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Effects of Intercropping with Hyperaccumulator Plants on Photosynthesis of Grape Seedlings under Cadmium Stress

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Abstract: The effects of intercropping with four hyperacculator plant species (*Galinsoga parviflora, Sigesbeckia orientalis, Solanum nigrum* and *Crassocephalum crepidioide*) on photosynthesis and soluble sugar content of grape seedlings under cadmium (Cd) stress were studied by a pot experiment. The results showed that intercropping treatments could increase the net photosynthetic rate (Pn), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and transpiration rate (Tr) of grape seedlings, and intercropping with *S. nigrum* increased most. The soluble sugar content in roots and shoots of grape seedlings significantly increased by intercropping compared to monoculture. Therefore, Intercropping could improve the photosynthesis and soluble sugar content of grape seedlings under Cd stress, and intercropping with *S. nigrum* effectively most improve the photosynthesis of grape seedlings.

Introduction

Cadmium (Cd) is a heavy metal element that has strong toxic effect on plants [1]. Excessive amount of Cd inhibit the growth and physiological processes especially photosynthesis of plants [2-4]. Grape is a worldwide important fruit and the production occupies a pivotal position of fruit[5], and it also can be polluted by Cd [6]. Different plants intercropping can increase the utilization efficiency of resources in plant, such as soil nutrients, water and light and change growth and photosynthetic characteristics [7]. The studies show that the heavy metal uptaking in non-hyperaccumulator plants reduces and the physiological processes changes when intercropping with hyperaccumulator plants [8-9]. Therefore, in this study, we used four Cd hyperaccumulator plant species *Galinsoga parviflora* [10], *Sigesbeckia orientalis* [11], *Solanum nigrum* [12], and *Crassocephalum crepidioides* [13] to intercrop with grape seedlings under Cd stress by a pot experiment to study the effects on on photosynthesis of grape seedlings.

Materials and Methods

Materials. In April 2016, the seeds of four hyperaccumulator plant species *G. parviflora, S. orientalis, S. nigrum* and *C. crepidioides* were collected from the surrounding farmland at Chengdu campus of Sichuan Agricultural University. Then, the seeds were put in the climate chamber to germination and further cultivation and transplanting. The experimental cultivar of grape is Kyoho, whose cutting seedlings were purchased from Longquanyi area seedlings base of Chengdu in May 2016. The soil for the experiment was collected from the surrounding farmland at Chengdu campus of Sichuan Agricultural University in April 2016.

Experimental Design. The experiment was conducted in Chengdu Campus of Sichuan Agricultural University from April to July 2016. In April 2016, the soil was air-dried and passed through a 6.72-mm sieve. 3 kg air-dried soil was weighed into each plastic pot (21 cm high, 20 cm in diameter), soaking uniformly by 5 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 May 2016, three uniform-sized cutting seedlings (the shoots were about 15 cm) of Kyoho grape were transplanted into pot for monoculture. Two uniform-sized seedlings (two ture leaves expanded) of each four hyperaccumulators and two grape seedlings were transplanted into each pot for intercropping. For each treatment with three replicates 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 grape seedling 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), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and transpiration rate (Tr). Then, grape seedlings were dug up and divided into three parts of root, stem and leave, then washed with tap water firstly, followed by deionized water. After that, the tissues of all plants were dried at 80 °C until constant weight, weighed, ground to < 0.149 mm to determined the soluble sugar contents [14].

Statistical Analyses. Statistical analyses were conducted using statistical software of SPSS 17.0. Date were analyzed by one-way ANOVA with least significant difference at 5% confidence level.

Results and Discussion

Net Photosynthetic Rate (Pn). Compared with the monoculture, intercropping with four hyperaccumulator plant species increased the Pn of grape seedlings under Cd stress (Fig. 1). The Pn of grape seedlings by intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* increased by 0.45% ($p > 0.05$), 27.26% ($p < 0.05$), 43.78% ($p < 0.05$) and 28.96% ($p < 0.05$) respectively compared to monoculture.

Stomatal Conductance (Gs). The Gs of grape seedlings were ranked in the following order: intercropping with *S. nigrum* > intercropping with *S. orientalis* > intercropping with *C. crepidioides* > intercropping with *G. parviflora* > monoculture (Fig. 2). According to the order, they were respectively 2.00 ($p < 0.05$), 2.21 ($p < 0.05$), 2.29 ($p < 0.05$) and 2.05 ($p < 0.05$) times of monoculture.

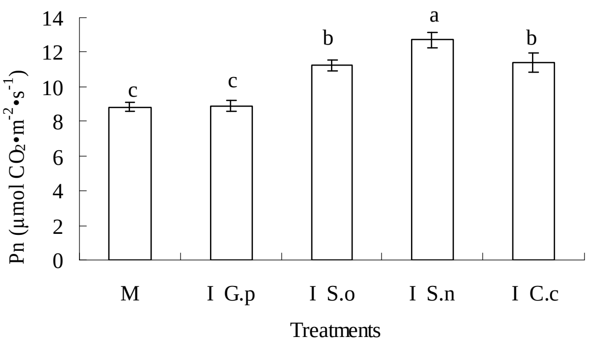

Fig. 1 Pn of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G.p = intercropping with *G. parviflora*, I S.o = intercropping with *S. orientalis*, I S.n = intercropping with *S. nigrum*, I C.c = intercropping with *C. crepidioides*.

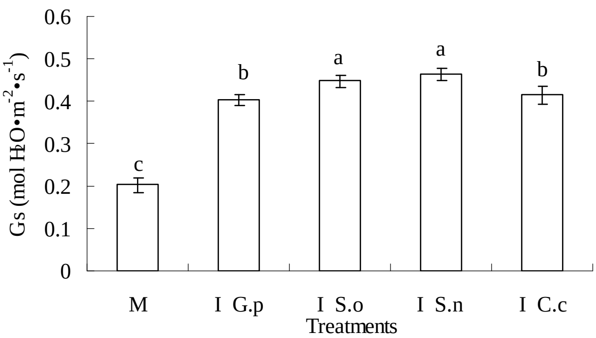

Fig. 2 Gs of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G.p = intercropping with *G. parviflora*, I S.o = intercropping with *S. orientalis*, I S.n = intercropping with *S. nigrum*, I C.c = intercropping with *C. crepidioides*.

CO_2 Concentration of Intercellular (Ci). Compared with the monoculture, the Ci of grape seedlings increased significantly by intercropping under Cd stress (Fig. 3, $p < 0.05$). The Ci of grape seedlings by intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* increased by 8.60%, 10.12%, 12.20% and 10.31% respectively compared to monoculture.

Transpiration Rate (Tr). Intercropping with four hyperaccumulators increased significantly the Tr of grape seedlings compared to monoculture under Cd stress (Fig. 4, $p < 0.05$). The Tr of grape seedlings were ranked as: intercropping with *S. nigrum* > intercropping with *S. orientalis* > intercropping with *C. crepidioides* > intercropping with *G. parviflora* > monoculture. The Tr of grape seedlings by intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* increased by 49.44%, 59.04%, 75.20% and 57.76% respectively compared to monoculture.

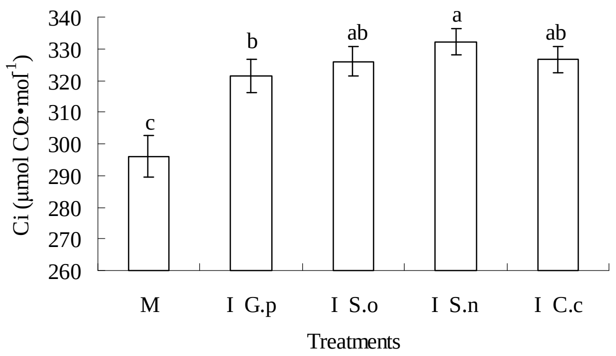

Fig. 3 Ci of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G.p = intercropping with *G. parviflora*, I S.o = intercropping with *S. orientalis*, I S.n = intercropping with *S. nigrum*, I C.c = intercropping with *C. crepidioides*.

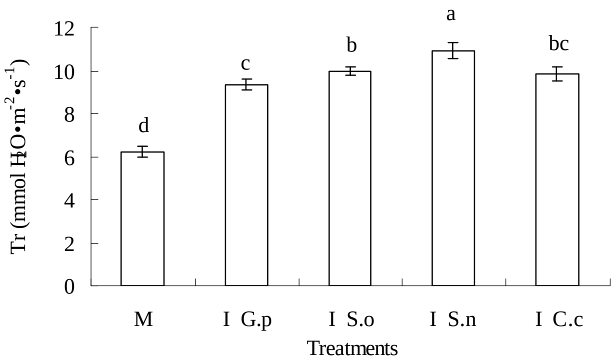

Fig. 4 Tr of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, I G.p = intercropping with *G. parviflora*, I S.o = intercropping with *S. orientalis*, I S.n = intercropping with *S. nigrum*, I C.c = intercropping with *C. crepidioides*.

Soluble Sugar Content of Grape Seedlings. Compared with the monoculture, intercropping with hyperaccumulator plants significantly increased the soluble sugar contents in roots and shoots of grape seedlings under Cd stress (Table 1, $p < 0.05$). Intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* increased the soluble sugar content in roots of grape seedlings by 129.89%, 53.16%, 9.77% and 18.97% compared to monoculture, respectively. The soluble sugar content in stems of grape seedlings was ranked as: intercropping with *G. parviflora* > intercropping with *S. orientalis* > intercropping with *S. nigrum* > intercropping with *C. Crepidioides* > monoculture. For the leaves of grape seedlings, the order was intercropping with *C. crepidioides* > intercropping with *S. orientalis* > intercropping with *G. parviflora* > intercropping with *S. nigrum* > monoculture. For the soluble sugar content in shoots of grape seedlings, intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* increased respectively by 18.80%, 13.50%, 3.28% and 19.71% compared to monoculture.

Conclusions

Under Cd stress, intercropping with four hyperaccumulators including *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* increased the Pn, Gs, Ci and Tr of grape seedlings and intercropping with *S. nigrum* increased most compared with the other three hyperaccumulators. The soluble sugar content in roots and shoots of grape seedlings significantly increased by intercropping compared to monoculture. Therefore, intercropping could improve the photosynthesis and soluble sugar content of grape seedlings under cadmium stress, and intercropping with *S. nigrum* effectively most improve the photosynthesis of grape seedlings.

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Table 1 Soluble sugar content in grape seedlings

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)	Shoots (mg/g)
Monoculture	0.348±0.008e	0.433±0.011c	0.602±0.010d	0.548±0.012c
Intercropping with *G. parviflora*	0.800±0.016a	0.576±0.010a	0.685±0.013b	0.651±0.012a
Intercropping with *S. orientalis*	0.533±0.009b	0.455±0.009b	0.697±0.012b	0.622±0.010b
Intercropping with *S. nigrum*	0.382±0.009d	0.437±0.011bc	0.628±0.012c	0.566±0.011c
Intercropping with *C. crepidioides*	0.414±0.006c	0.435±0.009bc	0.764±0.014a	0.656±0.011a

Values are mean ± SE. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 13.0 followed by the least significant difference test ($p < 0.05$).

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