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

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Abstract: To study the effects of intercropping on nutrient absorption of plants under cadmium (Cd) stress, 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 the nitrogen (N), phosphorus (P) and potassium (K) uptake of grape seedlings under Cd stress. When grape seedlings intercropped with four Bidens species, the total N and total K contents in grape seedlings decreased compared with the monoculture. Intercropping decreased the total P content in roots of grape seedlings except intercropping with *B. pilosa* and increased the total P content in shoots of grape seedlings except intercropping with *B. parviflora*. Compared with the monoculture, the soil available P and available K concentrations of intercropping decreased, but the soil alkali soluble N concentration of intercropping significantly increased. On the whole, intercropping with *B. pilosa* could most effectively improve the P absorption of grape seedlings and inhibit the N and K absorption of that. Therefore, intercropping with *B. pilosa* could benefit the nutrient absorption of grape seedlings under Cd stress.

Introduction

Intercropping as a major agricultural production model, could improve the production of composite plant population and the nutrient resources of soil [1]. Intercropping with white clover and perennial ryegrass in apple orchard improve the soil hydrolyzed nitrogen (N), soil available phosphorus (P) and soil available potassium (K) concentrations [2]. Natural grass in orchard significantly improves the available N, P and K concentrations in soil [3]. Intercropping with soybean or peanut in orchard also significantly improve the available nutrient concentrations of different layers of soil [4]. The rhizosphere effects occur in plants when intercropping to make a series of changes of physiology and biochemistry under heavy metal stress [5]. However, there are few studies on nutrient absorption of plants by intercropping with hyperaccumulator or accumulator plants under heavy metal stress. Bidens is one of Compositae, and some studies have shown that some species of it have accumulation ability of heavy metals such as Cd [6-8], but there is no study on nutrient absorption of fruit tree intercropping with Bidens species under heavy metal stress. Therefore, in this study, four Bidens species (*Bidens bipinnata*, *Bidens pilosa*, *Bidens parviflora* and *Bidens biternata*) were used to intercrop with grape seedlings under Cd stress, and the effects of intercropping with Bidens species on nutrient uptake of grape seedlings were studied. The aim of this study was to screen out the best Bidens species which could promote the nutrient absorption of grape.

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 farmland of Chengdu Campus of Sichuan Agricultural

University. Then, the seeds were put in the climate chamber to germinate and further cultivation and transplanting. 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%, and dug aperiodically to make soil mixed fully. In May 2016, three uniform-sized cutting seedlings (the shoots were about 15 cm) of Kyoho grape were transplanted into each pot for monoculture and two of them for intercropping, respectively. One uniform-sized seedling (two pairs leaves expanded) of each Bidens species were transplanted into each pot for intercropping. The five treatments in experiment were monoculture of grape, grape intercropped with *B. bipinnata*, grape intercropped with *B. pilosa*, grape intercropped with *B. parviflora* and grape intercropped with *B. biternata*. 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 grape seedlings 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 N, total P and total K contents [9]. 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 [9].

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

Total N Contents in Grape Seedlings. Compared with the monoculture, the total N contents in roots and shoots of grape seedlings decreased significantly ($p < 0.05$) by intercropping (Table 1). The total N content in roots and shoots of grape seedlings were ranked as monoculture > intercropping with *B. Pilosa* > intercropping with *B. Bipinnata* > intercropping with *B. parviflora* > intercropping with *B. Biternata*. The results indicate that intercropping with Bidens species could not promote the N absorption of grape seedlings under Cd stress, which might be that the N compete ability of Bidens species is higher than grape seedlings.

Table 1 Total N contents in grape seedlings under Cd stress

Treatments	Roots (g/kg)	Stems (g/kg)	Leaves (g/kg)	Shoots (g/kg)
Monoculture	9.89±0.21a	3.70±0.15a	14.52±0.40a	11.07±0.27a
Intercropping with *B. bipinnata*	8.93±0.30bc	3.59±0.16a	13.74±0.34bc	10.48±0.26b
Intercropping with *B. pilosa*	9.19±0.28b	3.61±0.17a	13.86±0.30b	10.55±0.23b
Intercropping with *B. parviflora*	8.56±0.22c	2.69±0.21b	13.34±0.32bc	10.19±0.28b
Intercropping with *B. biternata*	7.99±0.31d	2.55±0.24b	13.18±0.31c	10.29±0.25b

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

Total P Contents in Grape Seedlings. The total P content in roots of grape seedlings was ranked as intercropping with *B. pilosa* > monoculture > intercropping with *B. bipinnata* > intercropping with *B. parviflora*> intercropping with *B. Biternata* (Table 2). The total P content in shoots of grape seedlings increased by intercropping compared with the monoculture except intercropped with *B. parviflora*. When intercropped with *B. pilosa*, *B. biternata* and *B. bipinnata*, the total P content in

shoots of grape seedlings increased by 20.00% ($p < 0.05$), 10.00% ($p < 0.05$) and 2.86% ($p > 0.05$) respectively compared with the monoculture, indicating that intercropping with Bidens species could promote the P absorption of grape seedlings under Cd stress, and intercropping with *B. pilosa* could most effectively promote the P uptake in roots and shoots of grape seedlings.

Table 2 Total P contents in grape seedlings under Cd stress

Treatments	Roots (g/kg)	Stems (g/kg)	Leaves (g/kg)	Shoots (g/kg)
Monoculture	1.30±0.02ab	0.64±0.02b	0.73±0.01c	0.70±0.01c
Intercropping with *B. bipinnata*	1.29±0.06ab	0.56±0.04c	0.80±0.03b	0.72±0.03c
Intercropping with *B. pilosa*	1.33±0.04a	0.76±0.01a	0.87±0.01a	0.84±0.01a
Intercropping with *B. parviflora*	1.28±0.03ab	0.58±0.03c	0.69±0.02d	0.66±0.02d
Intercropping with *B. biternata*	1.25±0.01b	0.74±0.01a	0.79±0.02b	0.77±0.02b

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 Grape Seedlings. The total K contents in roots and shoots of grape seedlings decreased by intercropping compared with the monoculture (Table 3). When intercropped with *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata*, the total K content in roots of grape seedlings decreased by 35.31% ($p < 0.05$), 34.38% ($p < 0.05$), 41.91% ($p < 0.05$) and 39.66% ($p < 0.05$) respectively, compared with the monoculture, and decreased by 18.78% ($p < 0.05$), 7.62% ($p > 0.05$), 19.78% ($p < 0.05$) and 9.35% ($p < 0.05$), respectively shoots of that. It indicates that intercropping with four Bidens species could inhibit the total K absorption of grape seedlings under Cd stress. Among the four Bidens species, intercropping with *B. Pilosa* made the least decrease of total K absorption of grape seedlings.

Table 3 Total K contents in grape seedlings under Cd stress

Treatments	Roots (g/kg)	Stems (g/kg)	Leaves (g/kg)	Shoots (g/kg)
Monoculture	20.45±0.43a	9.53±0.32a	11.71±0.44a	11.02±0.39a
Intercropping with *B. bipinnata*	13.23±0.46b	7.12±0.67c	9.82±0.58b	8.95±0.61c
Intercropping with *B. pilosa*	13.42±0.60b	9.24±0.10a	10.52±0.87b	10.18±0.62ab
Intercropping with *B. parviflora*	11.88±0.23c	7.27±0.28c	9.50±0.43b	8.84±0.38c
Intercropping with *B. biternata*	12.34±0.44bc	8.29±0.36b	10.61±0.45b	9.99±0.41b

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. The changes of soil alkali soluble N, available P and available K concentrations got a significant level ($p < 0.05$) by intercropping compared with the monoculture (Table 4). The soil alkali soluble N concentration of intercropping significantly increased compared with the monoculture. When grape seedlings intercropped with *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata*, the soil alkali soluble N concentration increased by 8.29%, 16.79%, 11.69% and 11.91% respectively compared with the monoculture. The soil available P and available K concentrations of intercropping decreased compared to monoculture. When grape seedlings intercropped with *B. bipinnata*, *B. pilosa*, *B. parviflora* and *B. biternata*, the soil available P concentration decreased by 16.99%, 11.38%, 20.74% and 15.13% respectively, compared with the monoculture, and the soil available K concentration decreased by 36.76%, 19.58%, 39.48% and 28.31%, respectively. These results indicate that intercropping could improve the soil alkali soluble N concentration, but not improve the soil available P and available K concentrations. The treatment of grape seedlings intercropped with *B. pilosa* could maximally improve the soil alkali soluble N concentration and make the soil available P and available K concentrations reduce least.

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)
Grape monoculture	111.38±5.80c	30.66±0.55a	105.37±2.03a
Grape intercropped with *B. bipinnata*	120.61±1.20b	25.45±1.21bc	66.64±1.67d
Grape intercropped with *B. pilosa*	130.08±4.04a	27.17±1.90b	84.74±2.16b
Grape intercropped with *B. parviflora*	124.40±4.12ab	24.30±1.15c	63.77±4.21d
Grape intercropped with *B. biternata*	124.65±3.08ab	26.02±1.66bc	75.54±0.60c

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

Conclusions

When grape seedlings intercropped with four Bidens species, the total N and total K contents in grape seedlings decreased compared with the monoculture. Intercropping decreased the total P content in roots of grape seedlings except intercropping with *B. pilosa* and increased the total P content in shoots of grape seedlings except intercropping with *B. parviflora*. Compared with the monoculture, the soil available P and available K concentrations of intercropping decreased, but the soil alkali soluble N concentration of intercropping significantly increased. On the whole, intercropping with *B. pilosa* could most effectively improve the P absorption of grape seedlings and inhibit the N and K absorption of that. Therefore, intercropping with *B. pilosa* could benefit the nutrient absorption of grape seedlings under Cd stress.

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