increased total P content in roots of *S. photeinocarpum* by 34.15% ($p < 0.05$) and 33.23% ($p < 0.05$), respectively, increased total P content in stems by 33.50% ($p < 0.05$) and 6.50% ($p > 0.05$), respectively, and increased total P

content in leaves by 38.06% ($p < 0.05$) and 26.37% ($p < 0.05$), respectively.

Table 1 Total N contents in post generations of *S. photeinocarpum* under Cd stress

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)
FCK1	2.44±0.06c	1.88±0.06c	3.76±0.10bc
FRT1	2.91±0.11b	2.28±0.07b	3.82±0.13bc
FSC1	2.98±0.08b	2.38±0.10b	4.02±0.14b
MCK1	2.61±0.10c	2.41±0.13b	3.54±0.08c
MRT1	3.29±0.14a	2.74±0.07a	4.59±0.13a
MSC1	2.86±0.07b	2.70±0.11a	3.93±0.11b

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

Table 2 Total P contents in post generations of *S. photeinocarpum* under Cd stress

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)
FCK1	3.33±0.13c	2.25±0.07c	3.22±0.12e
FRT1	4.04±0.04b	2.55±0.08b	3.34±0.05e
FSC1	4.52±0.06a	3.17±0.09a	4.64±0.06c
MCK1	3.25±0.02c	2.00±0.05d	4.02±0.17d
MRT1	4.36±0.06a	2.67±0.14b	5.55±0.07a
MSC1	4.33±0.10a	2.13±0.10cd	5.08±0.06b

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

Total K Contents in Post Generations of *S. photeinocarpum*. The mutual grafting increased the total K contents in roots of post generations of *S. photeinocarpum* (Table 3). Compared with FCK1, FRT1 and FSC1 increased total K content in roots of *S. photeinocarpum* by 2.46% ($p > 0.05$) and 13.18% ($p < 0.05$), respectively. Compared with MCK1, MRT1 and MSC1 increased total K content in roots of *S. photeinocarpum* by 10.21% ($p > 0.05$) and 2.21% ($p > 0.05$), respectively. However, compared with the un-grafted *S. photeinocarpum*, the K contents in stems and leaves of post generations of mutual grafting *S. photeinocarpum* had no significant changes. So, mutual grafting had no obvious effects on K uptake in shoots of post generations of *S. photeinocarpum*.

Table 3 Total K contents in post generations of *S. photeinocarpum* under Cd stress

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)
FCK1	20.71±1.20b	16.86±0.21a	23.47±1.20a
FRT1	21.22±1.15ab	18.20±1.58a	24.11±1.26a
FSC1	23.44±0.61a	18.76±1.07a	24.34±1.77a
MCK1	20.86±1.19b	17.35±0.92a	23.23±1.09a
MRT1	22.99±0.89ab	18.94±1.50a	25.28±0.69a
MSC1	21.32±0.34ab	17.85±1.63a	23.43±1.51a

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

Soil Alkali Soluble N, Available P and Available K Concentrations. Compared with FCK1, FRT1 and FSC1 increased soil alkali soluble N concentration by 7.04% ($p > 0.05$) and 31.48% ($p < 0.05$), respectively, increased soil available P concentration by 2.08% ($p > 0.05$) and 30.06% ($p <$

0.05), respectively, and increased soil available K concentration by 9.09% ($p > 0.05$) and 45.16% ($p < 0.05$), respectively. Compared with MCK1, MRT1 and MSC1 increased soil alkali soluble N concentration by 7.47% ($p > 0.05$) and 3.86% ($p > 0.05$), respectively, increased soil available P concentration by 37.18% ($p < 0.05$) and 18.46% ($p < 0.05$), respectively, and increased soil available K concentration by 28.39% ($p > 0.05$) and 14.27% ($p < 0.05$), respectively.

Table 4 Soil alkali soluble N, available P and available K concentrations

Treatments	Alkali soluble N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
FCK1	74.58±2.18b	3.36±0.08d	102.53±4.71d
FRT1	79.83±4.84b	3.43±0.12d	111.85±6.32d
FSC1	98.06±5.88a	4.37±0.14b	148.83±5.74b
MCK1	90.91±3.10a	3.90±0.16c	130.37±7.65c
MRT1	97.70±2.63a	5.35±0.06a	167.38±6.52a
MSC1	94.42±3.49a	4.62±0.11b	148.98±4.43b

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 mutual grafting increased the total N, total P contents in roots, stems and leaves of post generations of *S. photeinocarpum*, which were benefit to the growth of *S. photeinocarpum*. However, mutual grafting had little effects on total K content in roots of post generations of *S. photeinocarpum*, and had no significant effects on total K content in stems and leaves of that. Mutual grafting increased the available P and available K concentrations, but had little effects on soil alkali soluble N concentration. Therefore, two ecotypes mutual grafting could promote the nutrient uptake of post generations of *S. photeinocarpum* under Cd stress.

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