Effects of Intercropping with Bidens Species on Photosynthesis of Grape Seedlings under Cadmium Stress

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Abstract: A pot experiment was conducted to study the effects of intercropping with four Bidens species (*Bidens bipinnata*, *Bidens pilosa*, *Bidens parviflora* and *Bidens biternata*) on photosynthesis and soluble sugar content of grape seedlings under cadmium (Cd) stress. The result showed that net photosynthetic rate (Pn), stomatal conductance (Gs), CO₂ concentration of intercellular (Ci) and transpiration rate (Tr) were decreased compared to monoculture. The soluble sugar content in roots and shoots significantly increased by intercropping compared to monoculture. Therefore, intercropping with four Bidens species inhibit the photosynthetic ability but improve the resistance to Cd stress of grapes.

Introduction

Cadmium (Cd), as a heavy metal element, can influence several physiological and biochemical processes such as water uptake, nutrient assimilation, photosynthesis, and respiration [1-2]. Some studies show that Cd stress result to photosynthesis inhibition for decreasing the contents of chlorophyll a and b [3]. Others indicate that Cd stress disturb the balance of phytohormone levels, and cause the decrease of photosynthetic capacity [4]. Grape as a major fruit in the world is also needed to be protected [5-6]. Some researches show appropriately intercropping can improve or reduce the photosynthetic performance of plants [7-8]. Intercropping different plants can reduce the biological stress and abiotic stress damage [9]. Bidens species are potential hyperaccumulators [10], and some studies show them can reduce the toxic effect of Cd on plants [11-12]. Therefore, in this study, we used four species of Bidens (*B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata*) to intercrop with grape seedlings under Cd stress by a pot experiment to study the effects on photosynthesis.

Materials and Methods

Materials. In April 2016, the seeds of four Bidens species *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata* 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 species of the experimental cultivar of grape is Kyoho which 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 CdCl₂·2.5H₂O) solution for 4 weeks [13]. All pots were watered every 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 true leaves expanded) of each four Bidens species 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 periodically 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 $400 \mu\text{mol}\cdot\text{mol}^{-1}$, temperature 30°C , light intensity $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The determination of photosynthetic parameters were net photosynthetic rate (Pn), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and transpiration rate (Tr) [14]. 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 $< 0.149 \text{ mm}$ to determined the soluble sugar contents.

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

Results and Discussion

Net Photosynthetic Rate (Pn). Intercropping with four *Bidens* species decreased the Pn of grape seedlings under Cd stress compared to monoculture (Fig. 1, $p < 0.05$). The Pn of grape seedlings were ranked in the following order: monoculture $>$ intercropping with *B. pilosa* $>$ intercropping with *B. bipinnata* $>$ intercropping with *B. biternata* $>$ intercropping with *B. parviflora*.

Stomatal Conductance (Gs). The Gs of grape seedlings intercropping with *B. parviflora* varied not obviously compared to monoculture under Cd stress (Fig. 2, $p < 0.05$), but intercropping with the other three *Bidens* species decreased the Gs of grape seedlings. The Gs of grape seedlings by intercropping with *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata* decreased by 25.80% ($p < 0.05$), 20.68% ($p < 0.05$), 0.97% ($p > 0.05$) and 48.18% ($p < 0.05$) respectively compared to monoculture.

Fig. 1 Pn of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, IB.bip = intercropping with *B. bipinnata*, IB.pil = intercropping with *B. pilosa*, IB.par = intercropping with *B. parviflora*, IB.bit = intercropping with *B. biternata*.

Fig. 2 Gs of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, IB.bip = intercropping with *B. bipinnata*, IB.pil = intercropping with *B. pilosa*, IB.par = intercropping with *B. parviflora*, IB.bit = intercropping with *B. biternata*.

CO_2 Concentration of Intercellular (Ci). Intercropping with four *Bidens* species decreased significantly the Ci of grape seedlings under Cd stress compared to monoculture (Fig. 3, $p < 0.05$). The Ci of grape seedlings were ranked in the following order: monoculture $>$ intercropping with *B.*

bipinnata > intercropping with *B. parviflora* > intercropping with *B. pilosa* > intercropping with *B. biternata*.

Transpiration Rate (Tr). Compared with the monoculture, the Tr of grape seedlings decreased significantly by intercropping under Cd stress (Fig. 4, $p < 0.05$). The Tr of grape seedlings were ranked as: monoculture > intercropping with *B. bipinnata* > intercropping with *B. pilosa* > intercropping with *B. parviflora* > intercropping with *B. biternata*. The Tr of grape seedlings by intercropping with *B. bipinnata*, *B. pilosa*, *B. parviflora*, and *B. biternata*, decreased by 9.92%, 19.39%, 32.16% and 35.93% 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 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, IB.bip = intercropping with *B. bipinnata*, IB.pil = intercropping with *B. pilosa*, IB.par = intercropping with *B. parviflora*, IB.bit = intercropping with *B. biternata*.

Fig. 4 Tr of grape seedlings. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$). M = monoculture, IB.bip = intercropping with *B. bipinnata*, IB.pil = intercropping with *B. pilosa*, IB.par = intercropping with *B. parviflora*, IB.bit = intercropping with *B. biternata*.

Soluble Sugar Content of Grape Seedlings. Intercropping significantly increased the soluble sugar content in roots and shoots of grape seedlings under Cd stress compared to monoculture (Table 1, $P < 0.05$). The soluble sugar content in roots of grape seedlings was ranked as: intercropping with *B. pilosa* > intercropping with *B. biternata* > intercropping with *B. parviflora* > intercropping with *B. bipinnata* > monoculture. Intercropping with *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata* increased the soluble sugar content in stems of grape seedlings by 11.38%, 22.57%, 24.89%, and 19.17% compared to monoculture, respectively. For the leaves of grape seedlings, the order was intercropping with *B. parviflora* > intercropping with *B. biternata* > intercropping with *B. bipinnata* > intercropping with *B. pilosa* > monoculture. For the soluble sugar content in shoots of grape seedlings, intercropping with *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata* increased respectively by 12.71%, 9.90%, 19.68% and 18.77% compared to monoculture.

Conclusions

In this study, intercropping with four *Bidens* species *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata* decreased the Pn, Gs, Ci and Tr of grape seedlings generally under Cd stress. Intercropping with four *Bidens* species increased the soluble sugar content in roots and shoots of grape seedlings under Cd stress. In conclusion, intercropping with four *Bidens* species inhibit the photosynthetic ability but improve the resistance to Cd stress of grape.

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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	0.518±0.081c	0.445±0.076e	0.751±0.234d	0.654±0.130c
Intercropping with <i>B. bipinnata</i>	0.575±0.135b	0.503±0.089d	0.867±0.259b	0.749±0.205b
Intercropping with <i>B. pilosa</i>	0.651±0.058a	0.575±0.123b	0.797±0.186c	0.726±0.162b
Intercropping with <i>B. parviflora</i>	0.637±0.128a	0.593±0.073a	0.907±0.183a	0.814±0.152a
Intercropping with <i>B. biternata</i>	0.639±0.082a	0.551±0.066c	0.899±0.137ab	0.805±0.130a

Values are mean ± SE. Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($p < 0.05$).

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