Effects of intercropping with *Youngia erythrocarpa*, *Conyza canadens* and *Mazus japonicus* on physiological and biochemical characteristics of *Galinsoga parviflora* under cadmium stress

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Abstract. A pot experiment was conducted to study the effects of intercropping with *Youngia erythrocarpa*, *Conyza canadens* and *Mazus japonicus* on physiological and biochemical characteristics of hyperaccumulator *Galinsoga parviflora* under cadmium (Cd) stress. The results showed that intercropping with *Y. erythrocarpa* (GIY), *C. canadens* (GIC) and *M. japonicus* (GIM) all increased the superoxide dismutase (SOD), peroxidase (POD), peroxidase (CAT) activity and the soluble protein content of *G. parviflora* compared with monoculture (MG). The photosynthetic pigment (chlorophyll a, chlorophyll b, total chlorophyll, carotenoid) content and chlorophyll a/b of *G. parviflora* increased after intercropping, too. Among all treatments, GIC had the highest antioxidant enzyme activity, soluble protein content and photosynthetic pigment content. As for photosynthetic characteristics, GIC had the highest net photosynthetic rate (Pn), followed by GIY, GIM and MG. Compared with MG, the stomatal conductance (Gs) and transpiration rate (Tr) of GIC and GIM all increased significantly while that of GIC had no significant change, moreover, GIC had significantly lower CO₂ concentration of intercellular (Ci) than other treatments, there was no significant difference in value of pressure deficit leaf (Vpdl) among all treatments. Therefore, under Cd stress, intercropping with *C. canadens* can improve the antioxidant enzyme activity and photosynthetic capacity of *G. parviflora* to the greatest extent, which was conducive to its remediation of Cd contaminated soil.

1. Introduction

Cadmium (Cd) is recognized as one of the most toxic metallic elements and is the most serious in soil heavy metal pollution which has a strong ability to migrate from the soil to the plant [1-2]. Cd is difficult to be degraded and removed after being absorbed by plants, and its accumulation in edible parts will pose a threat to human health [3], therefore, the remediation of Cd contaminated soil is imminent. Using super-enriched plants to extract and transfer Cd from soil is the most common remediation technology for Cd-contaminated soil [4]. At the same time, taking some agronomic measures to improve the tolerance and absorption capacity of existing super-enriched plants to Cd has become an important means to promote the development of phytoremediation technology [5].

Intercropping is one of the main agricultural production modes, which can improve the effective utilization of soil nutrients, water, light and other resources by crops [6]. Under heavy metal stress, due to the rhizosphere effect produced by plants, a series of physiological and biochemical changes
will take place in the body[7], plant roots can also secrete organic acids to form soluble complexes with heavy metals to reduce the bioavailability of heavy metals and reduce their damage to plants [8].

Galinsoga parviflora is a Cd-hyperaccumulator, but its biomass is small and its Cd extraction efficiency is relatively low [9]. In previous studies, it was found that mulching the straw of hyperaccumulator Youngia erythrocarpa [10], accumulator Conyza canadens [11] and tolerant plant Mazus japonicus [12] on soil surface could all greatly promote the growth of G. parviflora and increase its Cd accumulation capacity. In view of this, the effects of intercropping with the above three plants on the antioxidant enzymes and photosynthetic characteristics of G. parviflora were studied in order to screen the best plants for intercropping that can improve the tolerance of G. parviflora to Cd and provide reference for the remediation of Cd contaminated soil.

2. Materials and methods

2.1. Materials

The seeds of Y. erythrocarpa, C. canadens, M. japonicus and G. parviflora used in this experiment were collected from the same plant correspondingly in the farmland surrounding Chengdu Campus of Sichuan Agricultural University (30°42′N, 103°50′E), the potting soil was also taken from the same place.

2.2. Experimental design

The experiment was conducted from February to June 2019. The soil were air-dried and passed through a 5-mm sieve in February 2019, and then 3.0 kg of soil was placed into each pot (20 cm in height and 21 cm in diameter). Cd was added to make a final soil Cd concentration of 10 mg/kg with a heavy metal solution in the form of CdCl$_2$·2.5H$_2$O. The soils were mixed evenly and balanced for 4 weeks, and the soil moisture content was maintained at 80% of field capacity during this period. The seeds of G. parviflora were sown and put in the climate chamber to germination and further cultivation in March 2019. Then, the seedlings of Y. erythrocarpa, C. canadens and M. japonicus were transplanted together with G. parviflora seedlings respectively into the pots filled with Cd-containing soil when the four true leaves expanded.

There were four treatments in the experiment: Monoculture G. parviflora (MG), G. parviflora intercropped with Y. erythrocarpa (GIY), G. parviflora intercropped with C. canadens (GIC) and G. parviflora intercropped with M. japonicus (GIM). There were four seedlings of G. parviflora per pot for monoculture treatment and two seedlings of G. parviflora were transplanted into a pot with two seedlings of Y. erythrocarpa, C. canadens and M. japonicus respectively for each intercropping treatment. Each treatment was repeated for 3 times, the distance between the pots was 15 cm and was completely random. Watering frequently to meet the plant growth needs, during the whole growth process, the position between the pots was exchanged irregularly to weaken the influence of the marginal effect until the plants were harvested.

After the plants were mature (June 2019), the photosynthesis of G. parviflora in each treatment was measured by LI-6400 portable photosynthesis instrument whose photosynthetic parameters were as follows: CO$_2$ concentration 400 μmol/mol, temperature 30°C, light intensity 1000 μmol/m$^2$/s. The determination of photosynthetic parameters included net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), CO$_2$ concentration of intercellular (Ci) and value of pressure deficit leaf (Vpdl), each treatment was repeated three times. After that, the upper mature leaves of plants were collected to determine the contents of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid), the activity of superoxide dismutase (SOD), peroxidase (POD), peroxidase (CAT) and the content of soluble protein [13].

2.3. Statistical analyses

Statistical analyses were carried out by SPSS 17.0 statistical software. Data were analyzed by one-way analysis of variance with least significant difference (LSD) at 5% confidence level.
3. Results and discussion

3.1. Antioxidant enzyme activity and soluble protein content of G. parviflora

Intercropping with plants increased the antioxidant enzyme (SOD, POD and CAT) activity and the soluble protein content of G. parviflora (Table 1), which ranked as GIC > GIY > GIM > MG. The SOD, POD and CAT activities of GIC were significantly higher \((p < 0.05)\) than the other three treatments and increased by 20.59% \((p < 0.05)\), 23.50% \((p < 0.05)\) and 53.97% \((p < 0.05)\) respectively compared with MG. In terms of soluble protein content of G. parviflora, the GIC, GIY and GIM had increased by 20.28% \((p < 0.05)\), 17.82% \((p < 0.05)\) and 14.53% \((p < 0.05)\) on the basis of monoculture respectively.

| Treatment | SOD activity (U/g/min) | POD activity (U/g/min) | CAT activity (U/g/min) | Soluble protein content (mg/g) |
|-----------|------------------------|------------------------|------------------------|-----------------------------|
| MG        | 331.79±5.14c           | 1310.34±9.75c          | 103.05±4.24d           | 12.18±0.078b                |
| GIY       | 364.22±9.41b           | 1581.09±20.21b         | 139.89±3.54b           | 14.35±0.460a                |
| GIC       | 400.09±10.91a          | 1618.32±10.80a         | 158.67±4.95a           | 14.65±0.311a                |
| GIM       | 345.18±2.53bc          | 1565.67±7.58b          | 129.24±1.41c           | 13.95±0.346a                |

Means with the same letter within each column are not insignificantly different at 0.05 levels.

3.2. Photosynthetic pigment content of G. parviflora

Intercropping increased the content of photosynthetic pigments in G. parviflora plants to varying degrees under Cd stress (Table 2). The content of chlorophyll \(a\), chlorophyll \(b\) and total chlorophyll of G. parviflora with different treatments all ranked as GIC > GIY > GIM > MG, and the content of chlorophyll \(a\), chlorophyll \(b\) and total chlorophyll of GIC was increased by 19.28% \((p < 0.05)\), 11.70% \((p < 0.05)\) and 18.90% \((p < 0.05)\) respectively compared with MG. In terms of chlorophyll \(a/b\), GIM had the highest chlorophyll \(a/b\), while the difference between the other three treatments was not significant \((p > 0.05)\). The carotenoid content of GIC was the highest with a increments of 19.00% \((p < 0.05)\) compared with MG, followed by GIM and GIY whose carotenoid content increased by 12.72% \((p < 0.05)\) and 12.08% \((p < 0.05)\) compared with MG.

| Treatment | Chlorophyll \(a\) (mg/g) | Chlorophyll \(b\) (mg/g) | Total chlorophyll (mg/g) | Chlorophyll \(a/b\) | Carotenoid (mg/g) |
|-----------|--------------------------|--------------------------|--------------------------|---------------------|------------------|
| MG        | 1.800±0.034c             | 0.359±0.008c             | 2.159±0.042c             | 5.014±0.023b        | 0.621±0.005c     |
| GIY       | 2.031±0.018b             | 0.401±0.016ab            | 2.432±0.034b             | 5.065±0.152b        | 0.696±0.009b     |
| GIC       | 2.147±0.015a             | 0.420±0.015a             | 2.567±0.029a             | 5.112±0.144b        | 0.739±0.010a     |
| GIM       | 2.004±0.013b             | 0.367±0.011bc            | 2.371±0.024b             | 5.460±0.131a        | 0.700±0.016b     |

Means with the same letter within each column are not insignificantly different at 0.05 levels.

3.3. Photosynthetic characteristics of G. parviflora

Under Cd stress, intercropping treatments increased the Pn of G. parviflora compared with monoculture (Table 3), and the Pn of GIC, GIY and GIM increased by 24.21% \((p < 0.05)\), 7.97% \((p < 0.05)\) and 1.32% \((p > 0.05)\) on the basis of MG separately. The Gs of GIY and GIM were higher than that of MG while there was no significant difference \((p > 0.05)\) between GIC and MG in Gs. Only the Ci of GIM increased while the Ci of GIC was decreased by 17.91% \((p < 0.05)\) compared with MG. The Tr of G. parviflora was ranked as GIY > GIM > MG > GIC. As for the Vpd, there were no significant differences \((p > 0.05)\) between the four treatments.
Table 3. Photosynthetic characteristics of *G. parviflora*.

| Treatment | Pn (μmol CO$_2$/m$^2$/s) | Gs (mol H$_2$O/m$^2$/s) | Ci (μmol CO$_2$/mol) | Tr (mmol H$_2$O/m$^2$/s) | Vpdl (kPa) |
|-----------|--------------------------|-------------------------|----------------------|--------------------------|-------------|
| MG        | 5.321±0.080c             | 0.074±0.002c            | 371.60±9.57a         | 1.863±0.040c             | 2.386±0.021a|
| GIY       | 5.745±0.096b             | 0.097±0.05a             | 370.06±3.79a         | 2.426±0.078a             | 2.371±0.027a|
| GIC       | 6.609±0.039a             | 0.074±0.002c            | 305.05±2.28b         | 1.858±0.046c             | 2.398±0.011a|
| GIM       | 5.391±0.059c             | 0.080±0.001b            | 377.11±1.42a         | 2.002±0.007b             | 2.378±0.003a|

Means with the same letter within each column are not insignificantly different at 0.05 levels.

4. Conclusions

Under the condition of Cd contamination in soil, intercropping with plants all increased the antioxidant enzyme activity and the soluble protein content of *G. parviflora* compared with monoculture, the photosynthetic pigment content of *G. parviflora* increased after intercropping, too. And among all of treatments, GIC had the highest antioxidant enzyme activity, soluble protein content and photosynthetic pigment content. As for photosynthetic characteristics, the Pn of GIC was significantly higher than other treatments, followed by GIY, GIM and MG, compared with MG, the Gs and Tr of GIC and GIM all increased significantly while that of GIC had no significant change, in addition, GIC had significantly lower Ci than other treatments. To sum up, under Cd stress, intercropping with *C. canadensis* can improve the antioxidant enzyme activity and photosynthetic capacity of *G. parviflora* to the greatest extent, thus enhancing its tolerance to Cd and is conducive to its remediation of Cd contaminated soil.

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