Effects of Lettuce Intercropping with Post-Grafting Generation of *Galinsoga parviflora* on Soil Enzymes Activity under Cadmium Stress

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**Abstract:** The pot experiment was conducted to study the effects of lettuce intercropping with positive and negative grafting generations of two ecotypes (farmland and mining) of cadmium (Cd) hyperaccumulator plant *Galinsoga parviflora* on soil enzymes activity. The positive grafting: the rootstock was mining ecotype of *G. parviflora* (post generation was defined as mining-rootstock) and the scion was farmland ecotype of *G. parviflora* (post generation was defined as farmland-scion); the negative grafting: the rootstock was farmland ecotype of *G. parviflora* (post generation was defined as farmland-rootstock) and the scion was mining ecotype of *G. parviflora* (post generation was defined as mining-scion). The results show that only lettuce intercropping with mining-scion significantly increased the soil invertase activity, soil urease activity and soil catalase activity compared with the monoculture of lettuce. Therefore, intercropping lettuce with mining-scion could improve the ecological environment of Cd-contaminated-soil.

1. **Introduction**

In recent years, a large amount of heavy metals have entered the soil system due to the extensive mining and the use of chemical fertilizers and pesticides [1]. Heavy metals tend to accumulate in the soil, which not only destroys the physical and chemical properties of the soil, but also reduces the activity of soil enzymes [2]. Soil enzymes are mainly derived from soil microorganisms and plant root exudates [3]. Studies have shown that soil enzyme activity is closely related to soil fertility and can be used as an indicator of the pollution degree of soil by heavy metals [4]. It has been found that intercropping heavy metal hyperaccumulators with common plants can significantly increase the efficiency of soil remediation by hyperaccumulators [5]. Lettuce is an annual or biennial herb that is rich in nutrients, but it is not conducive to growth under cadmium stress [6]. *Galinsoga parviflora* is a widely distributed cadmium (Cd) hyperaccumulator and there are certain differences in the accumulation characteristics of Cd between the different ecotypes (mining and farmland) *G. parviflora* [7-8]. We have found the absorption of Cd in the post-grafting generations of the two ecotypes *G. parviflora* were significantly different. In view of this, this experiment intercropped lettuce with post-grafting generation of *G. parviflora*, and studied the effect of intercropping on the soil enzyme activity, in order to screen post-grafting generation of *G. parviflora* that can improve the soil enzyme activity and make reference to the production of lettuce.

2. **Materials and Methods**

**Materials Collection.** The seeds of mining ecotype *G. parviflora* and farmland ecotype *G. parviflora* were respectively collected from Tangjiashan lead-zinc mine (29°24’ N, 102°38’ E) and farmland of Ya’an campus farm of Sichuan Agricultural University (30°23’ N, 103°48’ E) in September, 2015, stored at 4 °C. The Tangjiashan lead-zinc mine locates in Hanyuan County, Sichuan Province, China, with a typical dry-hot valley climate. The farm of Sichuan Agricultural University locates in Yucheng County, Sichuan Province, China, with a humid subtropical monsoon
climate. Lettuce variety for Italy Resistant to Hot-Breaked Lettuce, purchased in JIEYANG BANGFENG SEED CO., LTD.

**Grafting.** In October 2015, the two ecotypes of *G. parviflora* seeds were grown at a temperature of 25 °C, illumination of 4000 lx and air humidity of 80%. When the height of *G. parviflora* seedlings were about 3 cm and 2 true leaves unfolded, transplanted into cadmium-free pots. After the *G. parviflora* height was about 10 cm, two ecological types of *G. parviflora* were grafted positively and negatively. (1) Un-grafted of farmland ecotype (post generation was defined as farmland). (2) Un-grafted of mining ecotype (post generation was defined as mining). (3) The positive grafting: the rootstock was farmland ecotype of *G. parviflora* (post generation was defined as farmland-rootstock) and the scion was mining ecotype of *G. parviflora* (post generation was defined as mining-scion). (4) The negative grafting: the rootstock was mining ecotype of *G. parviflora* (post generation was defined as mining-rootstock) and the scion was farmland ecotype of *G. parviflora* (post generation was defined as farmland-scion). All of the leaves of the rootstocks remained. The grafting method was cleft method. And the plastic band with a width of about 1 cm and a length of 20 cm was used for binding. Watering after grafting and keeping the soil moisture content was maintained at 80% of field capacity, and covering it with a mulching film and shade net. After 10 days, the mulching film and the shade net were gradually removed, and the tied plastic band was removed. When grafting treatments were completed, all *G. parviflora* were transplanted into cadmium-free pots, and collected the post generation seeds.

**Experimental Design.** The experiment was conducted in Chengdu Campus of Sichuan Agricultural University. In February 2016, after the cadmium-free soil was air-dried and crushed, 2.5 kg soil was weighed into each plastic pot (21 cm high, 20 cm in diameter). Soaking uniformly by 10 mg/kg Cd (in the form of CdCl₂·2.5H₂O) solution for 4 weeks. In March 2016, the post-grafting generations of *G. parviflora* were grown at a temperature of 25 °C, illumination of 4000 lx and air humidity of 80%. When the height of *G. parviflora* seedlings was about 3 cm and 2 true leaves unfolded, transplanted into Cd soil to intercrop with lettuce. 7 treatments were conducted: (1) monoculture of lettuce, (2) lettuce intercropped with farmland, (3) lettuce intercropped with mining, (4) lettuce intercropped with farmland-rootstock, (5) lettuce intercropped with mining-rootstock, (6) lettuce intercropped with farmland-scion and (7) lettuce intercropped with mining-scion. Intercropping treatments were planted with 3 lettuces and 1 *G. parviflora*. Monoculture treatments were planted 4 lettuces. Four replicates per treatment and the pots placed completely random. The soil moisture content was maintained at 80% of field capacity. After 60 days, collected soil separately and soil invertase activity, soil urease activity and soil catalase activity were measured [10].

**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

**Soil Invertase Activity.** Compared to the monoculture, only Inter. min-sc significantly increased the soil invertase activity, while the remaining treatments significantly reduced it (Fig. 1). And Inter. min minimized the soil invertase activity.

**Soil Urease Activity.** The soil urease activity was ranked in the following order: Inter. min > Inter. min-sc > Mon > Inter. min-rs > Inter. far-rs > Inter. far-sc > Inter. far (Fig. 2).

**Soil Catalase Activity.** Compared to the monoculture, only Inter. min-sc significantly increased the soil invertase activity, while the remaining treatments had little effect on it (Fig. 3).
Fig. 1 Soil invertase activity. The same letters within each column are not significantly different at $p < 0.05$. Mon = (monoculture of lettuce), Inter. far = (lettuce intercropped with farmland), Inter. min = (lettuce intercropped with mining), Inter. far-rs = (lettuce intercropped with farmland-rootstock), Inter. min-rs = (lettuce intercropped with mining-rootstock), Inter. far-sc = (lettuce intercropped with farmland-scion), Inter. min-sc = (lettuce intercropped with mining-scion).

Fig. 2 Soil urease activity. The same letters within each column are not significantly different at $p < 0.05$. Mon = (monoculture of lettuce), Inter. far = (lettuce intercropped with farmland), Inter. min = (lettuce intercropped with mining), Inter. far-rs = (lettuce intercropped with farmland-rootstock), Inter. min-rs = (lettuce intercropped with mining-rootstock), Inter. far-sc = (lettuce intercropped with farmland-scion), Inter. min-sc = (lettuce intercropped with mining-scion).

4. Conclusions

Intercropping lettuce with post-grafting generation of *G. parviflora* would affect the soil enzymes activity. And only lettuce intercropped with mining-scion significantly improved the soil invertase activity, soil urease activity and soil catalase activity. Therefore intercropping lettuce with mining-scion can improve the ecological environment of Cd contaminated soil and provide a theoretical basis for the production of lettuce.
Fig. 3 Soil catalase activity. The same letters within each column are not significantly different at $p < 0.05$. Mon = (monoculture of lettuce), Inter. far = (lettuce intercropped with farmland), Inter. min = (lettuce intercropped with mining), Inter. far-rs = (lettuce intercropped with farmland-rootstock), Inter. min-rs = (lettuce intercropped with mining-rootstock), Inter. far-sc = (lettuce intercropped with farmland-scion), Inter. min-sc = (lettuce intercropped with mining-scion).

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