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

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

[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@163.com, [c]627563308@qq.com, [d]lman@sicau.edu.cn

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

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Abstract: The effects of intercropping with four floricultural accumulator plants (*Helianthus annuus*, *Cosmos sulphureus*, *Cosmos bipinnata*, *Impatiens balsamina*) on photosynthesis of grape seedlings under cadmium (Cd) stress were studied by the pot experiment, and the photosynthetic pigment contents, photosynthetic characteristics and soluble sugar contents in grape were determined. The results showed that intercropping with *C. bipinnata* significantly improved the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of grape seedlings. The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), CO_2 concentration of intercellular (Ci) and value of pressure deficit leaf (Vpdl) were increased by intercropping with *C. bipinnata* compared with the monoculture. Intercropping with *C. bipinnata* significantly increased the soluble sugar content in leaves of grape seedlings. Therefore, intercropping with *C. bipinnata* could improve the photosynthesis of grape seedlings under Cd stress.

Introduction

With the development of society, heavy metal pollution has become an increasingly serious problem [1]. Cadmium (Cd), as one of heavy metals, inhibits the physiological processes of plants, especially photosynthesis [2-3]. Grape is a worldwide economic fruit tree, but it has been found that some vineyards have received heavy metals pollution, which inhibits the photosynthesis and yield of grapes [4]. Intercropping can change the distribution of light in the population and change the photosynthetic characteristics of the plants [5]. Studies have shown that intercropping systems are beneficial to plants photosynthesis and exhibit significant yield advantages [6]. Floricultural plants are rich in resources and belong to ornamental plants, intercropping with floricultural heavy metal-accumulator plants can repair the soil and improve the fruit tree photosynthesis [7]. Therefore, to enhancing the photosynthesis of grape under Cd stress, four floricultural Cd-accumulator plants *Helianthus annuus* [8], *Cosmos sulphureus* [9], *Cosmos bipinnata* [10], *Impatiens balsamina* [11] were used to intercrop with grape seedlings in Cd-contaminated soil and the effects of intercropping with four floricultural Cd-accumulator plants on photosynthesis of grape seedlings were studied.

Materials and Methods

Materials collection. The seeds of floricultural plants (*H. annuus*, *C. sulphureus*, *C. bipinnata*, *I. balsamina*) were collected from the farmland of Chengdu Campus of Sichuan Agricultural University and were put into the tray to germinate in April, 2016. The cultivar of grape is Kyoho with cutting seedlings. The fluvo-aquic soil samples were collected from the 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

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into pot for monoculture. One uniform-sized seedling (two pairs leaves expanded) of each floricultural plant and two grape seedlings were transplanted into each pot for intercropping. The five treatments in experiment were monoculture of grape, grape intercropped with *H. annuus*, grape intercropped with *C. sulphureus*, grape intercropped with *C. bipinnata* and grape intercropped with *I. balsamina*. Three 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 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). After that, the upper mature leaves of plants were collected to determine the photosynthetic pigment (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) contents [12]. 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 grape were determined by anthrone colorimetry with dry weight plant samples [12].

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 Contents in grape seedlings. The chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of grape seedlings were ranked in the following order: intercropping with *C. bipinnata* > intercropping with *H. annuus* > monoculture > intercropping with *I. balsamina* > intercropping with *C. sulphureu* (Table 1). Intercropping with *C. bipinnata* increased the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents by 28.25% (p < 0.05), 22.03% (p < 0.05), 26.56% (p < 0.05) and 35.06% (p < 0.05) respectively, compared with monoculture. The chlorophyll *a/b* of grape seedlings was ranked in the following order: intercropping with *C. sulphureu* > intercropping with *C. bipinnata* > intercropping with *H. annuus* > intercropping with *I. balsamina* > monoculture.

Table 1 Photosynthetic pigment contents in grape seedlings

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Chlorophyll *a*/*b*	Carotenoid (mg/g)
Monoculture	0.924±0.05b	0.345±0.02b	1.269±0.06b	2.678	0.231±0.01c
Intercropping with *H. annuus*	1.139±0.06a	0.410±0.02a	1.549±0.08a	2.778	0.275±0.01b
Intercropping with *C. sulphureus*	0.849±0.04c	0.297±0.01b	1.146±0.06c	2.859	0.216±0.01c
Intercropping with *C. bipinnata*	1.185±0.06a	0.421±0.02a	1.606±0.08a	2.815	0.312±0.02a
Intercropping with *I. balsamina*	0.886±0.04bc	0.326±0.02b	1.212±0.06bc	2.718	0.217±0.01c

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

Photosynthetic Characteristics of grape seedlings. Under Cd stress, intercropping with *C. bipinnata* increased the Pn, Gs, Tr and Vpdl of grape seedlings, and increased by 2.97% (p < 0.05), 23.26% ($p < 0.05$), 23.19% ($p < 0.05$) and 36.18% ($p < 0.05$) compared to monoculture, respectively (Table 2). Compared with the monoculture, intercropping had little effects on Ci of grape seedlings. The Pn, Gs, Ci, Tr and Vpdl of grape seedlings were ranked in the following order: intercropping with *C. bipinnata* > monoculture > intercropping with *C. sulphureu* > intercropping with *I. balsamina* > intercropping with *H. annuus*. Intercropping with *H. annuus* reduced the Gs, Tr and Vpdl of grape seedlings, and decreased by 28.91% ($p < 0.05$), 33.42% ($p < 0.05$) and 17.59% ($p < 0.05$) compared to monoculture, respectively.

Table 2 Photosynthetic characteristics of grape seedlings

Treatments	Pn (μmol $CO_2/m^2/s$)	Gs (mol $H_2O/m/s$)	Ci (μmol CO_2/mol)	Tr (mmol $H_2O/m^2/s$)	Vpdl (kPa)
Monoculture	12.78±0.63ab	0.460±0.02b	325.56±16.25a	8.02±0.44b	1.99±0.10b
Intercropping with *H. annuus*	11.36±0.58b	0.327±0.02c	306.43±15.52a	5.34±0.26c	1.64±0.08c
Intercropping with *C. sulphureus*	12.46±0.61ab	0.455±0.02b	317.38±15.76a	7.71±0.38b	1.93±0.11b
Intercropping with *C. bipinnata*	13.16±0.66a	0.567±0.03a	335.28±16.48a	9.88±0.47a	2.71±0.13a
Intercropping with *I. balsamina*	12.17±0.62ab	0.423±0.02b	311.26±15.27a	7.25±0.32b	1.75±0.09bc

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

Soluble Sugar Contents in grape seedlings. Compared with the monoculture, intercropping significantly increased the soluble sugar contents in roots of grape seedlings under Cd stress (Table 3). Intercropping with *H. annuus,* intercropping with *C. sulphureus,* intercropping with *C. bipinnata,* and intercropping with *I. balsamina* increased the soluble sugar contents in roots of grape seedlings by 43.83% ($p < 0.05$), 25.93% ($p < 0.05$), 63.58% ($p < 0.05$) and 34.57% ($p < 0.05$) compared to monoculture, respectively. Intercropping with *C. sulphureus* and intercropping with *C. bipinnata* significiantly reduced the soluble sugar content in stems of grape seedlings, decreased by 54.85% ($p < 0.05$) and 27.31% ($p < 0.05$) compared to monoculture, respectively. Compared with the monoculture, intercropping with *C. sulphureus* significantly increased the sugar content in leaves of grape seedlings under Cd stress, but other treatments reduced. The soluble sugar content in shoots of grape seedlings was ranked in the following order: intercropping with *C. sulphureu* > intercropping with *I. balsamina* > monoculture > intercropping with *H. annuus*> intercropping with *C. bipinnata.*

Table 3 Soluble sugar contents in grape seedlings

Treatments	Roots (%)	Stems (%)	Leaves (%)	Shoots (%)
Monoculture	0.324±0.02e	0.443±0.02a	0.780±0.04b	0.669±0.03b
Intercropping with *H. annuus*	0.466±0.02b	0.449±0.02a	0.706±0.04c	0.637±0.03c
Intercropping with *C. sulphureus*	0.408±0.02d	0.200±0.01c	0.950±0.05a	0.715±0.04a
Intercropping with *C. bipinnata*	0.530±0.03a	0.322±0.02b	0.530±0.03d	0.459±0.02d
Intercropping with *I. balsamina*	0.436±0.02c	0.457±0.02a	0.777±0.04b	0.683±0.03b

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, intercropping with *C. bipinnata* and intercropping with *H. annuus* improved the chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents of grape seedlings. Intercropping with *C. bipinnata* increased the Pn, Gs, Ci, Tr and Vpdl of grape seedlings, but other treatments reduced them, respectively. Compared with the monoculture, intercropping with *C. sulphureus* significantly increased the soluble sugar contents in leaves and shoots of grape seedlings. Intercropping with *C. bipinnata* significantly increased the soluble sugar contents in leaves of grape seedlings. Therefore, intercropping with *C. bipinnata* could improve the photosynthesis of grape seedlings under Cd stress.

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