# 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 postive 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 $CdCl_2 \cdot 2.5H_2O$) 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-scion 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).

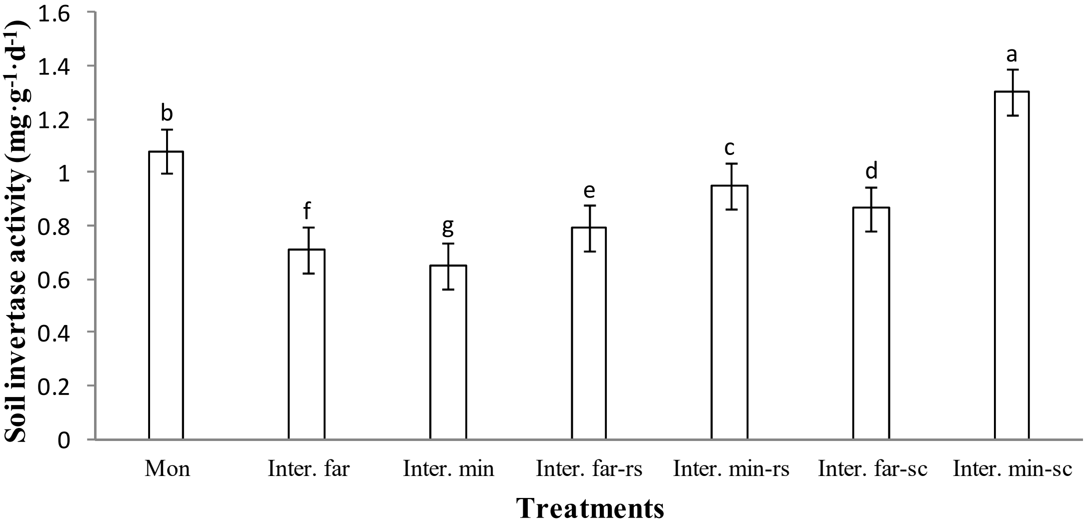

Fig. 1 Soil invertase activity. The same letters within each column are not significantly different at $p < 0.05$. Mon = (monoculture of lettuce), Inter. far = (lettuce intercropped with farmland), Inter. min = (lettuce intercropped with mining), Inter. far-rs = (lettuce intercropped with farmland-rootstock), Inter. min-rs = (lettuce intercropped with mining-rootstock), Inter. far-sc = (lettuce intercropped with farmland-scion), Inter. min-sc = (lettuce intercropped with mining-scion).

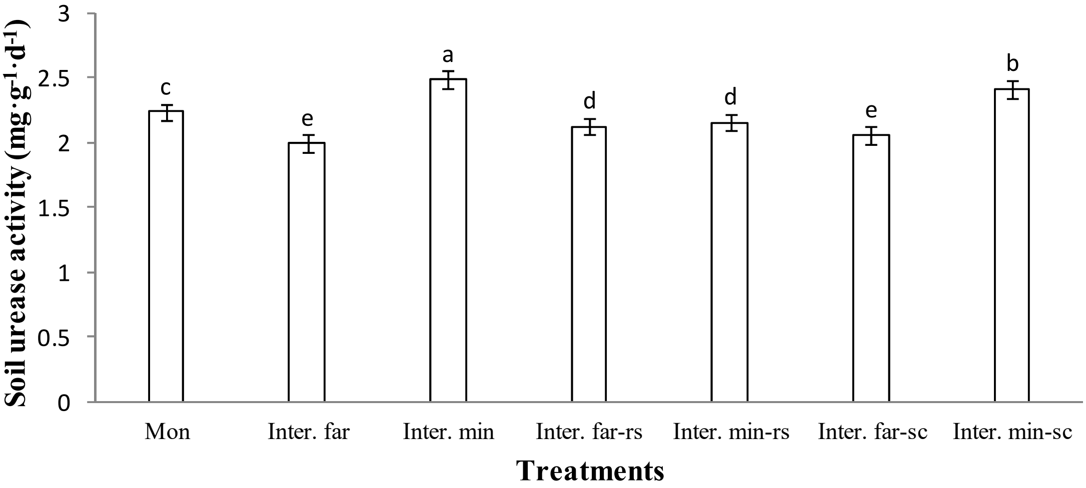

Fig. 2 Soil urease activity. The same letters within each column are not significantly different at $p < 0.05$. Mon = (monoculture of lettuce), Inter. far = (lettuce intercropped with farmland), Inter. min = (lettuce intercropped with mining), Inter. far-rs = (lettuce intercropped with farmland-rootstock),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

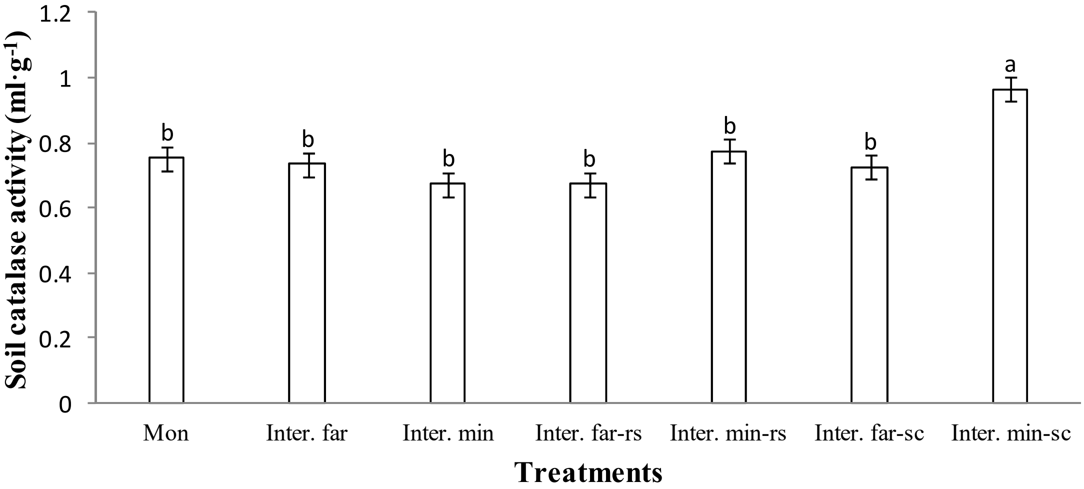

Fig. 3 Soil catalase activity. The same letters within each column are not significantly different at $p < 0.05$. Mon = (monoculture of lettuce), Inter. far = (lettuce intercropped with farmland), Inter. min = (lettuce intercropped with mining), Inter. far-rs = (lettuce intercropped with farmland-rootstock), 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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