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Effects of Intercropping with Post-Grafting Generation of *Galinsoga parviflora* on Photosynthesis of Lettuce under Cadmium Stress

Kewen Huang[1,a], Lijin Lin[2,b] and Ming'an Liao[1,c*]

[1]College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, China

[2]Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, China

[a]263733029@qq.com, [b]llj800924@qq.com, [c]lman@sicau.edu.cn

*Corresponding author. Kewen Huang and Lijin Lin contributed equally to this work.

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Abstract: The pot experiment was conducted to study the effects of intercropping with positive and negative grafting generations of two ecotypes (farmland and mining) of cadmium (Cd) hyperaccumulator plants *Galinsoga parviflora* on photosynthesis of lettuce. The positive grafting: the rootstock was mining ecotype of *G. parviflora* (post generation was defined as mining-rootstock) and the scion was farmland ecotype of *G. parviflora* (post generation was defined as farmland-scion); the negative grafting: the rootstock was farmland ecotype of *G. parviflora* (post generation was defined as farmland-rootstock) and the scion was mining ecotype of *G. parviflora* (post generation was defined as mining- scion). The results show that, compared with the monoculture of lettuce, only Intercropping with mining-scion significantly increased the chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of lettuce. In addition, the photosynthetic rate (Pn), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and transpiration rate (Tr) of lettuce all significantly increased by Intercropping with mining-scion and reached maximum in all treatments. Therefore, Intercropping with mining-scion could improve the photosynthesis of lettuce under Cd stress.

1. Introduction

Cadmium (Cd) is one of the most toxic heavy metals which is not easy to degrade in nature, with a high bioaccumulation coefficient [1]. The long-term accumulation of Cd in soil would affect the photosynthesis of plants, and even reduce the yield of economic crops [2-4]. Studies have shown that appropriate intercropping can change the distribution of light in the population, thereby increasing the plant's absorption and conversion of light [5]. Lettuce is an annual or biennial herb that is rich in nutrients and has a high food value [6]. However, the chlorophyll content of lettuce was significantly reduced under high Cd stress, which resulted in the decrease of photosynthesis [7]. *Galinsoga parviflora* is a widely distributed Cd hyperaccumulator that grows rapidly and has a strong vitality [8]. The study found that there are certain differences in the accumulation characteristics of Cd between the mining ecotype *G. parviflora* and the farmland ecotype *G. parviflora* [9]. A preliminary study of this experiment found that the absorption of Cd in the post-grafting generations of the two ecotypes *G. parviflora* were significantly different. In view of this, this experiment intercropped lettuce with post-grafting generation of *G. parviflora*, and studied the effect of intercropping on the photosynthetic characteristics of lettuce, in order to screen post-grafting generation of *G. parviflora* that can improve the photosynthesis of lettuce.

2. Materials and Methods

Materials collection. The seeds of mining ecotype *G. parviflora* and farmland ecotype *G. parviflora* were respectively collected from Tangjiashan lead-zinc mine (29°24' N, 102°38' E) and farmland of Ya'an campus farm of Sichuan Agricultural University (30°23' N, 103°48' E) in September, 2015, stored at 4 °C. The Tangjiashan lead-zinc mine locates in Hanyuan County, Sichuan Province, China, with an typical dry-hot valley climate. The farm of Sichuan Agricultural

University locates in Yucheng County, Sichuan Province, China, with an humid subtropical monsoon climate.

Lettuce variety for Italy Resistant to Hot-Breaked Lettuce, purchased in JIEYANG BANGFENG SEED CO. , LTD.

Grafting. In October 2015, the two ecotypes of *G. parviflora* seeds were grown at a temperature of 25 °C, illumination of 4000 lx and air humidity of 80%. When the seedlings of *G. parviflora* height was about 3 cm and 2 pieces of true leaves unfolded, transplanted into cadmium-free pots. After the *G. parviflora* height was about 10 cm, two ecological types of *G. parviflora* were grafted positively and negatively. (1) Un-grafted of farmland ecotype (post generation was defined as farmland). (2) Un-grafted of mining ecotype (post generation was defined as mining). (3) The postive grafting: the rootstock was farmland ecotype of *G. parviflora* (post generation was defined as farmland-rootstock) and the scion was mining ecotype of *G. parviflora* (post generation was defined as mining-scion). (4) The negative grafting: the rootstock was mining ecotype of *G. parviflora* (post generation was defined as mining-rootstock) and the scion was farmland ecotype of *G. parviflora* (post generation was defined as farmland-scion). All of the leaves of the rootstocks remained.The grafting method was cleft method. And the plastic band with a width of about 1 cm and a length of 20 cm was used for binding. Watering after grafting and keeping the soil moisture content was maintained at 80% of field capacity, and covering it with a mulching film and shade net. After 10 days, the mulching film and the shade net were gradually removed, and the tied plastic band was removed.When grafting treatments were completed, all *G. parviflora* were transplanted into cadmium-free pots, and collected the post generation seeds.

Experimental Design. The experiment was conducted in Chengdu Campus of Sichuan Agricultural University. In February 2016, after the cadmium-free soil was air-dried and crushed, 2.5 kg soil was weighed into each plastic pot (21 cm high, 20 cm in diameter). Soaking uniformly by 10 mg/kg Cd (in the form of $CdCl_2 \cdot 2.5H_2O$) solution for 4 weeks. All pots were watered each day to keep the soil moisture about 80%. In March 2016, the post-grafting generations of *G. parviflora* were grown at a temperature of 25 °C, illumination of 4000 lx and air humidity of 80%. When the seedlings of *G. parviflora* height were about 3 cm and 2 pieces of true leaves unfolded, transplanted into Cd soil to intercrop with lettuce. 7 treatments were conducted: (1) monoculture of lettuce, (2) lettuce intercropped with farmland, (3) lettuce intercropped with mining, (4) lettuce intercropped with farmland-rootstock, (5) lettuce intercropped with mining-rootstock, (6) lettuce intercropped with farmland-scion, (7) lettcue intercropped with mining-scion. Intercropping treatments were planted with 3 lettuces and 1 *G. parviflora*. Monoculture treatments were planted 4 lettuces. Four replicates per treatment and the pots placed completely random. The distance between pots was 15 cm, and the pot position exchanged aperiodically to weaken the impact of the marginal effects. The soil moisture content was maintained at 80% of field capacity until the plants were harvested. After 60 days, the photosynthesis of each lettuce was determined by using LI-6400 portable photosynthesis meter (LI-COR Inc., USA) in June 2016. The photosynthetic parameters of the photosynthesis meter were manual control CO_2 concentration 400 μmol/mol, temperature 30 °C, light intensity 1000 $\mu mol/m^2/s$. 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 then, the upper mature leaves of lettuce were collected to determine the photosynthetic pigment contents (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) [10].

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.

3. Results and Discussion

Photosynthetic Pigment Content in Lettuce. Compared to monoculture, only Intercropping with

mining-scion significantly increased the chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of lettuce by 5.57% ($p < 0.05$), 7.26% ($p < 0.05$), 5.80% ($p < 0.05$), respectively, and other treatments had little effect. For carotenoid content of lettuce, Intercropping with mining and Intercropping with farmland-scion significantly increased the carotenoid content of lettuce by 30.56% ($p < 0.05$) and 25.00% ($p < 0.05$) respectively compared to monoculture.

Table 1 Photosynthetic pigment content in lettuce

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
Monoculture	0.790±0.013ab	0.124±0.005ab	0.914±0.018ab	0.072±0.004cd
Intercropping with farmland	0.768±0.032ab	0.124±0.011ab	0.892±0.043ab	0.078±0.010bcd
Intercropping with mining	0.766±0.016ab	0.121±0.001ab	0.887±0.017ab	0.094±0.006a
Intercropping with farmland-rootstock	0.730±0.040b	0.113±0.006b	0.843±0.046b	0.066±0.003d
Intercropping with mining-rootstock	0.701±0.004b	0.114±0.001b	0.815±0.005b	0.085±0.002abc
Intercropping with farmland-scion	0.758±0.092ab	0.120±0.010ab	0.878±0.102ab	0.090±0.001ab
Intercropping with mining-scion	0.834±0.009a	0.133±0.001a	0.967±0.010a	0.080±0.008abc

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

Table 2 Photosynthetic characteristics of lettuce

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）
Monoculture	16.15±0.067b	0.272±0.034b	277.6±0.034b	3.413±0.134b	1.333±0.123a
Intercropping with farmland	15.65±0.032d	0.251±0.323d	273.5±0.023d	2.804±0.143c	1.312±0.982a
Intercropping with mining	11.06±0.043f	0.183±0.174f	267.5±0.014f	2.214±0.014d	1.254±0.042b
Intercropping with farmland-rootstock	8.75±0.012g	0.202±0.223e	265.5±0.013g	1.883±0.033e	1.152±0.034c
Intercropping with mining-rootstock	12.83±0.012e	0.173±0.214g	269.3±0.024e	1.734±0.094e	1.084±0.012d
Intercropping with farmland-scion	16.07±0.033c	0.264±0.313c	277.0±0.033c	3.685±0.033a	0.953±0.063e
Intercropping with mining-scion	16.24±0.024a	0.305±0.014a	296.9±0.024a	3.733±0.053a	0.952±0.014e

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

Photosynthetic Characteristics of Lettuce. Under Cd stress, the Pn and Ci of lettuce were ranked in the following order: Intercropping with mining-scion > Monoculture > Intercropping with farmland-scion > Intercropping with farmland > Intercropping with mining-rootstock > Intercropping with mining > Intercropping with farmland-rootstock. Compared to monoculture, in addition to Intercropping with mining-scion significantly increasted the Gs of lettuce other treatments all significantly reduced it. For the Tr of lettuce, Intercropping with farmland-scion and Intercropping with mining-scion significantly increased the Tr of lettuce by 7.97% ($p < 0.05$) and 9.38% ($p < 0.05$) respectively compareted to monoculture. And with the exception of Intercropping with farmland, all other treatments significantly reduced Vpdl of lettuce compared to monoculture.

4. Conclusions

Under Cd stress, Intercropping with mining-scion could significantly improve the chlorophyll a, chlorophyll b and total chlorophyll contents of lettuce, compared to monoculture. Besides, the Pn, Gs, Ci and Tr of lettuce all significantly increased by Intercropping with mining-scion and reached maximum in all treatments. Therefore, Intercropping with mining-scion could improve the photosynthesis of lettuce under cadmium stress.

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