

Research Article

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Cadmium (Cd) chloride affects the nutrient uptake and Cd-resistant bacterium reduces the adsorption of Cd in muskmelon plants

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Abstract: This study investigated the effect of cadmium (Cd) chloride on the uptake of N, P, and K and evaluate the effect of Cd-resistant bacterium “N3” on reducing the adverse effect of Cd in grafted and nongrafted plants. The shoot and total dry weights of the nongrafted muskmelon plants decreased under 50 and 100 μM Cd treatments. The scion and shoot dry weights of the grafted plants increased significantly, whereas their root dry weight increased by nearly onefold compared with those of the CK-grafted plants regardless of Cd concentration. The N, P, and K contents in the nongrafted plants decreased under Cd treatments but increased under 50 μM Cd treatment when inoculated with “N3”. The N, P, and K contents in the grafted plants were lower than those treated with only Cd. The grafted and nongrafted plants exhibited low Cd accumulation in the scion or shoot part compared with the root tissues. “N3” inoculation reduced the Cd concentration in all tissues of the grafted and nongrafted plants. Our results demonstrated great variation in Cd accumulation in the grafted and nongrafted muskmelon plants, thereby promoting food safety under Cd contamination conditions.

Keywords: *Burkholderia* species, cadmium adsorption, growing substrate, nutrient uptake, root growth

1 Introduction

Muskmelon is a warm season crop that is cultivated in arid and semi-arid regions of the world; it can be cultivated successfully in off-season [1]. China produces approximately 50% of the total amount of muskmelon globally [2]. Currently, muskmelon is one of the most important and fruity vegetables and has a huge potential demand around the world. Many studies have focused on the improvement of the muskmelon crop by breeding new cultivars and improving yields and nutritional qualities [3–5].

Grafting vegetable crops has become a well-developed and useful practice attributing to its horticultural advantages [6]. Currently, grafted plants are essential to vegetable production and have become a significant requirement in vegetable intensive seedling production, e.g., tomato, watermelon, muskmelon, and cucumber [7–9]. Grafting is widely used because of its advantages of limiting the effects of soil-borne pathogens, enhancing tolerance to abiotic stresses, contributing to the efficiency of nutrient uptake, and improving the fruit yield [10,11]. Plants must absorb enough nutrients to grow; for instance, rhizobial inoculants may induce high nutrient uptake in plants [12]. Grafting is a normal way of increasing the nutrient uptake in various horticultural plants [13]. Grafted plants exhibit better nutrient uptake because of the strong ability of the rootstock's roots to absorb nutrients from the soil. Therefore, regulating the assimilation of nutrients in the rootstock is vital for the grafted plant growth.

Cadmium (Cd) is one of the most toxic heavy metals that accumulates in the soil because of the application of chemical fertilizers and industrial waste contamination [14,15]. Cd can be absorbed not only by crop plants, such as rice, wheat, and maize, but also by vegetables, such as tomato, water spinach, and muskmelon, thereby posing a

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threat to human health [16–18]. Coping with Cd contamination in vegetable crops has become ever more important because vegetables are indispensable food for human beings all over the world. Despite the variation in Cd accumulation between and within plant species, most crops retain much of the Cd in their roots [19]. Therefore, the recent research on the mechanisms regulating Cd in plants helps with the development of a strategy for preventing Cd accumulation in grafted and nongrafted muskmelon plants. In the current work, results showed that Cd can stimulate the grafted plant growth when a pumpkin was used as the rootstock. Further experiments were performed to evaluate the effects of Cd on grafted and nongrafted plants. Moreover, whether Cd in the substrate affects the uptake of nutrients in grafted plants is still largely unknown. A Cd-tolerant bacterium “N3” was used and evaluated for its promoting effect on grafted and nongrafted plants under two Cd concentrations (50 and 100 μM) to analyze the accumulation of Cd in the melon plant. Our results expand our understanding on the potential of Cd-resistant bacteria to help plants reduce the Cd uptake when grown in substrates.

The objectives of the current study were (1) to investigate the effect of CdCl_2 on the uptake of N, P, and K and (2) to determine the effect of Cd-resistant bacterium “N3” on the Cd reduction and nutrient uptake in grafted and nongrafted muskmelon plants.

2 Materials and methods

2.1 Growing substrate

A 3:1:1 volume ratio of peat, vermiculite, and perlite was used to prepare the seedling substrates whose pH values were adjusted to pH 6.0 by using quick lime (CaO). The properties of the substrates were as follows [20]: available nitrogen (NH_4^+), 0.58 g kg^{-1} ; available phosphorus (P), 0.15 g kg^{-1} ; available potassium (K), 0.69 g kg^{-1} ; bulk density, 0.25–0.30 g cm^{-3} ; and total porosity, 85.8–87.0%.

2.2 Plant materials

Muskmelon (*Cucumis melo* L., cultivar “Cuimi”) and a rootstock were used. The hybrid was named “white pumpkin,” which was hybridized from “Chinese pumpkin” and “yellow pumpkin” (Chinese *Cucurbita moschata* ×

Indian *C. moschata*). White pumpkin is a popular rootstock, and it has been used in muskmelon grafting in Anhui Province, China. Seeds of the rootstocks were sown in substrate (23 g) in 6 cm diameter plastic pots, 7–8 days earlier than the seeds of the scions. Muskmelon seeds were also sown in substrate (23 g) in 6 cm-diameter plastic pots at a temperature range of 24–28°C and a relative humidity range of 85%–90% [20]. All the pots were placed under greenhouse conditions from January 10, 2018, to May 20, 2018, at the Anhui Academy of Agricultural Sciences.

2.3 Grafting method

Tongue approach grafting was used in this study in accordance with the previously described processes [9]. Grafting must be performed after the first true leaf develops in the rootstock and scion. The hypocotyls of both the rootstock and the muskmelon scion were cut. The two hypocotyls were clipped together. Afterward, the grafted plants were placed under a plastic film at 25–30°C and at more than 90% humidity without light. The grafted plants were exposed to sunlight 2–3 hours per day until the scions grew normally after 7 days. Two concentrations of Cd solutions (50 and 100 μM) were added to the grafted plant’s pot. A Cd-resistant bacterium, “SJN3” (abbreviated as “N3”, concentration of 10^8 CFU mL^{-1}), was inoculated separately twice into the 50 and 100 μM Cd(II) substrates at an interval of 7 days. This strain belonged to *Burkholderia* sp., with accession number KU736928 in the GenBank database (Figure 1). More than 10 grafted plants were used with three replacements.

2.4 Management of melon plants under greenhouse conditions

The nongrafted and grafted plants under different treatments were transferred to the greenhouse under regular management. All the plants were cultivated through surface irrigation with day and night temperatures of 24–28°C and 15–20°C [20], respectively.

2.5 Vegetable seedling sampling

A total of ten seedling plants were collected from each treatment and washed gently to remove the adhering

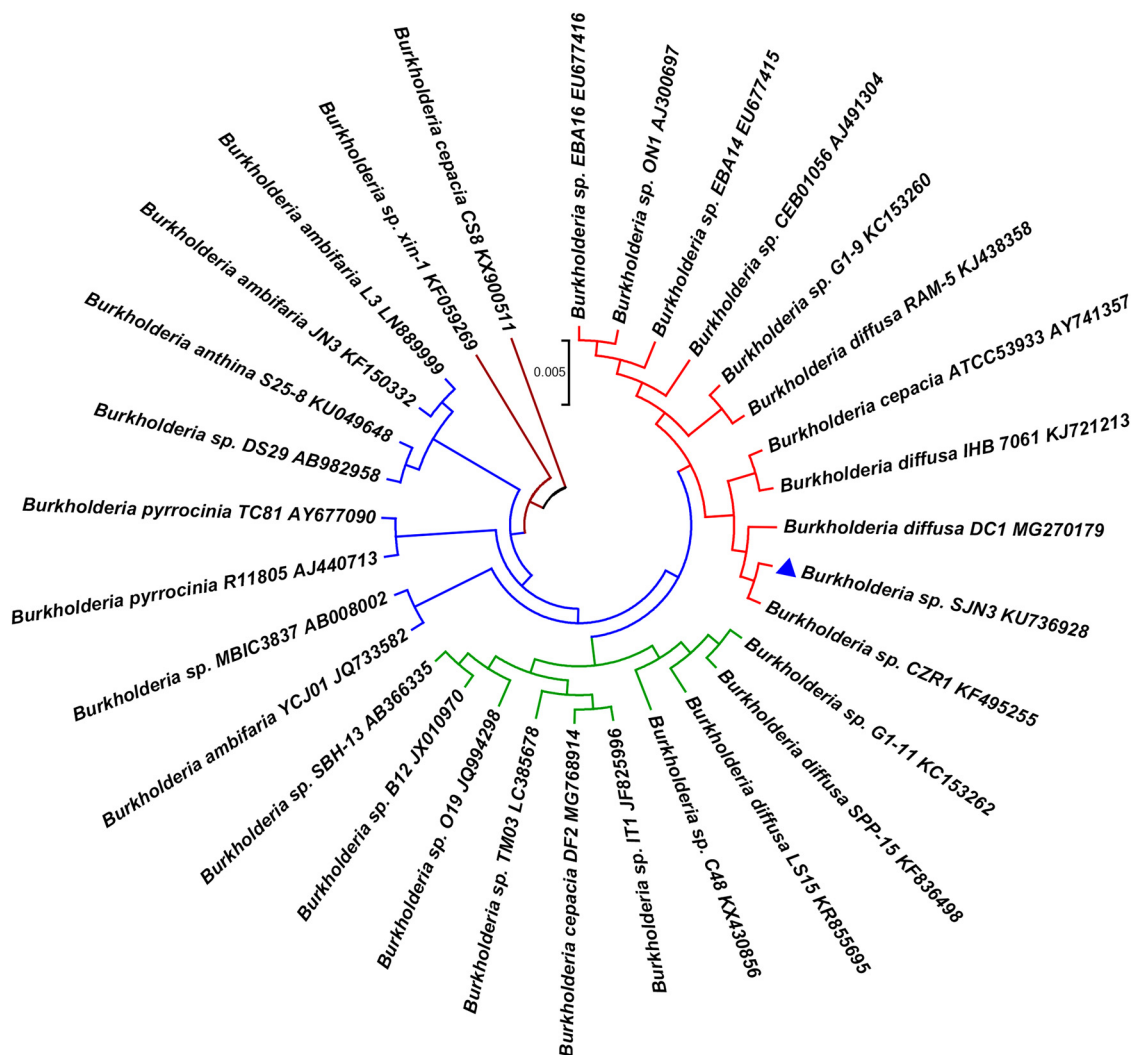


Figure 1: Phylogenetic tree was constructed based on 16S rDNA sequences among neighboring species using the neighbor-joining method with MEGA version 6.0 (www.megasoftware.net). Bootstrap percentage values were obtained from 2000 resamplings. The bar represents 0.005 substitutions per nucleotide position. Sequences with high similarity scores were downloaded from the NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) database.

substrate from the roots. The root was snipped from the grafted plant, and then the seedling shoots and roots were oven dried for 3 days at 70°C. Then, the shoot and root dry weights were measured and stored for further analysis.

2.6 Determination of N, P, and K and concentration of Cd

Dried plant samples were ground separately in a Wiley mill. Then, 0.5 g of the comminuted tissues was analyzed for N and K contents in accordance with a previously

described method [21]. The concentration of K was analyzed in the filtrate using a flame photometer. The total P of the plants was determined as previously reported [22]. Sulfuric acid (5 mL) was added into the digestive tract, and then 2 mL of hydrogen peroxide was added. After the intense reaction, heating was continued for 5 min to remove excess hydrogen peroxide. After cooling, the digested liquid was transferred into a 100 mL bottle. For Cd analysis, the grounded shoot and root samples were digested in a mixture of concentrated HNO_3 and HClO_4 (4:1, v/v). The Cd content in the grafted plants was determined with an atomic absorption spectrometer (TAS-986, Beijing, China) in accordance with the previously described procedures [23].

2.7 Statistical analysis

All data were analyzed by using one-way ANOVA followed by Fisher's least significant difference (LSD) at $p < 0.05$ (*) and $p < 0.01$ (**), respectively, and by Duncan's test at $p < 0.01$ using the SPSS software (version 19.0 Inc., USA).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Shoot and root dry weights in nongrafted plants

In this study, we explored the effect of Cd and Cd-resistant bacterium "N3" on N, P, and K uptake in grafted and nongrafted plants under the two concentrations of Cd. Cd is one of the hazardous heavy metals that restrains plant regeneration and growth [24–27]. The shoot dry weight and total dry weights of the nongrafted muskmelon plants decreased under 50 and 100 μM Cd treatments (Figure 2a). This result suggested that Cd inhibited the shoot growth of nongrafted muskmelon plants. Although the root dry weight increased by 16.07% under the 100 μM Cd treatment, the total dry weight (389.8 mg) in this treatment was still smaller than that of the control CK (400.3 mg), suggesting that Cd showed adverse effects on the growth of nongrafted muskmelon seedlings. Cd inhibits the growth and photosynthesis of melon cultivars [28] and reduces the biomass of pea plants [29].

Cd hinders the growth of many crops. In this study, a Cd-resistant bacterium "N3" was inoculated into the grafted and nongrafted plants. "N3" belongs to *Burkholderia* sp. (Figure 1) and shows a strong tolerance to Cd (100 μM Cd in liquid nutrients medium, Figure 3); it produces indole acetic acid or siderophores that can chelate irons [23,30,31]. This genus reportedly reduces the Cd toxicity and promotes the growth of tomato [32]. After inoculation with Cd-resistant bacterium "N3", the shoot, root, and total dry weights increased by 7.54%, 29.17%, and 8.90% under the treatment of 50 μM , respectively. Although the value of OD600 decreased with the increasing concentration of Cd, this strain showed remarkable tolerance to Cd (Figure 3). When

inoculated with "N3" at 100 μM , the dry weight and the total dry weight of the nongrafted plants did not increase, and the root dry weight significantly decreased (17.52%) (Figure 2a). In our work, inoculation with "N3" slightly increased the root and total dry weights in the nongrafted plants but decreased them in the grafted plants. This finding showed that under the treatment with 50 μM Cd, the inoculation with strain "N3" alleviated the suppression of nongrafted plants by Cd. However, the inoculation of strain "N3" could not alleviate the inhibitory effect of Cd on the nongrafted plants treated with 100 μM Cd. We speculated that the ability of "N3" to adsorb and immobilize Cd at 100 μM is insufficient. Our observations suggest that Cd-tolerant bacteria induce metal stress tolerance at specific Cd concentrations. Cd promoted the growth of grafted muskmelon plants, especially the root growth of rootstocks, and the effect varied at different Cd concentrations. However, at 100 μM Cd, the growth of the grafted plants was inhibited, especially the root growth of the rootstock. The total dry weights of the grafted seedling plants decreased after the inoculation of strain "N3" at the two concentrations, indicating that a part of the Cd ions was chelated by "N3". Thus, the stimulating effect of Cd on the growth of the grafted muskmelon plants was reduced. *Burkholderia* sp. can reportedly cause Cd stress alleviation due to the chelating metals, thereby improving phytoextraction [33]. We confirmed that "N3" can absorb Cd, reducing the uptake of Cd in the melon plants grown in the substrate. In accordance with a previous finding that cereal plants exhibit metal stress tolerance when Cd-tolerant bacteria are applied [34], our results suggested that heavy metal-tolerant bacteria are important for the plant growth and beneficial for reducing the Cd uptake in grafted and nongrafted plants.

3.2 Shoot and root dry weights in grafted plants

The grafting seedlings were treated with Cd and compared with the CK-grafted plants. Regardless of the treatment (50 or 100 μM), the scion, shoot, root, and total dry weights of the grafted plants significantly increased, whereas the root dry weight increased by nearly onefold (Figure 2b), suggesting that grafted plants grow remarkably when exposed to Cd. Various rootstocks have been used to increase the nutrient uptake in grafted vegetables; thus, grafting improves the plant growth [11,13,30].

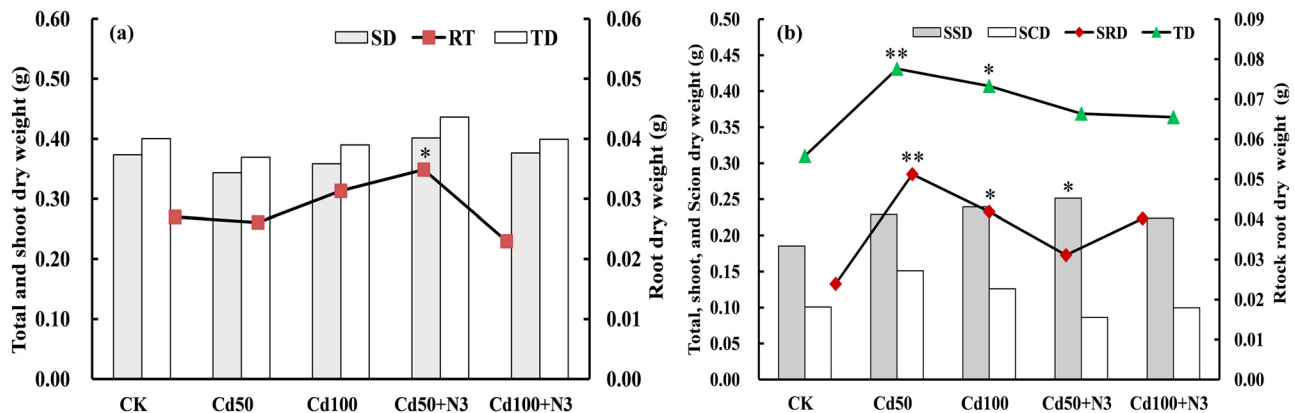


Figure 2: Shoot and root dry weights of (a) nongrafted and (b) grafted plants grown in substrates supplemented with 50 and 100 μM Cd. Significant differences were tested at $*p \leq 0.05$ and $**p \leq 0.01$. Values are the means $\pm$ standard deviations of three replicates. SD, shoot dry weight; RT, root dry weight; TD, total dry weight; SSD, rootstock shoot dry weight; SCD, scion dry weight; SRD, rootstock root dry weight.

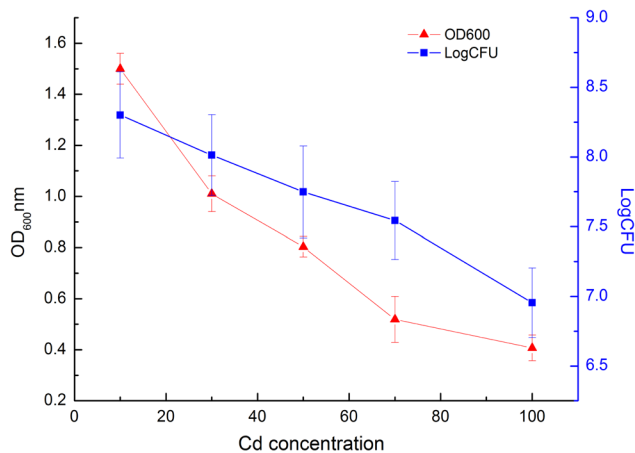


Figure 3: Values of $\text{OD}_{600 \text{ nm}}$ and Log CFU of bacterium “N3” under different Cd concentrations (μM) in the liquid medium.

However, we found that Cd positively stimulated the development of the grafted plants, especially the root growth of pumpkin rootstock. The plant root is a major organ that can absorb heavy metals from soil. We demonstrated that this rootstock can tolerate Cd by increasing the root growth to cope with the toxic metal (Cd). Under the 50 μM Cd treatment, the root dry weight increased by onefold compared with the CK-grafted plants. The scion, shoot, and total dry weights of the grafted plants significantly increased by 49.90%, 23.48%, and 39.08%, respectively. The dry weight of the stock root was significantly lower (22.29%), and the total dry weight was lower (5.85%), but the total dry weight was still higher than that of the control CK (31.39%) under the 100 μM Cd treatment than under the 50 μM Cd treatment.

The root dry weight (30.13%), stem weight (35.75%), and total dry weight (18.98%) of the grafted plants treated with 100 μM Cd and inoculated with strain “N3” increased, but the dry weight of the scion (16.96%) decreased compared with those of CK. Compared with the plants treated with 50 μM Cd, the scion dry weight (75.32%), root dry weight (64.76%), and total dry weight (16.89%) significantly reduced. At the same concentration, strain “N3” adsorbed Cd, thereby reducing the stimulating effect of Cd on the grafted plants. At the same time, the dry weight of the scions (46.18%), the root dry weight (34.73%), and total dry weight (10.43%) of rootstocks also decreased compared with those treated with 100 μM Cd (Figure 2b).

The root dry weight, shoot weight, and total dry weight of the grafted plants treated with 100 μM Cd and inoculated with strain “N3” increased by 68.34%, 20.82%, and 17.35%, respectively, compared with those of the control CK. The scion dry weight decreased by 51.58% in the 50 μM Cd-treated plants and decreased by 26.38% in the 100 μM Cd treatment. Compared with the 50 μM Cd + “N3” treatment, the root dry weight significantly increased by 29.37%. In the plants treated with 100 μM Cd, strain “N3” did not adsorb Cd, thereby showing the stimulating effect of Cd on the root growth of the grafted plants (Figure 2b). Our study confirmed that inoculation with “N3” reduces the adverse effect of Cd on nongrafted plants, suggesting that “N3” with Cd-resistant ability improves the muskmelon plant growth at the two Cd concentrations. However, at 100 μM Cd, only 44–50% of the total Cd accumulated at the roots. Although most plant species retain most of the Cd in the roots to limit the transportation of Cd and to prevent

excessive Cd accumulation into the shoot or seeds [19], our results suggest that Cd at excessive amounts spreads to the shoot tissues in nongrafted plants. Inoculation with “N3” reduced the Cd concentration in all plant tissues of the grafted and nongrafted plants (Figures 4a and 5a).

3.3 Uptake of N, P, and K in grafted and nongrafted plants

The N, P, and K contents in the nongrafted plants decreased after the addition of Cd. Compared with that in CK, the N in Cd50, Cd100, and Cd100 + “N3” decreased by 19.53%, 16.72%, and 11.20%, respectively, P decreased by 19.18%, 22.78%, and 10.81%, respectively, and K decreased by 20.12%, 18.19%, and 8.05%, respectively. Under the 50 μM Cd and “N3” inoculation treatments, N, P, and K increased by 1.49%, 1.48%, and 2.56%, respectively, indicating that “N3” reduced the toxic effect of Cd on the nongrafted plants. At 100 μM Cd, the inoculation of “N3” still affected the N, P, and K uptake in the nongrafted plants and decreased compared with that in the control (Table 1). In terms of the plant growth, inoculation with “N3” increased the uptake of the three elements in the nongrafted plants but reduced it in the grafted plants (Table 1).

The treatment with Cd increased the N, P, and K contents of the grafted plants. Compared with that in CK, the N in Cd50, Cd50 + “N3”, Cd100, and Cd100 + “N3” increased by 24.47%, 14.02%, 11.46%, and 4.80%,

respectively; P increased by 25.72%, 13.23%, 10.75%, and 5.10%, respectively; and K increased by 21.20%, 16.35%, 10.68%, and 4.38%, respectively. After the inoculation of “N3”, the N, P, and K contents of the grafted plants were lower than those of the grafted plants treated with Cd only (Table 1). In terms of the uptake of N, P, and K in the grafted and nongrafted plants, the treatment with Cd decreased the three elements in the nongrafted plants regardless of the Cd concentration. By contrast, Cd increased the uptake of the three elements in the grafted plants. A previous report proved that grafting enhances the copper tolerance of cucumber because of nutrient uptake [35]. However, the absorption of N, P, and K increased when the grafted plants were exposed to Cd, suggesting that Cd stimulates rootstock growth, especially root development. Many studies have reported that using rootstock can increase the nutrient uptake or tolerant abiotic stress in grafted plants [13,36–38]. The present work illustrates that the rootstock growth can also be triggered by abiotic stress factors, such as heavy metal Cd.

3.4 Cd concentration in grafted and nongrafted plants

The concentration of Cd in the seedling roots was greatly higher than that in the shoots of the nongrafted plants (Figure 4a). Compared with the nongrafted plant control, under the treatment of 50 μM Cd, the inoculation of “N3” reduced the Cd concentration in the roots and shoots of

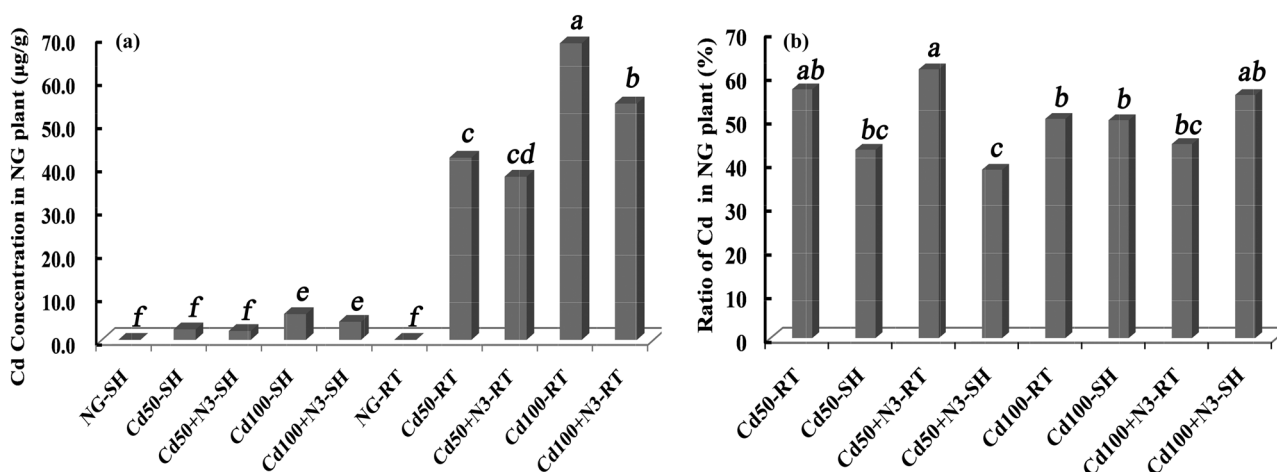


Figure 4: Concentrations of Cd in the shoot and root tissues of nongrafted plants. Significant differences tested according to Duncan's test at $p < 0.01$. (a) Cd concentration and (b) ratio of Cd in nongrafted plants. Values are the means $\pm$ standard deviations of three replicates. SH, shoot; RT, root.

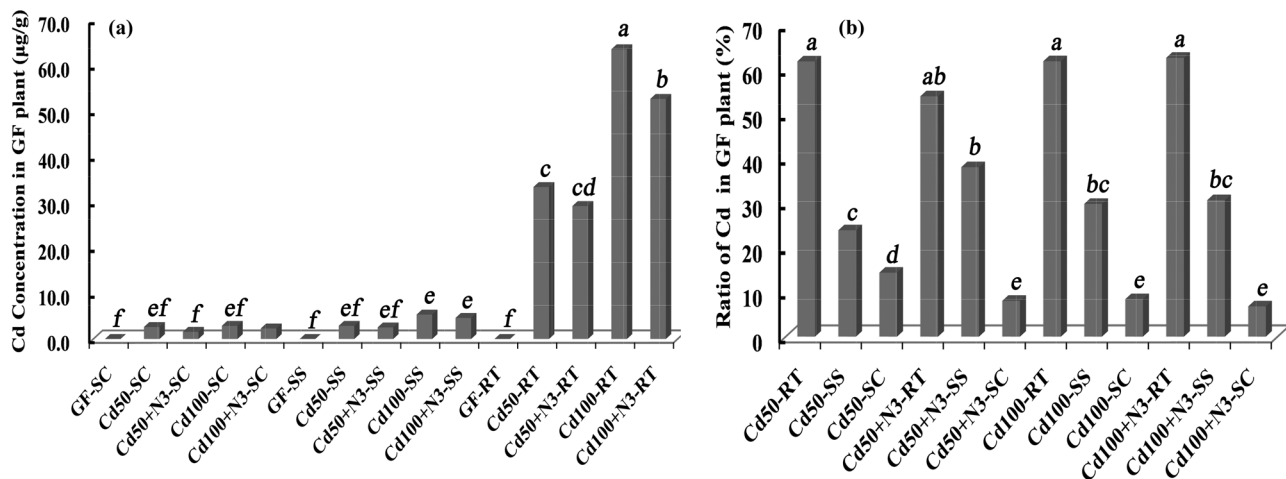


Figure 5: Concentrations of Cd in the shoot and root tissues of grafted plants. Significant differences tested according to Duncan's test at $p < 0.01$. (a) Cd concentration and (b) ratio of Cd in grafted plants. Values are the means $\pm$ standard deviations of three replicates. SC, scion; SS, rootstock shoot; RT, root.

Table 1: Total N, P, and K in grafted and nongrafted plants (mg g^{-1})

Treatment		CK	Cd50	Cd50 + N3	Cd100	Cd100 + N3
Total N	NG	14.17 $\pm$ 0.22	11.86 $\pm$ 0.14	14.39 $\pm$ 0.11	12.14 $\pm$ 0.10	12.75 $\pm$ 0.12
	GF	13.22 $\pm$ 0.16	16.46 $\pm$ 0.23	15.08 $\pm$ 0.20	14.74 $\pm$ 0.17	13.86 $\pm$ 0.15
Total P	NG	2.00 $\pm$ 0.05	1.68 $\pm$ 0.10	2.03 $\pm$ 0.08	1.63 $\pm$ 0.04	1.81 $\pm$ 0.03
	GF	2.08 $\pm$ 0.07	2.61 $\pm$ 0.06	2.35 $\pm$ 0.09	2.30 $\pm$ 0.09	2.19 $\pm$ 0.07
Total K	NG	21.10 $\pm$ 0.32	17.57 $\pm$ 0.15	21.64 $\pm$ 0.56	17.85 $\pm$ 0.19	19.53 $\pm$ 0.38
	GF	14.72 $\pm$ 0.21	17.84 $\pm$ 0.18	17.13 $\pm$ 0.26	16.30 $\pm$ 0.13	15.37 $\pm$ 0.27

CK, control treatments without addition of Cd; NG, nongrafted plant; GF, grafted plant.

the nongrafted plants by 11.70% and 18.05%, respectively. Under the treatment of 50 μM Cd, more than 56% of the Cd accumulated at the roots of the nongrafted plants. Under the 100 μM Cd treatment, the inoculation of "N3" reduced the Cd concentration in the roots (25.51%) and shoots (42.58%) of the nongrafted plants. However, under the treatment of 100 μM Cd, only 44–50% of Cd accumulated in the roots (Figure 4b), suggesting that Cd mainly accumulates in the root part at low Cd levels. Wilhelm et al. [39] found that in all three white lupine plants, the Cd concentration in the roots was much higher than that in the shoots. Similarly, rootstock reduces the Cd transport from the root to shoot tissues [40]. Our observations are consistent with their findings even though we used a different plant, indicating that the use of rootstock is a good way to reduce the heavy metal uptake. Moreover, it has been reported that the adsorption of Cd by the roots is a vital process in overall plant Cd accumulation, and the uptake of Cd in the roots has been confirmed in different plants [41–43].

The Cd content in the grafted plants was similar to that in the nongrafted plants. The Cd concentration in the roots was significantly higher than those in the shoots and scions (Figure 5a). More than 53–62.60% of Cd accumulated in the roots of the pumpkin rootstock (Figure 5b). This observation is also consistent with the previous conclusions that high amounts of Cd accumulate mainly in the roots [42,43]. A recent study has pointed out that grafted watermelon plant displays a strong capacity to inhibit the Cd uptake in the aerial parts [44]. Our observations suggest that Cd accumulates not only in nongrafted roots but also in grafted plant roots. In addition to the Cd concentration, the amount of Cd accumulation in the scion was also much less than those in the shoots and roots. Under the 50 μM Cd treatment, inoculation with "N3" reduced the Cd concentration in the scions, shoots, and roots of the grafted plants by 67.52%, 13.39%, and 14.34%, respectively. Under the 100 μM treatment, the inoculation with "N3" reduced the Cd concentration in the scions, shoots, and

roots of the grafted plants by 24.68%, 15.77%, and 20.70%, respectively. The accumulation of Cd decreased in the grafted and nongrafted plants when inoculated with strain “N3”. Our results clarified the potential queries regarding Cd immobilization by inoculating “N3” in the breeding substrates. We confirmed that inoculation with Cd-resistant bacterium “N3” showed potential in reducing the effects of Cd on the grafted and nongrafted plants.

4 Conclusion

Cadmium chloride stimulated the growth and the nutrient uptake of grafted muskmelon plants. Great variations in Cd accumulation existed in the grafted and nongrafted muskmelon plants under the two Cd conditions. Lower Cd accumulation was observed in the scion and shoot parts compared with that in the root tissues. Inoculation with Cd-resistant bacterium “N3” reduced the effects of Cd and decreased Cd adsorption in the grafted and nongrafted muskmelon plants. The results from the study provided insights into the impact of Cd on the N, P, and K uptake of the grafted and nongrafted muskmelon plants.

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Conflict of interest: No potential conflict of interest was reported by the authors.

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