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Influence of Substrate Composition and Container Size on the Growth of Tissue Culture Propagated Apple Rootstock Plants

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Abstract: Substrate composition and container size are considered crucial for apple rootstock grown in a plug seedling system. This study investigated the effect of substrate material's proportion and different container volumes on the growth of apple rootstock (M-9) plants propagated by tissue culture. In substrate composition, three different ratios of peat moss (PM): vermiculite (VL): perlite (PL) at 1:1:1 (S1), 1:2:3 (S2), 3:1:2 (S3) were used. For container size, plants were grown in 1000 mL (C1), 500 mL (C2), and 300 mL (C3) containers filled by 1:1:1 mixture of PM, VL and PL. In both cases, plants were treated eleven weeks in a green house. Our results demonstrate that the plant height, number of leaves, leaf area, shoot fresh weight and root fresh weight of apple rootstock were significantly higher in substrate composition S3 compared with S1 composition. However, chlorophyll content (SPAD) and photosynthesis rate were unaffected by variation of substrate composition. Furthermore, rootstock grown in C1 container showed plant height, number of leaves, leaf area, number of nodes, root length, shoot and root's fresh and dry weight were significantly higher than those grown in C2 and C3 volume containers. The chlorophyll content and transpiration rate were not significantly affected by the different container volumes. These results suggest that the substrate ratio 3:1:2 of PM:VL:PL and container size 1000 mL were more favorable than other treatments for initial growth and development of the tissue culture propagated apple rootstock plants.

Keywords: chlorophyll content; leaf area; number of leaves; photosynthesis rate; transpiration rate

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1. Introduction

In Korea, apples are cultivated on a large scale, and this has a great impact on the Korean economy. Approximately 33,600 ha land in Korea was used for apple cultivation in 2017 and 2018 [1]. Apple trees generally exist in the form of grafts of scion and rootstock [2]. The functions of rootstock are fixation, water and nutrient uptake, pest and disease protection, support to growth and development of scion and enhanced fruit yield and quality [3,4]. Hence, proper selection and production of desirable rootstock plants are essential for productive apple orchards [5]. Morphological traits in early stage are a good indicator for selecting productive apple plants [6]. Apple trees grafted on M-9 rootstock are most widely used in Korea because of its low-canopy and high-density planting system [7]. Furthermore, productivity of M-9 apple plant also depends on above ground plant length and stem thickness [8]. However, 30–60% seedlings as well as yield and quality of apple were reduced by different viral pathogens in Korea [9,10]. Mass production of virus-free

and uniform rootstock is needed to meet the demand of domestic apple industry. Tissue culture technique is usually applicable for rapid and abundant propagation of disease-free plants [11]. In addition, successful acclimatization into normal atmospheric condition is crucial for clonally propagated plants [12]. Furthermore, the expectation is to increase more than 50% supply of good quality apple seedlings in Korea compared presence by adopting culture technology in 2025 [13]. The plug seedling system may well be utilized for the production of healthy grafted apple plants with possible automation and lower management costs. Notably, horticultural substrate and container size greatly influence seedling quality, yield and production cost in the plug seedling system.

Horticultural substrates are use in nurseries as a replacement for soil for producing healthy and disease-free plants [14]. Substrate culture is popular day by day because of its water use efficiency and easy to control accurate root environment. The quality of substrate differs based on the composition of organic and inorganic materials, which leads to differ water and nutrient holding capacity [15]. It has a great impact on plant growth and development. Therefore, selecting suitable substrate composition is imperative to plant productivity. Organic material peat moss and inorganic materials vermiculite and perlite are most common ingredients in making substrates [16,17]. Peat moss is familiar as a growth media because of its air-filled porosity and ability to efficient oxygen diffusion [17]. It is naturally sterile and suppresses fungal disease that can afflict seedlings and plant. The water-physical properties of vermiculite are favorable for plant growth and promote its use as a substrate material [18]. Furthermore, perlite is widely preferred as a substrate because it reduces the risk of damping off, provide optimum balance between air and water in root zone and encourage faster root growth of a plant [19].

Container size is another factor for plant growth and development in nurseries. It facilitates above and below ground biomass production. A plant's photosynthesis also depends on container size and how long the plant remains in a container [20]. In addition, container size regulates the root growth of plants. Furthermore, plant physiology and shoot growth greatly depend on root growth and metabolic activities of a plant [20]. Particularly, container size is a vital factor for apple plants, because its rootstock is grown in a small pot and remains in a nursery long term before transplanting.

In general, high quality planting materials increase production cost including labor cost. To improve the cost efficiency in apple production in plug seedling system, knowledge about optimum substrate composition and container volume are necessary for growers. However, this information is still lacking. A better understanding of the substrate composition and container size effect can improve management cost in apple industry. For this reason, the objective of this study was to investigate the influence of substrate composition and container size on the growth parameters of apple rootstock plants propagated by tissue culture.

2. Materials and Methods

2.1. Growing Conditions

For this study, two experiments were performed separately at a time in a polyethylene-film covered greenhouse located at Kangwon National University, Chuncheon, South Korea (latitude, 37°48′ N, longitude, 127°52′ E) using clonally propagated apple rootstock (M-9) plants during 27 June 2017 to 12 September 2017. Rootstock were grown in container filled with substrate media (mixture of peat moss, vermiculite and perlite). The light intensity was measured by light sensor (LightScout Quantum Meter 3668I, Spectrum Technologies, Aurora, IL, USA) and maintained 150 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the experimental period. The temperature and relative humidity (RH) were recorded using data logger (WatchDog 1450, Spectrum Technologies, Aurora, IL, USA). Throughout the experiment, the average greenhouse temperature and relative humidity were 30 ± 5 °C and 72 ± 5% respectively. Plants were irrigated twice a day with tap water and once a week with nutrient solution (EC 1.3 $dS \cdot m^{-1}$, N-P-K-Ca-Mg = 13.1-3.5-5.0-7.0-2.0 $me \cdot L^{-1}$, Rose culture medium, Aichi Prefecture, Japan) throughout the growing period.

2.2. Treatments

2.2.1. Substrate Composition (Experiment 1)

Unique sizes of apple rootstock plants (15 cm tall) were selected and transplanted in same-size containers (7.5 cm wide, 16 cm tall) for growth (Figure 1B). All containers contained 500 mL substrate, but substrate composition was varied based on treatment. Three different substrate composition ratios of peat moss (PM): vermiculite (VL): perlite (PL) were maintained at 1:1:1 (S1), 1:2:3 (S2) and 3:1:2 (S3) for three different treatments.

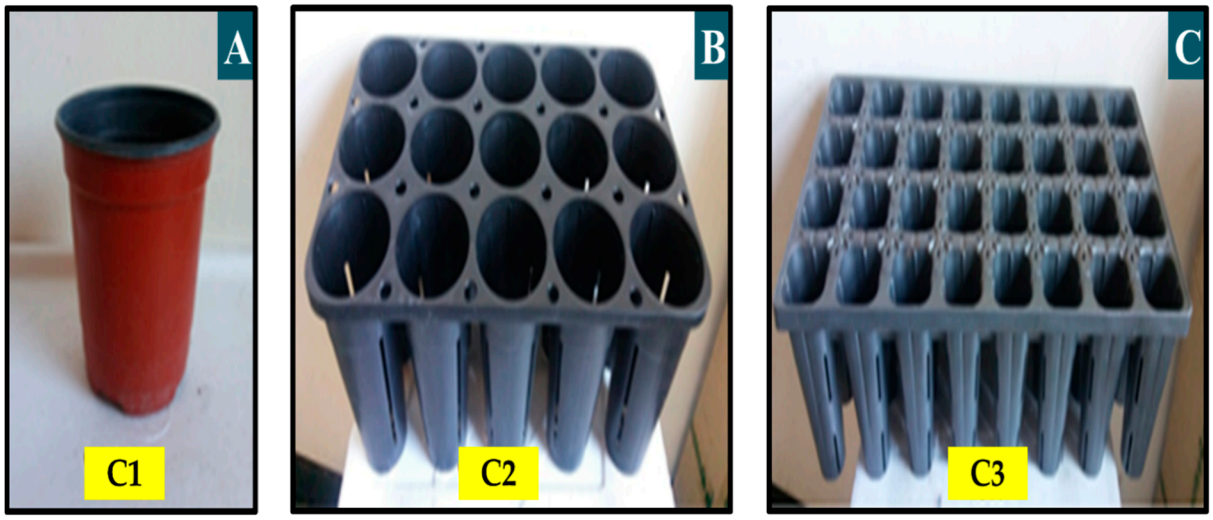

Figure 1. Different size of containers, (**A**) C1, 1000 mL; (**B**) C2, 500 mL; and (**C**) C3, 300 mL.

2.2.2. Container Size (Experiment 2)

In this experiment, 15 cm tall apple rootstock plants were selected and transplanted into a container for growth. Containers were filled with same composition of substrate media at 1:1:1 ratio of PM: VL: PL. However, the size of the container was different based on different treatments. Three types of containers indicated that C1 (1000 mL, 13 cm wide and 12 cm tall), C2 (500 mL, 7.5 cm wide and 16 cm tall) and C3 (300 mL, 5.5 cm wide and 17 cm tall) were selected for three treatments (Figure 1).

2.3. Measurement of Plant Growth Parameters

After eleven weeks of treatment, plant height was calculated from soil level to plant tip using a tape ruler. The number of leaves (except those less than 1 cm) and number of nodes were counted manually. Stem diameter was measured at 1 cm and 30 cm from the base of the plant using a digital caliper (CD-20APX; Mitutoyo Corp., Kanagawa, Japan). Chlorophyll content was measured by chlorophyll meter SPAD-502 (Konica-Minolta, Osaka, Japan). A portable photosynthesis meter (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) was used for measuring photosynthesis rate, stomatal conductance and transpiration rate in fully expanded leaves. Photosynthesis rates were measured at 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, 23 °C leaf temperature, 500 $\mu mol \cdot s^{-1}$ air flow and 400 $\mu mol \cdot mol^{-1}$ CO_2 in a leaf chamber.

Plants were uprooted from their container and then root was washed carefully by tap water to remove adhering materials and soil. Roots were separated from the plant. The root length of the plant was measured using a tape ruler. The leaf area of a plant was measured using a leaf area meter (Li-3100, Li-COR Inc., Lincoln, NE, USA). Shoot fresh weight and root fresh weight of plant were measured by an electronic balance (CUW420HX, CAS corporation, Korea). Shoot dry weight and root dry weight were measured after drying the plant samples in an oven (JEIO TECH OF-22GW, Korea) at 70 °C for 48 h.

2.4. Statistical Analysis

The experiment was conducted in a completely randomized design with five single plant replicates per treatment. Effects of treatments were analyzed using SAS program (Statistical analysis system, version 9.3, SAS Institute, Cary, NC, USA). Significant differences among the means were examined using ANOVA (Analysis of variance) followed by DMRT (Duncan's Multiple Range Test) at a 5% level ($p \leq 0.05$). The principal component analysis (PCA) was carried out using OriginLab 10.0 software (OriginLab, Northampton, MA, USA).

3. Results and Discussion

3.1. Effect of Substrate Composition (Experiment 1)

In Experiment 1, we used different substrate compositions, S1, S2 and S3, to improve plant growth and development of tissue culture-propagated apple plants (Figure 2). Plant height was significantly 11% taller in S3 compared with the S1 substrate composition (Figure 3A). Leaf number and leaf area were 5% and 11% higher, respectively, in S3 composition than in S1 (Figure 3B,C). The number of nodes was 20% higher in S3-treated plants compared with S1 (Figure 3D). Furthermore, shoot fresh weight was 23% and root fresh weight was 14% higher in plants those grown in S3 composition than those grown in S1 (Table 1). However, the number of leaves, leaf area, shoot fresh weight, root fresh weight and shoot dry weight were higher in S3-treated plants but significantly unaffected compared with those grown in the S2 substrate mixture (Figure 3 and Table 1). In addition, stem diameter at 1 cm and 30 cm above ground was not significantly different between the plants which were grown in S1, S2 and S3 compositions (Table 1).

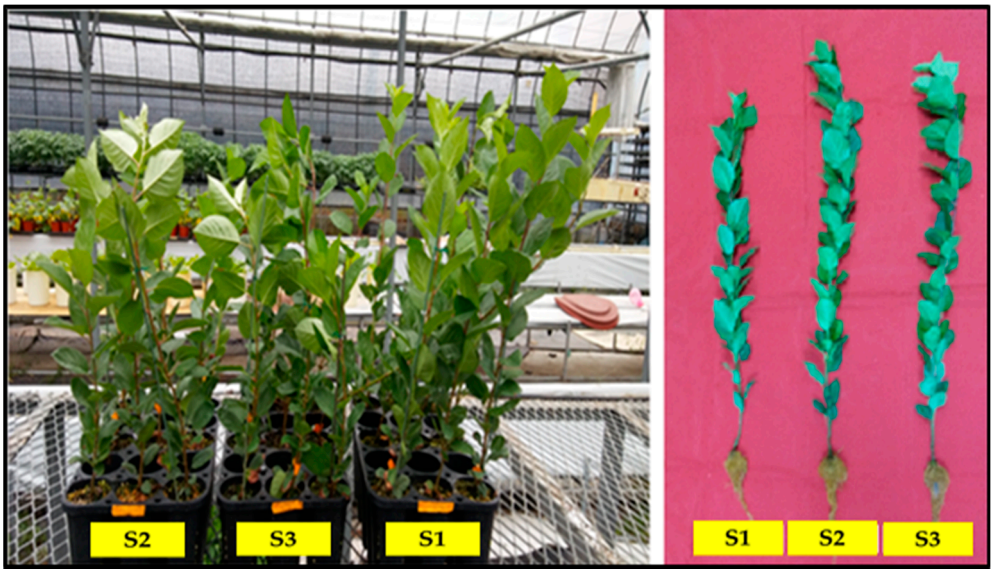

Figure 2. Effect of substrate composition on the growth of M-9 apple rootstock plants at 11 weeks after treatment in a greenhouse. S1, S2 and S3 indicate a 1:1:1, 1:2:3 and 3:1:2 ratio of peat moss: vermiculite: perlite, respectively.

In S3 substrate composition, proportion of peat moss is greater than perlite and vermiculite. May be the high concentration of peat moss in S3 substrate mixture is the reason for increased plant growth parameters in apple rootstock compared to other treatments. Kamrani et al. [17] reported that, as a substrate medium peat moss has favorable physiochemical properties for plant growth. In addition, its feature naturally freedom from pathogens and weed. Peat moss effect on apple rootstock is still unknown. However, other researchers showed that substrate with high concentrated peat moss is favorable for some vegetable plants. Sendi et al. [21] reported that five weeks after substrate treatment, high concentrated peat moss grown *Brassica oleracea* showed plant height, leaf number, leaf area,

shoot fresh weight and root fresh weight were significantly higher than those grown in low peat moss concentrated substrates. Plant height of lettuce, grown in only peat moss was taller than those grown in other substrate mixture such as compost, composted pine bark and humus [22]. Generally, in a substrate media peat moss provide nutrient, improve water holding capacity and buffering pH level which is helpful for plant growth and root development [16,23]. Furthermore, Vahdati et al. [24] showed, vigourness of the seedling depends on its rooting ability.

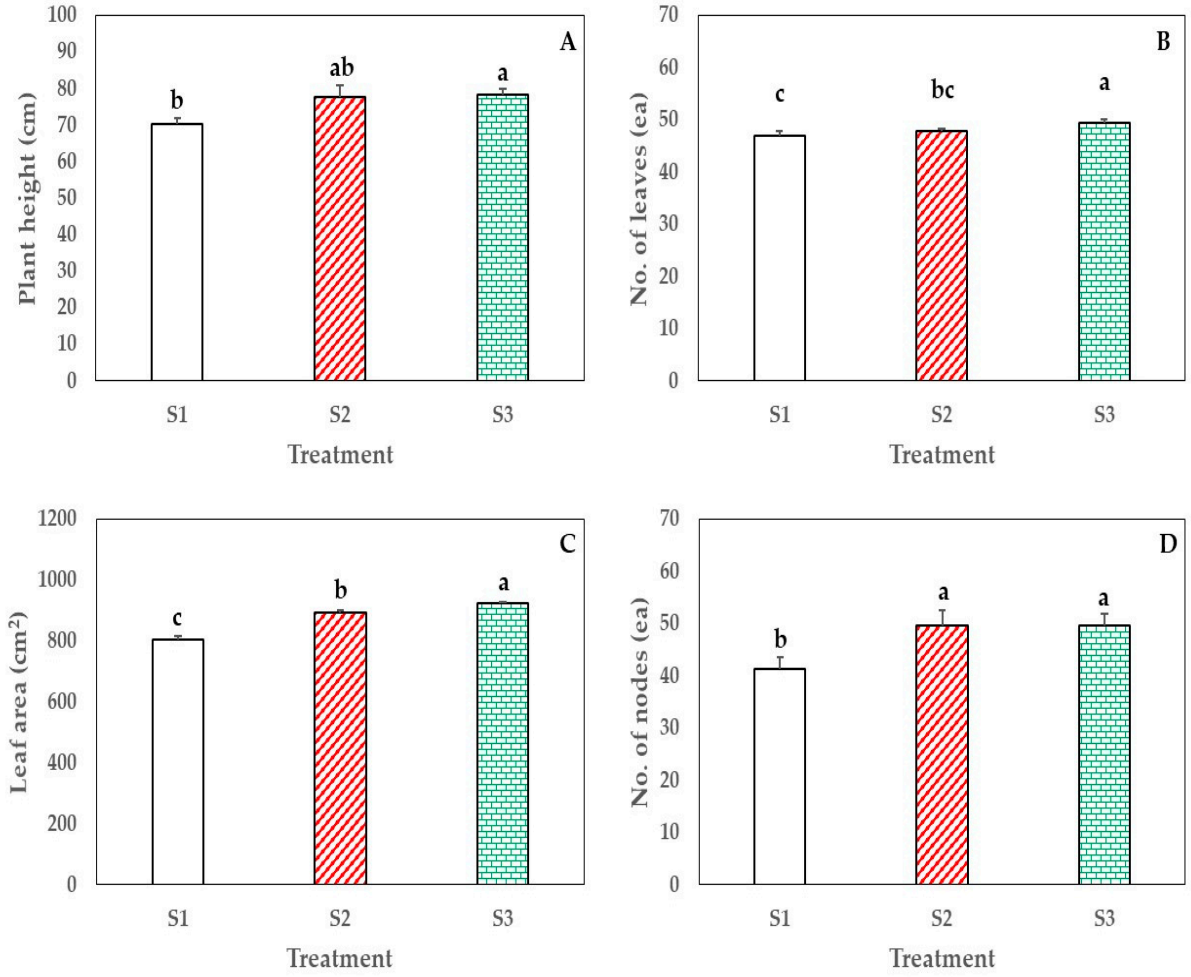

Figure 3. Effect of substrate composition on plant height (**A**), leaf number (**B**), leaf area (**C**) and node number (**D**) of M-9 apple rootstock plant at 11 weeks after treatment in a greenhouse. S1, S2 and S3 indicate 1:1:1, 1:2:3, and 3:1:2 ratio of peat moss: vermiculite: perlite, respectively. Lines above the bar represent the standard error of the mean (n = 5). Means above each bar followed by the same letters are not significantly different with Duncan's multiple range test (DMRT) at $p \leq 0.05$.

Table 1. Effect of substrate composition on stem diameter, root length, fresh weight and dry weight of M-9 apple rootstock plants at 11 weeks after treatment. S1, S2 and S3 indicate 1:1:1, 1:2:3, and 3:1:2 ratio of peat moss: vermiculite: perlite, respectively.

Treatment	SPAD (Value)	Photosynthesis ($\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$)	Conductance ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)
S1	38.9 ± 3.10 [z] a [y]	10.21 ± 1.21a	0.37 ± 0.08a	4.93 ± 0.32a
S2	34.8 ± 4.27a	11.02 ± 2.21a	0.38 ± 0.11a	4.93 ± 0.17a
S3	37.5 ± 3.41a	10.19 ± 1.89a	0.36 ± 0.12a	4.84 ± 1.81a

[z] Each value is the means ± standard error (n = 5). [y] Means within columns sharing the same letter are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

Photosynthesis is an indicator of plant strength. Different substrate combinations were not significantly affected in photosynthesis rate of apple rootstock plant (Table 2). Martin et al. [25] reported that photosynthesis rate of a plant greatly depends on it's received light intensity. In our experiment, light intensity was same for all plants grown in different treatments. In addition, although substrate mixture is different but substrate volume is same and rootstock of plant were in seedling stage. For these reasons, photosynthesis rate was similar in apple rootstock plant which were grown in different substrate combinations. Furthermore, at eleven weeks after treatment, SPAD, stomatal conductance and transpiration rate of apple rootstock plant were unaffected by different substrate composition (Table 2). It was happened due to unaffected photosynthesis rate. The PCA analysis also carried out to uncover the correlation of the different parameters with the different treatment groups of substrate composition (Figure 4). The elements of PC1 and PC2 described 57.39% and 42.61% of the variability in the data respectively. The PCA biplot presented clear segregation of the parameters into two groups. The results showed that photosynthesis rate, stomatal conductance, and transpiration rate were closely correlated to each other's compared to other variables. Several studies showed that the relation between photosynthesis, stomatal conductance and transpiration in a plant is interrelated and proportional [26–28]. Present study quite supports this hypothesis.

Table 2. Effect of substrate composition on SPAD, photosynthesis rate, stomatal conductance, and transpiration rate of M-9 apple rootstock plant at 11 weeks after treatment. S1, S2 and S3 indicate 1:1:1, 1:2:3, and 3:1:2 ratio of peat moss: vermiculite: perlite respectively.

Treatment	SPAD (Value)	Photosynthesis ($\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$)	Conductance ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)
S1	38.9 ± 3.10 [z] a [y]	10.21 ± 1.21a	0.37 ± 0.08a	4.93 ± 0.32a
S2	34.8 ± 4.27a	11.02 ± 2.21a	0.38 ± 0.11a	4.93 ± 0.17a
S3	37.5 ± 3.41a	10.19 ± 1.89a	0.36 ± 0.12a	4.84 ± 1.81a

[z] Each value is the means ± standard error (n = 5). [y] Means within columns sharing the same letter are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

3.2. Effect of Container Size (Experiment 2)

In Experiment 2, we focused on three different container sizes C1, C2 and C3 to improve plant growth of tissue culture propagated apple plant (Figure 5). Plant height in C1 size container was 6% and 12% significantly taller than those grown in C2 and C3 containers, respectively (Figure 6A). Information about the apple rootstock's response in different container sizes is still lacking. However, other researchers carried out experiments on the effect of container size on different tree and vegetable plants. *Parkia biglobosa* seedlings at ten weeks after treatment showed that plant height was taller in bigger-sized pots than those grown in a small-sized pot [29]. The plant height of sixteen-week-old *Eucalyptus citriodora* Hook seedlings was significantly taller in larger pot (5 L) compared with those grown in small pot (1 L) [30].

The number of leaves was 9% higher in C1 plants than those grown in C2 (Figure 6B). In addition, C1 treatment showed a 29% higher number of leaves than those grown in the C3 container. Leaf area in C1-treated plants was 12% significantly higher than C2-treated plants and 24% higher than C3-treated plants (Figure 6C). The node number of C1-treated plants was 20% significantly higher than those grown in C2 and C3 treatment (Figure 6D).

The stem diameter of C1-treated plants at 1 cm and 30 cm above ground was 25% greater than those plants were grown in C3 volume (Table 3). In addition, C2-treated plants showed more than 20% greater stem diameter at 1 cm and 30 cm above ground than those grown in C3 treatment. Shoot fresh weight of C1-treated plants was 21% higher than C2-treated plants and 38% higher than C3-treated plants. Shoot dry weight of C1-treated plants was 22% and 34% significantly higher than C2- and C3-treated plants, respectively. Dambreville et al. [31] reported that oilseed rape grown in a large pot showed a higher

number of leaves, leaf area, shoot dry weight and root dry weight compared with those grown in small pot.

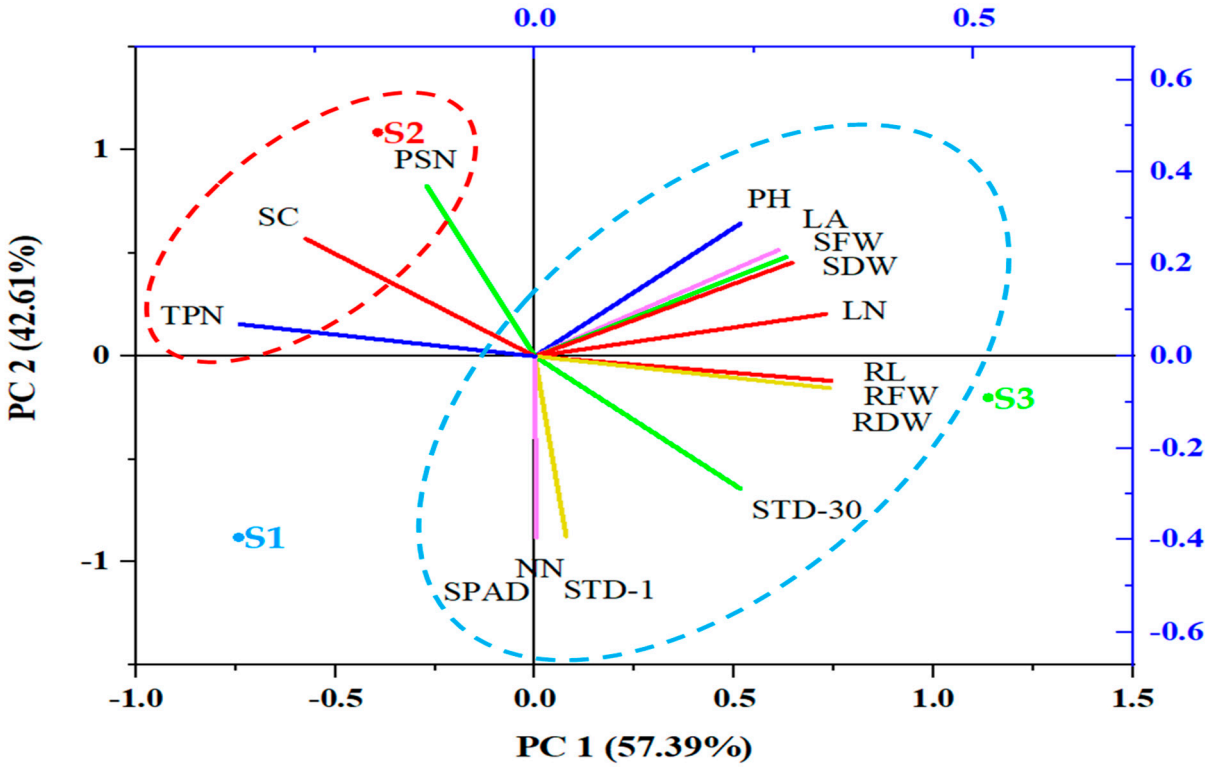

Figure 4. Principal component analysis (PCA) illustrates the variable treatment relationships among the three treatments of M-9 apple rootstock plant at 11 weeks after in a greenhouse. S1, S2 and S3 indicate 1:1:1, 1:2:3, and 3:1:2 ratio of peat moss: vermiculite: perlite, respectively. The lines starting from the central point of the biplots display the negative or positive associations of the different variables, and their proximity specifies the degree of correlation with specific treatment. PH, plant height; LN, leaf number; LA, leaf area; NN, node number; RL, root length; STD-1, stem diameter from 1 cm of ground; STD-30, stem diameter from 30 cm of ground; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; SPAD, SPAD value; PSN, photosynthesis rate; SC, stomatal conductance and TPN, transpiration rate.

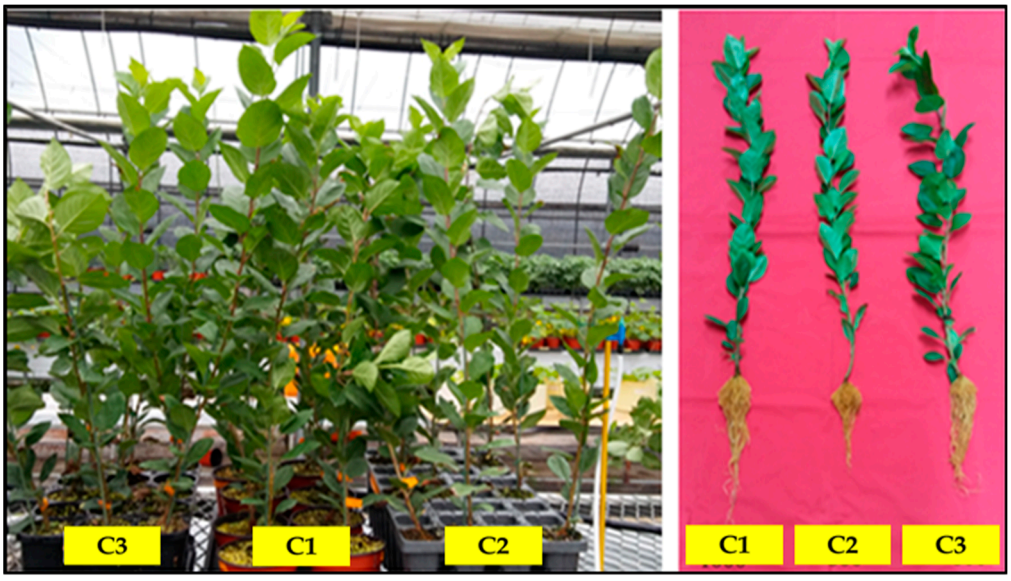

Figure 5. Effect of container size on the growth of M-9 apple rootstock plants at 11 weeks after treatment in a greenhouse. C1, C2 and C3 indicate container size 1000 mL, 500 mL and 300 mL, respectively.

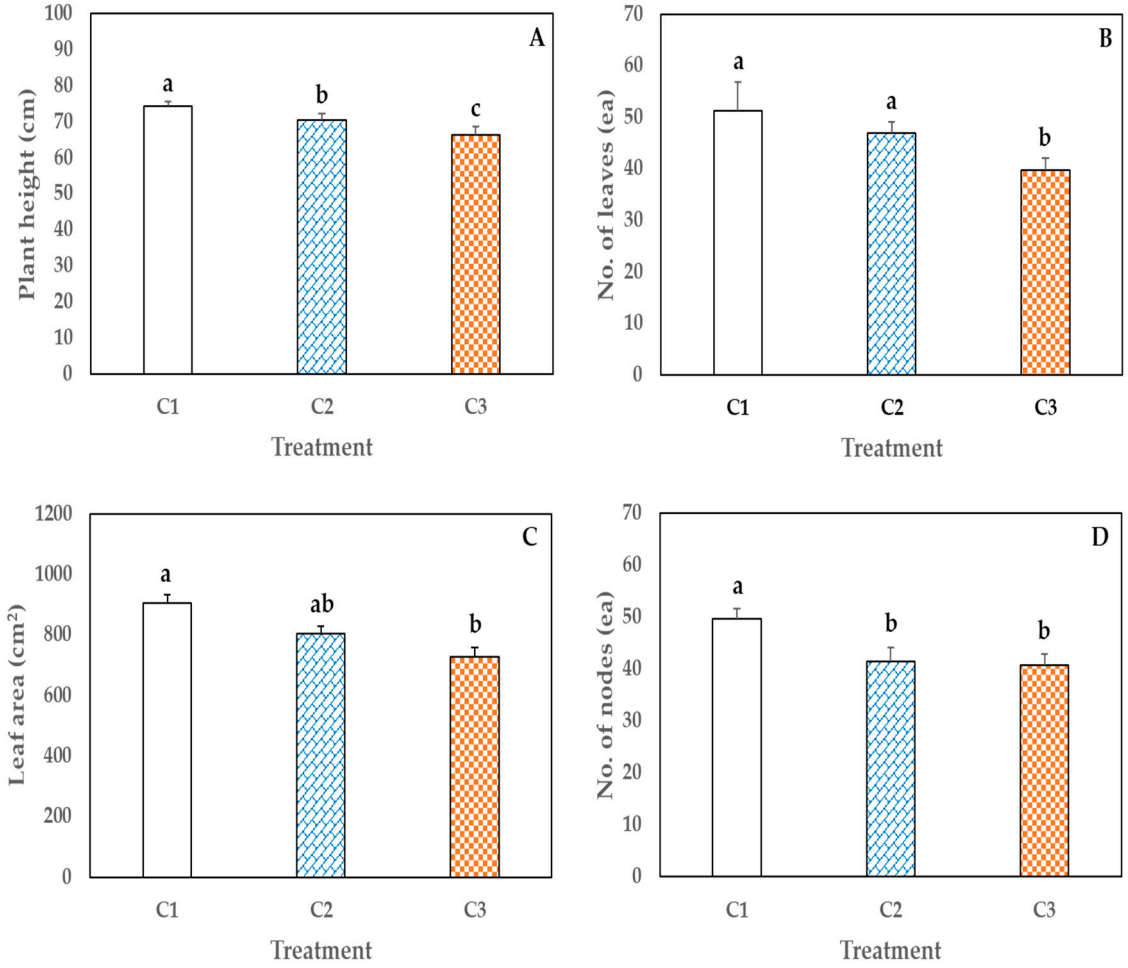

Figure 6. Effect of container size on plant height (**A**), leaf number (**B**), leaf area (**C**) and node number (**D**) of M-9 apple rootstock plant at 11 weeks after treatment in a greenhouse. C1, C2 and C3 indicate container size 1000 mL, 500 mL and 300 mL, respectively. Lines above bar represent the standard error of the mean (n = 5). Means above each bar followed by the same letters are not significantly different by Duncan's multiple range test (DMRT) at $p \leq 0.05$.

Table 3. Effect of container size on stem diameter, root length, fresh weight and dry weight of M-9 apple rootstock plants at 11 weeks after treatment. C1, C2 and C3 indicate container size 1000 mL, 500 mL and 300 mL, respectively.

Treatment	Stem Diameter from Ground (mm)		Root Length (cm)	Fresh Weight (g/plant)		Dry Weight (g/plant)	
	1 cm	30 cm		Shoot	Root	Shoot	Root
C1	6.8 ± 0.40 [z]a [y]	4.4 ± 0.30a	29.6 ± 2.12a	31.4 ± 1.18a	12.9 ± 0.46a	11.5 ± 0.27a	2.4 ± 0.74a
C2	6.1 ± 0.10a	4.5 ± 0.20a	20.5 ± 3.24b	25.9 ± 1.46b	9.2 ± 0.36b	9.4 ± 0.42b	2.0 ± 0.31a
C3	5.1 ± 0.30b	3.5 ± 0.20b	23.1 ± 2.33b	22.8 ± 3.24b	9.2 ± 0.47b	8.6 ± 0.31b	1.9 ± 0.22a

[z] Each value is the means ± standard error (n = 5). [y] Means within columns sharing the same letter are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

Understanding root growth and distribution in fruit trees was one of the most challenging and laborious aspects on plant growth and development. In this study, root length in C1-treated plants was 44% and 29% significantly higher than C2- and C3-treated plants, respectively (Table 3). Root fresh weight was 40% significantly higher in C1-treated plants than those grown in C2 and C3 treatment. Paltineanu [32] reported that root growth of six-year-old apple rootstock (M-9) was finest when soil depth was sufficient, compared with limited depth. Several studies showed the small size of container-restricted root growth of a plant [20,33]. Wang et al. [34] reported that space restricting the root growth of a plant affects nutrient acquisition. Because of reduced root growth and nutrient uptake,

carbohydrate accumulation also reduced in leaf and unbalanced the source: sink ratio in a plant [35,36]. In addition, the imbalanced source: sink ratio is responsible for reduction in shoot growth [37].

Photosynthesis rate and stomatal conductance were unaffected between the treatments (Table 4). Previous research reported that photosynthesis rates and stomatal conductance have a positive correlation on a plant [38]. Furthermore, SPAD and transpiration rate were not significantly different in plants grown in C1, C2 and C3 treatments (Table 4). SPAD and transpiration rate were unaffected due to the unaffected photosynthesis rate in our experiment. Chung et al. [39] reported that after five weeks of transplantation, photosynthesis rate and transpiration rate of in vitro-propagated M-9 apple rootstock had a positive correlation under various visible light spectrums. Kandel [40] and Shimomoto et al. [41] showed that the photosynthesis rate of a plant is positively correlated with stomatal conductance and transpiration rate. In this study, eleven weeks' duration of container size treatment in apple rootstock plant may be too short a period to show an effect on these physiological parameters. The PCA analysis presented a correlation between different parameters with the different treatment groups of container size (Figure 7). The elements of PC1 and PC2 described 90.24% and 9.76% of the variability in the data, respectively. Two separate groups of the parameters were observed from the PCA biplot. The graph showed that photosynthesis rate, stomatal conductance and transpiration rate were closely positively correlated to each other. Several studies showed that photosynthesis rate, stomatal conductance and transpiration rate in a plant are positively correlated [26–28].

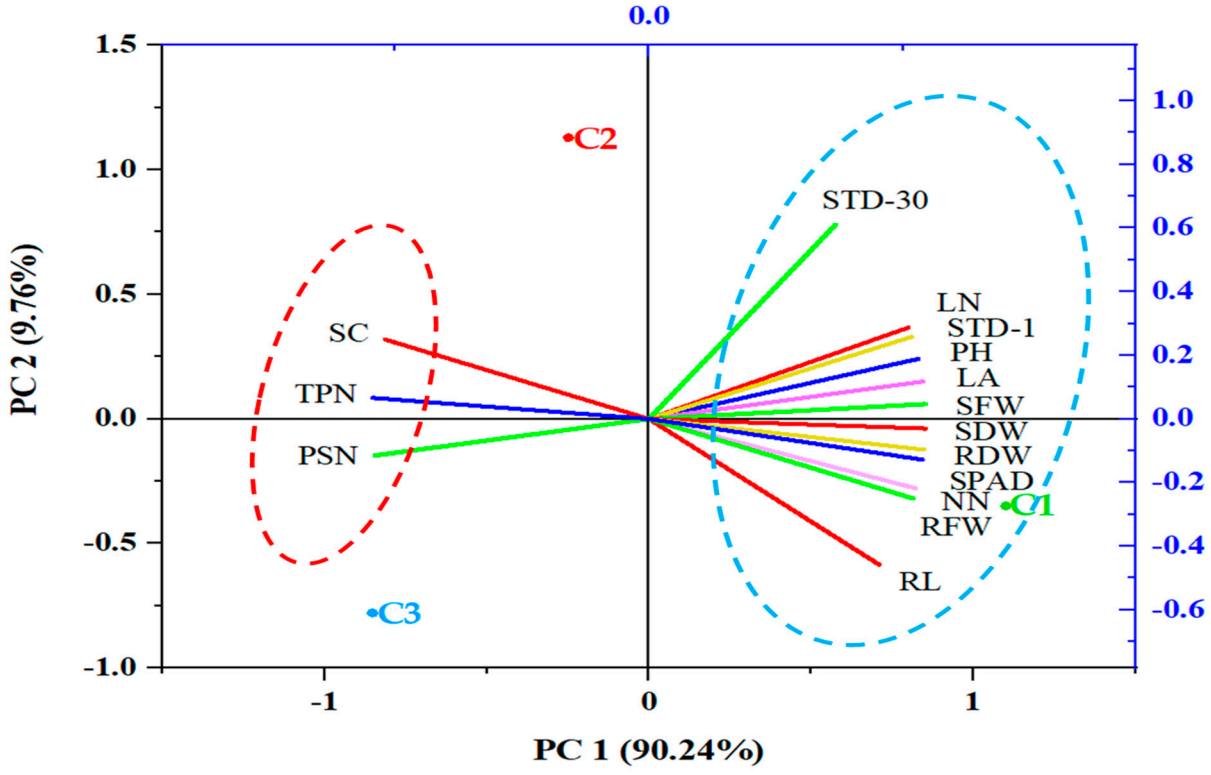

Figure 7. Principal component analysis (PCA) illustrates the variable treatment relationships among the three treatments of M-9 apple rootstock plant at 11 weeks after in a greenhouse. C1, C2 and C3 indicate container sizes of 1000 mL, 500 mL and 300 mL, respectively. The lines starting from the central point of the biplots display the negative or positive associations of the different variables, and their proximity specifies the degree of correlation with specific treatment. PH, plant height; LN, leaf number; LA, leaf area; NN, node number; RL, root length; STD-1, stem diameter from 1 cm of ground; STD-30, stem diameter from 30 cm of ground; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; SPAD, SPAD value; PSN, photosynthesis rate; SC, stomatal conductance and TPN, transpiration rate.

Table 4. Effect of container size on SPAD, photosynthetic rate, stomatal conductance, and transpiration rate of M-9 apple rootstock plant at 11 weeks after treatment. C1, C2 and C3 indicate container size of 1000 mL, 500 mL and 300 mL, respectively.

Treatment	SPAD	Photosynthesis ($\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$)	Conductance ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)	Transpiration ($mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$)
C1	42.0 ± 4.79 [z]a [y]	8.52 ± 1.86ab	0.35 ± 0.09a	4.80 ± 0.31a
C2	38.9 ± 3.96a	10.21 ± 1.12a	0.37 ± 0.03a	4.93 ± 0.09a
C3	38.3 ± 3.21a	11.50 ± 2.21a	0.37 ± 0.06a	4.97 ± 1.21a

[z] Each value is the means ± standard error (n = 5). [y] Means within columns sharing the same letter are not significantly different based on Duncan's multiple range test at $p \leq 0.05$.

4. Conclusions

Three different substrate composition and container size were separately examined in our research. Apple rootstock (M-9) plants were grown better in the substrate treatment, where the ratio of peat moss: vermiculite: perlite was 3:1:2. In addition, the container with a volume of 1000 mL was favorable for plant growth, as it showed higher plant height, leaf number, leaf area, fresh weight and dry weight compared with other treatments. Peat moss enhanced growth of apple rootstock plant. Too-small containers limit plant growth due to reduced root growth of the plant. These findings improved our understanding of the effect of substrate composition and container size on apple rootstock plants, and are useful to ensure plant quality and cost-effective large-scale production.

Author Contributions: J.K.K. and J.H.A. conceived and designed the experiments, carried out all the experimental works and data collection. M.R.A.S. analyzed the data, and drafted the final manuscript. Y.J.Y., S.J.P. and J.K.N. collected the data, and K.Y.C. supervised the study. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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