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Study on the Continuous Cropping Obstacles of 'Honey World' Melon (*Cucumis melon* L.) Seedlings Grafted on Three Types of Rootstocks

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Abstract: Grafting could reduce the obstacle effect of continuous cropping on melon. Melon was grafted two squash varieties and oriental pickling melon as rootstocks. When the melon seedlings were cultured with medium that had been used for continuous cropping twice, the medium exerted a significant inhibitory effect on the growth of both grafted and un-grafted seedlings, while the growth of the grafted seedlings was still better than that of the un-grafted seedlings. The grafted seedlings had a larger leaf area and greater above-ground fresh and dry weights. Using HPLC to analyze the phenolic compounds in the medium, the results showed that medium used for un-grafted melons mainly contained gallic acid and vanillic acid, and medium used for grafted melons mainly contained gallic acid and m-hydroxybenzoic acid. In terms of the level of total phenolic compounds, medium-cultured grafted melon produced using squash 'Xin Tu Zuo' as the rootstock had the highest content, followed by un-grafted plants and those that used oriental pickling melon 'Hwi Pei' as the rootstock, which had similar levels of total phenolic compounds; melon produced using squash 'Zhuang Shi' as the rootstock had the lowest content. In this study, the factor inhibited melon plant growth in continuous media was identified.

Keywords: Continuous Cropping Obstacles, Melon, Grafting, Phenolic Compounds

1. Introduction

Replant failure refers to the increase in the frequency of pests and diseases, increase in the miss-planted rate, suppression of plant nutrient growth, and decrease in fruit yield and quality caused by the continuous cultivation of the same crop on the same land under normal cultivation management. Replant failure may occur due to the effects of allelopathy between plants. Allelopathy is a phenomenon in which plants release metabolites into the environment to inhibit their own growth or that of their neighbors, and it plays a vital role in the changes in plant dominance and crop productivity [4, 5, 14]. The root system of melon is long and does not regenerate easily. Hence, it is susceptible to soil-borne diseases, and its continuous cropping is challenging. For facility cultivation of melons, problems caused by annual cultivation of melons, such as the lack of rainwater elution and poisoning because of the accumulation of root secretions in the soil—often leads to more serious continuous cropping obstacle problems, which in turn results in stunted growth of plants and poor fruiting. The root system of each melon crop has different adaptability to the soil environment, and grafting technology can be used to stabilize the growth of scion by using crops with strong adaptability as root stocks. In 1920, Japanese watermelon was grafted on bottle gourd or pumpkin rootstock to prevent Fusarium wilt. Miguel *et al.* and Yang *et al.* indicated that melon grafting on pumpkin rootstock can also effectively prevent *Fusarium oxysporum* infection [8, 13].

Phenolic acids, products of secondary metabolic reactions in plants, are related to disease resistance and reactive oxygen species scavenging systems in plants and can be excreted through the root, resulting in plant allelopathy. Because of differences between crops, their phenolic acid types and accumulation levels vary, gradually changing the physical and chemical properties of the soil. This affects crop growth not only directly but also indirectly by changing the microbial phase of the soil [3, 13]. Among the secretions of the cucumber root, the phenolic acid with the most significant inhibitory effect is 2,4-dichlorobenzoic acid [1]. Treating cucumbers and fig-leaf gourd (*Cucurbita ficifolia*) with 0.05–0.25 mol/L of cinnamic acid can gradually inhibit root elongation and reduce apical cell viability, and the extent of inhibition is significantly more severe in cucumbers than in *C. ficifolia* [10]. Because each crop has different sensitivity to phenolic acid, the grafting technique can be used to replace their root systems to avoid the negative effects of plant allelopathy. Qiao *et al*. and Zhou *et al*. are examples of successful grafting of cucumber on *C. ficifolia* with a high tolerance of cinnamic acid [10, 15]. The aforementioned studies focused on the improvement of low temperature tolerance. However, the better phenolic acid tolerance of the *C. ficifolia* root system mentioned implies that grafting of cucumber on *C. ficifolia* rootstock may improve the continuous cropping ability of the cucumber. In the present study, we used 'Honey World' melon as the scion and grafted its seedlings onto oriental pickling melon and squash rootstocks with better climate and soil adaptability in order to increase the grated seedlings' resistance to continuous cropping obstacles and to analyze phenolic acid composition of the medium causing continuous cropping obstacles.

2. Materials and Methods

2.1. Experimental Materials

'Honey World' melon (Known-You Seed, Kaohsiung, Taiwan) were grafted on the oriental pickling melon 'Hwi Pei' (Pingtung Hsin Da Seed Company, Pingtung, Taiwan), 'Zhuang Shi' squash (Known-You Seed), and 'Xin Tu Zuo' squash (He Sheng Seed Company, Tainan, Taiwan) rootstocks using top cleft grafting; the nongrafted own-rooted seedlings were used as the control group.

2.2. Experimental Methods

The experiment was conducted in two parts. The first cultivation site was the greenhouse of the Department of Horticulture, Chung Hsing University. The grafted seedlings were planted in 4-inch red plastic flowerpots after 7 days of hardening. They were irrigated with 80 mL of Yamazaki's Melon nutrient solution every 2 days. The medium used twice for continuous cropping of melons was used as the cultivation medium, while the control group was treated with a mixed cultivation medium comprising peat soil (Bio-Mix Potting substratum 003 B, Tref, The Netherlands), vermiculite, and pearl stone at an 8:1:1 (v/v/v) ratio.

The grafted seedlings were planted in 4-inch red plastic flowerpots after 7 days of curing. The gallic acid treatment group was not irrigated with any nutrient solution after colonization and before treatment to avoid interference, and only water was provided to maintain growth. After the treatment had started, 50 or 100 mg/L gallic acid was added to the nutrient solution, and the specimens were irrigated daily with 50 mL of the mixture solution. Each treatment was repeated three times, and each repetition involved two plants. Sampling analysis was performed on the 10th day following the colonization treatment.

2.3. Investigation Items and Methods

2.3.1. Plant Trait Investigation

a) Plant traits include scion length, stem diameter, above-ground fresh weight, above-ground dried weight, and number of leaves; the leaf area was measured by separating the leaves from the petioles and placing the leaves face up in the leaf area meter (LI-COR 3000A, LICOR Lincoln Neb.).
b) Chlorophyll value reading: With the sixth fully expanded, mature leaf from the growth point onwards selected, a chlorophyll meter (SPAD 502 Plus Chlorophyll Meter, Spectrum) was used to measure the chlorophyll content of any three random points, avoiding the leaf veins; the average value of the three taken values was taken as the measured chlorophyll content value. The measured value indicates the relative chlorophyll content or greenness of the leaves.
c) Degree of inhibition (%) formula: [(Pretreatment growth − posttreatment growth)/posttreatment growth] × 100%.

2.3.2. Analysis of Phenolic Compounds in the Medium After Fruit Harvesting

The medium materials were taken from the post-cultivation medium in Experiment 1, which was naturally air-dried, placed in a brown Kraft paper bag, and stored in a cool indoor place. Each treatment was repeated three times, and each repetition involved two pots.

a) Total phenolic content:

On the basis of the Office Method of Analysis of the Association of Office Analytical Chemist and the analysis methods used by Swain and Hill [11], 2.5 g of air-dried medium was weighed and added into a beaker, followed by the addition of 20 mL of deionized water. The mixture was shaken at room temperature in shaking water bath (SC12, Shinchan, New Taipei City, Taiwan) at 100 rpm for 1 h and allowed to stand for 30 min. The medium was filtered with filter paper (ADVANTEC No. 1) to obtain the extract. Next, 1 mL of extract was added into a glass test tube, and 5 mL of Folin–Ciocalteu phenol reagent (Merck) and 1 mL of saturated sodium carbonate aqueous solution were added subsequently. The mixture was shaken evenly and allowed to stand for 30 min. A spectrophotometer (Z-2300, Hitachi) was used to measure the absorbance at 750 nm. The measured absorbance was converted to the total phenol content using a standard curve made of gallic acid as the total phenol standard.

b) Phenolic acid analysis by high-performance liquid chromatography

First, 5 g of dry medium was weighed and placed into a beaker, followed by the addition of 10 mL of 95% EtOH and 10 mL of deionized water. The mixture was shaken at room temperature at 100 rpm for 1 h and allowed to stand for 30 min. Next, the extract was filtered with filter paper (ADVANTEC NO.1) and 0.45-μm Millipore Syringe Filters (HV) in sequence, and 20 μL of the filtrate was aspirated with a micro syringe and injected into the Hitachi L-2130 Intelligent Pump: the flow term was water: glacial acetic acid: methanol (v/v/v), the flow rate was 0.5 mL/min, the separation tube was Chromolith Performance RP-18 end capped (No.1.02129, Merck, Germany). Finally, the absorption wavelength of phenolic acid was measured at 250 nm (Hitachi RI Detector L-2490), and the flow time of each sample was 40 min.

Next, 0.01 g of each phenolic acid sample was weighed and added to 5 mL of alcohol and 5 mL of deionized water to prepare a 1000 mg/L mother solution. The mother solution was then diluted to 20, 40, 60, 80, and 100 times, and the delay time and peak area of each phenolic acid were analyzed using high-performance liquid chromatography (HPLC). A standard line was drawn to convert the content of different phenolic acids in the medium; the concentration conversion formula of each phenolic acid sample is presented in the appendix.

2.4. Statistical Analysis

Data statistics were designed by Completely randomized design, using Office Excel 2013 for graphing and PROC ANOVA (analysis of variance procedure) in SAS software package version 9.1 (SAS Insbitue, Cary, NC) for variance analysis (α = 0.05). Fisher's LSD was used to compare the means of each treatment.

3. Results

3.1. Cultivation Using a Continuous Cropping Medium

The vegetative growth situation of the ‘Honey World’ melon grafted seedlings after 7 and 14 days of cultivation using continuous cropping medium is presented in Table 1. After 7 days, the vegetative growth condition of the grafted seedlings differed from that of the own-rooted seedlings. The oriental pickling melon ‘Hwi Pei’, ‘Zhuang Shi’ squash, and ‘Xin Tu Zuo’ squash rootstock-grafted seedlings had significantly longer scion stem lengths (14.1, 12.4, and 12.6 cm, respectively) than did the own-rooted seedlings (7.5 cm). Between the experimental groups and the own-rooted seedlings, no significant difference was noted in scion stem diameter, which ranged from 2.89 to 3.46 mm. Between the treatment groups and the control group, no significant difference was noted in the number of leaves (range: 6–6.8 leaves). Additionally, no significant difference was noted between seedlings grafted on different rootstock, and all the grafted seedlings had more leaves than did the own-rooted seedlings (average of 5 leaves). The group with seedlings grafted on the oriental pickling melon ‘Hwi Pei’ rootstock had a significantly larger leaf area (174 cm^2) among the treatment groups. No significant difference was observed between the other three groups (range: 99.3–125 cm^2). The treated grafted seedlings and own-rooted seedlings groups both had significantly lower weight than did that of the control group; the reduction was 33.3%, 24.2%, 42.0%, and 53.7%, respectively. Regarding the comparison of rootstock-grafted seedlings, no significant difference was observed between the groups. All groups had a fresh weight ranging from 6.94 g to 8.21 g, with the control group having the lowest (4.73 g). The oriental pickling melon ‘Hwi Pei’ and ‘Zhuang Shi’ squash grafted seedlings and the own-rooted seedlings of the treatment groups had significantly lower dried weight compared with the control group; the reduction was 19.0%, 20.5%, and 13.0%, respectively; no significant difference was observed between the ‘Xin Tu Zuo’ squash grafted seedlings of the treatment groups and the control group; they had a dried weight of 0.75 and 0.86 g, respectively. Regarding the comparison of different rootstock grafting groups, the seedlings grafted on the oriental pickling melon ‘Hwi Pei’ and ‘Xin Tu Zuo’ squash rootstocks had significantly better dried weight accumulation, which was respectively 0.81 and 0.75 g. The seedlings grafted on the ‘Zhuang Shi’ squash rootstock followed closely in second with a dried weight of 0.66 g, and the own-rooted seedlings had the lowest dried weight of 0.43 g. Regarding the fresh weight inhibition ratio of the ‘Honey World’ melon top cleft grafted seedlings that were treated with the continuous cropping medium for 7 days, the own-rooted seedlings appeared to have the highest inhibition ratio (53.2%), which was significantly higher than that of the oriental pickling melon ‘Hwi Pei’, ‘Zhuang Shi’ squash, and ‘Xin Tu Zuo’ squash rootstock-grafted seedlings (33.3%, 23.3%, and 41.9%, respectively).

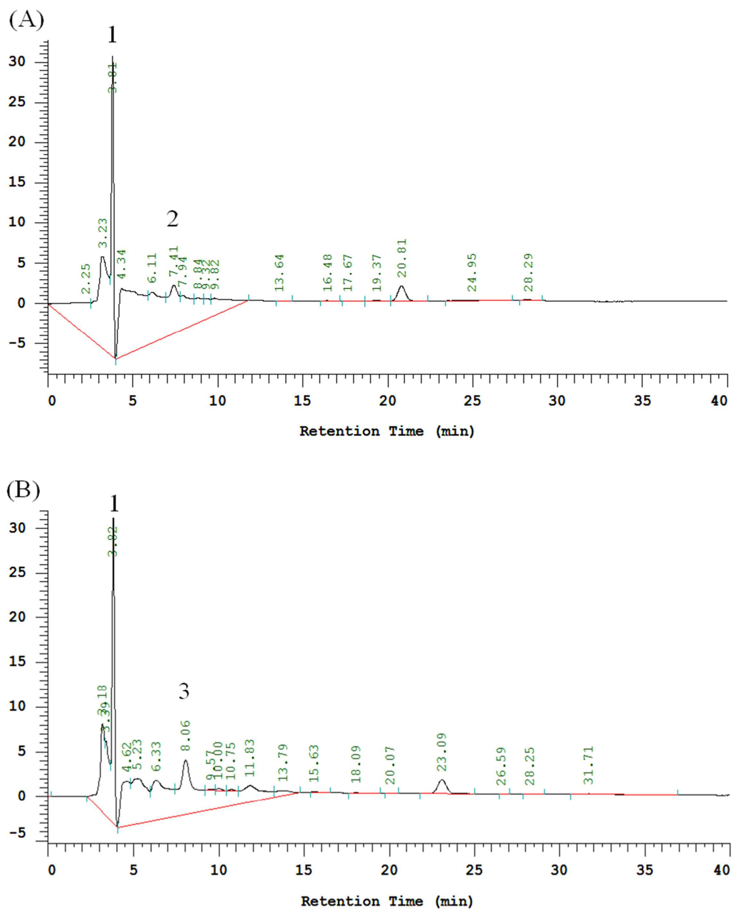
Table 1. *Influence of 7-day and 14-day continuous crop medium cultivation on the growth of the ‘Honey World’ melon grafted seedlings.*

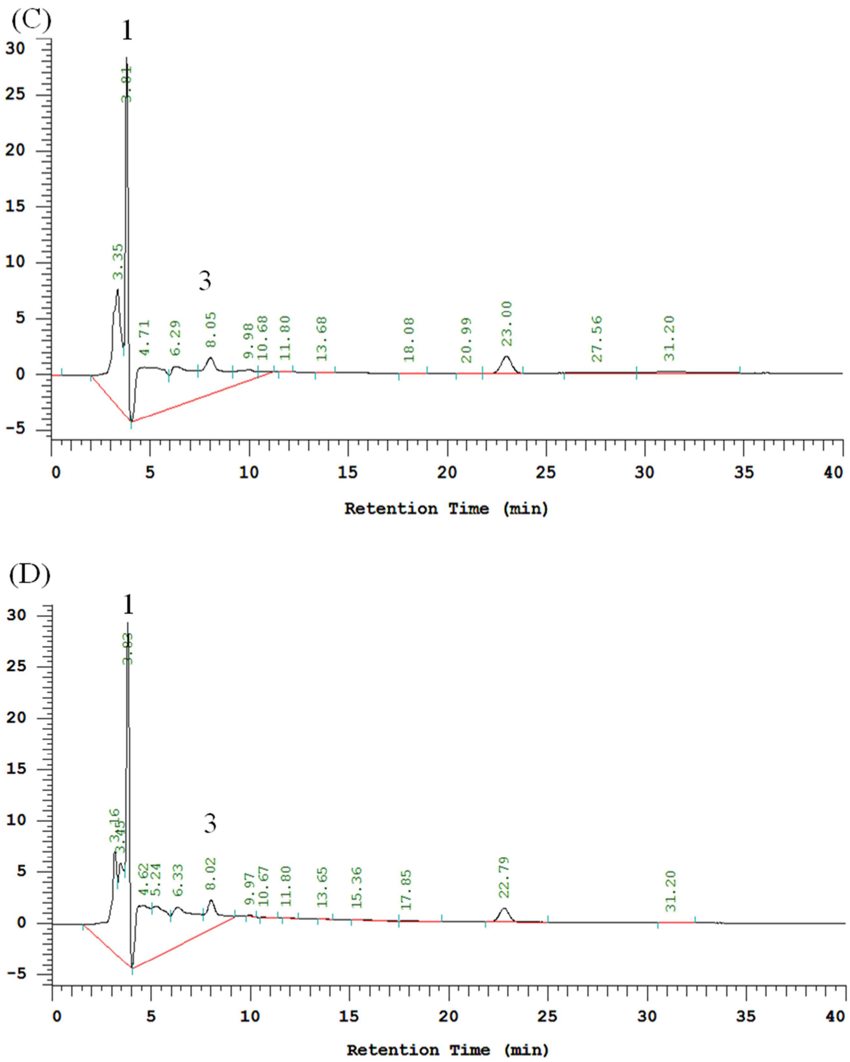
Rootstock species	Mediumy treatment	Scion length (cm)		Scion diameter (mm)		Number of leaves (No.)		Leaf area (cm2)		Fresh weight (g)		Dried weight (g)	
7 days													
‘Hwi Pei’ Oriental pickling melon	Yes	14.1	bc[z]	3.29	ab	6.5	ab	174	b	8.21	cd	0.81	b
	No	18.9	a	3.51	ab	6.8	a	219	a	12.3	a	1.00	a
‘Zhuang Shi’ squash	Yes	12.4	bc	3.48	ab	6.0	b	125	cd	6.94	d	0.66	cd
	No	15.8	ab	3.04	b	6.5	ab	170	b	9.10	bc	0.83	b
‘Xin Tu Zuo’ squash	Yes	12.6	bc	2.89	b	6.8	ab	123	cd	7.90	cd	0.75	bc
	No	13.6	bc	4.16	a	6.7	ab	147	bc	13.6	a	0.86	b
Own-rooted seedlings (control)	Yes	7.5	d	3.46	ab	5.0	c	99.3	d	4.73	e	0.43	e
	No	10.1	cd	3.99	a	5.0	c	128	cd	10.1	b	0.52	d

Rootstock species	Mediumy treatment	Scion length (cm)		Scion diameter (mm)		Number of leaves (No.)		Leaf area (cm2)		Fresh weight (g)		Dried weight (g)	
14 days													
‘Hwi Pei’ Oriental pickling melon	Yes	33.2	c	3.48	c	9.8	b	322	d	17.6	cd	1.91	de
	No	50.5	a	4.74	a	12.3	a	563	a	33.3	a	3.38	a
‘Zhuang Shi’ squash	Yes	33.5	c	3.73	bc	10.3	b	326	cd	19.4	c	2.13	de
	No	53.6	a	3.69	c	11.8	a	408	bc	26.8	b	2.99	ab
‘Xin Tu Zuo’ squash	Yes	35.0	bc	3.78	bc	10.3	b	361	cd	19.9	c	2.20	cd
	No	48.0	ab	4.21	abc	12.0	a	462	b	29.0	b	2.80	bc
Own-rooted seedlings (control)	Yes	25.4	c	4.00	bc	8.6	c	339	cd	13.8	d	1.58	e
	No	29.3	c	4.41	ab	9.5	bc	326	cd	19.7	c	2.00	de

[z] Means followed by different letters in the same columns in each growing period were significantly different by Fisher’s LSD test at 5% level.
[y] Treatment means replacement the original cultivation culture with the continuous cropping mediums that have been used twice for continuous cropping of own-rooted melon seedlings.

The results indicated that after the grafted seedlings of ‘Honey World’ melon were treated with the continuous cropping medium for 14 days, the said grafted seedlings exhibited growth inhibition. In terms of scion stem length, no significant difference was observed between the rootstock-grafted seedlings and the own-rooted seedlings of each treatment group; they all had a scion stem length of 25.4–33.5 cm. In terms of scion stem diameter, no significant difference was observed between the rootstock-grafted seedlings and the own-rooted seedlings of each treatment group; they all had a scion stem diameter of 3.48–4.00 mm. The grafted seedlings of the treatment group had significantly more leaves than the own-rooted seedlings (8.6 leaves); no significant difference was observed between the different rootstock-grafted seedlings; they all had 9.8–10.3 leaves. Leaf area was not significantly different between the different rootstock-grafted seedlings and the own-rooted seedlings of the treatment group; they all had a leaf area of 322–361 cm^2. The different rootstock-grafted seedlings of the treatment group had significantly better fresh weight accumulation than did the own-rooted seedlings (13.8 g); the different rootstock-grafted seedlings had a fresh weight accumulation of 17.6–19.9 g. The different rootstock-grafted seedlings of the treatment group had significantly better dried weight accumulation than did the own-rooted seedlings (1.58 g); no significant difference was observed between the different rootstock-grafted seedlings in terms of dried weight accumulation (range: 1.91–2.20 g).





*1: Gallic acid; 2: Vanillic acid; 3: m-Hydroxybenzoic acid

Figure 1. *Phenolic acid types in the medium after the cultivation of grafted melon seedlings analyzed using HPLC: (A) own-rooted seedlings; and (B) oriental pickling melon 'Hwi Pei' rootstocks; and (C) 'Zhuang Shi' squash rootstock; and (D) 'Xin Tu Zuo' squash rootstock.*

3.2. Qualitative and Quantitative Data of Phenolic Acids in the Postharvest Medium of Grafted Melon Seedlings

The HPLC analysis results of the phenolic acid types in the medium of the own-rooted seedlings of 'Honey World' melon were presented in Figure 1(A). A peak is obvious at 3.81 min (marked as 1), consistent with the retention time of gallic acid (3.93–3.99 min). Therefore, marking 1 was inferred to be gallic acid. A peak is observed at 7.41 min (marked as 2), consistent with the retention time of vanillic acid (7.19–7.50 min). Therefore, marking 2 was inferred to be vanillic acid.

The HPLC analysis results of the phenolic acid types in the medium of the oriental pickling melon 'Hwi Pei' grafted seedlings of 'Honey World' melon were presented in Figure 1(B). A peak is obvious at 3.82 min (marked as 1), which was inferred to be gallic acid. A peak is observed at 8.06 min (marked as 2), consistent with the retention time of m-hydroxybenzoic acid (7.77–8.23 min). Therefore, marking 2 was inferred to be m-hydroxybenzoic acid.

The HPLC analysis results of the phenolic acid types in the medium of the 'Zhuang Shi' squash grafted seedlings of 'Honey World' melon were presented in Figure 1(C). A peak is obvious at 3.81 min (marked as 1), which was inferred to be gallic acid, and at 8.05 min (marked as 2), which was inferred to be m-hydroxybenzoic acid.

The HPLC analysis results of the phenolic acid types in the medium of the 'Xin Tu Zuo' squash grafted seedlings of 'Honey World' melon are presented in Figure 1(D). A peak is obvious at 3.83 min (marked as 1), which was inferred to be gallic acid, and at 8.02 min (marked as 2), which was inferred to be m-hydroxybenzoic acid.

The types and contents of phenolic acids in the medium extract after fruit harvest analyzed with HPLC are presented in Table 2. In terms of gallic acid concentration, the medium of the 'Xin Tu Zuo' squash grafted seedlings obviously had the lowest concentration after harvest (21.4 mg/kg); the oriental pickling melon 'Hwi Pei' grafted seedlings, 'Xin Tu Zuo' squash grafted seedlings, and own-rooted seedlings did not differ significantly in terms of gallic acid concentration in the medium postharvest; they had a medium gallic acid concentration of 31.1, 33.1, and 34.4 mg/kg, respectively. Moreover, m-hydroxybenzoic acid was not detected in the medium after harvesting the own-rooted fruits. The postharvest m-hydroxybenzoic acid concentration in the medium was significantly higher in the oriental pickling melon ‘Hwi Pei’ (72.5 mg/kg) and ‘Xin Tu Zuo’ squash (74.4 mg/kg) grafted groups than that of ‘Zhuang Shi’ squash grafted group (62.8 mg/kg). Postharvest vanillic acid concentration in the own-rooted seedling medium was 39.7 mg/kg; vanillic acid was not detected in the medium of other rootstock-grafted groups after harvesting. The ‘Xin Tu Zuo’ squash grafted seedlings had the significantly highest postharvest total phenol concentration in the medium (263 mg/kg), followed by oriental pickling melon ‘Hwi Pei’ (222 mg/kg), control group (205 mg/kg), and ‘Zhuang Shi’ squash grafted seedlings (179 mg/kg).

Table 2. *HPLC analysis of the phenolic acid content of the medium after the cultivation of top cleft grafted ‘Honey World’ melon seedlings.*

Rootstock species	Gallic acid (mg/kg)		m-hydroxybenzoic acid (mg/kg)		Vanillic acid (mg/kg)	Total phenolic acid (mg/kg)	
‘Hwi Pei’ Oriental pickling melon	31.1	a[z]	72.5	a	-[y]	222	b
‘Zhuang Shi’ squash	21.4	b	62.8	b	-	179	c
‘Xin Tu Zuo’ squash	33.1	a	74.4	a	-	263	a
Own-rooted seedlings	34.4	a	-		39.7	205	b

[z] Means followed by different letters in the same columns were significantly different by Fisher’s LSD test at 5% level.
[y] The lack of a wave peak in the analysis spectrum indicates that a wave peak was not detected.

Table 3. *Influence of gallic acid treatment on the vegetative growth of the ‘Honey World’ melon top cleft grafted seedlings after 10 days.*

Rootstock	Concentration (mg/L)	Scion length (cm)		Scion diameter (mm)		Number of leaves (no.)		Leaf area (cm^2)		Chlorophyll (unit)		Fresh weight (g)		Dried weight (g)	
‘Hwi Pei’ Oriental pickling melon	0	72.9	ab[z]	5.39	ab	12.5	a	540	a	26.8	bc	31.3	bc	4.27	ab
	50	81.3	ab	5.43	ab	12.0	ab	639	a	29.7	ab	37.4	a	4.66	a
	100	63.7	b	5.74	a	11.0	b	549	a	26.1	c	33.6	bc	4.20	ab
‘Zhuang Shi’ squash	0	78.5	ab	4.77	bc	11.8	ab	563	a	30.7	a	31.2	bc	4.41	a
	50	90.0	a	4.22	c	11.7	ab	635	a	32.4	a	34.1	ab	4.47	a
	100	77.1	ab	5.03	abc	11.7	ab	552	a	32.1	a	31.0	bcd	4.18	abc
‘Xin Tu Zuo’ squash	0	67.0	b	5.19	ab	12.0	ab	506	a	30.1	a	30.5	cd	4.45	a
	50	75.0	ab	5.09	abc	12.3	ab	587	a	31.5	a	34.3	ab	4.63	a
	100	75.7	ab	4.99	abc	11.3	ab	577	a	30.7	a	33.8	b	4.86	a
Own-rooted seedlings (control)	0	74.7	ab	5.22	ab	12.7	a	503	a	27.0	bc	34.1	ab	4.52	a
	50	87.7	a	4.98	abc	12.7	a	600	a	31.5	a	32.7	bc	3.59	bc
	100	67.7	b	4.90	abc	11.3	ab	515	a	30.7	a	27.7	d	3.44	c

[z] Means followed by different letters in the same columns were significantly different by Fisher’s LSD test at 5% level.

3.3. Irrigation Treatment Using Gallic Acid

The vegetative growth of ‘Honey World’ melon grafted seedlings treated with 50 and 100 mg/L gallic acid for 10 days is presented in Table 3. Grafted seedlings treated with gallic acid did not exhibit significant growth inhibition, whereas the own-rooted seedlings did. No significant difference was observed in scion stem length between the treated grafted seedlings and the control group (63.7–81.3 cm, 77.1–90.0, and 67.0–75.7 cm, respectively). The own-rooted seedlings treated with 100 mg/L acid had a lower scion stem length of 67.7 cm. No significant difference was observed in scion stem diameter between the treated grafted seedlings, treated own-rooted seedlings, and control group (5.39–5.74, 4.22–5.03, 4.99–5.19, and 4.90–5.22 mm, respectively). The control group of the oriental pickling melon ‘Hwi Pei’ grafted seedlings group had significantly more leaves than did the 100 mg/L treatment group (12.5 leaves vs. 11.0 leaves). No significant difference was observed between the control group and treatment group of other rootstock-grafted seedlings and their corresponding own-rooted seedlings; they had approximately 11.8–11.7, 12.0–12.3, and 11.3–12.7 leaves. Leaf area was not significantly different between the control group and treatment group of rootstock-grafted seedlings (540–639 cm^2, 552–635 cm^2, 506–587 cm^2, and 515–600 cm^2, respectively). The 50 mg/L treatment group of oriental pickling melon ‘Hwi Pei’ rootstock-grafted seedlings had 3.6 units more chlorophyll than the 100 mg/L treatment group; the treatment groups of the own-rooted seedlings had 4.5 and 3.7 units more chlorophyll more than the control group. No significant difference was observed between the control group and the treatment group of the ‘Zhuang Shi’ squash and ‘Xin Tu Zuo’ squash rootstock-grafted seedlings (30.7–32.4 units and 30.1–31.5 units, respectively). With an above-ground fresh weight of 37.4 g, the 50 mg/L treatment group of the oriental pickling melon ‘Hwi Pei’ rootstock-grafted seedlings had a significantly higher above-ground fresh weight than did the other treatment groups; no significant difference was observed between the treatment groups and the control group of ‘Zhuang Shi’ squash rootstock-grafted the seedlings; they both had an above-ground fresh weight of 31.0–34.1 g. The treatment groups of the ‘Xin Tu Zuo’ squash rootstock-grafted seedlings had significantly higher above-ground fresh weight (34.3 and 33.8 g, respectively) than did that of the control group. No significant difference was observed between the different concentration treatment groups. The 100 mg/L treatment group of the own-rooted seedlings obviously had the lowest fresh weight of 27.7 g. No significant difference was observed in above-ground dried weight accumulation between the treatment group and the control group of the various rootstock-grafted seedlings (4.20–4.66 g, 4.18–4.47 g, and 4.45–4.86 g, respectively). The treatment group of the own-rooted seedlings had a significantly lower weight (3.59 g) than that of the control group (3.44 g). No significant difference was observed between the two treatment groups treated with mediums of different concentrations.

4. Discussion

4.1. Effects of the Continuous Cropping Medium on Grafted Seedlings

The occurrence of continuous cropping obstacles is mainly the result of the accumulation of pathogen and toxic substances caused by annual cultivation and improper fertilization. The decomposition of organic matter is accelerated because of the high temperature in the facility and the prevention of rainwater elusion by the rainwater shade; this causes the accumulation of toxic substances such as phenolic acid [2]. Lin treated melon seeds with an extract of medium used for continuous cropping of melon, which significantly inhibited seed germination; moreover, this inhibitory effect increased with the number of continuous cropping [6]. The results of seedling cultivation using the continuous cropping medium revealed that as the number of continuous cropping increased, the growth of seedlings declined, and the fresh and dried weight decreased significantly.

In the current study, the medium in which melons were cropped twice continuously was used to cultivate melon-grafted seedlings. The continuous cropping medium significantly inhibited the growth of grafted and own-rooted seedlings, and the inhibition ratio of own-rooted seedlings was significantly higher; our results were thus consistent with those of Lin et al., [6]. Meanwhile, grafting treatment can reduce the inhibitory effect of continuous cropping medium, and the vegetative growth of grafted seedlings in continuous cropping medium is significantly superior to that of the own-rooted seedlings in the same medium, indicated by larger leaf area and higher fresh and dry weights of above-ground parts. Among the different rootstocks, 'Xin Tu Zuo' squash obviously has the best fresh and dry weight accumulation. By using better root system obtained from grafting, seedlings could overcome the continuous cropping obstacles caused by continuous cropping medium.

Lu used cucumber-grafted pumpkin rootstock to reduce crop barriers [7]. The root system of the grafted pumpkin was stronger, and the nutrient uptake capacity was significantly better than that of own-rooted seedlings. In the agricultural production, the seedling stage is the most important part which crops laying the foundation for future harvest. In our study, the use of pumpkin rootstock can reduce the inhibitory effect of continuous cropping obstacles in the seedling stage and we did not observe any the soil-borne diseases happened during the test. The continuous cropping obstacle caused by the accumulation pathogen is suggested to be done in other research.

4.2. Analyzing the Postharvest-Cultivation Medium Using HPLC

Chen treated celery and Brassica seedlings with drip irrigation solution made from medium obtained from celery sand culture, resulting in the death of celery seedlings and the retardation of the growth of Brassica seedlings. This led to the inference that the substances causing the continuous cropping obstacle of celery may be water soluble. The type of phenolic acid in the continuous cropping medium that Chen analyzed through HPLC was similar to the pattern of gallic acid [2]. Lin analyzed the phenolic acids in the root and medium of two melon varieties using HPCL, and the results revealed that gallic acid and ρ-hydroxybenzoic acid were the dominant phenolic acids [6].

In our study, HPLC analysis of phenolic acids in the postharvest medium of melon indicated that the medium of the own-rooted seedlings may be dominated by gallic acid and vanillic acid, while the medium of other rootstock-grafted seedlings may be dominated by gallic acid and m-benzoic acid; these results agree with those obtained by Chen and Lin implying that gallic acid causes a substantial level of continuous cropping obstacle [2, 6]. The root system of 'Xin Tu Zuo' squash has higher total accumulated phenols; its root system is more developed and stronger, and the metabolism and secretion of the vigorously developed root system is higher [9]. The results indicated no significant difference between the self-root seedlings and oriental pickling melon "Qing Pi," indicating that the secretion of oriental pickling melon and cantaloupe roots may be similar, and the better growth of 'Xin Tu Zuo' squash rootstock-grafted seedlings in continuous cropping medium may be related to the stronger root system.

4.3. Irrigating the Grafted Seedlings Using Gallic Acid

According to the phenolic acid variety analysis results obtained based on the own-rooted seedling cultivation medium in previous studies and aforementioned experiments, we treated melon-grafted seedlings with 50 and 100 mg/L gallic acid. The results indicated no significant difference between the experimental groups (seedlings grafted on oriental pickling melon 'Hwi Pei' and squash rootstocks) and the control group. This indicated that gallic acid did not inhibit the grafted seedlings; however, the own-rooted seedlings exhibited significant growth inhibition, and the degree of inhibition increased with the increase of gallic acid concentration. Regarding the normal growth of grafted seedlings, the different tolerance of crops to phenolic acids may have overcome the growth inhibition exerted by phenolic acids. Xu grafted 'Jiashi' muskmelon using squash rootstock to mitigate the inhibitory effects of multiple phenolic acids on growth. In the phenolic acid treatment, the activity of reactive oxygen species–scavenging enzymes in the roots of grafted seedlings was significantly improved [12]. This implies that the better antioxidant capacity of the root system of pumpkin rootstock can resist the oxidative stress of cells caused by phenolic acid poisoning, leading to normal growth and metabolism. The maximum degree of tolerance to phenolic acid poisoning processed by pumpkin rootstock can be done in the future study for revealing the potential in practical application in the field.

5. Conclusion

Grafted seedlings cultivated in a continuous cropping medium can reduce the inhibitory effect of continuous cropping. We analyzed the types of phenolic acids in the medium after cultivation. The medium of own-rooted seedlings contained mainly gallic acid and vanillic acid, and the other treatments contained mainly gallic acid and m-hydroxybenzoic acid. The total phenolic content was the lowest in ‘Zhuang Shi’ squash. The grafted seedlings of each rootstock irrigated with gallic acid showed no adverse growth effect. However, the growth of the own-rooted seedlings was poor, indicating that gallic acid was the main inhibitory substance.

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