

Effects of reciprocal hybridization on cadmium accumulation in F1 hybrids of *Solanum diphyllum* and *Solanum nigrum*

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Abstract. A pot experiment was conducted to study the effects of reciprocal hybridization on cadmium (Cd) accumulation in F1 hybrid of *Solanum diphyllum* and *Solanum nigrum* by Cd treatment (the final soil Cd concentration was 10 mg/kg). The results showed that the Cd content of F1 hybrid was between the parents. In stems, leaves and shoots, the Cd content of DN (*S. diphyllum* male × *S. nigrum* female) and ND (*S. nigrum* male × *S. diphyllum* female) F1 hybrid increased by 14.09% and 17.34%, 8.08% and 11.14%, 9.5% and 12.04% respectively compared with *S. nigrum*, and decreased by 13.34% and 10.87%, 6.52% and 3.88%, 7.44% and 5.0% respectively compared with *S. diphyllum*, with no significant difference in roots. Therefore, the accumulation ability of Cd in F1 hybrids was improved to a certain extent compared to *S. nigrum*, but decreased to a certain extent compared to *S. diphyllum*.

1 Introduction

With the development of industry, environmental problems come one after another. Heavy metal pollution in soil threatens human's health and life, so it is urgent to solve this problem. Cadmium is one of the heavy metal elements, and excessively high concentration of Cd will affect redox balance, nutrient absorption, reproductive growth and other aspects of plants [1]. In the first national soil pollution survey in China, it was found that by 2014, Cd was one of the main pollutants in cultivated land, forest land, grassland and unused land [2]. Zheng et al. [3] investigated in rural areas of Shaanxi Province in 2015 and found that Cd pollution in the province was serious. Li [4] found that the Cd content in the road green belt in the south of Shenyang was relatively high. Deng et al. [5] found that Cd content in different types of soil in Guangxi Zhuang Autonomous Region was higher than the standard. All kinds of facts indicated the seriousness of soil Cd pollution. Therefore, how to solve the problem of Cd pollution in a green and effective way is the top priority.

Solanum nigrum, a one-year to perennial wild herb of the nightshade family, is mostly grown on the roadside or in the fields. It can not only be used for the remediation of Cd-polluted soil, but also is a traditional Chinese herbal medicine in China, which has great development value [6-7]. Solanine is rich in *S. nigrum*, which is widely used in the treatment of tumors due to its cytotoxicity [8-9]. Studies by Huang et al. [10] have found that solanine has the effect of inhibiting the proliferation of SGC-7901 cells and promoting cell apoptosis in gastric cancer. In addition, the fruit has certain edible value, can be made into drinks, canned, fruit wine, etc [11].

Solanum diphyllum is a variety of *S. nigrum*, mainly

distributed in Taiwan. It is a small evergreen shrub with bright fruit, which has certain ornamental value [12]. Besides that, it is found that *S. diphyllum* has certain medicinal value. Guo et al. [13] studied the antioxidant and anti-inflammatory activities in vitro of the fruit extract of *S. diphyllum*, providing a basis for the development of natural antioxidants and anti-inflammatory agents.

To study the influence of interbreeding on F1 Cd accumulation in progeny with *S. diphyllum* and *S. nigrum* as parents plays an important role in increasing species that can be used in phytoremediation technology, which is conducive to the treatment of Cd pollution. In addition, the utilization of the medicinal value of F1 hybrids has positive significance for the treatment of diseases, but the changes of its medicinal properties need to be further studied.

2 Materials and method

2.1 Plant and soil

The mature seeds of *S. diphyllum* and *S. nigrum* plants were collected from the farmland around the Yucheng District, Ya'an City, Sichuan Province, China in 2018, 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. diphyllum* and *S. nigrum* 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

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capacity. The hybridizing treatments were *S. diphyllum* male × *S. nigrum* female (henceforth DN) and *S. nigrum* male × *S. diphyllum* female (henceforth ND). 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, 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 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*, DN and ND 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*, DN and ND, 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 CdCl₂·2.5H₂O 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 Biomass and Cd content determination

In August 2019, isolating the roots, stems and leaves of all plants, and washing them with tap water and deionized water separately. The single organ would be air dried at 110°C for 15 min, then at 75°C to a constant weight. Subsequently, the biomass of each dried organ was measured, then, they were finely grounded, digested in HNO₃/HClO₄ (4:1, v/v), and determined the Cd contents with iCAP 6300 ICP spectrometer. The Cd extraction by plant was calculated as follows: Cd extraction= biomass × Cd content.

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 Cd content in plants

The Cd contents in roots of *S. nigrum* were significantly ($p < 0.05$) higher, but in stems, leaves, and shoots of which were significantly ($p < 0.05$) lower than that of *S. diphyllum*. Both the DN and ND F1 hybrids showed a significant increase of Cd content in different organs except for roots compared with *S. nigrum* parent ($p < 0.05$), while a decrease in that relative to *S. diphyllum* parent (Table 1). The DN F1 hybrid increased Cd content in stems, leaves, and shoots by 14.09% ($p < 0.05$), 8.08% ($p < 0.05$), and 9.5% ($p < 0.05$) relative to *S. nigrum* parent, but decreased that in these organs by 13.34% ($p < 0.05$), 6.52% ($p < 0.05$), and 7.44% ($p < 0.05$) relative to *S. diphyllum* parent. While the ND F1 hybrid exhibited an increase of Cd content in stems, leaves, and shoots by 17.34% ($p < 0.05$), 11.14% ($p < 0.05$), and 12.04% ($p < 0.05$) relative to *S. nigrum* parent, but a decrease by 10.87% ($p < 0.05$), 3.88% ($p > 0.05$), and 5.0% ($p > 0.05$) relative to *S. diphyllum* parent. Cd content in roots of DN and ND F1 hybrids showed no significant difference between each other, and also with *S. nigrum* parent.

Table 1. Cd content in plants.

Treatments	Roots (mg/kg)	Stems (mg/kg)	Leaves (mg/kg)	Shoots (mg/kg)
<i>S. nigrum</i>	38.73±0.92a	27.33±1.38c	50.38±0.87c	42.94±1.09c
<i>S. diphyllum</i>	34.85±0.98b	35.98±1.37a	58.25±1.09a	50.64±1.20a
DN	37.02±0.83ab	31.18±0.84b	54.45±0.48b	46.87±0.52b
ND	38.05±1.17a	32.07±0.37b	55.99±1.32ab	48.11±0.89ab

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 Cd extraction amount of plants

In the Cd-contaminated soil, the Cd extraction of *S. diphyllum* was significantly higher in stems, leaves and shoots ($p < 0.05$). Both the DN and ND F1 hybrids showed a significant increase of Cd extraction in different organs except for roots compared with *S. nigrum* parent ($p < 0.05$), while a decrease in that relative to *S. diphyllum* parent. No significant difference of Cd extraction by roots existed between all the tested treated samples ($p > 0.05$) (Table 2). The Cd extraction by stems, leaves, shoots of DN and ND F1 hybrids increased by 20.82% and 28.32% ($p < 0.05$), 12.70% and 17.94% ($p < 0.05$), 14.38% ($p > 0.05$) and 20.08% ($p < 0.05$), respectively, compared to *S. nigrum* parent, but decreased by 23.27% and 18.51% ($p < 0.05$), 11.11% ($p < 0.05$) and 17.94% ($p > 0.05$), 14.07% ($p < 0.05$) and 9.78% ($p > 0.05$), respectively, relative to *S. diphyllum* parent. The Cd extraction by roots of DN F1 hybrid decreased by 3.03% and 6.21% ($p > 0.05$), respectively, compared to *S. nigrum* and *S. diphyllum*, and ND F1 hybrid increased by 9.03% ($p > 0.05$) and 5.46%

($p > 0.05$), respectively, compared to *S. nigrum* and *S. diphyllum*.

4 Conclusions

To sum up, the Cd content and Cd extraction of DN and ND F1 hybrids were between the two parents after reciprocal hybridization with *S. diphyllum* and *S. nigrum* as parents. Different organs showed different performance. Compared with *S. nigrum*, reciprocal hybridization significantly increased the Cd content and Cd extraction in stems, leaves and shoots, while compared with *S. diphyllum*, the Cd content and Cd extraction significantly decreased. In roots, there was no significant difference between the F1 hybrids and the parents. Therefore, under the stress of Cd, the Cd accumulation ability of the F1 hybrid was between *S. nigrum* and *S. diphyllum*. It was improved to a certain extent compared to *S. nigrum*, but decreased to a certain extent compared to *S. diphyllum*. F1 hybrids play active roles in the restoration of Cd-polluted soil.

Table 2. Cd extraction amount of plants.

Treatments	Roots ($\mu\text{g}/\text{plant}$)	Stems ($\mu\text{g}/\text{plant}$)	Leaves ($\mu\text{g}/\text{plant}$)	Shoots ($\mu\text{g}/\text{plant}$)
<i>S. nigrum</i>	36.32 \pm 1.99a	24.01 \pm 1.60c	92.92 \pm 4.17c	116.92 \pm 5.77c
<i>S. diphyllum</i>	37.55 \pm 3.25a	37.81 \pm 2.18a	117.81 \pm 4.84a	155.62 \pm 7.02a
DN	35.22 \pm 1.58a	29.01 \pm 1.67b	104.72 \pm 2.47b	133.73 \pm 4.13b
ND	39.60 \pm 0.85a	30.81 \pm 1.33b	109.59 \pm 3.61ab	140.40 \pm 4.93ab

Values are means (\pm 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.

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