Effects of Intercropping with Hyperaccumulator plants on Nutrient Uptake of Grape Seedlings under Cadmium Stress

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Abstract: A pot experiment was carried out to study the effects of intercropping with four cadmium (Cd) hyperaccumulator plants (*Galinsoga parviflora*, *Sigesbeckia orientalis*, *Solanum nigrum*, *Crassocephalum crepidioides*) on nutrient uptake of grape seedlings under Cd stress. When grape seedlings intercropped with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides*, the total nitrogen (N), total phosphorus (P) and total potassium (K) contents in grape seedlings decreased compared with the monoculture under Cd stress. Grape intercropped hyperaccumulator plants had no significant effects or reduced the soil alkali soluble N, soil available P and soil available K concentrations. Therefore, intercropping with hyperaccumulator plants decreased the nutrient absorption of grape seedlings under Cd stress.

Introduction

With increasing industrialization and disturbance of natural biogeochemical cycles, the problem of heavy metals' pollution is becoming more and more serious [1]. Cadmium (Cd) is one of the most problematic non-essential heavy metals, which is not conducive to plant growth [2]. Grape is widely cultivated around the word and global grape orchards have increased rapidly in recent years [3]. However, the current individual grape orchards have been subject to heavy metal pollution, which to some extent inhibited the growth of grapes [4].

Intercropping could make full use of light, heat, water, soil and other resources [5]. The study found that intercropping grass in the orchard line could prevent water loss, fertilize the soil and promote fruit tree growth [6-7]. In addition, intercropping with Cd-hyperaccumulator plants is confirmed to be beneficial to plant uptake of nutrients in Cd-contaminated soil [8]. *Galinsoga parviflora* [9], *Sigesbeckia orientalis* [10], *Solanum nigrum* [11] and *Crassocephalum crepidioides* [12] are Cd-hyperaccumulator plants. In this study, *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* were used to intercropped with grape seedlings in Cd-contaminated soil, and the effects of intercropping with hyperaccumulator plants on nutrient uptake of grape seedlings were studied. The aim of this study was to screen out the best hyperaccumulator plant which could promote the nutrient absorption of grape.

Materials and Methods

Materials. In April, 2016, the seeds of hyperaccumulator plants (*G. parviflora*, *S. orientalis, S. nigrum* and *C. crepidioides*) 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 huperaccumulator plant were transplanted into each pot for intercropping. The five treatments in experiment were monoculture of grape, grape intercropped with *G. parviflora*, grape intercropped with *S. orientalis*, grape intercropped with *S. nigrum* and grape intercropped with *C. crepidioides*. 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 nitrogen (N), total phosphorus (P) and total potassium (K) contents [13]. 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 [13].

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. Intercropping significantly decreased the N content in stems of grape seedlings compared with the monoculture (Table 1). The N content in leaves of grape seedlings was ranked as: monoculture > intercropping with *G. parviflora* > intercropping with *S. orientalis* > intercropping with *S. nigrum* > intercropping with *C. crepidioides*. Compared with the monoculture, intercropping with *C. crepidioides* had little effect on the N content in roots of grape seedlings, and the other treatments significantly decreased the N contents in their roots ($P < 0.05$). Intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* significantly decreased the N contents in shoots of grape seedlings, which decreased by 6.68% ($P < 0.05$), 16.18% ($P < 0.05$), 21.28% ($P < 0.05$), and 21.20% ($P < 0.05$), respectively, compared with the monoculture.

Table 1 Total N contents in grape seedlings under Cd stress

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)	Shoots (mg/g)
Monoculture	10.61±0.21a	4.67±0.32a	14.67±0.93a	11.37±0.72a
Intercropping with *G. parviflora*	6.80±0.56d	2.97±0.17c	14.06±1.07b	10.61±0.79b
Intercropping with *S. orientalis*	8.66±0.77c	4.26±0.15b	11.75±0.75c	9.53±0.69c
Intercropping with *S. nigrum*	9.98±0.53b	3.12±0.23c	11.69±0.88c	8.95±0.74d
Intercropping with *C. crepidioides*	10.58±0.68a	3.04±0.26c	11.58±1.31c	8.96±0.73d

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. Intercropping significantly decreased P content in leaves of grape seedlings compared with the monoculture (Table 2). The P content in stems of grape seedlings was ranked as: monoculture > intercropping with *S. nigrum* > intercropping with *G. parviflora* > intercropping with *S. orientalis* > intercropping with *C. crepidioides*. Compared with the monoculture, intercropping with *C. crepidioides* had little effect on the P content in roots of grape seedlings, and the other treatments significantly decreased the P content of its roots ($P < 0.05$). Intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* significantly decreased the P content in shoots of grape seedlings, which decreased by 23.19% ($P < 0.05$), 21.93% ($P < 0.05$), 18.60% ($P < 0.05$), and 26.41% ($P < 0.05$), respectively compared with the monoculture.

Table 2 Total P contents in grape seedlings under Cd stress

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)	Shoots (mg/g)
Monoculture	1.394±0.07a	0.623±0.08a	0.988±0.07a	0.871±0.32a
Intercropping with *G. parviflora*	1.348±0.08b	0.575±0.01b	0.709±0.03cd	0.669±0.29bc
Intercropping with *S. orientalis*	1.294±0.07c	0.568±0.05b	0.731±0.01bc	0.680±0.19bc
Intercropping with *S. nigrum*	1.293±0.06c	0.616±0.02a	0.749±0.08b	0.709±0.24b
Intercropping with *C.crepidioides*	1.395±0.13a	0.540±0.06c	0.688±0.07d	0.641±0.18c

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. Intercropping significantly decreased the K content in leaves of grape seedlings compared with the monoculture (Table 3). The K content in stems of grape seedlings was ranked as: monoculture > intercropping with *S. orientalis* > intercropping with *S. nigrum* > intercropping with *G. parviflora* > intercropping with *C. crepidioides*. Compared with the monoculture, intercropping with *C. crepidioides* had little effect on the K content in roots of grape seedlings, and the other treatments significantly decreased the K contents in their roots ($P < 0.05$). Intercropping with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides* significantly decreased the K contents in shoots of grape seedlings, which decreased by 33.13% ($P < 0.05$), 12.96% ($P < 0.05$), 20.00% ($P < 0.05$), and 30.35% ($P < 0.05$), respectively, compared with the monoculture.

Table 3 Total K contents in grape seedlings under Cd stress

Treatments	Roots (mg/g)	Stems (mg/g)	Leaves (mg/g)	Shoots (mg/g)
Monoculture	17.63±0.62a	9.28±0.58a	12.54±0.53a	11.50±0.62a
Intercropping with *G. parviflora*	16.09±0.71b	6.02±0.51b	8.43±0.47d	7.69±0.79d
Intercropping with *S. orientalis*	12.70±0.64c	8.76±0.75a	10.59±0.57b	10.01±0.82b
Intercropping with *S. nigrum*	12.65±0.58c	6.25±0.62b	10.58±0.68b	9.20±0.44c
Intercropping with *C.crepidioides*	18.04±0.86a	4.99±0.56c	9.39±±0.61c	8.01±0.68d

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. Compared with the monoculture, intercropping had little effect on alkali soluble N concentration of soil (Table 4). When the grap seedlings intercropped with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. Crepidioides*, the available P concentrations of soil significantly reduced, which reduced by 18.03% ($P < 0.05$), 27.06% ($P < 0.05$), 17.63% ($P < 0.05$), and 16.98% ($P < 0.05$), respectively, compared with the monoculture. The available K concentration of soil was ranked as grape intercropped with *C. crepidioides* > grape monoculture > grape intercropped with *S. nigrum* > grape intercropped with *S. orientalis* > grape intercropped with *G. parviflora*.

Conclusions

When grape seedlings intercropped with *G. parviflora*, *S. orientalis*, *S. nigrum* and *C. crepidioides*, the total N, total P and total K contents in grape seedlings decreased compared with the monoculture under Cd stress. Grape intercropped hyperaccumulator plants had no significant effects or reduced the soil alkali soluble N, soil available P and soil available K concentrations. These results indicate that intercropping with hyperaccumulator plants decreased the nutrient absorption of grape seedlings under Cd stress.

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	115.82±2.51a	32.22±1.07a	117.50±3.52a
Grape intercropped with *G. parviflora*	114.51±3.77a	26.41±0.98b	77.45±2.24c
Grape intercropped with *S. orientalis*	122.83±1.46a	23.50±1.32c	92.47±3.21b
Grape intercropped with *S. nigrum*	116.61±3.87a	26.54±2.41b	114.98±4.68a
Grape intercropped with *C.crepidioides*	116.20±3.79a	26.75±1.33b	119.95±3.79a

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

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