Effects of reciprocal hybridization on physiological characteristics in F1 hybrids of Solanum nigrum and Solanum diphyllum under cadmium stress

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Abstract. A pot experiment was conducted to study the effects of cross-hybridization on biomass, photosynthetic pigment content, antioxidant enzyme activity and soluble protein content of *Solanum nigrum* and *Solanum diphyllum*. The results showed that under cadmium (Cd) stress, the plant biomass, photosynthetic pigment content, antioxidant enzyme activity and soluble protein content of hybrid ND (*S. nigrum* male × *S. diphyllum* female) and DN (*S. diphyllum* male × *S. nigrum* female) were all higher than those of *S. nigrum. S. diphyllum* was optimal, and hybrid ND was superior to hybrid DN. The POD activity of *S. nigrum* was higher than that of *S. diphyllum*, and the POD activity of hybrid ND and DN was significantly higher than that of parent.

1 Introduction

Cadmium (Cd) is one of the five toxic elements of heavy metals, which can inhibit cell division to prevent plant growth and affect cell structure [1]. About 1.0×10^7 hm^2 of farmland soil in China has been polluted by heavy metal Cd [2]. Cd has various chemical forms in soil. Compared with other heavy metals, Cd is more easily absorbed by plants, and its activity is related to soil redox environment [3]. At present, the agricultural soil polluted by heavy metals in China accounts for about 16.7% of the planting area of crops, and more than 40% of the soil is polluted by Cd [4]. Soil Cd contamination due to concealment, hysteresis and non-biodegradable, excessive accumulation in plant body inhibits plants on the absorption of nutrients [5], disrupt the normal metabolism, reduce plant chlorophyll content in the body, destruction of respiration and photosynthesis in plants and so on, which affect crop yield, quality and safety [6]. It is easy to be absorbed and enriched by crops in production activities, which seriously affects the photosynthesis and quality of crops. *Solanum nigrum* is a plant of the *Solanaceae* and it is a good medicinal plant with functions such as clearing away heat and detoxifying [7], promoting blood circulation, reducing swelling and diuresis [8]. This study simulated Cd-contaminated soil to investigate the effects of Cd stress on photosynthetic pigment content, antioxidant enzyme activity and soluble protein of *S. nigrum*, *Solanum diphyllum* and its hybrids, in order to further reveal their physiological characteristics.

2 Materials and method

2.1 Plant and soil

The mature seeds of *S. nigrum and S. diphyllum* plants were from the farmland around the Yucheng District, Ya'an City, Sichuan Province, China. Then the seeds were air-dried and stored at 4°C. The soil used in the experiment was a fluvo-aquic soil.

2.2 Hybridizing treatment

In April 2019, the mature seeds of *S. nigrum and S. diphyllum* were sown in the hole tray to be germinated, then transplanted to clean soil when they were about 5 cm high and with two fully expanded true leaves, and watered irregularly to ensure that soil moisture kept at 80% field capacity. The hybridizing treatments were *S. nigrum* male × *S. diphyllum* female (henceforth ND) and *S. diphyllum* male × *S. nigrum* female (henceforth DN). The hybridizing experiment was carried out as follow: The day before plant flowering, the tweezers was used to poke the female petals and remove and clean the anthers, and then the female inflorescences were placed into plastic bags for isolation. Within 1-3 days after castration, at the noon, we opened the bags again, and applied a small amount of male pollens onto the female stigma evenly, and then the female inflorescences were placed into plastic bags for isolation again. About a week after hybridization, when the petals tended to fade and the young fruit formed, we removed the bags and marked it. Subsequently, we collected the matured seeds, air-dried, and stored them separately at

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4°C. Apart from that, the F1 generation of *S. nigrum* seeds was marked as *S. nigrum*, and F1 generation of *S. diphyllum* seeds was *S. diphyllum*.

2.3 Seedling

In June 2019, The F1 seeds of *S. nigrum*, *S. diphyllum*, ND and DN were sown in uncontaminated potting soil, which was put in a 15 cm × 18 cm (height × diameter) plastic basin. Each pot had 3 kg of soil and with 30 seeds in it. These seeds were covered with 2 mm soil, and the soil moisture content was kept at 80% of the field capacity. Then these seedlings were transplanted when they were about 10 cm high.

2.4 Cd treatment

In June to September 2019, the Cd treatment experiment was conducted. There were four treatments: *S. nigrum*, *S. diphyllum*, ND and DN, each treatment was repeated three times, but before that, the uncontaminated soil would be air dried, ground, and sieved with 5 mm sieves. 3 kg soil was put in a 15 cm × 18 cm (height × diameter) plastic basin, mixed with $CdCl_2 \cdot 2.5H_2O$ saturated solution until the final soil Cd concentration was 10 mg/kg, and then incubated for 4 weeks. Soil moisture was kept at 80% of field capacity. In July 2019, seedlings growing neatly of various F1 were selected and transplanted to the plastic basin with Cd contaminated soil, and each plastic basin had 4 seedlings. All the plants were placed in transparent awnings, and irregularly watered to keep soil moisture was at 60% field capacity. The plants would be harvested when they were in full-bloom stage.

2.5 Chemical analysis and biomass determination

In August 2019, collecting fresh mature leaves to quantify the photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid) and soluble protein, and determining the antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)] activities [9].

2.6 Statistical analyses

Statistical analyses were conducted using SPSS 20.0 (IBM, Chicago, IL, USA). Data were subjected to one-way analysis of variance, followed by least significant difference test (5% confidence level).

3 Results and discussion

3.1 Biomass of plants

Under high Cd stress, the biomass of various organs of F1 hybrids seriously differed from their parents (Table 1). The roots, stems, leaves, and shoots biomasses of DN and ND were higher than *S. nigrum* parent, but lower than *S. diphyllum* parent. DN F1 hybrids had increased roots, stems, leaves, and shoots biomasses by 14.82%, 5.92%, 4.80%, and 4.81% ($p > 0.05$), respectively relative to *S. nigrum* parent, while ND F1 hybrids increased by 11.09% ($p > 0.05$), 9.45%, 6.13%, and 7.20% ($p < 0.05$), respectively. Compared with *S. diphyllum*, DN F1 hybrids had decreased roots, stems, leaves, and shoots biomasses by 11.70%, 11.51%, 4.90%, and 7.16% ($p > 0.05$), respectively, while ND F1 hybrids increased by 3.25% ($p > 0.05$), 8.56% ($p < 0.05$), 3.21% ($p > 0.05$), and 5.04% ($p > 0.05$), respectively. Moreover, no significant difference of organ biomass existed between DN and ND ($p > 0.05$). Biomass of *S. diphyllum* was the best.

Table 1. Biomass of plants.

Treatments	Roots (g/plant)	Stems (g/plant)	Leaves (g/plant)	Shoots (g/plant)
S. nigrum	0.938±0.029b	0.878±0.014c	1.844±0.051b	2.722±0.065c
S. diphyllum	1.077±0.063a	1.051±0.021a	2.022±0.045a	3.073±0.066a
DN	0.951±0.021ab	0.930±0.028bc	1.923±0.028ab	2.853±0.057bc
ND	1.042±0.054ab	0.961±0.030b	1.957±0.018a	2.918±0.049ab

Values are means (±SE) of three replicate pots. Different lowercase letters within a column indicate significant differences based on one-way analysis of variance followed by the least significant differences at the 5% confidence level.

3.2 Photosynthetic pigment content in plants

Significant differences of photosynthetic pigment content existed between the parents ($p < 0.05$), and the hybrid seems seriously affected the photosynthetic pigment content in plants. Compared with *S. nigrum*, the contents of chlorophyll *a* and chlorophyll *b* in DN and ND F1 hybrids increased by 1.52% ($p > 0.05$) and 18.59% ($p < 0.05$), 0.41% ($p > 0.05$) and 24.17% ($p < 0.05$), respectively. Their total chlorophyll and carotenoid contents increased by 1.23% ($p > 0.05$) and 21.32% ($p < 0.05$), 4.06% ($p < 0.05$) and 11.17% ($p < 0.05$), respectively. However, compared with *S. diphyllum*, the contents of chlorophyll *a* and chlorophyll *b* were reduced by 15.62% ($p < 0.05$) and 1.43% ($p > 0.05$), 30.03% and 13.47% ($p < 0.05$). Their total chlorophyll and carotenoid content scores decreased by 19.91% ($p < 0.05$) and 4.02% ($p > 0.05$), 10.28% ($p < 0.05$) and 1.9% ($p > 0.05$).

Table 2. Photosynthetic pigment content in plants.

Treatments	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
S. nigrum	2.033±0.049b	0.724±0.013c	2.757±0.062b	0.394±0.009c
S. diphyllum	2.446±0.056a	1.039±0.033a	3.485±0.022a	0.457±0.007a
DN	2.064±0.042b	0.727±0.019c	2.791±0.061b	0.410±0.016bc
ND	2.411±0.076a	0.899±0.026b	3.345±0.101a	0.438±0.015ab

Values are means (±SE) of three replicate pots. Different lowercase letters within a column indicate significant differences based on one-way analysis of variance followed by the least significant differences at the 5% confidence level.

3.3 Antioxidant enzyme activity and soluble protein content of plants

As shown in Table 3, the SOD activity, CAT activity and soluble protein content of *S. diphyllum* were significantly higher than those of *S. nigrum*, and POD activity was significantly lower than that of *S. nigrum* ($p < 0.05$). Compared with *S. nigrum*, the POD activity and SOD activity of DN and ND F1 hybrids increased by 482.27% and 539.11% ($p < 0.05$), 5.59% ($p > 0.05$) and 15.3% ($p < 0.05$), respectively. Their CAT activity and soluble protein content increased by 31.85% and 53.23% ($p < 0.05$), 4.19% and 6.6% ($p > 0.05$), respectively. Compared with *S. diphyllum*, the POD activity of DN and ND F1 hybrids increased by 141.53% and 539.11% ($p < 0.05$), but their SOD activity, CAT activity and soluble protein content decreased by 128.9% (p < 0.05) and 22.36% ($p > 0.05$), 19.01% and 5.87% ($p < 0.05$), 8.52% ($p < 0.05$) and 6.41% ($p > 0.05$), respectively.

Table 3. Antioxidant enzyme activity and soluble protein content of plants.

Treatments	POD activity (U/g/min)	SOD activity (U/g)	CAT activity (mg/g/min)	Soluble protein content (mg/g)
S. nigrum	1777±70d	232.54±8.63c	4.330±0.155d	12.88±0.61b
S. diphyllum	4284±76c	345.37±9.44a	7.049±0.069a	14.67±0.49a
DN	10347±320b	245.55±5.44c	5.709±0.115c	13.42±0.05b
ND	11357±309a	268.13±4.91ab	6.635±0.093b	13.73±0.21ab

Values are means (±SE) of three replicate pots. Different lowercase letters within a column indicate significant differences based on one-way analysis of variance followed by the least significant differences at the 5% confidence level.

4 Conclusions

According to the experiment, under Cd stress, the biomass, photosynthetic pigment content, SOD activity, CAT activity and soluble protein of ND and DN were higher than *S. nigrum* but lower than *S. diphyllum*. *S. diphyllum* had the highest plant biomass, photosynthetic pigment content, SOD activity, CAT activity and soluble protein, while *S. nigrum* had the lowest. The POD activity of *S. nigrum* was higher than that of *S. diphyllum*, POD activity of hybrid ND and DN was significantly higher than that of parents. The plant biomass, photosynthetic pigment content, antioxidant enzyme activity and soluble protein of hybrid ND were all higher than that of hybrid DN. Therefore, parent *S. diphyllum* is optimal, and hybrid ND is superior to hybrid DN.

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