agronomy

MDPI

Article

An Optimal Combination of the Propagation Medium and Fogging Duration Enhances the Survival, Rooting and Early Growth of Strawberry Daughter Plants

Hao Wei [1,†], Chen Liu [1,†] and Byoung Ryong Jeong [1,2,3,*]

1 Department of Horticulture, Division of Applied Life Science (BK21 Plus Program), Graduate School of Gyeongsang National University, Jinju 52828, Korea; oahiew@gmail.com (H.W.); chenliu215@gmail.com (C.L.)
2 Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, Korea
3 Research Institute of Life Science, Gyeongsang National University, Jinju 52828, Korea
* Correspondence: brjeong@gnu.ac.kr; Tel.: +82-010-6751-5489
† These authors contributed equally to this work.

Received: 7 March 2020; Accepted: 9 April 2020; Published: 12 April 2020

check for updates

Abstract: Runner propagation is an important process in strawberry cultivation, which influences plant survival and fruit yield during the commercial production. In South Korea, this process is carried out on plug tray benches that are set off the ground in greenhouses, rather than in open fields. During the propagation, the propagation media and fogging systems play an important role in the survival and rooting of daughter plants. The aim of this study is to investigate the influences of the different types of propagation media and various fogging treatments on the rooting and early growth of strawberry daughter plants. Two strawberry cultivars—'Maehyang' and 'Seolhyang'—in a glasshouse, grown in four different propagation media-a peat moss-based mixture (PBM), rockwool cube (RWC), granular rockwool (GRW), and a coir-based mixture (CBM)—combined with five fogging durations, 0, 3, 6, 9 and 12 days were tested. PBM with 9–12 days of fogging was the most ideal combination for the rooting and early growth of daughter plants of the two strawberry cultivars, which increased the ratio of survival and rooting, number of new leaves, root length and maximised the fresh and dry weights of the aboveground and belowground runner biomass. However, fogging treatments shorter than 9 days led to a lower survival rate and rooting. RWC and GRW are not recommended for young plants of these two strawberry cultivars because their strong water-retentive properties could subject the root to water stress, which can lead to root rotting. The purpose of this study is to provide a reference for strawberry growers to improve the quality of plants and efficiency of production during the propagation period.

Keywords: cutting; humidity; survival ratio; soilless cultivation; substrate

---

1. Introduction

In South Korea, strawberry production has reached to 208,699 tons in 2017. The total area of production is about 6435 hectares, 6290 of which were protected cultivation, while only 145 hectares were field cultivation. Almost all strawberries are now propagated with plugs in South Korea [1]. With the fast development of the strawberry industry, strawberry propagation has become an integral part of the strawberry production process. Botanically, strawberry (*Fragaria x ananassa*) is a hybrid species that propagates by either seeds, or vegetatively by runners that produce daughter plants that are identical to the mother plant. Nearly all strawberries are propagated asexually in the Korean strawberry industry to maintain the true characteristics of the cultivar [2]. Cutting propagation is an efficient method for producing strawberry daughter plants of uniform sizes. But many commercial strawberry farms still use traditional propagation methods, such as runner pegging using the pin. Runner pegging takes freshly rooted runners that branch out from mother plants and anchors them on the substrate for further growth and root development. After rooting, a complete strawberry plant can be obtained. This process can be done in open fields or on plug trays in greenhouses. Because of the advantages of reducing pesticide requirement and soilborne diseases, facilitating mechanised operations, lowering fertiliser and water requirement and increasing survival rate and yield, plug seedling propagation has been widely used for vegetables, ornamental plants, tissue culture plants and trees [3]. Poling and Maas [4] mentioned that using plug seedlings to produce strawberry transplants can improve the efficiency while reducing diseases and insect pests. They also found that intermittent misting for 10 to 12 days was effective for rooting daughter plants. Currently, almost all strawberries in South Korea are produced in greenhouses, and therefore seedlings are produced on special strawberry plug trays. In the first period of strawberry production on plug trays, runners with 2–3 leaves are pinned down in special plug trays on either side of the mother plant. When the runners have rooted, they are separated from the mother plants. Because the runners are well-rooted before they are separated from the mother plant, there is no need for high humidity conditions as there are the cutting process used in this method. However, because of the different rooting times of runners, the size of daughter plants is not uniform. Unequal runner sizes will influence bloom and fruit production of strawberries after the runners are moved out of plug trays and planted in open fields [5–7]. Recently, this method has been gradually replaced by the cutting method. First, daughter plants with similar sizes are separated from their mother plants. It is necessary to leave at least 1 cm of the daughter plants, so that it serves as an anchor at the moment of planting [8]. In this method, the size of the cuttings can be selected, thus greater uniformly sized plants to be in the same plug tray [9]. This method can improve the quality of the runners and fruit production in the subsequent cultivation. It can also standardize the production.

A successful rooting of daughter plants depends on various factors such as the growth media, air humidity and quality of plant-lets [10]. The growth media plays an important role in the rooting process of runners. The growth media helps to provide moisture, support, nutrients and aeration for growing plants and aids in the proper growth and development of the plants. Thus, the growth media can affect the survival and rooting rates of the runner tips, as well as the subsequent growth and fruit production of the plants [11,12]. Both the physical properties of the media such as the structure, texture, porosity, water-holding and drainage capacities, bulk density and aeration as well as the chemical properties such as the pH, cation exchange capacity and organic matter, influence the performance of the plants in the media. At present, the commonly used soilless cultivation or cutting media are mainly divided into organic media and inorganic media. The mixed organic media made of peat moss, coir, vermiculite, perlite, etc., is widely used in horticultural production. A common advantage of mixed media is that the proportion of components can be adjusted to meet the needs of a particular crop. Rockwool is a kind of inorganic medium, which has the characteristics of light weight, strong water-holding ability, and can be cut according to any shape, so it is also widely used in soilless cultivation.

Humidity is also crucial for the rooting of the runner tips. Once the runner tips are cut off from the mother plants, the water supply is limited, while the continued transpiration loses water vapor through the stomata and the incision. Until the root system is established, water absorbed from the medium and the atmospheric moisture are not sufficient for the plant, and water supply from intermittent misting or artificial fogging method is necessary. Supplementary fogging is an effective way to guarantee high survival and rooting rates for the daughter plants. With fogging, not only is the surface of the young leaves always kept with a water film, which ensures that the plants do not die because of water loss before rooting, but also the medium is kept moist to create an optimal environment for root formation, ensuring a higher possibility of rooting and survival. Most previous studies used a water spray or misting method, but fogging system used in this study employs an ultrasonic oscillator to shake the water into particles which are smaller than 20 μm in diameter. The small water particles are more easily suspended in the air, and cause less condensation to form water droplets on the surface of the leaves, thus blocking the gas exchange of the leaves etc. Fogging duration is also important. If it is shorter than the necessary duration, fogging may not work effectively, whereas if it is longer than the necessary duration, fogging may lead to root rot or mildew and affect the survival rate. When the water content in the medium is too high, it will cause water stress to the plants. The degree of stress can be reflected by measuring the content of hydrogen peroxide or antioxidant enzyme [13,14].

Jafarnia, Giménez and Bartczak, etc. [15–17] have extensively studied the influence of the growth medium on the growth and development of strawberry runners and mother plants. Jafarnia [15] found that for both 'Fresno' and 'Selva' strawberries, 100%:0 and 60%:40% perlite/peat moss are the best propagation media for strawberry production in Iran. By comparing the growth of two strawberry cultivars 'Elsanta' and 'Honeoye' in different media (mixture of peat with pine bark (1:1 v/v), coconut fibre, mixture of brown coal with disintegrated rockwool (2:1 v/v) and rockwool blocks), Bartczak et al. [17] found that rockwool was the most useful substrate for the production of strawberry plants. Durner and Rowley [3,8] mentioned the high humidity required for rooting of strawberry runners. However, the effects of the propagation medium combined with the humidity on the growth and development of strawberry runners during the cutting propagation period have yet to be investigated.

'Maehyang' and 'Seolhyang' are two popular strawberry (*Fragaria* × *ananassa*) cultivars in South Korea. The general characteristics of 'Maehyang' and 'Seolhyang' include high vigour and erect type in growth. They are suitable for forcing culture because it shows weak dormancy, high yield and fruit quality [18]. Compared with 'Seolhyang', 'Maehyang' has harder fruits and they are better for long-term storage. Therefore, 'Maehyang' is mainly cultivated for the export markets and 'Seolhyang' is cultivated mostly for the Korean domestic market [19]. These two cultivars account for 62.7% of the total strawberry production in South Korea [20]. To provide a planting guidance at an early stage of strawberry production for growers, this experiment investigated the effects of the propagation medium and the duration of fogging on the rooting and early growth of daughter plants of these two strawberry cultivars.

2. Materials and Methods

2.1. The Experiment Locations and Experimental Conditions

This experiment was carried in a glasshouse at Gyeongsang National University (GNU), Jinju, South Korea (35°09′ N, 128°05′ E). The glasshouse environment during the experiment period is shown in Figure 1.

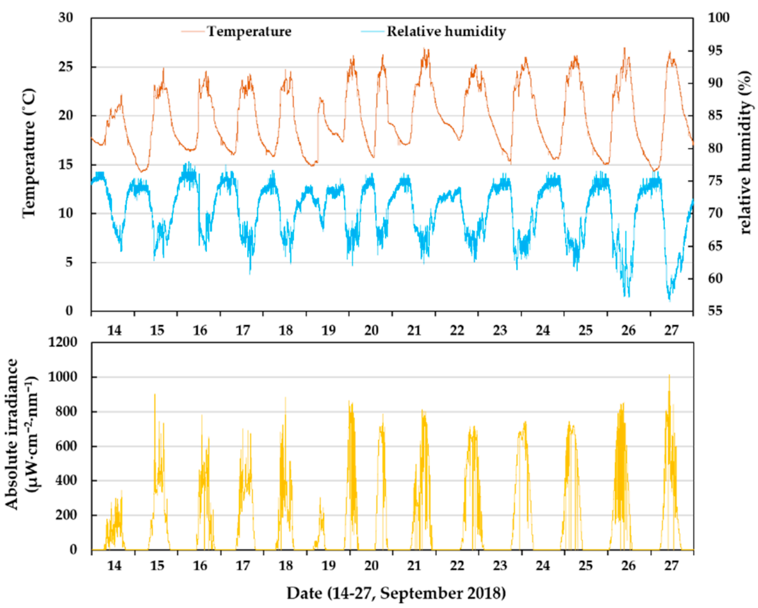
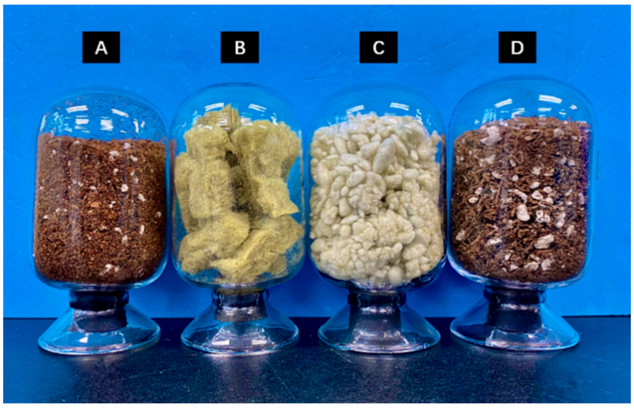


Figure 1. The temperature, relative humidity and solar radiation in the glasshouse during the experiment duration (14–27 September 2018).

2.2. Plant Materials and Propagation Media

All the unrooted runner materials of 'Maehyang' and 'Seolhyang' used in this study were selected from one strawberry farm. All selected runners were of similar size and each runner had three leaves. Four common media (Figure 2) available in South Korea were selected for the experiment: a peat moss-based mixture (PBM, Bas Van Buuren Substrates, De Lier, The Netherlands), a commonly used medium for strawberries; rockwool cube (RWC, Korea UR Media, Co., Seoul, Korea), a medium that is marketed as a rooting medium; granular rockwool (GRW, Korea UR Media, Co., Seoul, Korea) having similar properties as the RWC, but with more large-sized pores; and a coir-based mixture (CBM, Tosilee Medium, Shinan Grow Co., Jinju, Korea), a popular propagation medium produced and used in South Korea. The four media can be divided into two categories: PBM and CBM are organic media. PBM is a mixture of 80% peat moss, 10% coir dust and 10% perlite. CBM is a mixture of 50% coir dust, 25% peat moss, 10% perlite, 10% vermiculite and 5% zeolite. Although these two premixed media cost more than RWC and GRW (Figure 2), they can be formulated according to the requirements, where their physical and chemical properties are reasonably consistent. RWC and GRW, two forms of rockwool, made from spinning molten basaltic rock into fine fibres which are then formed into a range of cubes, blocks, growing slabs and granular products. Both RWC and GRW are pure inorganic substrates. The physicochemical property of the four common propagation media in South Korea are also shown in Figure 2.



Medium	Bulk Density ($g{\cdot}cm^{-3}$)	Air Space (%)	Water-Holding Ability ($g{\cdot}cm^{-3}$)	pH	EC ($dS{\cdot}m^{-1}$)	Price ($\${\cdot}m^{-3}$)
A PBM	**0.23**	**59.3**	**0.33**	**6.02**	**0.26**	**243.6**
B RWC	**0.07**	**81.5**	**0.72**	**7.58**	**0.03**	**188.8**
C GRW	**0.15**	**90.1**	**0.61**	**8.45**	**0.09**	**140.7**
D CBM	**0.24**	**48.8**	**0.29**	**5.83**	**0.23**	**218.7**

Figure 2. Physical morphology, physicochemical property, and cost of the four common propagation media in South Korea. (**A**) A peat moss-based mixture (PBM); (**B**) rockwool cube (RWC); (**C**) granular rockwool (GRW); (**D**) a coir-based mixture (CBM).

2.3. Fogging System and Experimental Design

A special system was developed for the experiment such that the effects of the fogging as well as the propagation media on plants could be studied. Initially, a bench to support the propagation media and plants was built with steel bars. Cell trays (21-Zigpot/21 cell tray, Daeseung, Jeonju, Korea) filled with one of the four media with daughter plants stuck in them were laid out on the benches. For each fogging group, there were eight trays (4 media × 2 cultivars) placed side by side.

Daughter plants were separated from the mother plants with around 3 cm of the runner left on the crown, and this runner was pegged into the medium leaving the crown of the runner exposed to the air. The medium was drenched completely with a nutrient solution (the greenhouse multipurpose nutrient solution (in mmol L^{-1} NH_4^+ 1.0, NO_3^- 10.6, $H_2PO_4^-$ 1.2, K^+ 5.1, Ca^{+2} 3.1, Mg^{+2} 1.0, and SO_4^{-2} 0.3, and in µmol·L^{-1} Fe^{+3} 43.6, BO_3^{-3} 22.6, Mn^{+2} 9.4, Zn^{+2} 1.5, and MoO_4^- 0.5) with electrical conductivity (EC) 1.6 dS·m^{-1}, adjusted pH 5.8) and a layer of polyethylene (PE) film was put over (30 cm from the top of the plant) to cover the whole system. A fogging system (UH-303, JB Natural Co. Ltd., Gunpo, Korea) connected to a polyvinyl chloride (PVC) pipe of a 10 cm diameter with holes was used to evenly distribute fog inside the PE film. The fogging time was from 6:00 to 18:00 every day, and each fogging duration was 30 min, and the interval was also 30 min.

Five fogging groups were set with a duration of 0, 3, 6, 9 or 12 days, respectively (Figure 3). All groups formed a line on the bench. In each group, daughter plants were grown in four growth media (4 media × 2 cultivars × 21 runners/tray = 168 runners/fogging group). Runners were anchored on the four media in 21-cell trays each, where two trays were filled with each medium, with each tray holding one cultivar. Seven plants out of each 21-cell tray were randomly selected as a repeat or an observation unit. The first group was located at one side of the bench, the farthest from the fogging system, where fogging was totally absent from the beginning to the end of the experiment. The second group was fogged for 3 days, the third group for 6 days, and so on in a similar fashion for the rest of the groups.

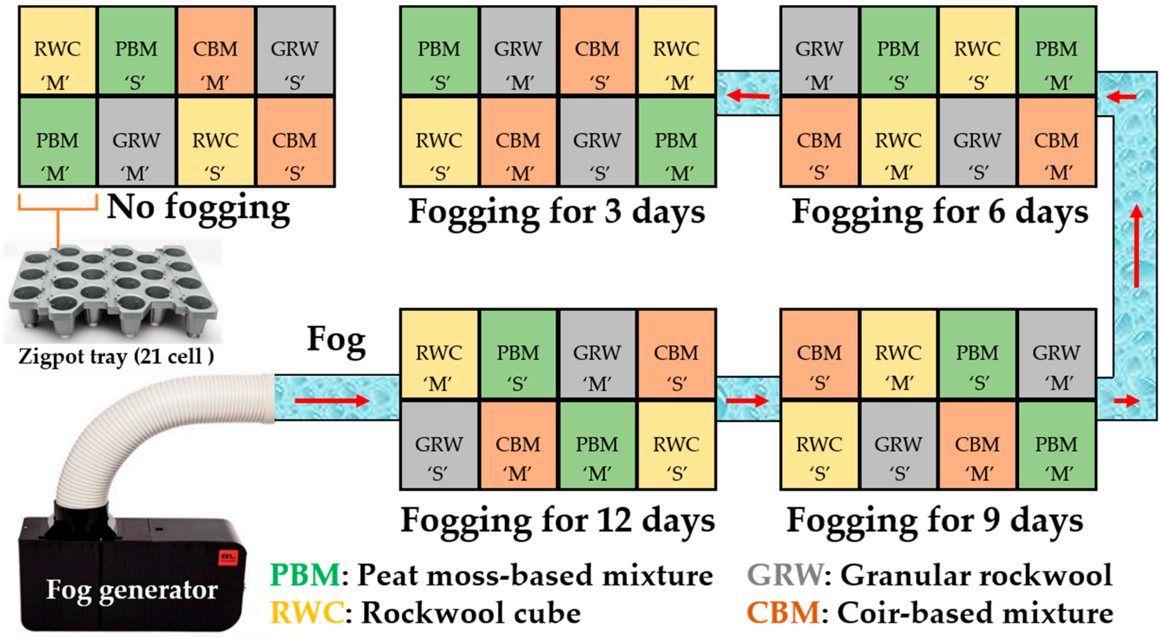


Figure 3. Experimental design to study the effects of the growth medium and fogging duration on the rooting and early growth of daughter plants of strawberry 'Maehyang' ('M') and 'Seolhyang' ('S').

2.4. *Measurements of the Growth Parameters*

To calculate the percentages of survival, rooting and newly formed leaves, an observation was made every 3 days. Runners with dry leaves and root were considered dead, and when the root extended into the medium, plants were judged to be rooted. The proportion of rooted plants to the total number of plants was recorded as the rooting percentage, and the proportion of living plants to the total number of plants was recorded as the survival percentage. The leaf grows and develops until it is fully unfolded as new trifoliate. The proportion of the number of new leaves to the total number of plants was recorded as the ratio of new leaves. Growth parameters, including the number of roots, length of the shoot and root, as well as fresh and dry weights of the shoot and root were measured after 15 days. The length of the longest root was measured after the medium was cleaned with running water. Dry weights of the shoot, root and whole plant were measured after 72 h of drying in a drying oven (FO-450M, Jeio Technology Co. Ltd., Daejeon, Korea) at 70 °C.

2.5. Measurement of Hydrogen Peroxide

High humidity environment after fogging treatment may cause water stress on plants, especially the stress on root after rockwool media was saturated with water, so the content of hydrogen peroxide (H_2O_2), an important signal molecule in response to stress was measured. The spectrophotometric determination of H_2O_2 was carried out according to the method described by Christou et al. [21]. Root samples (0.1 g) were homogenised in 1 mL of 0.1% trichloroaceticacid (TCA) and centrifuged at 10,000 rpm for 15 min. Then, 0.5 mL of the supernatant was mixed with a 10 mM phosphate buffer (0.5 mL, pH 7.0) and 1 mL potassium iodide (1M). The mixture was incubated at room temperature for 30 min, after which the absorbance was measured at 390 nm. The H_2O_2 content was determined from the standard calibration curve.

2.6. Measurement of Activities of Antioxidant Enzymes

Similarly, in the stress condition, the activity of various antioxidant enzymes in plants will also change. Through the determination of the activity of four antioxidant enzymes in the root of plants, the influence of different media and different fogging duration on plants will be further determined. For the analysis of antioxidant enzymes, 0.1 g fresh root samples were homogenized in a 1.5 mL ice-cold 50 mM phosphate buffer (pH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA), 0.05% triton X-100, and 1 mM polyvinylpyrrolidone (PVP). The extracts were centrifuged at 13,000 rpm for 20 min at 4 °C and the supernatant was employed for the analysis of enzyme activities. The superoxide dismutase (SOD) activity was estimated by following the nitro blue tetrazolium (NBT) inhibition methods according to the protocol of Giannopolitis and Ries [22]. The activity of the catalase (CAT) enzyme was measured based on the method of Cakmak and Marschner [23]. The guaiacol peroxidase (GPX) activity was determined based on the amount of enzyme required for the formation of tetraguaiacol per minute, following the methods of Shah et al. [24]. The ascorbate peroxidase (APX) activity was assayed following the methods of Nakano and Asada [25].

2.7. Statistical Analysis

The experiment was carried out with three replicates in a randomized complete block design, using seven runners in each observation unit. The replications were randomly located to eliminate the effects of the position within a controlled environment. A multi-way analysis of variance (ANOVA) with the SAS program (Statistical Analysis System, V.9.1, Cary, NC, USA) was applied to statistically analyse the collected data (Factors: medium, fogging, and cultivar). Tukey's multiple range test was adopted to test the significant differences between the different means. The interactions were analysed by the main factors of medium, fogging and cultivar.

3. Results

3.1. Survival Percentage

The effects of the medium, fogging duration and cultivar on the survival of strawberry daughter plants are showed in Table 1. Neither the propagation medium nor the cultivars affected the survival, but fogging duration significantly affected the survival of plants. There also have some interactive effects between the media and fogging durations.

To show the survival percentage changes over time in different media more clearly, changes in survival were recorded in Figures 4 and 5 for each strawberry cultivar. The only exception was in GRW, where a few strawberry 'Seolhyang' daughter plants survived without fogging, but they were all in a poor condition. Fogging for 9 or 12 days could guarantee a 100% survival percentage of plants grown in any of the four media. However, the plants grown with 3 or 6 days fogging treatments were dying after the fogging stopped. Without fogging, dead plants were found at every inspection time until almost all of them died.

Table 1. The effects of the medium, fogging duration and cultivar on the survival of strawberry daughter plants after 15 days treatment.

	Survival						
F-test	C [z]	M	F	C * M	C * F	M * F	C * M * F
	NS [y]	NS	***	NS	NS	**	NS

[z] C, cultivar; M, medium; and F, fogging duration. [y] NS, not significant. *, **, and ***, significant at $p = 0.05$, 0.01, or 0.001, respectively.

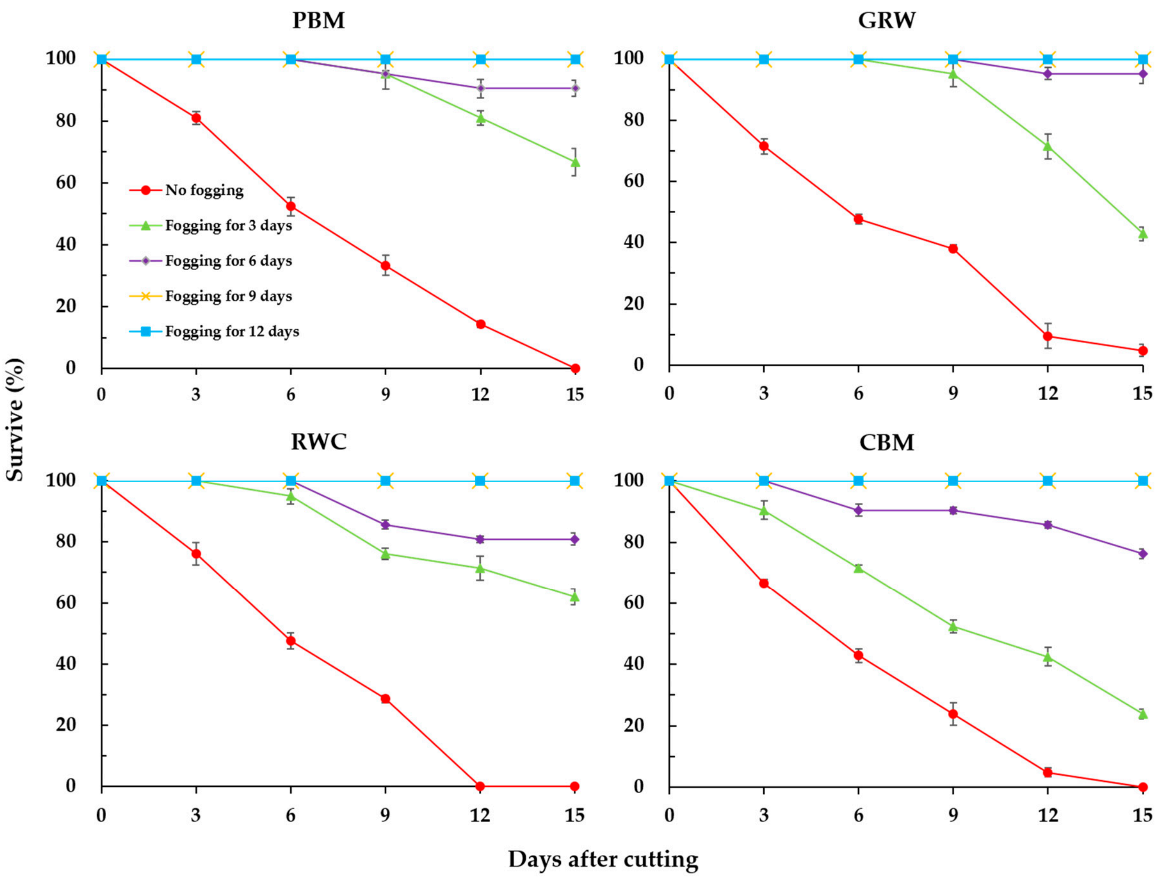


Figure 4. The effects of the growth medium on the survival percentage of daughter plants of strawberry 'Maehyang' with 0, 3, 6, 9 and 12 days fogging treatments. Error bars represent the SEs of the three observation units ($n = 3$). PBM, a peat moss-based mixture; RWC, rockwool cube; GRW, granular rockwool; and CBM, a coir-based mixture.

3.2. Percentage of Rooting and Ratio of Newly-Grown Leaves

As Table 2 shows, no plants rooted without fogging. Even with only 3 days of fogging, about half of the daughter plants developed roots. Especially in the PBM medium, the rooting percentage reached to 70–90%. Fogging for at least 6 days ensured a 100% rooting percentage in both cultivars. With longer fogging duration, plants developed a greater number of newly formed leaves except for 'Seolhyang' daughter plants grown in RWC. The 'Seolhyang' daughter plants had a lower ratio of newly formed leaves after 12 days of fogging than after 9 days of fogging in RWC. A 12-day fogging guaranteed that the ratio of newly formed leaves was higher than 80%.

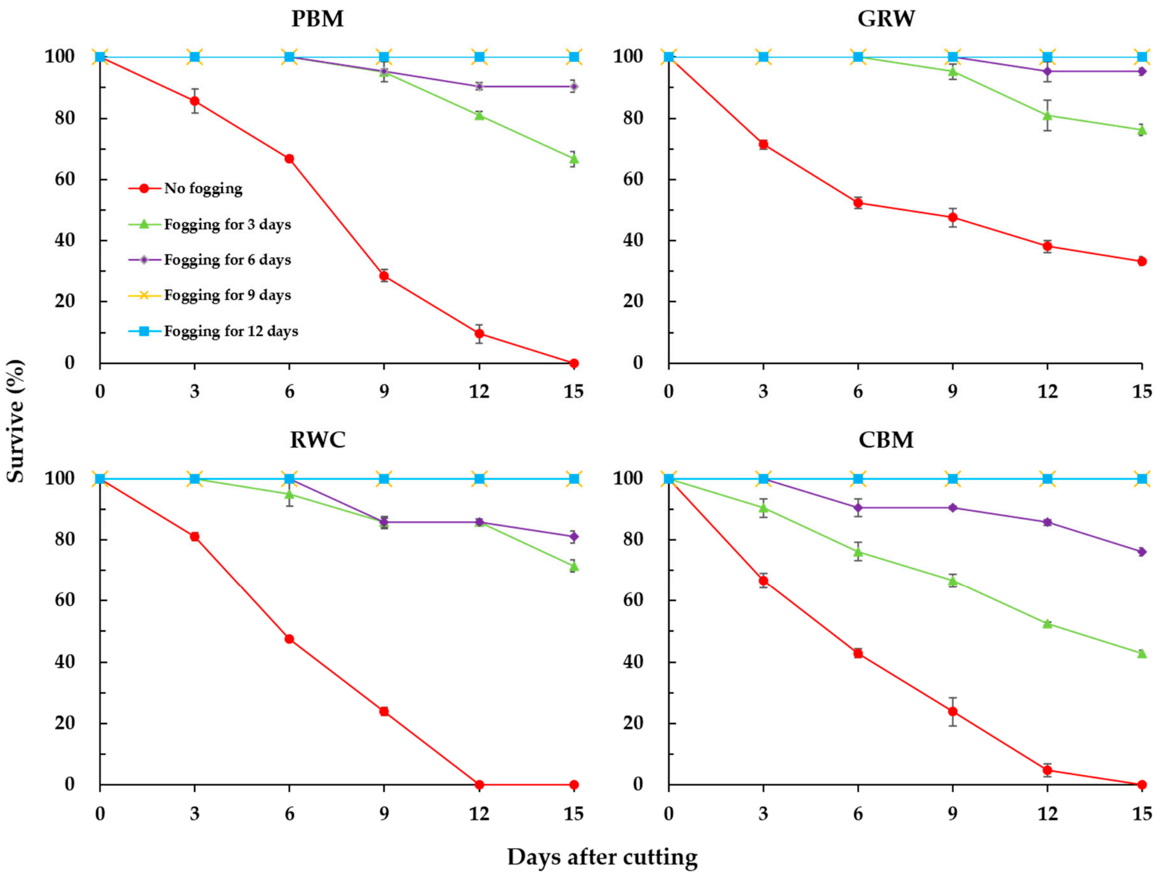


Figure 5. The effects of the growth medium on the survival percentage of daughter plants of strawberry 'Seolhyang' in the 0, 3, 6, 9 and 12 days fogging treatments. Error bars represent the SEs of the three observation units (n = 3). PBM, a peat moss-based mixture; RWC, rockwool cube; GRW, granular rockwool; and CBM, a coir-based mixture.

Table 2. The percentages of rooting and ratio of new leaves of strawberry runners affected by the fogging duration, medium and cultivar. The experiment was carried out in a glasshouse at GNU (35°09′ N, 128°05′ E) on 14–27 September 2018.

Cultivar	Duration of Fogging (Day)	Rooting (%)				Ratio (%) of New Leaves			
		PBM	RWC	GRW	CBM	PBM	RWC	GRW	CBM
'Maehyang'	0	0.0 e ^{z}	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
	3	86.6 b	53.3 d	66.7 cd	73.3 c	66.7 c	40.0 d	40.0 d	53.3 c
	6	100.0 a	100.0 a	100.0 a	100.0 a	40.0 d	80.0 bc	80.0 bc	66.7 c
	9	100.0 a	100.0 a	100.0 a	100.0 a	66.7 c	53.3 c	53.3 c	66.7 c
	12	100.0 a	100.0 a	100.0 a	100.0 a	80.0 bc	80.0 bc	80.0 bc	80.0 bc
'Seolhyang'	0	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
	3	73.3 c	53.3 d	66.7 cd	73.3 c	26.7 d	26.7 d	33.3 d	33.3 d
	6	100.0 a	100.0 a	100.0 a	100.0 a	60.0 c	53.3 c	40.0 d	80.0 bc
	9	100.0 a	100.0 a	100.0 a	100.0 a	80.0 bc	106.0 ab	53.3 c	73.3 c
	12	100.0 a	100.0 a	100.0 a	100.0 a	86.7 bc	80.0b c	120.0 a	106.0 ab

^{z} According to Tukey's test, lowercase letters at $p \leq 0.05$ indicate significant differences between different treatments. PBM, a peat moss-based mixture; RWC, rockwool cube; GRW, granular rockwool; and CBM, a coir-based mixture.

3.3. Growth Parameters

Groups without fogging or with 3 days of fogging had low percentages of survival, rooting and newly grown leaves, so it was meaningless to measure the subsequent growth data. Thus, the growth parameters were measured for groups that were fogged for 6 days or longer (Table 3). For strawberry 'Maehyang', PBM with a 12-day fogging enhanced the length of roots (47.9% greater than with

6 days of fogging), root fresh weight (57.3% greater than with 6 days of fogging) and root dry weight (52.1% greater than with 6 days of fogging), shoot fresh weight (56.6% greater than with 6 days of fogging) and shoot dry weight (53.3% greater than with 6 days of fogging). Strawberry daughter plants grown in CBM with 9-day or 12-day fogging also performed well, but there was still a gap between those grown in CBM and PBM. RWC and GRW are not recommended for strawberry root growth, as daughter plants grown in these two media had worse root appearances and shorter root lengths than those grown in the other two media (Figure 6). Strawberry 'Seolhyang' with a 9-day fogging performed the best when grown in CBM, especially in root length (53.6% greater than with 6 days of fogging) and biomass (24.0% greater than with 6 days of fogging). With a 12-day fogging, PBM resulted in the highest quality daughter plants, especially in the root quality (fresh and dry weights increased 39.7% and 43.5%, and length increased 31.0% compared to those fogged for 6 days).

Table 3. The effects of the growth medium, fogging duration and cultivar, on the length, fresh weight and dry weight of the strawberry daughter plants after 15 days.

Cultivar	Medium	Duration of Fogging (Day)	Shoot			Root		
			Length (cm)	Fresh Weight (g)	Dry Weight (g)	Length (cm)	Fresh Weight (g)	Dry weight (g)
'Maehyang'	PBM	6	16.90 e [z]	5.17 e	1.26 f	5.05 d	1.84 e	0.35 e
		9	18.07 e	7.21 de	1.63 d	7.92 b	2.31 d	0.43 c
		12	23.83 b	11.93 a	2.70 a	9.69 a	4.31 a	0.73 a
	RWC	6	22.80 ab	6.66 e	1.76 c	3.87 e	1.72 e	0.39 d
		9	20.03 d	8.27 d	1.86 c	6.81 bc	3.85 b	0.68 a
		12	25.30 a	9.98 c	2.27 b	7.36 bc	2.17 d	0.41 c
	GRW	6	18.43 e	5.81 e	1.40 e	4.52 de	1.88 e	0.37 e
		9	22.47 c	6.71 e	1.50 e	6.43 c	2.95 cd	0.50 bc
		12	24.87 ab	10.69 b	1.82 c	7.72 b	3.00 cd	0.47 bc
	CBM	6	18.87 de	6.69 e	1.61 d	6.64 bc	2.10 de	0.42 c
		9	21.93 cd	6.52 e	1.36 f	7.71 b	2.14 d	0.36 e
		12	21.93 cd	8.77 d	1.67 d	9.18 b	3.20 c	0.52 b
'Seolhyang'	PBM	6	22.97 bc	6.56 c	1.47 d	6.49 bc	2.42 cd	0.35 d
		9	22.07 bc	5.69 cd	1.40 d	7.85 b	2.40 cd	0.41 cd
		12	27.43 a	9.57 a	2.09 a	9.41 ab	4.01 a	0.62 a
	RWC	6	19.77 c	3.83 d	1.38 d	3.39 d	1.42 d	0.41 cd
		9	23.20 b	8.07 b	1.88 ab	6.42 c	3.05 c	0.38 d
		12	24.27 b	5.04 cd	1.78 c	3.45 d	1.87 d	0.34 d
	GRW	6	23.93 b	7.01 c	1.82 ab	3.75 d	2.64 c	0.43 cd
		9	19.30 d	6.67 c	1.47 d	4.99 cd	2.50 cd	0.47 c
		12	23.87 b	8.51 b	1.77 c	6.72 bc	3.03 c	0.47 c
	CBM	6	17.33 d	3.89 d	1.41 d	4.70 cd	1.76 d	0.33 d
		9	21.03 c	8.21 b	1.73 c	10.07 a	3.60 b	0.56 b
		12	24.37 b	8.28 b	1.81 ab	9.19 ab	3.50 b	0.55 b

[z] According to Tukey's test, lowercase letters at $p \leq 0.05$ indicate significant differences between different treatments. PBM, a peat moss-based mixture; RWC, rockwool cube; GRW, granular rockwool; and CBM, a coir-based mixture.

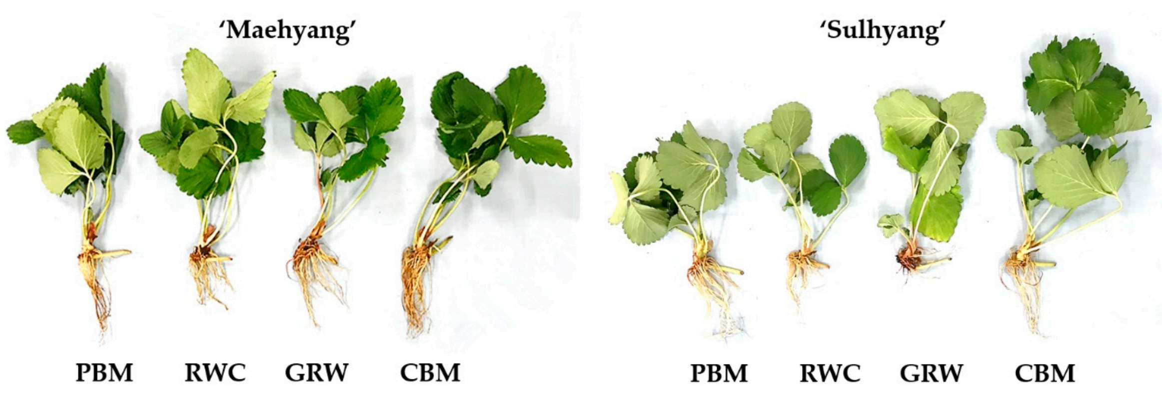


Figure 6. The effects of the growth medium on the morphology of strawberry 'Maehyang' and 'Seolhyang' daughter plants grown with a 12-day fogging. PBM, a peat moss-based mixture; RWC, rockwool cube; GRW, granular rockwool; and CBM, a coir-based mixture.

3.4. Plants Morphology

The morphology of the Strawberry 'Maehyang' and 'Seolhyang' daughter plants affected by the different media of the 12-day fogging treatment are shown in Figure 6. The differences in above-ground part were not obvious, but the roots of daughter plants grown in PBM and CBM were significantly better developed than those grown in RWC and GRW. Rotten roots were found in RWC and GRW with a 12-day fogging.

3.5. Hydrogen Peroxide Content and Activities of Antioxidant Enzymes

The two cultivars of strawberry daughter plants treated with 9 and 12-days fogging all reached to 100% rooting and survival, and the strawberry daughter plants treated with 12-days fogging showed better growth parameters, but rotten roots were found in RWC and GRW. In order to determine the influence of four media on root development, we analysed the hydrogen peroxide (H_2O_2) content in the roots of two cultivars of strawberry daughter plants treated with 12-days fogging (Figure 7). The results showed that the H_2O_2 content in RWC and GRW was significantly higher than that in PBM and CBM. There was no significant difference between PBM and CBM. This indicates that the roots grown in the two kinds of rockwool were under stress.

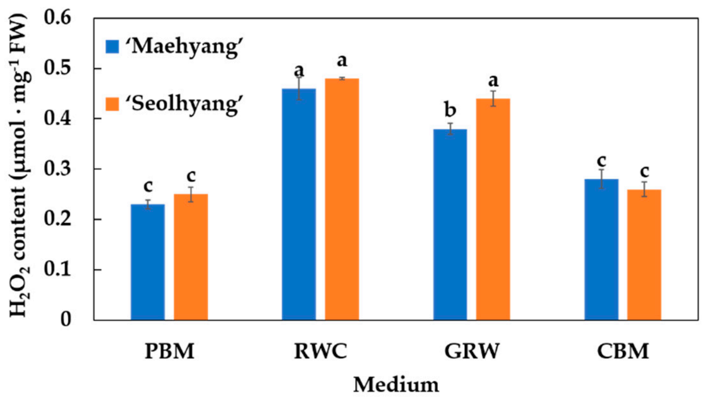


Figure 7. The effects of growth medium on the hydrogen peroxide (H_2O_2) content of two cultivars of strawberry 'Maehyang' and 'Seolhyang' daughter plants grown with a 12-day fogging. The error bars represent the SEs of biological replicates ($n = 3$). According to Tukey's test, lowercase letters at $p \leq 0.05$ indicate significant differences between different treatments.

The activities of four antioxidant enzymes were also measured and analysed (Figure 8). The activities of four antioxidant enzymes in the roots of strawberries daughter plants all showed a similar effect to H_2O_2 contents in four different media. The activity of superoxide dismutase (SOD) in RWC and GRW was significantly higher than that in PBM and CBM, 'Maehyang' grown in PBM showed lowest activity. The activity of catalase (CAT) was the highest in both cultivars grown in RWC and 'Maehyang' grown in GRW. The 'Seolhyang' grown in CRW was lower but still significantly higher than the plants grown in PBM and CBM. Strawberry 'Seolhyang' grown in RWC had the highest activity of ascorbate peroxidase (APX). There was some difference between PBM and CBM but both were lower than RWC and GRW. The guaiacol peroxidase (GPX) activities were also similar to that of the previous three enzymes in the four media, the plants grown in RWC and GRW were still significantly higher than that grown in PBM and CBM. These results indicated that the roots of strawberry grown in two kinds of rockwool had increased activity of enzymes due to resistance to stress, and further demonstrated the plants grown in the two types of rockwool were under stress.

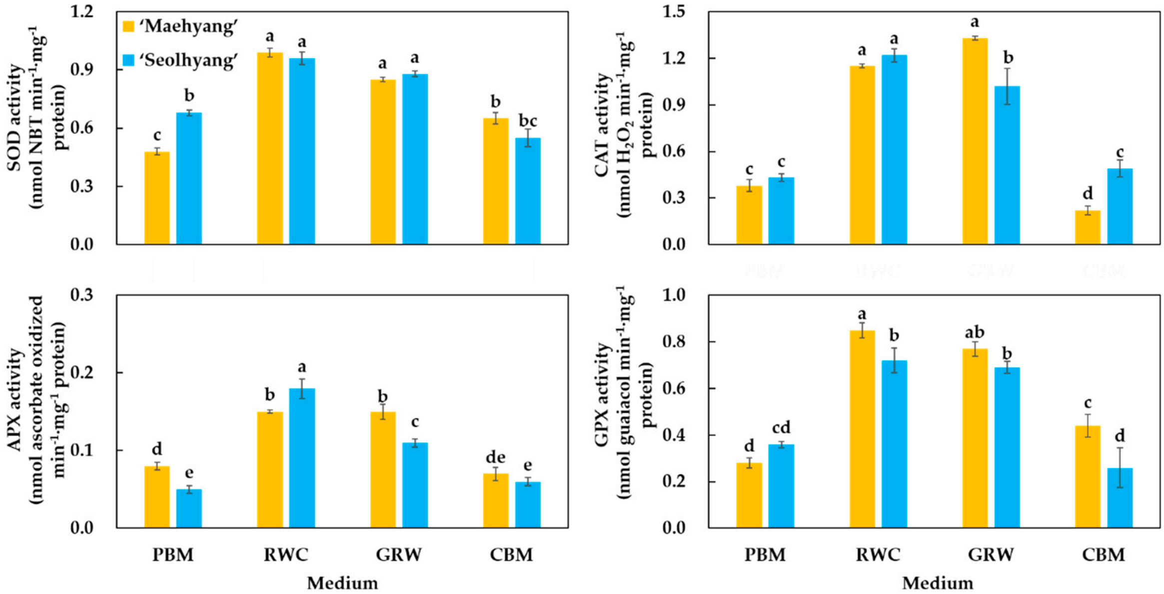


Figure 8. The effects of growth medium on the activities of four antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX and guaiacol peroxidase, GPX) in two cultivars of strawberry 'Maehyang' and 'Seolhyang' daughter plants grown with a 12-day fogging. The error bars represent the SEs of the biological replicates ($n = 3$). According to Tukey's test, lowercase letters at $p \leq 0.05$ indicate significant differences between different treatments.

4. Discussion

It can be seen that from the environmental conditions recorded during the experiment, except the cloudy days on the first day and the sixth day, the temperature, humidity and light change evenly, so it can be regarded as the environment change in the glasshouse has little influence on the experimental results. Through analysing the results, it was found that 100% plants survived in any medium tested in our experiment as long as the fogging duration was more than 9 days. Thus 9-day fogging guaranteed the highest survival rate of strawberry runners. In 3-day or 6-day fogging treatment, a few plants survived in RWC and GRW. Some plants even survived without any fogging in GRW. One possible reason for this observed survival of plants is that both GRW and RWC have excellent water-holding and aeration characteristics [26]. Moreover, GRW has a higher number of large pores among particles that are more conducive to rooting than RWC, thus some plants even survived in the absence of fogging GRW.

There is a correlation between rooting and survival. When the strawberry runners began to root, the new roots absorbed water and nutrients from the medium. After being fogged for 3 days, half of the runners rooted. However, the small number of newly developed roots were too weak to supply sufficient water and nutrients needed by the leaves. If fogging was stopped after 3 days, the runners withered and died. Kim et al. [27] made a study on growth and rooting rate of 'Maehyang' strawberry as affected by irrigation method on cutting propagation. They found that the rooting ratio of 'Maehyang' was significantly higher in the overhead irrigation and fog irrigation treatments. The rooting percentage reached to 77.8 and 91.7% after 4 and 8 days of fog irrigation treatment. Their study showed that rooting rate increased with longer fogging duration. Although their results were not completely consistent with our results because of the differences in equipment and environment, our study also proved that longer fogging duration (that is 9 days) promoted stronger rooting system of strawberry runners and guaranteed 100% survival rate.

In order to reduce water loss caused by transpiration, it is very important to maintain a high humidity when rooting vegetative stems [28]. Rowley et al. [8] described in detail how strawberry plug plants are produced in the United States. They noted that it is necessary to mist the runners during the rooting duration of strawberry. This is consistent with the results of our study, that is, the survival percentage of plants was very low without fogging or with short fogging duration. Rowley et al. [8] also found that a rooting environment with high humidity is prone to fungal rots. In our study, root rot was also found in strawberry plants grown in RWC and GRW after fogging for 12 days, especially in the 'Seolhyang' cultivar (Figure 7). The maximum water retention of the rockwool growing medium can reach 90% [29], which is much higher than that of common mixed media.

Major factors such as abiotic stresses-heavy metals, drought, water-logging, salt, low temperature, etc., can limit crop productivity and yield. These abiotic stresses are associated with the production of certain deleterious chemical entities termed reactive oxygen species (ROS), which include hydrogen peroxide (H_2O_2), superoxide radical (O^{2-}), hydroxyl radical (OH^-), etc., [30]. Gratão et al. [31] suggested that in order to prevent or alleviate injuries from ROS, plants have evolved an antioxidant defence system that includes non-enzymatic compounds, such as ascorbate, glutathione, tocopherol, carotenoids, flavonoids and enzymes such as SOD, CAT, POX, APOX, GR and PPO. When plants are subjected to the abiotic stress, these changes in ROS content and activity of enzymes can reflect whether the plant is in the normal state of growth. Both GRW and RWC have excellent water-holding characteristics. We speculate that the excellent water-holding characteristics of RWC causes the plant roots to be under water stress after a long period of fogging treatment and thus reducing the growth of strawberry cuttings. The results showed that the number of roots per cutting and root length were significantly improved (30.8%) in the two organic mixtures treatments compared with those in the rockwool. The main influencing factor could be that the high water-retention capacities of rockwool caused water stress in the roots of plants under the condition of continuous high humidity with fogging. The subsequent determination of hydrogen peroxide (H_2O_2) content and activity of enzymes in the roots did reveal that the roots were under stress (Figures 7 and 8). Hall et al. [32] also found that the root respiration was inhibited after sunflower was subjected to water stresses. Rotten roots were found with daughter plants grown in RWC and GRW with a 12-day fogging, probably also because of the high water-retention capacities of the rockwool. Similar to the findings of Rowley et al. [8], the high humidity rooting environment is prone to fungal rots. Liao and Lin [33] reviewed the physiological adaptation of crop plants to flooding stresses. Excess water produces anoxic soil conditions within a few hours [34]. Plant roots, consequently, suffer hypoxia or anoxia. A low respiratory capacity leads to anaerobic metabolism of roots, which consequently causes ethanolic fermentation of the cell. Then, gas exchange will be out of balance among the shoots, roots and leaves. Flooding causes a significant decrease in the capacity for root gas exchange in most flood-intolerant plants. It can be seen that the ratio of new leaves in both PBM and CBM increases with the increase of the duration of fogging, but in the two types of rockwool, the abnormal result was that the new leaves in 9-day fogging were less than in 6-day. We speculate that it is the water stress of two types of rockwool on the root that also affects the development of new leaves. We conclude that this is why RWC and GRW are not suitable for cutting propagation in the fogging tunnels with a high humidity. Baisak et al. [14] also found that water stress caused a rapid increase in the activity of SOD and APX in wheat leaves. Decline in the efficacy of the H_2O_2 decomposing system is probably responsible for the oxidative damage occurring in water-stressed wheat leaves. On the other hand, the high water retention properties of rockwool may be an advantage in the low air humidity environment for cutting propagation. But for the herbaceous plants like strawberries, which tend to wilt when they are separated from their mother plants, obviously a low air humidity environment cannot sustain strawberries survival. The rockwool may be used in cutting propagation of the herbaceous plants with thicker waxy layers or the woody plants.

From the point of view of the media constituents, compared to the inorganic media of RWC and GRW, the two organic mixtures, PBM and CBN, contain a nutrient charge and a wetting agent. Low levels of fertilizers containing minor nutrients are not required initially for germination or rooting [35]. Moreover, the pH of these two organic media is faintly acidic, which is better for strawberry growth than the two kind of alkalescency rockwool [36,37]. Olympios [38] mentioned that compared with an organic mixed matrix, an inorganic matrix such as rockwool has a high disposal cost after use. Tehranifar et al. [39] reported that growth media with peat and cocopeat showed less malformed fruits in soilless cultivation of strawberry. Gislerød H.R. [40] introduced the physical conditions of propagation media and their influence on the rooting of cuttings in detail, where two types of compressed peat and one type of rockwool were compared during poinsettia cutting process.

About the cultivar, 'Maehyang' and 'Seolhyang' have similar traits but also have certain differences. Previous studies [41–44] have found that these two cultivars responded differently to different treatments. These differences are shown at the seedling stage, which lead to slightly different responses between the two cultivars in different fogging treatments in this study.

5. Conclusions

The experimental results and production practices show that PBM is the most appropriate medium for the cutting propagation of strawberry 'Maehyang' and 'Seolhyang' plants when using the fogging system, and 9–12 days of fogging is the most appropriate duration. Rockwool is not suitable for use in high air humidity environment for cutting propagation of strawberry 'Maehyang' and 'Seolhyang'. PBM combined with a 9 or 12-day fogging significantly enhanced the growth of young plants, making them healthy and strong. After calculating the cost and profit, the growers can make a reasonable production plan based on the valuable results of this study.

Author Contributions: Conceptualization, B.R.J. and H.W.; methodology, B.R.J. and H.W.; software, H.W. and C.L.; validation, B.R.J.; formal analysis, B.R.J. and H.W.; investigation, H.W. and C.L.; resources, B.R.J.; data curation, H.W. and C.L.; writing—original draft preparation, H.W. and C.L.; writing—review and editing, B.R.J. and H.W.; visualization, H.W. and C.L.; supervision, B.R.J.; project administration, B.R.J. and H.W.; funding acquisition, B.R.J. and H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Agrobio-Industry Technology Development Program, Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea (Project No. 315004-5).

Acknowledgments: Hao Wei and Chen Liu were supported by a scholarship from the BK21 Plus Program, Ministry of Education, Korea.

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

References

1. Korea Agro-Fisheries & Food Trade Corporation. 2017. Available online: http://www.at.or.kr/article/apko361000/list.action (accessed on 7 March 2020).
2. Husaini, A.M.; Neri, D. (Eds.) *Strawberry: Growth, Development and Diseases*; CABI: Wallingford, UK, 2016.
3. Durner, E.F.; Poling, E.B.; Maas, J.L. Recent advances in strawberry plug transplant technology. *Hort. Technol.* **2002**, *12*, 545–550. [CrossRef]
4. Poling, E.B.; Maas, J.L. Strawberry plug transplant technology. *Acta Hortic.* **1998**, *513*, 393–402. [CrossRef]
5. Hughes, H. The effects of planting time, runner size and plant spacing on the yield of strawberries. *J. Hortic. Sci.* **1967**, *42*, 253–262. [CrossRef]
6. Freeman, J.A.; Pepin, H.S. Influence of plant size, date of digging and duration of cold storage on the growth of strawberry plants. *Can. J. Plant Sci.* **1971**, *51*, 267–274. [CrossRef]
7. Crawford, T.D.; Himelrick, D.G.; Sibley, J.L.; Pitts, J.A. Effect of runner plantlet size on performance of strawberry plug plants. *Small Fruits Rev.* **2000**, *1*, 15–21. [CrossRef]
8. Rowley, D.; Black, B.; Drost, D. *Strawberry Plug Plant Production*; Plants, Soils, and Climate Faculty Publications, USU Extension: Logan, UT, USA, 2010; pp. 1–5.
9. Lieten, F. Short cut strawberry propagation. *Grow.* **1994**, *121*, 35.
10. Treder, W.; Tryngiel-Gac, A.; Klamkowski, K.; Masny, A. Evaluation of efficiency of a nursery system for production of strawberry potted plants in protected conditions. *Infrastrukt. Ekol. Teren. Wiej.* **2014**, *4*, 1333–1341.
11. Peyvast, G.H.; Noorizadeh, M.; Hamidoghli, J.; Ramezani-Kharazi, P. Effect of four different substrates on growth, yield and some fruit quality parameters of cucumber in bag culture. *Int. Symp. Grow. Media* **2005**, *779*, 535–540.

12. Gao, H.B.; Zhang, T.J.; Lv, G.Y.; Zhang, G.H.; Wu, X.L.; Li, J.R.; Gong, B.B. Effects of different compound substrates on growth, yield and fruit quality of cucumber. *Int. Symp. Veg. Saf. Hum. Health* **2006**, *856*, 173–180. [CrossRef]
13. Jiang, M.; Zhang, J. Role of abscisic acid in water stress-induced antioxidant defense in leaves of maize seedlings. *Free Radic. Res.* **2002**, *36*, 1001–1015. [CrossRef]
14. Baisak, R.; Rana, D.; Acharya, P.B.; Kar, M. Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. *Plant Cell Physiol.* **1994**, *35*, 489–495.
15. Jafarnia, S.; Hatamzadeh, A.; Tehranifar, A. Effect of different substrates and varieties on yield and quality of strawberry in soilless culture. *Adv. Environ. Biol.* **2010**, *4*, 325–329.
16. Giménez, G.; Andriolo, J.L.; Janisch, D.; Godoi, R. Closed soilless growing system for producing strawberry bare root transplants and runner tips. *Pesqui. Agropecuária Bras.* **2008**, *43*, 1757–1761. [CrossRef]
17. Bartczak, M.; Pietrowska, M.; Knaflewski, M. Effects of substrate on vegetative quality of strawberry plants (*Fragaria x ananassa* Duch.) produced by soilless method. *Folia Hortic.* **2007**, *19*, 39–46.
18. Kim, T.I.; Jang, W.S.; Choi, J.H.; Nam, M.H.; Kim, W.S.; Lee, S.S. Breeding of strawberry 'Maehyang' for forcing culture. *Hortic. Sci. Technol.* **2004**, *22*, 434–437.
19. Kim, T.I.; Jang, W.S.; Nam, M.H.; Lee, W.K.; Lee, S.S. Breeding of strawberry 'Sulhyang' for forcing culture. *IHC* **2006**, *2006*, 231.
20. Han, K.S.; Kim, S.C.; Lee, Y.B.; Kim, S.C.; Im, D.H.; Choi, H.K.; Hwang, H. Strawberry harvesting robot for bench-type cultivation. *J. Biosyst. Eng.* **2012**, *37*, 65–74. [CrossRef]
21. Christou, A.; Manganaris, G.A.; Fotopoulos, V. Systemic mitigation of salt stress by hydrogen peroxide and sodium nitroprusside in strawberry plants via transcriptional regulation of enzymatic and non-enzymatic antioxidants. *Environ. Exp. Bot.* **2014**, *1074*, 6–54. [CrossRef]
22. Giannopolitis, C.N.; Rios, S.K. Superoxide dismutases: Purification and quantitative relationship and soluble protein in seedlings. *Plant Physiol.* **1977**, *59*, 315–318. [CrossRef]
23. Cakmak, I.; Marschner, H. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol.* **1992**, *98*, 1222–1227. [CrossRef]
24. Shah, K.; Kumar, R.G.; Verma, S.; Dubey, R.S. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Sci.* **2001**, *161*, 1135–1144. [CrossRef]
25. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **1981**, *22*, 867–880.
26. Bussell, W.; Mckennie, S. Rockwool in horticulture, and its importance and sustainable use in New Zealand. *N. Z. J. Crop. Hort.* **2004**, *32*, 29–37. [CrossRef]
27. Kim, H.M.; Kim, H.M.; Jeong, H.W.; Lee, H.R.; Jeong, B.R.; Kang, N.J.; Hwang, S.J. Growth and rooting rate of 'Maehyang' strawberry as affected by irrigation method on cutting propagation in summer season. *Prot. Hortic. Plant Fact.* **2018**, *27*, 103–110. [CrossRef]
28. Okunlola, A.I. The effects of cutting types and length on rooting of *Duranta repens* in the nursery. *Glob. J. Hum. Soc. Sci. Geogr. Geo. Sci. Environ. Disaster Manag.* **2003**, *13*, 1–5.
29. De Swaef, T.; Verbist, K.; Cornelis, W.; Steppe, K. Tomato sap flow, stem and fruit growth in relation to water availability in rockwool growing medium. *Plant Soil* **2012**, *350*, 237–252. [CrossRef]
30. Choudhury, S.; Panda, P.; Sahoo, L.; Panda, S.K. Reactive oxygen species signaling in plants under abiotic stress. *Plant Signal. Behav.* **2013**, *8*, e23681. [CrossRef]
31. Gratão, P.L.; Polle, A.; Lea, P.J.; Azevedo, R.A. Making the life of heavy metal-stressed plants a little easier. *Funct. Plant Biol.* **2005**, *32*, 481–494. [CrossRef]
32. Hall, A.J.; Connor, D.J.; Whitfield, D.M. Root respiration during grain filling in sunflower: The effects of water stress. *Plant Soil* **1990**, *121*, 57–66. [CrossRef]
33. Liao, C.T.; Lin, C.H. Physiological adaptation of crop plants to flooding stress. *Proc. Natl. Sci. Counc. ROC(B)* **2001**, *25*, 148–157.
34. Gambrell, R.P.; Patrick, W.H. Chemical and microbiological properties of anaerobic soils and sediments. In *Plant Life Anaerob. Environ*; Hook, D.D., Crawford, R.M.M., Eds.; John Wiley & Sons Ltd.: Collingwood, MI, USA, 1978; pp. 375–423.

35. Beyl, C.A.; Trigiano, R.N. *Plant Propagation Concepts and Laboratory Exercises*; CRC Press: Boca Raton, FL, USA, 2011.
36. Regagba, Z.; Choi, J.M.; Latigui, A.; Mederbal, K.; Latigui, A. Effect of various mg concentrations in nutrient solution on growth and nutrient uptake response of strawberry (Fragaria×ananassa duch.) 'Seolhyang' grown in soilless culture. *J. Biol. Sci.* **2014**, *14*, 226–236.
37. Takeda, F. Strawberry production in soilless culture systems. *Int. Symp. Grow. Media Hydroponics* **1997**, *19*, 289–296. [CrossRef]
38. Olympios, C.M. Soilless media under protected cultivation rockwool, peat, perlite and other substrates. *Acta Hortic. 1992. 323*, 215–234. [CrossRef]
39. Tehranifar, A.; Poostchi, M.; Arooei, H.; Nematti, H. Effects of seven substrates on qualitative and quantitative characteristics of three strawberry cultivars under soilless culture. In Proceedings of the XXVII International Horticultural Congress-IHC2006: International Symposium on Advances in Environmental Control, Automation, Seoul, Korea, 13–16 August 2006; pp. 485–488.
40. Gislerød, H.R. Physical conditions of propagation media and their influence on the rooting of cuttings. *Plant Soil* **1983**, *75*, 1–14. [CrossRef]
41. Jun, H.J.; Jun, E.H.; Kang, S.I.; Bae, K.H. Effect of cold treatment for mother plants of new strawberry cultivars bred in Korea on the production of runners and daughter plants. *Prot. Hortic. Plant Fact.* **2015**, *24*, 8–12. [CrossRef]
42. Choi, H.G.; Hwang, M.R.; Choi, K.S.; Kang, N.J. Effect of application of salicylic acid on the plant growth and fruit quality in strawberry. *J. Agric. Life Sci.* **2018**, *52*, 11–22. [CrossRef]
43. Kang, D.I.; Jeong, H.K.; Park, Y.G.; Hwang, S.J.; Jeong, B.R. Effect of nutrient solution strength and duration of nutrient starvation on growth and flowering of two strawberry cultivars. *J. Agric. Life Sci.* **2019**, *53*, 19–28. [CrossRef]
44. Kim, D.Y.; Kim, S.; Kang, Y.I.; Yun, H.K.; Yoon, M.K.; Kim, T.I.; Choi, J.M. Effect of runner cutting time on growth and yield during nursery of strawberry (cv. 'Maehyang' and 'Seolhyang'). *Prot. Hortic. Plant Fact.* **2012**, *21*, 385–391. [CrossRef]