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Effects of Intercropping with Post-Grafting Generation of Two Ecotypes of *Galinsoga parviflora* on Nutrient Uptake of *Lactuca sativa* under Cadmium Stress

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Abstract: The effects of intercropping with two ecotypes of *Galinsoga parviflora* and their post-grafting generations on nutrient uptake of *Lactuca sativa* were investigated through pot experiment under the cadmium stress. The results showed that intercropping with post-grafting generation of *G. parviflora* of farmland ecotype as rootstock (FR) had the best effect on increasing P content in *L. sativa* roots, while intercropping with post-grafting generation of *G. parviflora* of mine ecotype as scion (MS) could increase the P content in *L. sativa* shoots most effectively. The root content of K in the monoculture *L. sativa* was the highest, and the highest shoots K content was found in the *L. sativa* intercropping with *G. parviflora* of farmland ecotype (FCK). As far as the grafting method is concerned, intercropping with post-grafting generation of *G. parviflora* of mine ecotypes are used as scions and farmland ecotypes as rootstocks is more conducive to *L. sativa* nutrient uptake. Intercropping with MS can increase soil available N and K.

1. Introduction

Lactuca sativa, a nutrient-rich leafy vegetable, has been studied and confirmed to be adversely affected in soils contaminated by heavy metals [1]. In agricultural production, intercropping can not only increase the yield of plants, reduce the occurrence of pests and diseases, but also improve the soil environment and increase the uptake of nutrients by plants [2-3]. *Galinsoga parviflora* is a cadmium hyperaccumulator [4]. Different ecotypes of *G. parviflora* not only show differences in the absorption or tolerance of heavy metals, but they are often significantly different in response to environmental factors. Grafting is a common agronomic practice and studies have shown that grafting leads to changes in DNA methylation, produces reversible genetic changes in genomic function, and may have morphological, physiological, and ecological consequences [5-6]. Therefore, in this experiment, the Cd-hyperaccumulator *G. parviflora* with two ecotypes was grafted, and two ecotypes of *G. parviflora* and their post-grafting generations were intercrossed with *L. sativa* to study their effects on *L. sativa* nutrient uptake.

2. Materials and Methods

Materials. The seeds of the *G. parviflora* used in this experiment were collected from farms (farmland ecotype) at Ya'an Campus, Sichuan Agricultural University (30°23' N, 103°48' E) and Tangjiashan Lead-Zinc Mine (mine ecotype) at Hanyuan County, Ya'an City, Sichuan Province (29°24' N, 102°38' N). The climatic types and soil physicochemical properties of the two places refer to the results of Lin et al. [7]. The soil samples used in the pot experiment were paddy soil that were collected from the Chengdu campus farm of the Sichuan Agricultural University (29° 59′ N, 102° 59′ E), pH 7.35, organic matter 41.38 g/kg, total nitrogen 3.05 g/kg, total phosphorus 0.31 g/kg, total potassium 15.22 g/kg, alkaline nitrogen 165.30 mg/kg, available phosphorus 5.87 mg/kg, available potassium 187.03 mg/kg, Cadmium not detected. The basic physical and chemical properties of the soil and the determination of heavy metal content are based on references [8]. *L. sativa* variety is Italian lettuce, with a year-round, resistant to twitching and heat resistance, purchased from Baofeng Seed Company.

Grafting Methods. In October 2015, the two ecotypes of *G. parviflora* were collected and sowed. When the seedling height is about 3 cm and two true leaves are spread, they are transplanted into the pot. Afterwards, two ecotypes of *G. parviflora* were grafted when the height of *G. parviflora* was about 10 cm. The specific grafting method is as follows: (1) Farmland ecotypes are not grafted: The *G. parviflora* seedlings were transplanted directly, and then the seeds were collected for preservation as *G. parviflora* of farmland ecotype (FCK). (2) Mine ecotypes are not grafted: The *G. parviflora* seedlings were transplanted directly, and then the seeds were collected for preservation as *G. parviflora* of mine ecotype (MCK). (3) Mine ecotypes are used as rootstocks and farmland ecotypes as scions for grafting: Two ecotypes of *G. parviflora* seedlings were cut from about 6 cm above the ground. The mine ecotype *G. parviflora* seedlings were used as rootstocks and the farmland ecotype *G. parviflora* shoots (4 cm) were grafted as scions. Keep rootstock leaves and buds. After that, collect rootstock seeds as post-grafting generation of *G. parviflora* of mine ecotype as rootstock (MR) and collect scion seeds as post-grafting generation of *G. parviflora* of farmland ecotype as scion (FS). (4) Farmland ecotypes are used as rootstocks and mine ecotypes as scions for grafting: Two ecotypes of *G. parviflora* seedlings were cut from about 6 cm above the ground. The farmland ecotype *G. parviflora* seedlings were used as rootstocks and the mine ecotype *G. parviflora* shoots (4 cm) were grafted as scions. Keep rootstock leaves and buds. After that, collect rootstock seeds as post-grafting generation of *G. parviflora* of farmland ecotype as rootstock (FR) and collect scion seeds as post-grafting generation of *G. parviflora* of mine ecotype as scion (MS). During the grafting process, the soil used was free from heavy metal pollution.

Experimental Design. The experiment was conducted in the greenhouse of the Sichuan Agricultural University in Chengdu campus. Air-dried soil (2.5 kg) was weighed and placed into each polyethylene pot (21 cm high, 20 cm diamete). Cd was added to the soil samples as analytical reagent $CdCl_2 \cdot 2.5H_2O$ at the concentration of 10 mg/kg [9], and then the soil was thoroughly mixed. Natural balance for 4 weeks, occasionally turning soil mixing, so that the soil is fully mixed. In March 2016, the collected *G. parviflora* seeds and purchased *L. sativa* seeds were sown and nursed. In April 2016, when the seedlings of *G. parviflora* were about 3 cm high and 2 true leaves were unfolded, they were transplanted into pots with *L. sativa*. Seven treatments were applied in the experiment: *L. sativa* monoculture (CK), intercropping with FCK, intercropping with MCK, intercropping with FR, intercropping with MR, intercropping with FS, intercropping with MS. Monoculture treatment four *L. sativa* per pot, intercropping treatments were planted with three *L. sativa* and one *G. parviflora* per pot. Repeat 4 times for each treatment, completely randomized design with 15-cm spacing between pots, timely weeding, pest control, and no disease. After 60 d(June 2016), the entire plants of each pot were harvested, roots and shoots were separated, washed, dried, crushed, and the P and K content were determined [8]. Then collect the soil, air dry, crush, measure the soil alkaline N, available P and available K content [8].

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.

3. Results and Discussion

P Content in *L. sativa*. Table 1 shows that under cadmium stress, the P content in *L. sativa* in intercropping with FCK, FR, MR and MS were significantly higher than monoculture ($P < 0.05$). And the root P content in *L. sativa* was increased by 21.34%, 38.21%, 17.87% and 14.39% compared to the monoculture. In terms of shoots, compared with monoculture, except for intercropping with FS, which significantly reduced the P content in shoots of *L. sativa*, other treatments significantly increased the P content in in shoots of *L. sativa*. Furthermore, the P content in shoots of *L. sativa* intercropping with FCK, MCK, FR, MR and MS increased by 23.54%, 7.53%, 19.77%, 14.12% and 38.04% compared with the monoculture.

Table 1 P content in *L. Sativa*

Treatments	Roots (mg/g)	Shoots (mg/g)
CK	0.403±0.022 c	0.531±0.007 e
FCK	0.489±0.026 b	0.656±0.027 b
MCK	0.449±0.004 bc	0.571±0.006 d
FR	0.557±0.017 a	0.636±0.015 bc
MR	0.475±0.022 b	0.606±0.011 c
FS	0.332±0.017 d	0.467±0.002 f
MS	0.461±0.029 b	0.733±0.006 a

Different lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 17.0 followed by the least significant difference test ($P < 0.05$). CK = monoculture, FCK = intercropping with *G. parviflora* of farmland ecotype, MCK = intercropping with *G. parviflora* of mine ecotype , FR = intercropping with post-grafting generation of *G. parviflora* of farmland ecotype as rootstock, MR = intercropping with post-grafting generation of *G. parviflora* of mine ecotype as rootstock, FS = intercropping with post-grafting generation of *G. parviflora* of farmland ecotype as scion, MS = intercropping with post-grafting generation of *G. parviflora* of mine ecotype as scion. Same as below.

K Content in *L. sativa*. Table 2 shows that under the cadmium stress, the root K content of all intercropping treatments in *L. sativa* was significantly lower than that of the control (monoculture) ($P < 0.05$). In terms of shoots, intercropping with FR and FS reduced the shoots K content of *L. sativa* compared to monoculture. However, K content in the shoots of *L. sativa* intercropping with FCK, MCK, MR, and MS was significantly increased compared with the monoculture, which increased by 33.25%, 16.66%, 24.77%, and 31.17%, respectively.

Table 2 K content in *L. Sativa*

Treatments	Roots (mg/g)	Shoots (mg/g)
CK	44.570±1.127 a	74.016±0.211 e
FCK	20.355±0.705 cd	98.630±0.071 a
MCK	19.607±0.352 d	86.348±0.388 d
FR	21.252±0.564 c	70.379±0.423 f
MR	19.308±0.634 d	92.352±0.352 c
FS	28.890±0.092 b	69.682±1.409 f
MS	12.183±0.423 e	97.085±0.705 b

Available Nutrients in Soil. Table 3 shows that in cadmium-contaminated soil, the content of soil alkaline N in *L. sativa* intercropping with MS was significantly higher than that of the monoculture, and it was 11.25% higher than that of the monoculture, but the content of alkaline N in other treatments was not significantly different from the monoculture ($P > 0.05$). Compared with the monoculture, *L. sativa* intercropping with the two ecotypes of *G. parviflora* and their post-grafting generations had no significant effect on the content of available P in the cadmium-contaminated soil ($P > 0.05$). Under the condition of cadmium pollution, the available K content in the soil of *L. sativa* intercropping with MS increased by 8.15% compared with the monoculture ($P < 0.05$), and there was no significant difference in available K content between the other treated soils and the control (monoculture).

4. Conclusions

Under the cadmium stress, after six intercropping treatments, the results showed that intercropping with two ecotypes of *G. parviflora* and their post-grafting generations could affect the nutrient uptake of *L. sativa*. In general, with the exception of intercropping with FS, other treatments can improve the nutrient uptake of *L. sativa*. Among them, intercropping with FR had the best effect on increasing P content in *L. sativa* roots, while intercropping with MS could increase the P content in *L. sativa* shoots most effectively. For K content, the root content of K in the monoculture *L. sativa* was the highest, and the highest shoots K content was found in the *L. sativa* intercropping with FCK. As far as the grafting method is concerned, intercropping with post-grafting generation of *G. parviflora* of mine ecotypes are used as scions and farmland ecotypes as rootstocks is more conducive to *L. sativa* nutrient uptake. On the other hand, intercropping with MS can increase soil available N and K. It may be that the root exudates produced by the *L. sativa* intercropping with *G. parviflora* affected the physicochemical properties of the plant rhizosphere soil, increased soil enzyme activities in the rhizosphere soil, and improved soil fertility. The change of soil available nutrient content is also one of the reasons that affect the absorption of *L. sativa* nutrients.

Table 3 Available nutrients in the soil

Treatments	Alkaline nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
CK	54.741±3.762b	1.211±0.070 a	91.365±1.756 b
FCK	56.375±1.520 ab	1.135±0.023 a	87.651±0.014 b
MCK	58.975±1.237 ab	1.107±0.070 a	87.641±3.511 b
FR	54.950±1.485 ab	1.080±0.116 a	87.641±3.511 b
MR	59.325±2.227 ab	1.097±0.077 a	88.882±1.756 b
FS	54.600±3.960 b	1.129±0.016 a	87.646±0.007 b
MS	60.900±0.990 a	1.138±0.074 a	98.813±1.756 a

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