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Research article

Variations in pH significantly affect cadmium uptake in grafted muskmelon (*Cucumis melo* L.) plants and drive the diversity of bacterial communities in a seedling substrate

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ABSTRACT

Substrates are fundamental prerequisites for growing grafted seedlings. In this study, substrates with different pH levels (5.0, 5.5, 6.0, 6.5, 7.0, and 8.0) were set up to elucidate the effect of pH on cadmium (Cd) uptake in grafted muskmelon (*Cucumis melo* L.) plants. Bacterial diversity was also investigated. Results showed that pH and high Cd concentration greatly affected the growth of grafted plants. The chlorophyll content of the muskmelon leaves decreased at 100 μM Cd. The majority of the Cd ions accumulated in the rootstock rather than in the shoot tissue in all of the treatments. The shoots and roots showed the highest Cd content at pH 5.5 and the lowest Cd content at pH 8.0 regardless of the Cd concentration. The operational taxonomic units belonging to *Proteobacteria* and *Bacteroidetes* were significantly ($p < 0.05$) enriched at different substrate pH levels compared with those at pH 5.0. The operational taxonomic units belonging to the phyla *Firmicutes*, *Acidobacteria*, and *Chloroflexi* were significantly decreased. The available nitrogen, phosphorus, Cd, and pH were strongly linked to bacterial community compositions. On the contrary, the available potassium was weakly correlated with the bacterial structure. This study demonstrates that pH greatly affects Cd uptake in grafted muskmelon plants and predicts microbial community structures in breeding substrates with different pH levels. Our results suggest that Cd accumulation in grafted plants can be reduced by setting the appropriate substrate pH. This work can serve as a reference for growing high-quality grafted plants and ensuring food safety in the presence of Cd contamination.

1. Introduction

Grafting of vegetable crops is a well-developed practice that has been intensively used in the last 50 years because of its various horticultural advantages (Edelstein et al., 2014). The percentage of vegetables cultivated through grafting has increased worldwide, and these vegetables are under intensive production (Lee et al., 2010). At present, grafting plays an essential role in vegetable production and addresses an important requirement in the intensive seedling production of vegetable crops, such as watermelon, muskmelon, and tomato (Kubota, 2008; Lee et al., 2010; Mohamed et al., 2014). Grafting reduces the adverse effects of soil-borne pathogens (Huang et al., 2016) and enhances abiotic stress tolerance and nutrient uptake efficiency, along with fruit quality and yield (Edelstein et al., 2011). However, several other factors, such as rootstock, concentrations of minerals in growing substrates, grafting techniques, and field management, affect the yielding attributes (Rouphael et al., 2010). Among these factors, a growing substrate directly influences the growth of grafted vegetable crops (Martínez-Ballesta et al., 2008). Thus, the optimum physical and chemical characteristics of a substrate for seedling plant growth should be determined.

In our previous study, we reported that a substrate composed of different proportions of peat, vermiculite, and pearlite can be used to grow nongrafted and grafted seedlings (Zhang et al., 2017a). However, substrates usually feature inconsistent physical properties (e.g., electrical conductivity and water holding capacity) (De Grazia et al., 2004)

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because their different components, such as turf, are derived from various sources and show different attributes (e.g., particle size, bulk density, porosity, and pH) (Kechavarzi et al., 2010; Lishtvan et al., 2017). pH is an important consideration during substrate selection for seedling production (Rincón et al., 2005) because it maintains the physicochemical characteristics of substrates. Considering these viewpoints, we explored the most suitable substrate pH for growing grafted plants.

Cadmium (Cd) is a highly toxic heavy metal that accumulates in soil via chemical fertilizer or pesticide application and industrial waste contamination (Wei et al., 2016). In China, more than 16.8% of the total cropland is contaminated with various heavy metals, including Cd (Bashir et al., 2018). Cd can be absorbed by crop plants and may threaten food safety (Dourado et al., 2014). For example, rice, wheat, and vegetables are greatly exposed to Cd (Luo et al., 2011; Yang et al., 2010). Cd accumulation in soil is one of the most serious environmental hazards, and this phenomenon exacerbates pollution, affects crop production, and threatens human health (Jiang et al., 2008). Strategies to cope with the Cd contamination of vegetable crops have become increasingly important because vegetables are a staple food in many parts of the world. Although microbes drive many important biogeochemical cycles not only in soils but also in plants, the pattern of microbial diversity in growing substrates is rarely described. Microbial communities can be used as indicative markers for the assessment of cultivating healthy seedlings. Other issues in growing seedlings include determining whether microbial structures will respond to variations in pH in growing substrates and uncovering the changes in the response of microbes to Cd in substrates.

Muskmelon (*Cucumis melo* L.) is a crop growing in warm season, suited in arid and semi-arid regions, and can be cultivated all year round (Pandey et al., 2016). For example, China produces 50% of the total amount of muskmelon (Vendruscolo et al., 2017), and cultivating muskmelon is an important approach to increase income. Considering that muskmelon is an important horticultural crop and has great potential and demand worldwide, many researchers attempted to improve this crop by breeding new cultivars, enhancing quality, and increasing soluble sugar content (Dehghani et al., 2012; Murakami et al., 2017; Zhang et al., 2010). The key step is to obtain a suitable substrate for growing grafted muskmelon. However, whether substrate pH or leaf chlorophyll content affects the growth of grafted muskmelon remains unknown. Studies have rarely evaluated the Cd uptake potential of grafted plants grown in substrates with different pH levels.

In this study, we varied substrate pH and selected a commercial rootstock to characterize the effect of pH on the early growth of grafted muskmelon plants. We also evaluated whether substrate pH affected Cd uptake in the grafted plant. We subjected the selected seedlings to phenotypic analyses for shoot and root traits and rhizobacterial community analyses through v4-v5 16S rRNA amplicon pyrosequencing. This study improved our understanding of the role of substrate pH in the growth of grafted plants and provided new insights into grafting plants for intensive seedling production.

2. Materials and methods

2.1. Preparation of substrates

Our seedling substrate was composed of peat:vermiculite:perlite at a ratio of 3:1:1 (v/v). The total density of the mixed substrate was 0.25–0.30 g cm^{-3}, the total porosity was 85.8%–87.0%, and the initial pH of peat was 5.0. The water holding capacity was 2.4 ml cm^{-3} to 3.0 ml cm^{-3}, and the moisture content of the substrate was 50%–55%. Substrate pH was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, and 8.0 by using quick lime composed of calcium oxide (Sangon Biotech, Shanghai, Co., Ltd.). The initial properties of the substrate were as follows: 0.58 g kg^{-1} available nitrogen (NH_4^+) (AN), 0.15 g kg^{-1} available phosphorus (AP), and 0.69 g kg^{-1} available potassium (AK). W1F, W2F, W3F, W4F, W5F, and W6F represented pH 5.0, 5.5, 6.0, 6.5, 7.0, and 8.0, respectively.

2.2. Plant materials

Muskmelon (*C. melo* L., cultivar 'Cuimi') plants, which exhibit growth retardation in the substrate when 50 or 100 μM Cd was added, were used as the nongrafted control in a greenhouse from October 01, 2017, to March 10, 2018, at the Anhui Academy of Agricultural Sciences. The hybrid of 'White pumpkin,' which is one of the most popular rootstocks commercially used in muskmelon grafting in Anhui Province, China, was obtained from a cross between 'Chinese pumpkin' and 'Yellow pumpkin' (Chinese *Cucurbita moschata* × India *C. moschata*) and used as the rootstock.

2.3. Sowing seeds

Seeds of the rootstock were sown in 23 g of substrate in 6 cm-diameter plastic pots under greenhouse conditions at the Anhui Academy of Agricultural Sciences 7–8 days earlier than the scion seeds to meet uniform shoot diameters for the grafting experiment. Muskmelon seeds were also sown in 23 g of substrate in 6 cm-diameter plastic pots under greenhouse conditions. The environmental conditions for germination were set at 24 °C–28 °C temperature and 85%–90% relative humidity.

2.4. Grafting method and management under greenhouse conditions

The tongue approach was used for grafting. In brief, grafting was performed after the first true leaf developed in the rootstock and scion. The hypocotyls of the rootstock and the muskmelon scion were cut with a sharp and gauzy blade and clipped together. Then, the grafted plants were placed under a plastic film at 25 °C–30 °C and more than 90% humidity in darkness. The grafted plants were exposed to sunlight 2–3 h per day until the scions were alive and normally grown after 7 days. Two Cd solution concentrations (50 and 100 μM) were added to the grafted plants of different substrate pH levels. More than 10 grafted plants in three replicates were used. The grafted plants under different treatments were transferred to the greenhouse in accordance with regular management. A N:P:K (1:3:3) solution (10 ml) was regularly supplemented in the substrate (after every 8 days) to satisfy the nutrient requirement of a growing grafted plant. The plants were cultivated through surface irrigation with day and night temperatures of 24 °C–28 °C and 15 °C–20 °C, respectively.

2.5. Vegetable seedling sampling

Ten grafted seedling plants were sampled from each treatment. Fresh leaves were collected from each of the 10 plants and stored at 4 °C before analysis. The roots were snipped from the grafted plant. Then, the shoots and the roots were oven dried for 3 days at 70 °C. The dried shoots and roots were measured for dry weight and stored for further analyses. More than 10 grafted plants in three replicates were used.

2.6. Detection of chlorophyll in leaves

The chlorophyll contents in the leaves of the grafted muskmelon plants were detected using a Konica Minolta SPAD-502 Plus chlorophyll meter (Konica Minolta, Inc., Japan) as previously described (Akiyama et al., 2001; Zhang et al., 2017a).

2.7. Determination of total phosphorus amount and Cd concentration

The total amount of phosphorus in the dried grafted plants was determined as previously described (Abbasi et al., 2011). The oven-dried samples (shoots and roots) were ground (0.5 mm) for analysis. Then, the ground shoot (200 mg) and root (100 mg) samples were digested in a solution of concentrated HNO_3 and $HClO_4$ (4:1, v/v). Subsequently, the volume was added to 10 ml by using double deionized water. The Cd content in the grafted plants was determined using an atomic absorption spectrometer (TAS-986, Beijing, China) in accordance with the method reported by Jiang et al. (2008). Reagent blank and analytical duplicates were arranged to ensure accuracy in the analysis process.

2.8. Microbial DNA extraction

The DNA was extracted from 0.5 g of fresh rhizosphere substrate in triplicate from each plastic pot by using FastDNA isolation kits (MP Biomedicals, USA) in accordance with the manufacturer's instructions. In addition, the quantity and quality of DNA extracts were determined using a NanoDrop™ 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA was stored at −80 °C for further analyses.

2.9. 16S rRNA gene amplification and Illumina MiSeq sequencing

Illumina MiSeq sequencing was performed as previously described (Shen et al., 2013). The primers F515 (5′-GTGCCAGCMGCCGCGG-3′) and R907 (5′—CCGTCAATTCMTTTRAGTTT-3′) were used to amplify the bacterial 16S rRNA genes (V4—V5). Polymerase chain reactions (PCRs) were performed as previously described (Sun et al., 2015). PCR was performed in 50 μl under the following conditions: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The PCR products were purified using a Qiagen gel extraction kit (Qiagen, Germany). Sequencing was performed using an Illumina MiSeq platform at a commercial biotechnology company (Biozeron, http://www.biozeron.com, Shanghai, China).

2.10. Sequence analysis

After pyrosequencing was performed, the technicians of Sino GenoMax wrote scripts to filter the sequences (Hu et al., 2012). The sequences were initially screened and eliminated from further analysis in accordance with the following criteria: length shorter than 200 bp, presence of a homopolymer longer than 6 bp, substantial mismatching bases in primer or barcodes, or quality score below 25 by using Sino GenoMax. The sequence with a high relative abundance in each operational taxonomic unit (OTU) was selected using the nucleotide nonredundant database of the National Center for Biotechnology Information in accordance with a previous report (Sun et al., 2015). These OTUs were determined using Mothur (http://www.mothur.org), and 97% identities were found. The most abundant sequence was chosen as a typical OTU (Sun et al., 2015).

2.11. Statistical analysis

The species richness of the samples was estimated using Chao1 and Shannon indexes, which were calculated using QIIME (Zhou et al., 2018). Principal component analysis (PCA) was performed to analyze the interrelationships between microbial communities of different samples (Wanapaisan et al., 2018). The LEfSe analysis, which emphasizes biological relevance and statistical significance, was performed as previously reported (Zhang et al., 2013). Canonical correspondence analysis (CCA) was conducted to analyze the effect of setting factors (e.g., pH, AN, AP, AK, and Cd^{2+} ion) on the bacterial community (Zhou et al., 2018). Data were analyzed through one-way ANOVA. The Shannon index was used to compare substrate bacterial number diversity. Mean analysis was conducted using one-way ANOVA followed by Fisher's LSD test at $p < 0.05$ (*) and $p < 0.01$ (**) by using SPSS version 19.0 (SPSS Inc., USA).

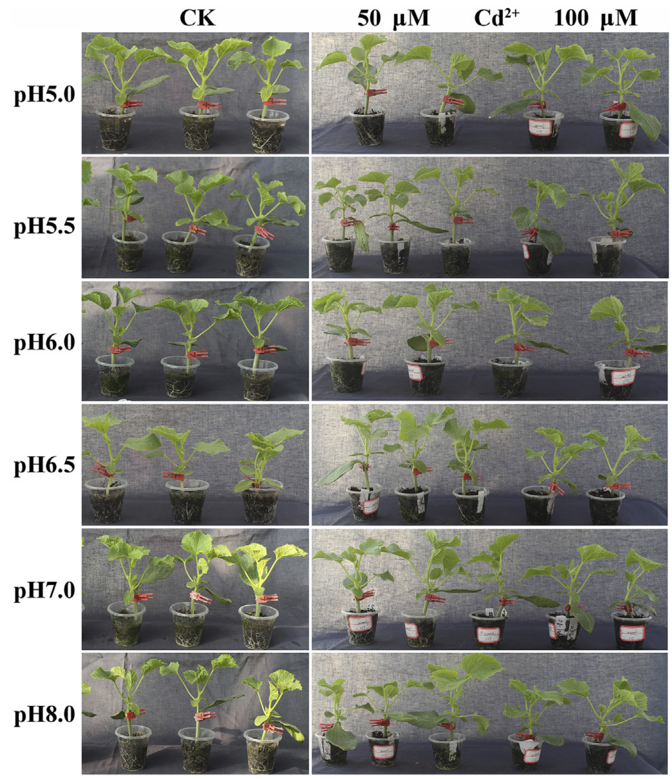

Fig. 1. Grafted plants grown in six treatments (pH 5.0, 5.5, 6.0, 6.5, 7.0, and 8.0). Two groups of substrates were supplemented with 50 and 100 μM Cd. Six pH levels were set. The initial pH of the substrate was 5.0, and this level was adjusted with quick lime to pH 5.5, 6.0, 6.5, 7.0, and 8.0. After the grafted plants were sampled, the pH levels of the grown substrates were detected.

3. Results

3.1. Change in substrate pH

After the grafted seedlings grew, the pH of all of the substrates, except those with pH 5.0, decreased. The reduction rate in the substrate with pH 6.0 was the most significant among the samples. The reduction in the substrate with pH 8.0 was also significant. Supplementing the substrates with 50 or 100 μM Cd exerted no significant effect on the substrate pH (Supplemental Fig. 1a).

3.2. Grafted plant growth analysis at six pH levels

The control plants grown at six pH levels were excellent and showed no significant difference in appearance (Fig. 1). The grafted muskmelon treated with a low Cd concentration (50 μM) at pH 6.5 grew well but grew weakly at pH 8. At a high Cd concentration (100 μM), all of the treated grafted muskmelon plants were relatively weaker than the control and the plants exposed to a low Cd concentration (50 μM).

3.3. Chlorophyll content

The addition of 50 μM Cd in the substrate increased the chlorophyll content of the leaves compared with that of the control. The chlorophyll content of the plants at a high Cd concentration (100 μM) decreased in the substrates treated with pH 6.0 and 8.0 (Supplemental Fig. 1b). In general, the chlorophyll content of each set was the same. In particular, the highest chlorophyll content was found at pH 6.5, whereas the lowest chlorophyll contents were detected at pH 5.0 and 8. At a high Cd concentration, the chlorophyll content of the muskmelon leaves decreased but not to a significant level.

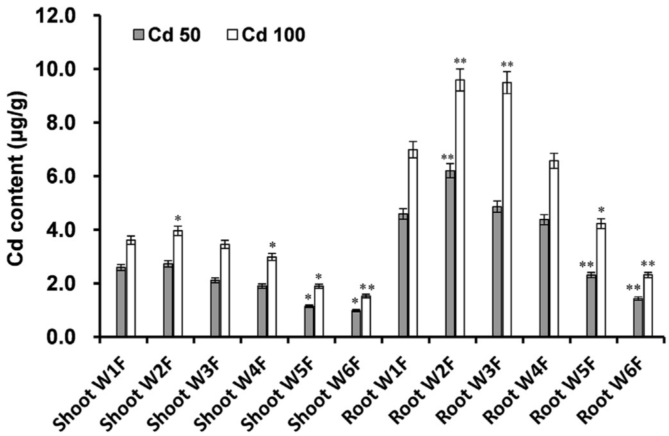

Fig. 2. Cd concentrations in the shoot and root tissues of the grafted plants grown in six treatments. Significant differences were tested with Fisher's protected LSD at $*p \leq 0.05$ and $**p \leq 0.01$. Values were written as means ± standard deviations of three replicates. W1F, W2F, W3F, W4F, W5F, and W6F represented pH 5.0, 5.5, 6.0, 6.5, 7.0, and 8.0, respectively.

3.4. Shoot and root dry weights

Among the six pH levels, pH 5.5 showed the lowest shoot dry weights (380.4 mg) in the high Cd treatment (Supplemental Fig. 2a). The treatment with pH 8.0 showed a high shoot dry weight (540.11 mg) in the control. The root dry weights were the same among the six treatments without Cd addition. However, the root dry weights (34.7 mg) significantly ($p < 0.05$) decreased at pH 6.5 at 100 µM Cd compared with that of the control (44.53 mg). The highest root dry weight was 48.25 mg at pH 6.5 without the addition of Cd (Supplemental Fig. 2b). The addition of 100 µM Cd affected the root growth of the grafted muskmelon plants.

3.5. Cd concentration in grafted plants

The absorption of Cd in the shoots and roots of the grafted plants fluctuated on the basis of the change in substrate pH (Fig. 2). The concentration of Cd was the highest in roots and shoots at pH 5.5 (2.73 and 3.96 µg g^{-1}, respectively) and 6.19 and 9.59 µg g^{-1} in roots at 50 and 100 µM, respectively. Although the Cd content in the shoots and roots decreased at pH 6.0, the absorbed amount of Cd was similar to that at pH 5.5. The Cd concentrations of the shoots were 2.11 and 3.45 µg g^{-1} at 50 and 100 µM, respectively, and the Cd concentrations of the roots were 4.85 and 9.49 µg g^{-1} at 50 and 100 µM, respectively. However, when pH was 8.0, the Cd contents in the shoots (0.97 and 1.52 µg g^{-1}) and roots (1.43 and 2.31 µg g^{-1}) were low at 50 and 100 µM, respectively.

In both Cd concentration treatments, the amount of Cd in the roots was significantly higher than that in the shoots at the same pH level. Increasing the pH decreased the root-to-shoot ratio of Cd. In the 50 µM-treated grafted plants, the lowest root-to-shoot ratio was 0.52 (root, pH 8.0: shoot, pH 5.5), whereas the highest ratio was 6.36 (root, pH 5.5: shoot pH 8.0) (Table 1). In the 100 µM-treated grafted plants, the lowest root-to-shoot ratio was 0.58 (root, pH 8.0: shoot, pH 5.5), whereas the highest ratio was 6.30 (root, pH 5.5: shoot, pH 8.0) (Table 2). After analyzing the 100 µM–50 µM treatment ratios, we found that the largest value was 2.16 (root, pH 6.0: shoot, pH 5.0) (Supplemental Table 1). All of the root-to-shoot ratios of Cd at 100 µM–50 µM were higher by 1.

3.6. Diversities of bacterial communities

The diversity index of the W2-50, W2-100, and W3-50 treatments had higher bacterial Shannon indexes than those of the other treatments (Supplemental Fig. 3a). The Su1 and Su6 treatments had the lowest bacterial Shannon indexes. The Chao index of the W3-50, W5-100, and W5F treatments yielded the highest bacterial Chao indexes among 18 samples, whereas the Su1 and Su6 treatments had the lowest bacterial Chao index (Supplemental Fig. 3b).

3.7. Compositions of bacterial communities

Before the seedlings were grown, the abundance of the members of *Proteobacteria* was the lowest and accounted for 38.62% when the initial substrate pH was 5.0 (Fig. 3). Increasing the pH gradually increased the count to 64.64% during seedling growth. At the end of the grafting experiment, this phylum increased to its maximum and accounted for 67.84% at pH 7.0 at 50 µM Cd. In other treatments, the change in bacterial count (50%–60%) was not highly significant. The members of the phylum *Firmicutes* accounted for 39.68% in the initial substrate of pH 5.0. Increasing the pH level significantly decreased the bacterial count to 10.20% at Su6 with pH 8.0. At 100 µM, the abundance of bacteria from *Firmicutes* was 3.95% when the substrate pH was increased to 8.0. Increasing the pH level also significantly increased the abundance of the members of *Bacteroidetes* from 3.51% at the initial substrate to 20.99% at pH 6.0. In other treatments, the abundance of bacteria considerably increased. The Cd concentration did not affect the abundance of the members of *Bacteroidetes*.

Changes in the abundance of *Actinobacteria* fluctuated relative to pH. The value was only 6.90% at pH 5.0. The highest value was 10.37% at pH 7.0, whereas the lowest value was 3.49% in the substrate with 50 µM Cd. At 50 µM, the highest count (9.44%) was found at pH 6.0. At 100 µM, the highest level of *Actinobacteria* was 10.21% at pH 6.0. *Gemmatimonadetes* also significantly increased. However, such increase only accounted for 1.41% in the initial substrate and had the lowest value of 0.79% at pH 8.0. The treatments that produced the highest abundance of this phylum were W5-100 (pH 7.0, 100 µM Cd), W5F (pH 7.0), and W5-50 (pH 7.0, 50 µM Cd), which accounted for 9.18%, 8.96%, and 7.58%, respectively.

Cyanobacteria accounted for 0.68% at the initial substrate at pH 5.0 and gradually decreased as pH increased. However, this outcome was undetected in the initial substrate at pH 8.0. The highest proportion (10.49%) was detected in the 50 µM-treated grafted plants with a substrate pH of 8.0. The difference between other treatments was not evident and ranged from 2.50% to 6.00%. *Chloroflexi* accounted for 2.67% in the initial substrate at pH 5.0. The lowest value (0.86%) was found in the substrate at pH 8.0, and the highest value (5.27%) was detected at pH 6.0 with 50 µM Cd treatment. The pH level slightly affected this phylum.

The abundance of the phylum *Acidobacteria* gradually decreased from 5.25% to 0.69% as substrate pH increased. After the grafted plants were grown, the abundance of this phylum decreased from 5.62% to 0.54%. This phylum decreased in abundance from 6.29% to 1.04% at 50 µM Cd and increased from 7.51% to 1.09% at 100 µM Cd. The heatmap results showed the high abundance of bacteria (Supplemental Fig. 4). The increase in pH considerably enhanced the abundance of *Bacteroidetes* and *Actinobacteria*.

3.8. PCA and LEfSe analyses

PCA revealed that the bacterial community compositions were separated by pH level (Supplemental Fig. 5). Four groups were clustered through LEfSe analyses, and 83 supposed microbes were selected and considered the key ones in the four types of substrates. The microbiota of each substrate in each group was compared through LEfSe to identify the specific microbial groups associated with different pH levels between Su1 and W-100 treatments (Fig. 4). A few OTUs were also identified, and they varied between the four treatments in the different pH substrate groups.

Table 1
Ratio of Cd content in the grafted muskmelon root and shoot at 50 μM treatment.

Shoot Root		5.0	5.5	6.0	6.5	7.0	8.0
		2.59 ± 0.06	2.73 ± 0.09	2.11 ± 0.05	1.90 ± 0.03	1.14 ± 0.02	0.97 ± 0.04
5.0	4.58 ± 0.05	1.77 ± 0.02[a]b	1.68 ± 0.04b	2.17 ± 0.06 ab	2.42 ± 0.05b	4.02 ± 0.11 ab	4.70 ± 0.10b
5.5	6.20 ± 0.01	2.39 ± 0.04a	2.27 ± 0.09a	2.94 ± 0.07a	3.27 ± 0.03a	5.43 ± 0.13a	6.36 ± 0.15a
6.0	3.15 ± 0.04	1.22 ± 0.08bc	1.16 ± 0.06bc	1.49 ± 0.04	1.66 ± 0.08bc	2.76 ± 0.08bc	3.24 ± 0.08
6.5	4.37 ± 0.10	1.69 ± 0.06b	1.60 ± 0.01b	2.07 ± 0.02b	2.30 ± 0.05b	3.83 ± 0.09b	4.48 ± 0.11b
7.0	2.31 ± 0.11	0.89 ± 0.03c	0.85 ± 0.03c	1.09 ± 0.04c	1.22 ± 0.06bc	2.02 ± 0.05c	2.37 ± 0.09c
8.0	1.43 ± 0.07	0.55 ± 0.05c	0.52 ± 0.05c	0.68 ± 0.06cd	0.75 ± 0.02c	1.25 ± 0.02cd	1.47 ± 0.05cd

[a] Number = Cd in root/Cd in shoot. Same letters indicate non-significant differences.

4. Discussion

4.1. Variations in pH level and chlorophyll content

Grafting is a unique and practical technology that allows growers to breed robust seedlings (Justus and Kubota, 2010; Penella et al., 2017), and the substrate is the main component for the production of grafted plants (Ribeiro et al., 2007). Analyzing the effect of substrate pH on the growth of grafted muskmelon plants can help grow healthy seedlings. In this study, no significant change was found among the six substrates with six pH levels at pH 5.0, 5.5, 6.5, and 7.0. Large variations were observed at pH 6.0 and 8.0 (Supplemental Fig. 1a). The grafted plants grew well in all of the six treatments with different pH levels, suggesting that rootstock was adaptive to pH variation. A similar observation was reported in the grafted tomato plants, whose dry biomass does not remarkably vary at different pH levels (Borgognone et al., 2013). Nevertheless, our results suggested that rootstock could adapt to a larger range of pH, even though the rootstocks were different. The addition of Cd had no significant effect on the growth of the grafted muskmelon in all of the six substrates. However, the addition of 50 μM Cd increased the chlorophyll content (Supplemental Fig. 1b), whereas the addition of 100 μM Cd decreased the chlorophyll content at pH 6.0 and 8.0. Cd can affect photosynthesis and inhibit enzymatic activity in plants (Ji et al., 2017; Pereira de Araújo et al., 2017). Sunflower photosynthetic processes are considerably affected only in plants grown in the presence of Cd (Di Cagno et al., 2001). Our observations indicated that a high Cd concentration suppressed plant chlorophyll content and impaired plant root growth (Supplemental Fig. 1b).

4.2. Cd concentration and uptake between shoots and roots

Cd is a hazardous heavy metal (Karri et al., 2018) that threatens human health and restrains the regeneration and growth of plants (Belimov et al., 2005; Li et al., 2017; Xiao et al., 2015). Our study showed that the Cd concentrations in the shoot and root tissues varied considerably among the different pH levels (Fig. 2). The highest Cd content was obtained at pH 5.5 in the shoot and roots, and this value was close to the Cd content at pH 6.0. This result suggested that these pH levels were favorable to substrates that absorbed Cd for grafted muskmelon plants. The Cd adsorption capacity of bamboo charcoal increases as pH increases, and the optimum pH for Cd removal is 8.0 (Wang et al., 2010). Conversely, our study demonstrated that less Cd was detected in the shoots and roots at pH 8.0 (Fig. 2), indicating that the increase in substrate pH level decreased the Cd uptake of the grafted muskmelon plant because of the ability of high pH to immobilize Cd. Moreover, grafting can reduce Cd uptake in muskmelon. Grafted watermelon exhibits a strong capacity to inhibit Cd accumulation in aerial parts (Shirani Bidabadi et al., 2018). Our results addressed the scientific queries regarding Cd immobilization by regulating pH levels in breeding substrates. We confirmed that a low pH could increase the Cd activity, whereas a high pH in substrates immobilized Cd.

The concentration of Cd was higher in the root than in the shoot tissue at all of the pH levels in this study. Similarly, Wilhelm et al. (2000) observed that the Cd concentrations in the roots are much higher than those in the shoots of three white lupine plants. Although we used different plants, the same conclusion was obtained. The adsorption of Cd by the roots is considered a key process in the overall plant Cd accumulation, and the active uptake of Cd into roots has been demonstrated in various plants (Chan and Hale, 2004). Our study further revealed that Cd in the shoots and roots of the grafted muskmelon plants was positively correlated with substrate pH. These observations indicated that the variations in Cd uptake in the grafted muskmelon plants grown in the substrates were directly correlated with pH. Using biochar to increase soil pH can enhance Cd immobilization (Bashir et al., 2018), suggesting that Cd uptake in plants can be reduced by increasing substrate pH. Therefore, a decrease in Cd in the shoots and roots of a grafted plant at pH 8.0 is likely due to the immobilized Cd in substrates, considering that pH is an important parameter affecting the mobility of heavy metals in soils (Houben et al., 2013). Our results suggested that regulating the appropriate pH in substrates could help obtain safe high-quality grafted muskmelon plants even when they were grown in Cd-contaminated substrates or soils.

4.3. Diversities of bacterial communities in the grown substrate

Soil pH is an important parameter to study the biological communities in soils (Zhalnina et al., 2015). Under field or greenhouse conditions, soil pH can be adjusted to high or low by using quick lime or

Table 2
Ratio of Cd content in the grafted muskmelon root and shoot at 100 μM treatment.

Shoot Root		5.0	5.5	6.0	6.5	7.0	8.0
		3.61 ± 0.11	3.96 ± 0.09	3.45 ± 0.13	2.98 ± 0.10	1.89 ± 0.08	1.52 ± 0.04
5.0	6.99 ± 0.06	1.94 ± 0.05[a]ab	1.76 ± 0.02 ab	2.03 ± 0.05 ab	2.34 ± 0.01 ab	3.69 ± 0.10b	4.60 ± 0.18b
5.5	9.59 ± 0.04	2.66 ± 0.05a	2.42 ± 0.05a	2.78 ± 0.06a	3.21 ± 0.09a	5.07 ± 0.14a	6.30 ± 0.14a
6.0	9.49 ± 0.05	2.63 ± 0.03a	2.40 ± 0.07a	2.75 ± 0.04a	3.18 ± 0.08a	5.01 ± 0.08a	6.24 ± 0.09a
6.5	6.57 ± 0.07	1.82 ± 0.07 ab	1.66 ± 0.02 ab	1.90 ± 0.05 ab	2.20 ± 0.05 ab	3.47 ± 0.11b	4.32 ± 0.08b
7.0	4.22 ± 0.03	1.17 ± 0.03b	1.07 ± 0.03b	1.22 ± 0.03b	1.41 ± 0.03b	2.23 ± 0.05c	2.78 ± 0.06c
8.0	2.31 ± 0.04	0.64 ± 0.01bc	0.58 ± 0.01bc	0.67 ± 0.01bc	0.77 ± 0.05bc	1.22 ± 0.07cd	1.52 ± 0.03d

[a] Number = Cd in root/Cd in shoot. Same letters indicate non-significant differences.

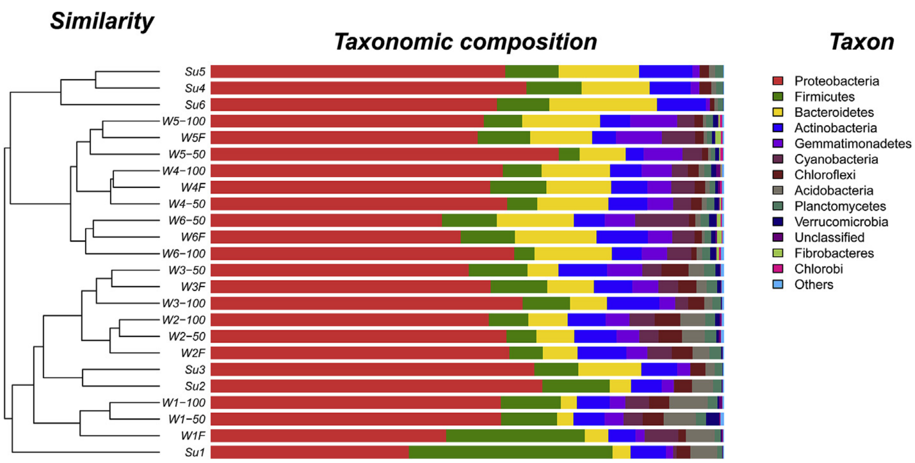

Fig. 3. Compositions of bacterial communities in all of the substrates illustrated using Treebar at the phylum level.

humic acid in acidic pH (Malik et al., 2018; Wang et al., 2009; Zhou et al., 2019). The diversity of soil bacterial communities can be predicted through soil pH (Fierer and Jackson, 2006; Khaled et al., 2018). In our study, Shannon and Chao index analyses indicated that the substrate at pH 5.0 showed substantially decreased bacterial richness. The predominant bacterial phyla detected through high-throughput sequencing were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Gemmatimonadetes*, and *Chloroflexi* (Fig. 3). Our results reflected the main phyla present in the substrate and corresponded to those of previous studies based on agricultural soils (Embarcadero-Jiménez et al., 2016). These results suggested that the predominant bacteria colonized the soil and the grown substrate. *Proteobacteria copiotroph* is favored by nutrient-rich conditions with a high carbon content (Newton and McMahon, 2011). The abundance of *Acidobacteria* in soil is often high under low organic matter conditions and negatively correlated with soil pH (Jones et al., 2009). Our study also demonstrated that several OTUs belonging to *Proteobacteria* and *Bacteroidetes* were significantly enriched at different substrate pH levels. On the contrary, *Firmicutes*, *Acidobacteria*, and *Chloroflexi*, which participate in the degradation of plant-derived compounds, such as cellulose (Wang et al., 2018), significantly decreased as the substrate pH increased (Fig. 3). Soil pH or spatial distances are mainly determined by soil bacterial diversity (Tripathi et al., 2018; Zhang et al., 2017b). LEfSe analyses (Fig. 4) revealed that bacterial communities from each treatment significantly differed. In particular, the number of specific microbial groups (in blue) in W-50 was more than that in the three other treatments, suggesting that bacteria in W-50 not only responded to Cd stress but also positively adapted to various pH substrates. The number of microbial groups (in green) in W-100 was lower than that in the other treatments, suggesting that a high Cd concentration could significantly reduce the bacterial OTUs. After the seedlings were grown without adding Cd, the number of microbial groups (in purple) in the substrates (WF) was more than that in the

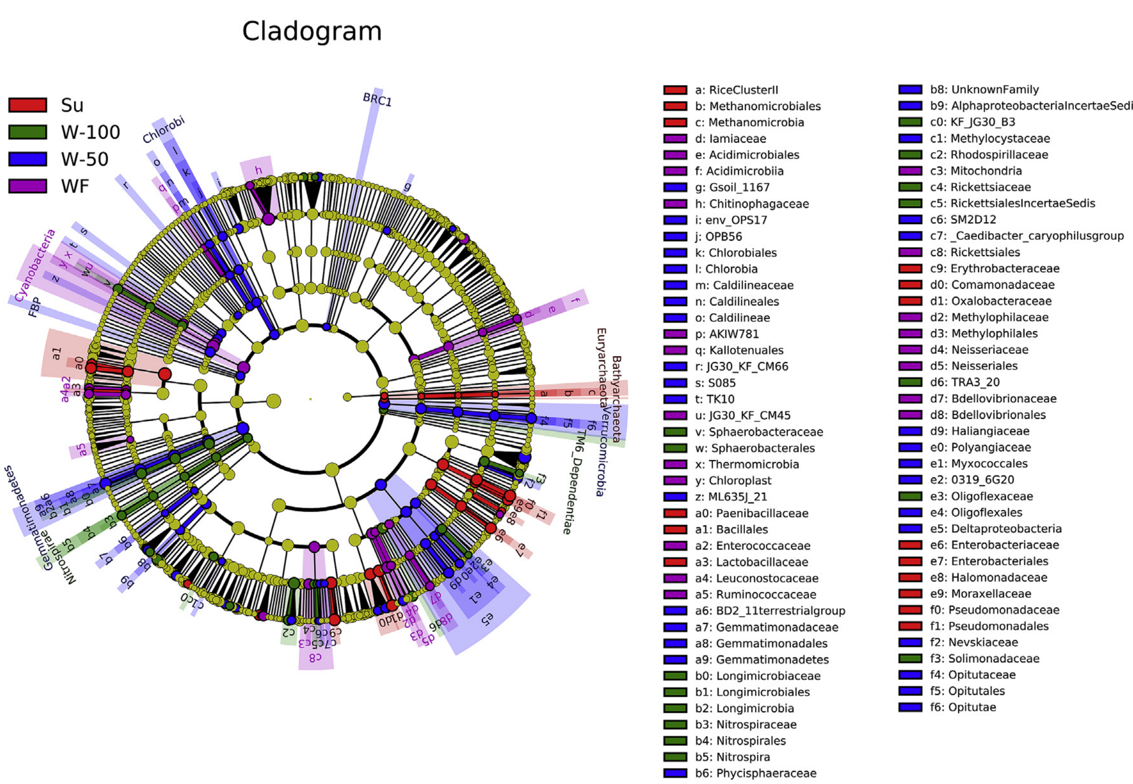

Fig. 4. LEfSe analysis of all of the substrates. Four groups were illustrated with different colors. Each color indicated the key bacterial phylum in each treatment.

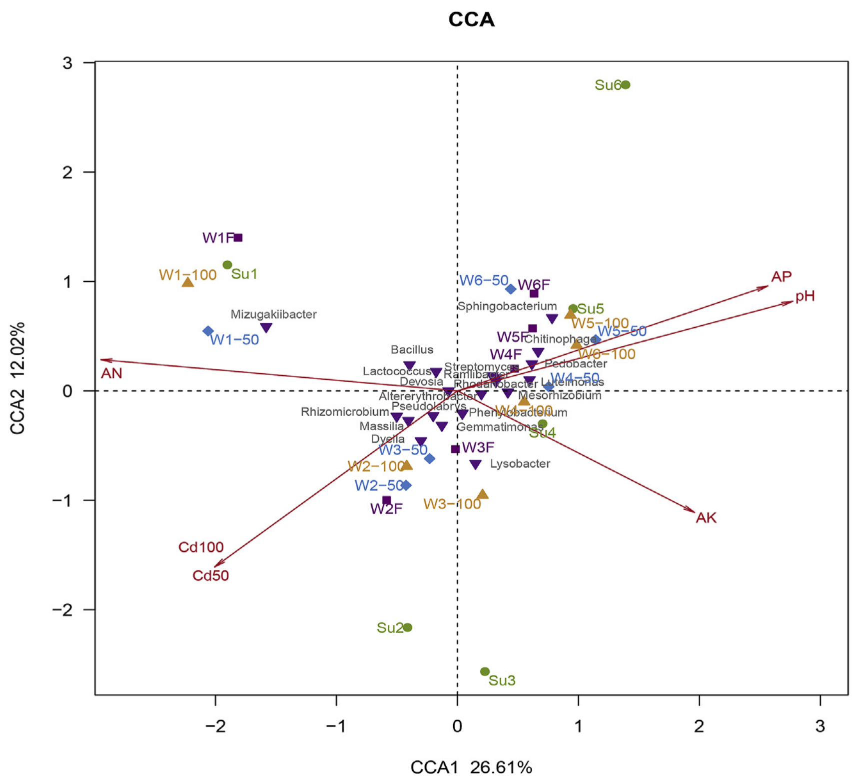

Fig. 5. Canonical correspondence analysis of bacterial communities in different substrates related to environmental variables. Environmental factors included available nitrogen (AN), available phosphorus (AP), available potassium (AK), Cd concentration, and pH.

initial substrate (Su) used before the seedlings were grown, implying that numerous beneficial bacteria would colonize the rhizosphere of seedling plants. In summary, our findings provided evidence that the pH of substrates influenced the microbial diversity in the breeding substrates. Another study showed that pH is a strong universal predictor of bacterial community structures in acidic soils and alkaline sediments (Jinbo et al., 2012). Our study demonstrated that pH affected microbial community structure in the whole root rhizosphere of grafted muskmelon plants grown in breeding substrates. The CCA results showed that pH, AN, AP, and Cd were strongly linked to bacterial community compositions, whereas AK was weakly correlated with the bacterial structure (Fig. 5) in the substrates with different pH levels. Notably, pH exhibited the strongest correlation with the microbial community in the substrates. Similar to soil condition, bacterial diversity is predominantly determined or positively correlated with pH (Sun et al., 2015; Yuan et al., 2018). This result confirmed that pH contributed to the formation of microbial community compositions not only in soils but also in breeding substrates. This study provided baseline information for growing seedlings in substrates with different pH levels.

5. Conclusion

This study investigated the effects of pH on Cd accumulation in grafted muskmelon plants grown in a substrate. Cd accumulation greatly varied in the grafted muskmelon plants studied under low and high pH conditions. The pH level of the substrate played an important role in Cd uptake and accumulation in the grafted muskmelon plants. Cd accumulation in the shoots of the grafted plants was lower than that in the roots. The significant reduction in Cd accumulation at high pH might be mainly due to a decrease in Cd mobility in the substrates. *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* were significantly enriched ($p < 0.05$) at different substrate pH levels. On the contrary, *Chloroflexi* and *Acidobacteria* significantly decreased. The pH levels, AP, AN, and Cd were strongly correlated with the bacterial composition in the substrates. Our study suggested that we could significantly reduce Cd accumulation in grafted muskmelon plants by selecting the appropriate substrate pH level. This work provided insights into the effect of substrate pH and Cd concentration on the growth of grafted plants and could serve as a reference for growing safe and high-quality grafted plants under Cd-contaminated conditions.

Conflicts of interest

The authors confirm that this article content has no conflict of interest.

Authors' contributions

ZJ and WPC conceived and designed the experiments. ZJ, WPC, THM, XQQ, ZSJ and JHK performed the experiments. ZJ and WPC analyzed the data. ZJ and WPC wrote the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.03.013.

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