INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19F–194/2020/23–6–1051–1060
DOI: 10.17957/IJAB/15.1385
http://www.fspublishers.org

Full Length Article

Nutrient Uptake and Bacterial Structures Response to Varied pH Substrates in Grafted Melon Plant

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Received 10 December 2019; Accepted 20 December 2019; Published 02 April 2020

Abstract

Nutrient uptake is one of the most important processes for plant growth and soil microbes regulate the processes of nutrient cycling. We studied the influence of varied pH substrates on grafted melon (*Cucumis melo* L.) plants' growth and root bacterial communities. Six pH levels of substrates and two pumpkin rootstocks were selected to evaluate the impact of pH on the grafted melon plants' growth. Nutrient uptakes in nongrafted and grafted plant were measured, and Illumina MiSeq sequencing was performed to analyze the microbial community structures. pH considerably influenced the nutrient uptake of grafted melon plants. Using two rootstocks can significantly increase the nitrogen (N), phosphorus (P), and potassium (K) absorption in the scion or rootstock section of the grafted seedling plant regardless of pH levels in substrates, and many nutrients were absorbed at the pH range of 6.0 to 6.5. Few nutrients were taken up under low (5) or high pH (8). N, P, and K in grafted plant increased by 3.87-, 4.42- and 3.60-fold higher than those in nongrafted melon plant. The phyla *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Gemmatimonadetes* were the most predominant bacteria. The pH values and available N and P were considerably linked to bacterial community compositions. Our data demonstrated that pH played a vital role in the nutrient uptake of grafted melon plants and shifts bacterial structures in substrates when using pumpkin as rootstocks. Our study provides an example illustrating the importance of nutrient uptake of grafted seedlings in varied pH substrates.

Keywords: Bacterial communities; Grafted plant; Nutrient uptake; pH values; Substrate

Introduction

The plant–soil system is one of the most important components not only in agricultural but also in natural ecosystems (Zhang *et al.* 2017a). At present, plant–soil system is related to the inorganic nutrition of plants, such as movement of nutrients to roots or leaves, which is vital for plant growth (Okamoto *et al.* 2016). Many interactions exist between nutrient supplies, and the transport of one nutrient affects another's absorption and utilization (Zhang *et al.* 2017a). Nutrients such as nitrogen (N), phosphorus (P), and potassium (K) are the essential elements that limit plant growth in various natural ecosystems, including soil and water environments (Lambers *et al.* 2008). N and P are the most important nutrients for plant growth and development in soil (Dordas 2008; Glæsner *et al.* 2019) but also widely considered to limit primary productivity in tropical plants (Turner *et al.* 2018). Therefore, nutrient uptake in plants under different conditions must be analyzed to improve plant growth, especially facing varied pH substrates.

Melon plant (*Cucumis melo* L.) is widely known as an economically important crop and has a growing acceptance contributing to a huge potential need in markets worldwide (Silberstein *et al.* 1999). Fresh-cut melon has a good taste after several days of storage (Aguayo *et al.* 2008). However, bitter melon considerably improves glucose metabolism and ameliorates obesity (Snee *et al.* 2011). Numerous commercial melon varieties and few wild-types can be continuously cultivated under greenhouse or field conditions (Pandey *et al.* 2018). Consequently, considerable development has been made toward breeding melon varieties not only in China but also in other countries, such as Turkey, Iran, Egypt, Korea, and USA in the past decades (Wang *et al.* 2018). Approximately half of the total amount of melon is produced in China (Vendruscolo *et al.* 2017), and approximately 8 million t of melon are being supplied annually since 2000 (Yang *et al.* 2007). Melon cultivation has now become an important way to increase income. At present, many research groups attempted to establish new varieties of melon crops by improving fruit quality and enhancing flavor (Zhang *et al.* 2010b; Murakami *et al.* 2017). Along with the considerable demand for melon consumption is melon seedling production and planting also have considerable requirement.

Melons are increasingly being produced under greenhouse conditions, thereby contributing to improved fruit quality, early harvest, and high yield (Huang *et al.* 2017; Murakami *et al.* 2017). However, the melon plant cannot be cultivated in the greenhouse soil constantly because of soil-borne pathogens, especially those caused by *Fusarium* spp. (Gava and Pinto 2016; Seo and Kim 2017). Thus, melon production has relied on the grafting of their seedlings to rootstocks to produce high yield. At present, grafting plays not only an essential but also has an extremely important role in melon production and becomes a necessary requirement (Kubota 2008; Lee *et al*. 2010; Mohamed *et al.* 2014). Grafting is not only used to control soil-borne diseases but also to enhance plant vigor and prolong the harvesting period, thereby increasing fruit yield and quality (Nawaz *et al.* 2017). For instance, melon scions can be grafted onto rootstocks to control root-knot nematode infection or improve abiotic stress tolerance (Liu *et al.* 2015). However, although grafting with squash rootstock from an interspecific hybrid can accelerate fruit development, it diminishes the fruit quality of melon (Wenjing *et al.* 2015). The N use efficiency of watermelon scion can be improved using pumpkin rootstock (Nawaz *et al.* 2017). Thus, rootstocks have a considerable potential function in regulating melon grafting plants. At present, improving nutrient uptake and use efficiency in crop plants in various environmental conditions still remains a challenging aim (Zhang *et al.* 2010a). A suitable rootstock must be selected to improve nutrient acquisition for grafting melon plant growth. Here, we attempted to use two rootstocks to explore nutrient uptake in grafted melon plant.

Instead of soil condition, the properties of substrate directly influence the grafted vegetable plants' growth (Martínez-Ballesta *et al.* 2008). Substrate can be composed of peat, vermiculite, and pearlite or other suitable materials (Tang *et al.* 2010; Zhang *et al.* 2017b). For instance, spent mushroom substrates can be used as a component of growth media for tomato, lettuce, or melon seedlings (Tam and Wang 2015). These works have made valuable attempts in substrate production. However, the substrate's physical and chemical characteristics will influence seedling growth undoubtedly. The effect of substrate on the growth of seedling plants have been evaluated (Tam and Wang 2015; Marín-Guirao *et al.* 2016; Yang *et al.* 2018). Although high or low pH is a vital criterion during substrate preparation for seedling production (Ryan *et al.* 2016), the coping mechanisms of grafted plants against varied pH substrates still remains unclear. Thus, the properties of substrates must be further determined, and research on the substrates may provide valuable information on how to improve seedlings. Nevertheless, the nutrient uptake of the grafted plants in substrates with varied pH values has been rarely explored. Hence, we attempted to analyze the impact of pH on grafted melon plants and clarify further whether microbial community structures would respond to pH in substrates.

We satisfied the requirements in the production of grafted melon plants and increase yield to satisfy market demand only by carefully selecting the suitable properties of substrates and rootstocks. Additional comprehensive studies on melon grafting plants must be conducted to illustrate the role of nutrient uptake among different rootstocks. Therefore, the main objectives of this study were (1) to evaluate whether different pH values in substrate improve nutrient uptake in grafted melon plant by using two different rootstocks and (2) confirm whether microbial structures respond to the selected substrates and rootstocks in grafted melon plants. Our study broadened our current perception in the effect of pH in the production of grafted plants and provided further insights into rootstock selection to improve nutrient use efficiency.

Materials and Methods

Plant materials

Melon (*Cucurbita melo* L., cultivar "Cuimi") was used in this study under greenhouse condition from June 05, 2017, to May 30, 2018, at the basement of Anhui Academy of Agricultural Sciences. Two rootstocks, namely, "yellow pumpkin" (India *C. moschata*, abbreviated as YF) and a hybrid of "white pumpkin," which was selected from hybrid progenies between one variety of "Chinese pumpkin" and another one "yellow pumpkin" (Chinese *C. moschata* crossed with India *C. moschata* (WF), were used in this study.

Substrate preparation

In this study, experimental seedling substrate was initially composed of peat, vermiculite, and perlite (3:1:1, v/v). The substrate pH was 5.0 and altered to six levels (5.0, 5.5, 6.0, 6.5, 7.0, and 8.0; abbreviated as Su1 to Su6, respectively) by using calcium oxide. Before the use of the substrate, its moisture content was adjusted to 50%–55%. The mixed substrate contained 0.58, 0.15, and 0.69 g kg^{-1} available N (NH_4^+, AN), P (AP), and K (AK, respectively. Two rootstocks, namely, WF and YF that grew in the six pH substrates were abbreviated as W1F to W6F and Y1F to Y6F, respectively.

Melon scion and rootstock preparation

A total of 23 g substrate was placed in a 6 cm diameter plastic pots, and the seeds of the rootstock were sown in pots under greenhouse conditions. The rootstock was prepared 7 days earlier than melon. The melon seeds were also sown in the same plastic pots with the same amount of substrate. The greenhouse conditions were kept at the temperature ranging from 24°C to 28°C. During the seed sprouting time, the humidity was adjusted between 85 - 90% for germination.

Grafting and greenhouse management

Tongue approach, which is common method that used in grafting, was used for grafting. A stand of grafting was processed as follow. When the first true leaf unfolded in the scion and rootstock, grafting was performed. The hypocotyls of the rootstock were first cut in the pots, and then the melon scions were cut and clipped together with rootstock by using a plastic clip. Then, the grafted plants were kept in a chamber and covered with a plastic film at the temperature ranging from 25°C to 30°C. Meanwhile, humidity was maintained at > 90%, and the seedlings were placed in the dark. After 7 days, the grafted plants were exposed to sunlight 2 or 3 h per day. All grafted plants were alive and then transferred to the greenhouse. To meet the later growth, we added a nutrient solution (composed of N, P, and K at a ratio of 1:3:3, 10 mL) in the substrate (8 days per time). The grafted plants were under greenhouse management, and water was sprinkled by surface irrigation. The temperature ranges were 24°C to 28°C at daytime and 15°C to 20°C at night. More than 10 grafted plants were sampled and used in 3 replicates for further analysis.

Melon seedling sampling

Ten nongrafted and grafted seedling plants were collected from different treatments. The rootstock sections from the grafted point were snipped, and the scion sections were also kept. Then, the scions and the rootstocks were oven dried for 3 days at 70°C until their weight remained constant. Afterward, the shoots and roots were measured and stored for further analysis. More than ten grafted melon plants in three replicates were used for further analysis.

Determination of total N, P and K amount

Dried non-grafted and grafted plant samples were ground to powder separately in a Wiley mill. In the grafted plant, scion and rootstock sections were ground separately. Subsequently, 0.5 g comminuted tissues was analyzed for N and K contents following a previously reported method (Rashid *et al.* 2016). The total K content was analyzed in the filtrate by using flame atomic absorption spectrophotometry. The total P content of the plants was determined according to a previously reported method (Abbasi *et al.* 2011). Approximately 5 mL of H_2SO_4 was added into the digestive tract. Then, 2 mL of H_2O_2 was added. After intense reaction, heating was continued for 5 min to remove excess H_2O_2. After cooling, the digested liquid was transferred into a 100 mL bottle.

Microbial DNA extraction

In each plastic pot, approximately 0.5 g fresh rhizosphere substrate was used to extract total DNA. In this study, Fast DNA kits (MP Biomedicals, U.S.A.) was used according to the manufacturer's instructions. Afterward, a Nano Drop™ 2000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA) was used to determine DNA quantity and quality. To improve our analysis, we stored the DNA samples at -80°C.

Gene amplification and sequencing

To amplify the 16S rRNA V4–V5 genes from bacteria, we used the primers F515 (5′ -GTGCCAGCMGCCGCGG-3′) and R907 (5′ -CCGTCAATTCMTTTRAGTTT-3′). The DNA samples that were previously stored at −80°C were used as templates, and the polymerase chain reactions (PCRs) were operated following a previously reported method (Sun *et al.* 2015) under the following conditions (50 μL). In brief, the reaction was allowed to proceed at the initial temperature of 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and finally, at 4°C. To increase the quality of analysis further, we purified the PCR products (Qiagen Gel Extraction Kit, Germany) as the stand processes. Illumina MiSeq sequencing was handled as previously described (Shen *et al.* 2013). Then, sequencing was accomplished (SRP234750) using an using an Illumina MiSeq platform (Biozeron Company, http://www.biozeron.com, Shanghai, China).

Sequence analysis

When pyrosequencing was finally accomplished, the sequences were analyzed by the following processes, and several standards were set to screen the sequences (Hu *et al.* 2012). On the basis of the sequencing results, we used the following criteria: sequences with the length of < 200 bp were initially filtered and excluded from further analysis, a homopolymer longer than 6 bp were removed, and substantial mismatching bases or barcodes or those with a quality score of <25 as detected by Sino GenoMax were excluded. The sequence of each operational taxonomic unit (OTU) exhibiting high relative abundance was further analyzed using the nucleotide database according to a previously reported method (Sun *et al.* 2015). The Mothur software was used to determine and analyze these OTUs (http://www.mothur.org), and 97% identities were covered. A typical OTU was considered according to its abundance in sequences (Sun *et al.* 2015).

Statistical analysis

Chao 1 and Shannon indices were estimated to evaluate the bacterial richness of the samples by using the software QIIME according to a method that was described previously (Zhou *et al.* 2018). To analyze the functions between microbial communities of all substrate samples, we performed principal component analysis (Pcoa) following a previously reported method (Wanapaisan *et al.* 2018). LEfSe analysis was performed to highlight biological

relevance and uncover the important microbial groups in the samples (Zhang *et al.* 2013). Main environmental factors, including pH, AN, AP, and AK, were chosen to analyze their effect on bacterial community by using canonical correspondence analysis (CCA) (Zhou *et al.* 2018). The sequence data have been deposited in the Read Archive database under request for release. Data were collected, and mean analysis was performed using EXCEL. The data were also analyzed using one-way ANOVA (Fisher's LSD test) and Pearson correlation coefficient at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***) by using the SPSS software version 19.0 (SPSS Inc., USA).

Results

Nutrient uptake in scion and rootstock

The growth performances between grafted melon plants were insignificant when two rootstocks were used. However, the grafted plants in WF rootstock were slightly stronger than those of YF rootstock in six treatments (Fig. 1a and b). Significantly differences were detected in terms of nutrient adsorption. The highest N and P contents of the scions in the WF rootstock were 3.29 and 0.39 mg plant^{-1} at the pH of 6.5, respectively (Table 1). Meanwhile, the highest K content was 2.42 mg plant^{-1} at pH 5.5 and 6.5. N content in scion was the lowest at the pH of 5, which was 2.08 mg plant^{-1}, while the P and K contents were the lowest at the pH of 6. The highest N, P, and K contents in WF rootstock section were 2.77, 0.41, and 2.27 mg plant^{-1} at the pH of 6, respectively. The lowest N content of rootstock was 1.98 mg plant^{-1} at the pH of 5, and the lowest P and K contents were 2.06 and 1.44 mg plant^{-1} at the pH of 8, respectively.

Among the grafted plants by using YF rootstocks, the highest N, P, and K contents in scions were 3.19, 0.33, and 2.26 mg plant^{-1} at the pH values of 6.5, 5.0, and 6.5, respectively. The lowest N, P, and K contents in scions were 2.39, 0.24, and 1.42 mg plant^{-1} at the pH of 8, respectively. The highest N and P contents in YF rootstock sections were 2.26 and 0.36 mg plant^{-1} at the pH of 5.5, respectively. Meanwhile, the highest K content was 1.45 mg plant^{-1} at the pH of 6.5, and the lowest N, P, and K contents in rootstock sections were 2.39, 0.24, and 1.42 mg plant^{-1}, at the pH of 8, respectively. The lowest N content was 1.43 mg plant^{-1} at the pH of 7. The lowest P and K contents were 0.15 and 0.84 mg plant^{-1} at the pH of 8, respectively (Table 1).

The nutrient content ratio of scion to rootstock was calculated in WF and YF rootstocks. The distribution ratio of N in scions and rootstocks was > 1.0, except for WF rootstocks at the pH of 6, which was 0.77. The P proportion in the scions and rootstocks was < 1.0, and only that in the YF rootstocks were >1 at the pH values of 5, 7, and 8. The K distribution ratio in scions and rootstocks was >1.0, except for WF rootstocks at the pH of 6, which was 0.62. In the grafted seedlings of YF rootstocks, the K content in the scions and rootstocks constantly increased (Table 1).

Nutrient uptake in non-grafted and grafted plants

Among the six pH substrates, the N content in non-grafted plants was between 1.22 mg plant^{-1} to 1.67 mg plant^{-1}, and the highest content was 1.67 mg plant^{-1} at the pH of 6.5. Meanwhile, the total P content ranged from 0.12 and 0.23 mg plant^{-1}, and the highest content was observed at the pH of 6. The lowest K content was observed between 0.94 and 1.90 mg plant^{-1}, and highest one was found at the pH of 6.5. The lowest N and P contents were observed at the pH of 8.0 for non-grafted seedlings, while the lowest K content was found at the pH of 5.0 (Table 2).

Among the grafted plants of WF rootstocks, the highest total N and P contents were observed at the pH of 6.5, which were 5.73 and 0.79 mg plant^{-1}, respectively. Meanwhile, the highest K content was observed at the pH of 5.5, which was 4.65 mg plant^{-1}. In the grafted plants of YF rootstocks, the highest N, P, and K contents were 5.41 mg plant^{-1} at the pH of 6.5, 0.67 mg plant^{-1} at the pH of 5.5, and 3.72 mg plant^{-1} at the pH of 6.5, respectively. The lowest total N, P, and K contents in the grafted seedlings of the two rootstocks were all observed at the pH of 8. The N, P, and K contents in grafted seedlings were significantly higher than those in non-grafted plant. Grafting with two rootstocks (WF and YF) increased the N, P, and K uptake by 3.3-, 3.5-, and 2.4-fold, respectively. At the pH of 5, the highest N, P, and K values were 3.87-, 4.42-, and 3.60-fold when YF rootstocks were used to graft seedlings (Table 2).

Pearson correlation analysis showed that in the two rootstocks (WF and YF), pH was negatively correlated with root dry weight and N, P, and K contents (Table 3). P was negatively and strongly correlated with pH in the scion of YF rootstock (**$P < 0.01$). When WF was used as the rootstock, the dry weight and N of scions was significantly correlated with P and K (**$P < 0.01$). The P content was significantly correlated with K in scions and rootstocks (**$P < 0.01$). The N contents in the scions and rootstocks were significantly correlated with P in rootstocks and K in the whole plant by using YF as the rootstock. The P content in the rootstock was significantly correlated with K in rootstock (**$P < 0.01$). The P content in the scion was significantly correlated with that of rootstock. Meanwhile, the P and K contents in the rootstock were significantly correlated (**$P < 0.01$). The K content in scion was significantly correlated (*$P < 0.05$) with that in the rootstock (Table 3).

Bacterial community diversities

As shown in Fig. 2, the Chao index reflected the structure of bacterial community in the substrate. The initial Chao index of the substrate was significantly lower than that of the substrate after seedling raising (Fig. 2a). Regardless of the pH value, the abundance of bacterial community in the substrate of the two rootstocks increased, especially in the rhizosphere of the grafted seedlings of yellow-seeded rootstocks. Compared with the Shannon index, the difference

Table 1: Comparison of nutrient uptake in grafted scion and two rootstocks plants

Treatment	Nitrogen (mg plant^{-1})		Ratio	Phosphorus (mg plant^{-1})		Ratio	Potassium (mg plant^{-1})		Ratio
	Scion	Rootstock		Scion	Rootstock		Scion	Rootstock	
W1F (5.0)	2.08 ± 0.09	1.98 ± 0.05	1.05	0.26 ± 0.05	0.31 ± 0.04	0.83	1.67 ± 0.09	1.67 ± 0.08	1.00
W2F (5.5)	3.04 ± 0.11	2.41 ± 0.06	1.26	0.37 ± 0.03	0.40 ± 0.03	0.91	2.42 ± 0.06	2.23 ± 0.05	1.08
W3F (6.0)	2.12 ± 0.08	2.77 ± 0.03*	0.77	0.23 ± 0.02	0.41 ± 0.04*	0.56	1.40 ± 0.03	2.27 ± 0.06*	0.62
W4F (6.5)	3.29 ± 0.07*	2.44 ± 0.09	1.35	0.39 ± 0.03*	0.39 ± 0.03	0.97	2.42 ± 0.05*	2.14 ± 0.07	1.13
W5F (7.0)	3.16 ± 0.04	2.42 ± 0.08	1.30	0.33 ± 0.02	0.34 ± 0.06	0.97	2.38 ± 0.08	1.82 ± 0.05	1.31
W6F (8.0)	2.26 ± 0.06	2.06 ± 0.06	1.10	0.24 ± 0.05	0.26 ± 0.02	0.93	1.52 ± 0.05	1.44 ± 0.04	1.05
Y1F (5.0)	3.07 ± 0.08	2.14 ± 0.04	1.44	0.33 ± 0.06*	0.32 ± 0.05	1.06	1.96 ± 0.06	1.41 ± 0.06	1.40
Y2F (5.5)	2.86 ± 0.09	2.26 ± 0.10	1.26	0.30 ± 0.07	0.36 ± 0.06	0.84	1.92 ± 0.05	1.43 ± 0.05	1.34
Y3F (6.0)	2.75 ± 0.08	2.08 ± 0.10	1.32	0.30 ± 0.08	0.31 ± 0.03	0.99	1.81 ± 0.02	1.27 ± 0.06	1.43
Y4F (6.5)	3.19 ± 0.10	2.21 ± 0.09	1.45	0.26 ± 0.06	0.31 ± 0.05	0.85	2.26 ± 0.06*	1.45 ± 0.09*	1.56
Y5F (7.0)	2.40 ± 0.09	1.43 ± 0.08	1.67	0.24 ± 0.07	0.19 ± 0.02	1.26	1.65 ± 0.03	0.95 ± 0.08	1.73
Y6F (8.0)	2.39 ± 0.10*	1.53 ± 0.09*	1.57	0.24 ± 0.05	0.15 ± 0.03*	1.54	1.42 ± 0.04*	0.84 ± 0.06*	1.70

Ratio = scion / rootstock. * Significant at the $P < 0.05$ level

Two rootstocks, namely, WF and YF that were grown in six pH substrates were abbreviated form as W1F to W6F and Y1F to Y6F, respectively

Table 2: Nutrient uptake in non-grafted and grafted seedlings

Treatment		Nitrogen (mg plant^{-1})	Ratio	Phosphorus (mg plant^{-1})	Ratio	Potassium (mg plant^{-1})	Ratio
Non-grafted	NG1 (5.0)	1.35 ± 0.09	/	0.15 ± 0.10	/	0.94 ± 0.10	/
	NG2 (5.5)	1.47 ± 0.08	/	0.20 ± 0.08	/	1.31 ± 0.11	/
	NG3 (6.0)	1.59 ± 0.06	/	0.23 ± 0.09	/	1.67 ± 0.09	/
	NG4 (6.5)	1.67 ± 0.12	/	0.18 ± 0.11	/	1.90 ± 0.08	/
	NG5 (7.0)	1.63 ± 0.11	/	0.15 ± 0.10	/	1.66 ± 0.10	/
	NG6 (8.0)	1.22 ± 0.07	/	0.12 ± 0.10	/	1.39 ± 0.09	/
Grafted	W1F (5.0)	4.06 ± 0.08	3.02	0.57 ± 0.09	3.90	3.34 ± 0.12	3.57
	W2F (5.5)	5.45 ± 0.09	3.70	0.78 ± 0.07	3.88	4.65 ± 0.11	3.54
	W3F (6.0)	4.89 ± 0.11	3.08	0.64 ± 0.11	2.74	3.67 ± 0.13	2.20
	W4F (6.5)	5.73 ± 0.09	3.43	0.79 ± 0.14	4.39	4.56 ± 0.10	2.41
	W5F (7.0)	5.59 ± 0.12	3.42	0.67 ± 0.12	4.40	4.20 ± 0.12	2.53
	W6F (8.0)	4.32 ± 0.13	3.51	0.50 ± 0.10	4.11	2.96 ± 0.08	2.13
	Y1F (5.0)	5.20 ± 0.10	3.87	0.65 ± 0.08	4.42	3.37 ± 0.15	3.60
	Y2F (5.5)	5.13 ± 0.07	3.48	0.67 ± 0.02	3.31	3.35 ± 0.13	2.55
	Y3F (6.0)	4.84 ± 0.12	3.04	0.61 ± 0.09	2.61	3.08 ± 0.11	1.85
	Y4F (6.5)	5.41 ± 0.09	3.24	0.58 ± 0.05	3.21	3.72 ± 0.13	1.96
	Y5F (7.0)	3.83 ± 0.08	2.34	0.44 ± 0.07	2.85	2.59 ± 0.09	1.57
	Y6F (8.0)	3.92 ± 0.11	3.19	0.39 ± 0.11	3.21	2.26 ± 0.07	1.62

The two rootstocks, namely, WF and YF that were grown in six pH substrates were abbreviated as W1F to W6F and Y1F to Y6F, respectively

of bacterial community abundance between initial pH substrate and post-nursery was not as significant as that of Chao index. However, after breeding the seedlings, the abundance of bacterial community in substrate significantly increased (Fig. 2b).

Among the six initial substrates, *Rhodanobacter* increased significantly from 0.40% in Su6 to 14.26% in W5F (Fig. 3). The number of *Bacillus* decreased from 14.61% in Su1 to 2.56% in W2F. The proportion of *Lactococcus* population in the initial substrate was low, thereby increasing from 2.59% in Su1 to 11.73% in W1F. *Rhizomicrobium* population had a high number in the initial substrate (Su1–Su6) and high number in the low-pH substrate (6.64% in W2F and 5.24% in Y1F). The *Chloroplast* population content in the initial substrate (Su1–Su6) was extremely low, and the total distribution content was between 0.01 and 0.60%. After the breeding of seedling, the number of *Chloroplast* increased to 6.14% in W1F and 6.27% in Y3F. The initial substrate content of *Devosia* population was also relatively low, which was only 1.03% of Su1, and increased to 3.55% of Y2F and 3.44% of W2F after seedling were produced (Fig. 3).

The Venn diagram showed that 191 common OTUs were found in all substrates. Y3F showed the highest number of OTUs, while the lowest was Su6. The pH value was between 6 and 7, and the OTU of bacteria was at a high level, especially for grafted seedlings of YF rootstocks (Fig. 4a). Through Pcoa analysis, with the increase in the pH value, each treatment showed evident regional distribution. pH was the main factor that regulated the OTU numbers in the six substrates (Fig. 4b).

The heat map analysis showed that the community abundance of the initial substrate was low, while the structure abundance of bacterial community of yellow-seeded grafted rootstocks increased most significantly (Fig. 5). Regardless of whether the pH increased or not, the main bacterial populations in the substrate were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Gemmatimonadetes*. The abundances of the bacterial community in the base of the pH value were significantly lower than those in the base after seedling raising. The rhizosphere bacterial communities of the two rootstocks showed similar trends with the increase in pH.

CCA and LEfSe analyses

The CCA analysis showed that the structure of bacterial community was significantly correlated with pH, N, and P

Table 3: Pearson correlations of pH values and dry weights of scion, rootstock part, and root with nutrients

Variables	WSD	WSHD	WRD	WNS	WNR	WPS	WPR	WKS	WKR	
	0.086	0.222	-0.253	0.136	-0.082	-0.127	-0.508	-0.075	-0.473	pH
		0.676	0.850*	0.986**	0.146	.949**	0.352	0.979**	0.291	WSD
YSD	-0.180		0.817*	0.765	0.784	0.612	0.661	0.613	0.649	WSHD
YSHD	-0.481	0.817*		0.874*	0.557	0.881*	0.772	0.870*	0.720	WRD
YRD	-0.513	0.659	0.442		0.272	0.944**	0.432	0.962**	0.384	WNS
YNS	-0.702	0.702	0.877*	0.523		0.179	0.820*	0.142	0.845*	WNR
YNR	-0.792	0.373	0.783	0.277	0.894*		0.516	0.970**	0.462	WPS
YPS	-0.952**	0.020	0.327	0.446	0.634	0.766		0.423	0.995**	WPR
YPR	-0.884*	0.281	0.723	0.273	0.818*	0.961**	0.812*		0.361	WKS
YKS	-0.631	0.729	0.907*	0.398	0.945**	0.824*	0.482	0.797		
YKR	-0.849*	0.497	0.834*	0.407	0.936**	0.966**	0.761	0.966**	0.913*	
	pH	YSD	YSHD	YRD	YNS	YNR	YPS	YPR	YKS	

WSD, white rootstock scion dry weight; WSHD, white rootstock shoot dry weight; WRD, white rootstock root dry weight; WNS, N content in scion; WNR, N content in rootstock section; WPS, P content in scion; WPR, P content in rootstock section; WKS, K content in scion; WKR, K content in rootstock section. The same abbreviations are defined for yellow rootstock

Correlation is significant at the $^{**}P < 0.01$ level and $^{*}P < 0.05$ level

but showed weak correlation with K (Fig. 6a). pH was significantly correlated with not only the increase but also the decrease in most bacterial communities, followed by the N and P contents, which were also significantly correlated with the change in bacterial community structure. However, the change in K was slightly weak, which can affect the structure of bacterial communities (Fig. 6b).

The functional groups of bacterial communities in different substrates varied in numbers and groups. Red color represents the microbial groups that played an important role in the initial substrates, but the total number in Su was small. Meanwhile, green and blue colors represented the important bacterial communities in WF rootstocks and YF rootstocks after the end of seedling cultivation (Fig. 7). The bacterial community that played an important role in the substrate of YF rootstock was much higher than that of WF rootstock.

Discussion

In vegetable production, grafting has become a standard and useful practice to breed robust seedlings to overcome soil-borne diseases (Kubota 2008; Lee *et al.* 2010; Mohamed *et al.* 2014; Nawaz *et al.* 2017). The present study showed that melon grafted to the two rootstocks (WF and YF) can significantly increase N, P, and K uptake regardless of the pH levels in the substrates. Nutrient uptake is a major factor determining plant growth and affecting productivity (Pii *et al.* 2015). Plants can acquire N, P, and K through their roots from the soil under inorganic and organic forms to meet plant growth (Kirk 2001; Owen *et al.* 2015; Raven *et al.* 2018), which is especially important in grafted plants. The results of the present study showed that different pH substrates significantly affect nutrient uptake not only in non-grafted melon plants but also in grafted melon plants. Soil pH shows remarkable impact on nutrient uptake by crop plants (Bouain *et al.* 2019), and changing the pH in soil can improve nutrient status (Agegnehu *et al.* 2016; Oladele *et al.* 2019). The data of the present study suggested that pH can affect nutrient uptake in substrate ranging from 5 to 8.

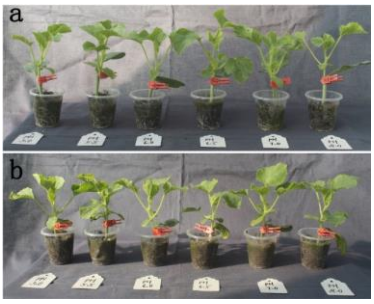

Fig. 1: Grafted melon plants from white rootstock (**a**) and yellow rootstocks (**b**) grown in six treatments (pH range of 5.0 to 8.0)

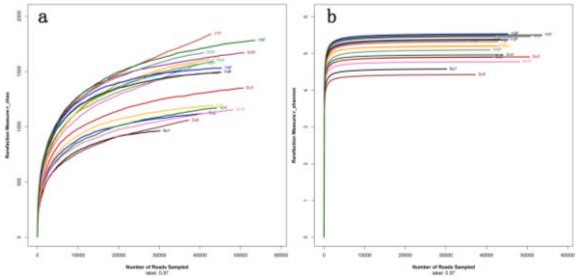

Fig. 2: Chao (**a**) and Shannon (**b**) indices of bacteria in all treatments

Su1 to Su6, W1F to W6F, and Y1F to Y6F represented the pH values of 5.0, 5.5, 6.0, 6.5, 7.0, and 8.0 in the initial substrate, white rootstock, and yellow rootstock, respectively

Additional nutrients were absorbed at the pH ranging 6.0 to 6.5 but decreased amount were taken under low (5.0) or high pH (8.0) regardless in scion or rootstock sections (Table 1). On the other hand, our finding showed that pH of substrate should be properly regulated to a fine range to breed seedlings. pH affects plant nutrient uptake, and a suitable pH range should be considered (Smith *et al.* 2004; Tu *et al.* 2018). This finding will be helpful in seedling substrate production.

Plant root and leaf traits explain approximately 50% of the variability in nutrient concentration (Wendling *et al.* 2016). In the present work, we also analyzed the nutrient

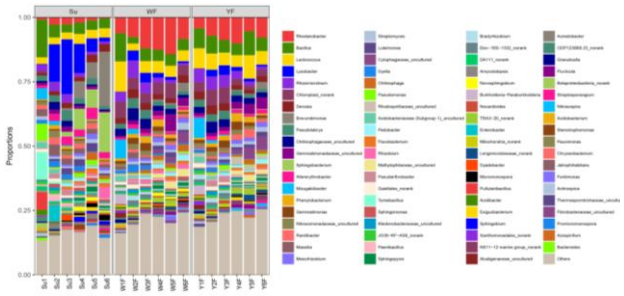

Fig. 3: Bacterial community compositions in all substrates at genus level

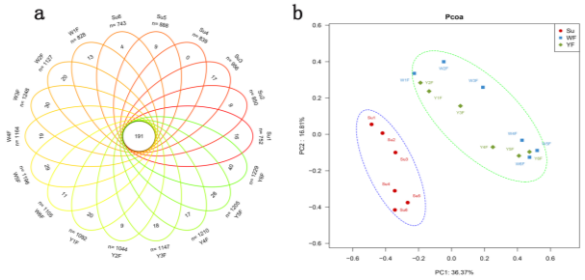

Fig. 4: OTU Venn analysis (**a**) and Pcoa analysis (**b**) of bacteria in different treatments

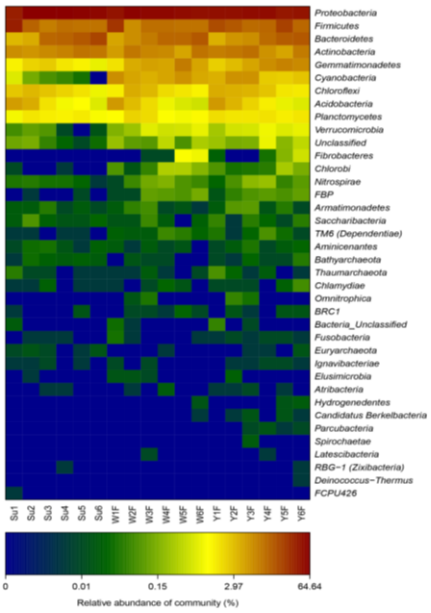

Fig. 5: Heatmap analysis of bacterial communities at phylum level

distribution in the whole grafted plants. The N and K ratios in the scion and rootstock was maintained at >1. The P ratio was <1 in the white rootstock but >1 in the YF rootstock at high pH setting. Although P and K uptake in plants are similar to N assimilation (Wu *et al.* 2005; Gómez *et al.* 2019), the N content in the scions and rootstocks in the present study was significantly correlated with P in rootstocks and K in whole plant by using YF as the rootstock. N availability in soil can positively affect P

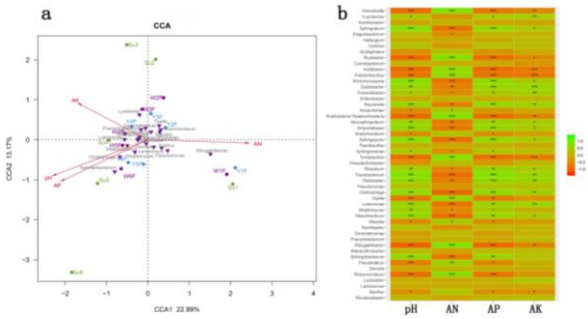

Fig. 6: Canonical correspondence analysis of bacterial communities in different substrate samples and environmental nutrient factors. (**a**) Heatmap of correlations (**b**). *, **, and *** indicated significant correlations at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Available N (AN), available P (AP), available K (AK), and pH were used

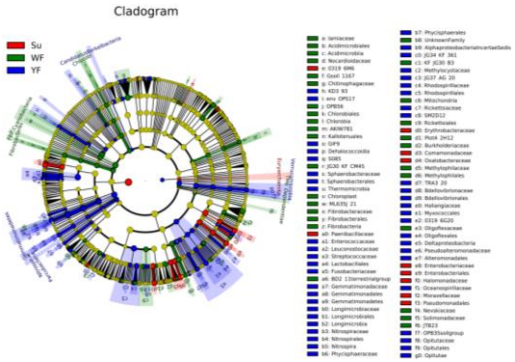

Fig. 7: Diagrammatic presentation of LEfSe analysis in three substrates. Three groups were illustrated with red (Su), green (WF), and blue (YF) colors. Each marked color group exhibited the important bacterial phyla in three treatments

resorption in plant leaves (Yan *et al.* 2018). Our data indicated that N affected P and K uptake in the scions and rootstocks in varied pH substrates.

The N and K concentrations were maintained high in the scion section instead of in the root (Table 1). By contrast, the P content was low in the scion. Soper *et al.* (2019) found no evidence confirming that N status is strongly linked to P acquisition. Alternatively, P is critical for plant growth but also is one of the most difficult nutrients for plants to acquire (Vance *et al.* 2003; Rose *et al.* 2016). P deficiency affects the accumulation and partitioning of other nutrients, such as rice plant grown in soil without P, exhibiting decreased accumulation of N and K in above-ground part (Guerriero and Cai 2018). In the present study, P content in rootstock section was not only significantly correlated with K content (**$P < 0.01$) but also with P content in the scion in the YF rootstock (Table 3). As long as the plants can adapt their root system according to nutrient conditions (Wendling *et al.* 2016; Borden *et al.* 2019), proper pH value of substrate will be helpful to produce grafted plants. The P content was low in the scion, whereas those of N and K were high in the scion than in the rootstock, thereby suggesting that additional N

and K were absorbed and transported to the scion section in grafted plants. Scion leaf size is the main factor contributing to biomass, and leaf dry weight is increased by N uptake (Terrer *et al.* 2018). Our data also indicated that additional N and K were required by the growth of scions; otherwise, grafted plant growth will be restricted by low N availability.

Grafting is a tool that can increase the low nutrient stress tolerance of crops (Pradeep *et al.* 2015; Martínez-Andújar *et al*. 2017). Similar to the results of these reports, in the present study, compared with non-grafted melon plants, those grafted with two rootstocks showed significantly increased N, P, and K absorption in the scion or the rootstock section of the grafted seedling plant. Our data showed that the N, P, and K contents in grafted plant can be increased by 3.87-, 4.42-, and 3.60-fold higher than those in non-grafted melon plant, especially in terms of increased P uptake (Table 2). Our data suggested that using pumpkin rootstock will be a useful practice to increase nutrient status in grafted plants. The genome sequences of pumpkin species (*Cucurbita* spp.) have provided evidence supporting an allotetraploid of *Cucurbita* from two progenitors. Upregulated genes involved in defense and heat responses are also present in hybrid root (Sun *et al.* 2017). These findings may explain the strong ability of pumpkin to absorb nutrients in the substrates. Few rootstocks can increase scion growth and yield but do not differentially respond to decreased N rates (Suchoff *et al.* 2019). On the contrary, other study has pointed out that the influence of grafting on nutrient uptake in pepper depends not on the rootstock genotype but on the combination of rootstock/scion (Ropokis *et al.* 2019). However, in the present study, the two rootstocks showed remarkable improvement in nutrient uptake (Table 2). Hence, pumpkin rootstocks have strong ability to absorb nutrients from the substrate, thereby providing additional elements to the scion. Ren *et al*. (2018b) reported that the grafted cucumber grew better than non-grafted treatment, and compatible pumpkin rootstocks promotes plant growth, which may due to strong ability for cell proliferation and increased efficiency in carbohydrate metabolism. In summary, these findings can be continuously applied in exploring nutrient traits in other rootstock plant species.

Microbes play key roles in plant nutrient acquisition, N and C cycling, and soil formation (Heijden *et al.* 2015). The present results on the Chao and Shannon indices indicated that the substrate at the pH of 5 showed decreased richness of bacteria. After the breeding of grafted plant, Shannon and Chao indices in the two rootstocks increased in six pH substrates (Fig. 2). Soil bacterial diversity (Shannon index) and richness (Chao index) are important criteria for microbial community analysis (Lupwayi *et al.* 2018; Fu *et al.* 2019). As shown in Fig. 3, using two rootstocks showed the similar microbial structures among six pH substrates. However, the structures of these two rootstocks was considerably different from the initial structure of microbial communities, thereby indicating that breeding seedlings changed the structures of microbial communities in the substrates by the same pumpkin rootstock. In the three substrates (*i.e.*, Su, WF, and YF), the most predominant bacteria phyla included *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Gemmatimonadetes* (Fig. 5), thereby indicating that these phyla were the basic groups in the treatment. However, these phyla are the most abundant ones not only in soil but also in water and sediment (Cheng *et al.* 2016; Hou *et al.* 2018). *Actinobacteria* is recognized as a dominant bacterial population in soils, and *Proteobacteria* is also the predominant phylum in agricultural soils (Lupwayi *et al.* 2018). However, in the present study, the phyla *Chloroflexi* and *Acidobacteria* were not abundant in the substrates as previously reported (Hou *et al.* 2018). In contrast to previous results, *Verrucomicrobia* and *Chlorobi* only showed varied abundance in WF and YF substrates but not in Su1 to Su6. However, in other studies, the two phyla were identified as the main groups in the reactors in biological nutrient removal or in shrimp cultural enclosure ecosystems (Hou *et al.* 2017; Liu *et al.* 2019). This result may be attributed to the different conditions of substrates, and rootstock root system play functions in shaping microbial structures in substrates. These findings will be helpful in discriminating healthy seedlings when using different rootstocks.

According to CCA analysis, pH is the most important factor that determines the bacterial structures. The majority of bacterial groups increasing or decreasing were affected by the substrate pH (Fig. 6b). pH plays an important role not only in the shift of microbial community structures in sludge fermentation (Maspolim *et al.* 2015) but also in shaping soil bacterial structures in the soil active layer. pH also has a strong effect on the structure of soil microbial communities at all scales (Ren *et al.* 2018a). Similar to these reports, our data also suggested that pH was the first priority in selection of substrates for breeding seedlings, thereby indicating that in the substrates, most microbial communities will be affected and are easily regulated by setting proper pH values. AN and AP also remained strongly correlated with bacterial community compositions, whereas AK was weakly linked with the bacterial structures. N availability affects the microbial community that is involved in N cycling (Li *et al.* 2019). P is another principal factor in shaping microbial community composition (Liu *et al.*, 2018; Samaddar *et al.* 2019). The findings of the present study suggested that AN and AP still played important roles in shaping microbial diversity in the breeding substrates when producing grafted seedling by using two selected rootstocks. Our study provides an example illustrating the importance of nutrient uptake in grafted seedlings.

Conclusion

The different pH levels of substrate played important roles in nutrient uptake and accumulation in the grafted melon plants. Significant nutrient uptake was observed in grafted melon plants in all six pH levels of the substrates. The most

predominant bacterial phyla were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Gemmatimonadetes*. On the contrary, phyla such as *Chloroflexi* and *Acidobacteria* decreased in the substrates. The pH, AN, and AP were significantly correlated with the bacterial community structures in the varied pH substrates.

Acknowledgments

We thank Yu Cao, undergraduate student from the School of Horticulture in Anhui Agricultural University, for his assistance in the experiment. We also want to acknowledge the people in the experimental basements. This work was financially supported by the National Natural Science Foundation of China (Grant No. 31701968), the Anhui Key Research and Development Project (201904a06020004), and the Second Level Youth Development Fund from Anhui Academy of Agricultural Sciences.

References

Abbasi MK, S Sharif, M Kazmi, T Sultan, M Aslam (2011). Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plants. *Plant Biosyst* 145:159–168

Agegnehu, G, PN Nelson, MI Bird (2016). Crop yield, plant nutrient uptake and soil physicochemical properties under organic soil amendments and nitrogen fertilization on Nitisols. *Soil Till Res* 160:1–13

Aguayo E, VH Escalona, F Artés (2008). Effect of hot water treatment and various calcium salts on quality of fresh-cut 'Amarillo' melon. *Postharv Biol Technol* 47:397–406

Borden KA, SC Thomas, ME Isaac (2019). Variation in fine root traits reveals nutrient-specific acquisition strategies in agroforestry systems. *Plant Soil*, doi: 10.1007/s11104-019-04003-2

Bouain N, G Krouk, B Lacombe, H Rouached (2019). Getting to the root of plant mineral nutrition: Combinatorial nutrient stresses reveal emergent properties. *Trends Plant Sci* 24:542–552

Cheng J, G Jing, L Wei, Z Jing (2016). Long-term grazing exclusion effects on vegetation characteristics, soil properties and bacterial communities in the semi-arid grasslands of China. *Ecol Eng* 97:170–178

Dordas C (2008). Role of nutrients in controlling plant diseases in sustainable agriculture: A review. *Agron Sustain Dev* 28:443–460

Fu X, J Wang, UM Sainju, F Zhao, W Liu (2019). Soil microbial community and carbon and nitrogen fractions responses to mulching under winter wheat. *Appl Soil Ecol* 139:64–68

Gava CAT, JM Pinto (2016). Biocontrol of melon wilt caused by *Fusarium oxysporum* Schlect f. spp. *melonis* using seed treatment with *Trichoderma* spp. and liquid compost. *Biol Cont* 97:13–20

Glæsner N, FVD Bom, S Bruun, T McLaren, FH Larsen, J Magid (2019). Phosphorus characterization and plant availability in soil profiles after long-term urban waste application. *Geoderma* 338:136–144

Gómez MI, S Magnitskiy, LE Rodríguez (2019). Nitrogen, phosphorus and potassium accumulation and partitioning by the potato group Andigenum in Colombia. *Nutr Cycl Agroecosyst* 113:349–363

Guerriero G, G Cai (2018). Interaction of nano-sized nutrients with plant biomass: A review, *In*: *Phytotoxicity of Nanoparticles*, pp:135–149. Springer Nature, Switzerland

Heijden MGAVD, SD Bruin, L Luckerhoff, RSPV Logtestijn, K Schlaeppi (2015). A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J* 10:389-399

Hou D, Z Huang, S Zeng, J Liu, S Weng, J He (2018). Comparative analysis of the bacterial community compositions of the shrimp intestine, surrounding water and sediment. *J Appl Microbiol* 125:792–799

Hou D, Z Huang, S Zeng, J Liu, D Wei, X Deng, S Weng, Z He, J He (2017). Environmental factors shape water microbial community structure and function in shrimp cultural enclosure ecosystems. *Front Microbiol* 8; Article 2359

Hu M, X Wang, X Wen, Y Xia (2012). Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. *Bioresour Tchnol* 117:72–79

Huang Y, W Li, L Zhao, T Shen, J Sun, H Chen, Q Kong, MA Nawaz, Z Bie (2017). Melon fruit sugar and amino acid contents are affected by fruit setting method under protected cultivation. *Sci Hortic* 214:288–294

Kirk GJD (2001). Plant-mediated processess to acquire nutrients: Nitrogen uptake by rice plants. *Plant Soil* 232:129–134

Kubota C (2008). Use of grafted seedlings for vegetable production in north America *Acta Hortic* 770:21–28

Lambers H, JA Raven, GR Shaver, SE Smith (2008). Plant nutrient-acquisition strategies change with soil age. *Trends Ecol Evol* 23:95–103

Lee JM, C Kubota, SJ Tsao, Z Bie, PH Echevarria, L Morra, M Oda (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci Hortic* 127:93–105

Li S, C Peng, T Cheng, C Wang, L Guo, D Li (2019). Nitrogen-cycling microbial community functional potential and enzyme activities in cultured biofilms with response to inorganic nitrogen availability. *J Environ Sci* 76:89–99

Liu B, J Ren, Y Zhang, J An, M Chen, H Chen, C Xu, H Ren (2015). A new grafted rootstock against root-knot nematode for cucumber, melon, and watermelon. *Agric Sustain Dev* 35:251–259

Liu H, H Sun, M Zhang, Y Liu (2019). Dynamics of microbial community and tetracycline resistance genes in biological nutrient removal process. *J Environ Manage* 238:84–91

Liu J, Z Yang, P Dang, H Zhu, Y Gao, VN Ha, Z Zhao (2018). Response of soil microbial community dynamics to *Robinia pseudoacacia* L. afforestation in the loess plateau: A chronosequence approach. *Plant Soil* 423:327–338

Lupwayi NZ, WE May, DA Kanashiro, RM Petri (2018). Soil bacterial community responses to black medic cover crop and fertilizer N under no-till. *Appl Soil Ecol* 124:95–103

Marín-Guirao JI, P Rodríguez-Romera, B Lupión-Rodríguez, F Camacho-Ferre, JC Tello-Marquina (2016). Effect of Trichoderma on horticultural seedlings' growth promotion depending on inoculum and substrate type. *J Appl Microbiol* 121:1095–1102

Martínez-Andújar C, JM Ruiz-Lozano, IC Dodd, A Albacete, F Pérez-Alfocea (2017). Hormonal and nutritional features in contrasting rootstock-mediated tomato growth under low-phosphorus nutrition. *Front Plant Sci* 8; Article 533

Martínez-Ballesta MC, L López-Pérez, M Hernández, C López-Berenguer, N Fernández-García, M Carvajal (2008). Agricultural practices for enhanced human health. *Phytochem Rev* 7:251–260

Maspolim Y, Y Zhou, C Guo, K Xiao, WJ Ng (2015). The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation. *Bioresour Technol* 190:289–298

Mohamed FH, KEA El-Hamed, MWM Elwan, MNE Hussien (2014). Evaluation of different grafting methods and rootstocks in watermelon grown in Egypt. *Sci Hortic* 168:145–150

Murakami K, N Fukuoka, S Noto (2017). Improvement of greenhouse microenvironment and sweetness of melon (*Cucumis melo* L.) fruits by greenhouse shading with a new kind of near-infrared ray-cutting net in mid-summer. *Sci Hortic* 218:1–7

Nawaz MA, L Wang, Y Jiao, C Chen, L Zhao, M Mei, Y Yu, Z Bie, Y Huang (2017). Pumpkin rootstock improves nitrogen use efficiency of watermelon scion by enhancing nutrient uptake, cytokinin content, and expression of nitrate reductase genes. *Plant Growth Regul* 82:233–246

Okamoto S, R Tabata, Y Matsubayashi (2016). Long-distance peptide signaling essential for nutrient homeostasis in plants. *Curr Opin Plant Biol* 34:35–40

Oladele SO, AJ Adeyemo, MA Awodun (2019). Influence of rice husk biochar and inorganic fertilizer on soil nutrients availability and rain-fed rice yield in two contrasting soils. *Geoderma* 336:1–11

Owen D, AP Williams, GW Griffith, PJA Withers (2015). Use of commercial bio-inoculants to increase agricultural production through improved phosphrous acquisition. *Appl Soil Ecol* 86:41–54

Pandey S, WA Ansari, N Atri, B Singh, S Gupta, KV Bhat (2018). Standardization of screening technique and evaluation of muskmelon genotypes for drought tolerance. *Plant Genet Res* 16:1–8

Pii Y, T Mimmo, N Tomasi, R Terzano, S Cesco, C Crecchio 2015. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol Fert Soils* 51:403–415

Pradeep K, E Menahem, C Mariateresa, F Emanuela, C Giuseppe (2015). Grafting affects growth, yield, nutrient uptake, and partitioning under cadmium stress in tomato. *HortScience* 50:1654–1661

Rashid MM, M Jahan, KS Islam (2016). Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. *Rice Sci* 23:119–131

Raven JA, H Lambers, SE Smith, M Westoby (2018). Costs of acquiring phosphorus by vascular land plants: Patterns and implications for plant coexistence. *New Phytol* 217:1420–1427

Ren B, Y Hu, B Chen, Y Zhang, J Thiele, R Shi, M Liu, R Bu (2018a). Soil pH and plant diversity shape soil bacterial community structure in the active layer across the latitudinal gradients in continuous permafrost region of Northeastern China. *Sci Rep* 8; Article 5619

Ren Y, Q Xu, L Wang, S Guo, S Shu, N Lu, J Sun (2018b). Involvement of metabolic, physiological and hormonal responses in the graft-compatible process of cucumber/pumpkin combinations was revealed through the integrative analysis of mRNA and miRNA expression. *Plant Physiol Biochem* 129:368–380

Ropokis A, G Ntatsi, C Kittas, N Katsoulas, D Savvas (2019). Effects of temperature and grafting on yield, nutrient uptake, and water use efficiency of a hydroponic sweet pepper crop. *Agronomy* 2019; Article 110

Rose TJ, A Mori, CC Julia, M Wissuwa (2016). Screening for internal phosphorus utilisation efficiency: Comparison of genotypes at equal shoot P content is critical. *Plant Soil* 401:79–91

Ryan WD, RF Paul, RP Sonali, RA William (2016). Evaluating calibrachoa (*Calibrachoa* ×*hybrida* Cerv.) genotype sensitivity to iron deficiency at high substrate pH. *HortScience* 51:1452–1457

Samaddar S, P Chatterjee, J Truu, R Anandham, S Kim, T Sa (2019). Long-term phosphorus limitation changes the bacterial community structure and functioning in paddy soils. *Appl Soil Ecol* 134:111–115

Seo Y, YH Kim (2017). Pathological interrelations of soil-borne diseases in cucurbits caused by *Fusarium* species and *Meloidogyne incognita*. *Plant Pathol J* 33:410–423

Shen C, J Xiong, H Zhang, Y Feng, X Lin, X Li, W Liang, H Chu (2013). Soil pH drives spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol Biochem* 57:204–211

Silberstein L, I Kovalski, R Huang, K Anagnostou, MMK Jahn, R Perl-Treves (1999). Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers. *Sci Hortic* 79:101–111

Smith BR, PR Fisher, WR Argo (2004). Water-soluble fertilizer concentration and pH of a peat-based substrate affect growth, nutrient uptake, and chlorosis of container-grown seed geraniums. *J Plant Nutr* 27:497–524

Snee LS, VR Nerurkar, DA Dooley, JT Efird, AC Shovic, PV Nerurkar (2011). Strategies to improve palatability and increase consumption intentions for *Momordica charantia* (bitter melon): A vegetable commonly used for diabetes management. *Nutr J* 10; Article 78

Soper FM, MK Nasto, BB Osborne, CC Cleveland (2019). Nitrogen fixation and foliar nitrogen do not predict phosphorus acquisition strategies in tropical trees. *J Ecol* 107:118–126

Suchoff DH, CC Gunter, JR Schultheis, RL Hassell, FJ Louws (2019). The effect of grafting on nitrogen use in determinate field-grown tomatoes. *J Hortic Sci Biotechnol* 94:102–109

Sun H, S Wu, G Zhang, C Jiao, S Guo, Y Ren, J Zhang, H Zhang, G Gong, Z Jia, F Zhang, J Tian, WJ Lucas, JJ Doyle, H Li, Z Fei, Y Xu (2017). Karyotype stability and unbiased fractionation in the paleo-allotetraploid *Cucurbita* genomes. *Mol Plant* 10:1293–1306

Sun R, XX Zhang, X Guo, D Wang, H Chu (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biol Biochem* 88:9–18

Tam NV, CH Wang (2015). Use of spent mushroom substrate and manure compost for honeydew melon seedlings. *J Plant Growth Regul* 34:417–424

Tang M, Zl Bie, MZ Wu, HP Yi, JX Feng (2010). Changes in organic acids and acid metabolism enzymes in melon fruit during development. *Sci Hortic* 123:360–365

Terrer C, S Vicca, BD Stocker, BA Hungate, RP Phillips, PB Reich, AC Finzi, IC Prentice (2018). Ecosystem responses to elevated CO_2 governed by plant-soil interactions and the cost of nitrogen acquisition. *New Phytol* 217:507–522

Tu C, T He, X Lu, Y Luo, P Smith (2018). Extent to which pH and topographic factors control soil organic carbon level in dry farming cropland soils of the mountainous region of Southwest China. *Catena* 163:204–209

Turner BL, T Brenes-Arguedas, R Condit (2018). Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature* 555:367–370

Vance CP, C Uhde-Stone, DL Allan (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytol* 157:423–447

Vendruscolo EP, LFC Campos, A Seleguini, APB Martins, SFD Lima (2017). Economic viability of muskmelon cultivation in different planting spacing in Brazil central region. *Rev Fac Nac Agric Medellín* 70:8319–8325

Wanapaisan P, N Laothamteep, F Vejarano, J Chakraborty, M Shintani, C Muangchinda, T Morita, C Suzuki-Minakuchi, K Inoue, H Nojiri, O Pinyakong (2018). Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium. *J Hazard Mater* 342:561–570

Wang YL, LY Gao, SY Yang, YB Xu, HY Zhu, LM Yang, Q Li, JB Hu, SR Sun, CS Ma (2018). Molecular diversity and population structure of oriental thin-skinned melons, *Cucumis melo* subsp. *agrestis*, revealed by a set of core SSR markers. *Sci Hortic* 229:59–64

Wendling M, L Büchi, C Amossé, S Sinaj, A Walter, R Charles (2016). Influence of root and leaf traits on the uptake of nutrients in cover crops. *Plant Soil* 409:419–434

Wenjing G, Z Xin, JH Donald (2015). Grafting with an interspecific hybrid squash rootstock accelerated fruit development and impaired fruit quality of galia melon. *HortScience* 50:1833–1836

Wu SC, ZH Cao, ZG Li, KC Cheung, MH Wong (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma* 125:155–166

Yan T, XT Lü, JJ Zhu, K Yang, LZ Yu, T Gao (2018). Changes in nitrogen and phosphorus cycling suggest a transition to phosphorus limitation with the stand development of larch plantations. *Plant Soil* 422:385–396

Yang B, G Yonghong, W Chunling, L Xuewen (2007). Melon production in China. *Acta Hortic* 731:493–500

Yang L, H Cao, Q Yuan, S Luoa, Z Liu (2018). Component optimization of dairy manure vermicompost, straw, and peat in seedling compressed substrates using simplex-centroid design. *J Air Waste Manage Associat* 68:215–226

Zhang C, S Li, L Yang, P Huang, W Li, S Wang, G Zhao, M Zhang, X Pang, Z Yan, Y Liu, L Zhao (2013). Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nat Commun* 4; Article 2163

Zhang F, J Shen, J Zhang, Y Zuo, L Li, X Chen (2010a). Rhizosphere processes and management for improving nutrient use efficiency and crop productivity: implications for China. *Adv Agron* 107:1–32

Zhang FS, J Shen, YG Zhu (2017a). Nutrients: Interactions in soil-plant system. *In*: *Encyclopedia of Soil Science*, pp:1594–1596, Lal R (ed.). CRC Press, Boca Raton, Florida, USA

Zhang J, Y Wang, P Wang, QA Zhang, C Yan, F Yu, J Yi, L Fang (2017b). Effect of different levels of nitrogen, phosphorus, and potassium on root activity and chlorophyll content in leaves of *Brassica oleracea* seedlings grown in vegetable nursery substrate. *Hortic Environ Biotechnol* 58:5–11

Zhang W, H Hao, L Ma, C Zhao, X Yu (2010b). Tetraploid muskmelon alters morphological characteristics and improves fruit quality. *Sci Hortic* 125:396–400

Zhou H, G Wang, M Wu, W Xu, X Zhang, L Liu (2018). Phenol removal performance and microbial community shift during pH shock in a moving bed biofilm reactor (MBBR). *J Hazard Mater* 351:71–79