horticulturae

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Exogenous Treatments to Enhance Splice-Grafted Watermelon Survival

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Abstract: This study evaluated the use of splice grafting as a propagation strategy for watermelon. In experiment 1, the treatments consisted of sucrose, antitranspirant A, antitranspirant B, auxin (indole-3-butyric acid (IBA)) at two concentrations (10 and 20 mg·L^{-1}), plus a water control. The survival (%) of splice-grafted watermelon plants differed due to the number of days after grafting and treatment ($p < 0.0001$, for both). At 21 days after grafting, plants treated with sucrose and antitranspirant A, and sucrose and antitranspirant A with 10 mg·L^{-1} auxin had 90% and 88% survival, respectively, whereas the graft survival was 18% for plants treated with water. Experiment 2 included the three top performing treatments from experiment 1 and a water control treatment, applied to both root-intact and root-excised rootstocks. There was a significant difference in survival (%) of splice-grafted watermelon due to root treatments, exogenous treatments, and the number of days after grafting ($p < 0.0001$, for all). At 21 days after grafting, survival for root-excised grafted plants was 11% lower compared to root-intact plants. Plants treated with sucrose and antitranspirant A, and sucrose and antitranspirant A with 10 mg·L^{-1} auxin had 87% and 86% survival, respectively, whereas plants treated with water had 14% survival. The external application of auxin applied to rootstock seedlings does not appear to be cost-effective; however, other products should be evaluated.

Keywords: auxin; abscisic acid; carbohydrate; cotyledon; root-excision; rootstock regrowth

Citation: Devi, P.; DeVetter, L.W.; Lukas, S.; Miles, C. Exogenous Treatments to Enhance Splice-Grafted Watermelon Survival. *Horticulturae* **2021**, *7*, 197. https://doi.org/10.3390/horticulturae7070197

Academic Editor: Federica Caradonia

Received: 28 June 2021
Accepted: 14 July 2021
Published: 16 July 2021

1. Introduction

Grafting in watermelon (*Citrullus lanatus*) production has emerged as a promising abiotic and biotic stress management strategy. However, the adoption of the practice is limited in the U.S., in part due to the additional costs involved in producing grafted watermelon with the commonly used one-cotyledon grafting method. The one-cotyledon method requires more labor during and after grafting to scout and remove undesirable rootstock regrowth from the watermelon transplants, both in the greenhouse and in the field [1–3]. The cost of grafted watermelon transplants with currently available methods can be up to five times greater than nongrafted plants [4], with labor representing 48% to 60% of the total cost in a manual grafting operation [5,6]. Further, Lewis et al. [6] reported that the number of watermelon plants that can be grafted with the one-cotyledon method is about 150 plants/h, whereas 300 solanaceous plants/h can be splice-grafted (both cotyledons removed from the rootstock). Additionally, there is no rootstock regrowth with splice grafting because meristem tissue lies below the axillary bud at the base of the cotyledon and is completely removed [7,8]. The watermelon grafting process needs to become more efficient and cost effective in order to produce the large number of plants required by growers, especially for medium- and large-scale farms in the U.S. that use approximately 7160 plants per hectare (based on spacings of 1.5 m by 0.9 m) [9]. Splice grafting could significantly increase grafting efficiency for watermelon, however, the success rate of splice

grafting watermelon is very low in comparison with grafting methods where at least one cotyledon remains intact on the rootstock [7,8].

Upon cutting both the rootstock and the scion, water, phytohormones, and carbohydrate transport cease at the graft junction and a wound healing pathway is activated. Grafting success depends on the development of vascular tissue (xylem and phloem) and the reconnection between the rootstock and the scion [10–12]. Carbohydrates from the rootstock play a pivotal role in the formation of callus and the cellular differentiation that forms the connection of vascular bundles at the graft interface [13–15]. In the case of watermelon, cell division is inhibited during tissue reunion due to limited carbohydrate levels in the rootstock hypocotyl when both cotyledons are removed [2,3,16]. In contrast, tomato (*Solanum lycopersicum*) and other solanaceous crops have higher levels of carbohydrates in the hypocotyl and their grafting success is very high (>95%) with splice grafting [1–3]. For healing, newly grafted plants are placed in low light conditions for at least 3 days and the synthesis of new carbohydrates is limited under these conditions; thus the grafted seedling is reliant on stored carbohydrates for survival [3].

The root excision of the rootstock prior to grafting could conserve carbohydrates in the rootstock hypocotyl. Sabatino [17] reported that the hypocotyl with roots removed had greater nutrient reserves than when roots were intact, and this played a major role in sustaining the grafted seedling during healing. Root excision from the rootstock before grafting, and rerooting immediately after grafting has been used with a more than 95% survival in cucurbit grafting using the one cotyledon method [18,19]. As both graft healing and root growth require energy, excising the roots could allow energy reserved in the rootstock to be used for graft healing, leading to improved graft success [3,20,21]. Further, Guan and Zhao [18] reported that root-excised grafted muskmelon (*Cucumis melo*) seedlings showed rapid root regeneration at 8 days after grafting (DAG), and reached similar root length and surface area as the root-intact plants at 16 DAG. There are also reports that root-excised seedlings grew faster than root-intact grafted seedlings immediately after transplanting [22]. A study by Zhao et al. [23] reported that root excision treatments did not affect aboveground growth or the root characteristics of grafted plants except for a significant increase in stem diameter, when seedless watermelon cv. Melody was one-cotyledon grafted onto *Cucurbita maxima* × *C. moschata* rootstock cvs. Marvel, Super Shintosa, and Root Power.

The carbohydrate level in the rootstock hypocotyl can also be increased by the drench applications of sucrose solution to rootstock seedlings before splice grafting and this resulted in increased watermelon grafting success [7]. Another greenhouse study conducted by Devi et al. [8] found that the survival of splice-grafted watermelon seedlings 21 DAG was 91% for plants when rootstock seedlings received sucrose and antitranspirant solutions before grafting, compared with 67% for plants receiving 2% sucrose alone and 25% for plants that received only water. This study also found that when the plants were treated with a different antitranspirant solution without sucrose, the survival was only 70%. Antitranspirant products that contain abscisic acid (ABA) can reduce scion transpiration by entering the leaf and inducing stomatal closure by manipulating the ABA signaling pathway [24–26]. Reducing scion transpiration during the time of vascular connection between the rootstock and the scion during healing is crucial for grafting survival [7,8].

In addition to carbohydrates and ABA, other phytohormones play an important role in successful grafting [12,27]. Several studies have reported that phytohormones such as auxin and cytokinin induce the initiation and proliferation of callus and new vascular tissues by promoting cell division and/or cell differentiation [28,29]. Cotyledons are an important source of auxin, and cotyledon-derived auxin promotes graft formation in young plants [30–32]. Auxin is transported from the leaves to the roots [33], thus severing the vascular tissues in the stem via grafting impedes its movement and accumulation in the root system. Shimomura and Fujihara [34] showed that the application of the synthetic auxin 1-naphthale-neacetic acid (NAA) to the scion apices stimulated vascular reconnection during the grafting of cactus (*Notocactus submammulosus*) plants. In Arabidopsis (*Arabidopsis*

thaliana), auxin accumulation at the graft union was followed by cell differentiation and vascular reconnection between the rootstock and the scion [35]. In another study with Arabidopsis, Matsuoka et al. [36] showed that hypocotyl graft reunion was inhibited when cotyledons were removed, or when an auxin inhibitor was transiently applied on the cotyledon. In vegetable grafting, Sachs [37] reported that xylem formation across the graft was blocked when the majority of the shoot was removed in a grafted pea (*Pisum sativum*), but the exogenous application of auxin in the place of the missing shoot allowed xylem tissue to form. A similar result was found when seedless watermelon cv. Yellow Buttercup QV766 cuttings were treated with auxin (indole-3-butyric acid (IBA)) [38]. In cucumber (*Cucumis sativus*) hypocotyl cuttings, root formation and growth were inhibited when cotyledons were completely removed [39], further emphasizing the importance of auxin transport.

The overall objective of this study was to determine if the exogenous applications of auxin in combination with sucrose and antitranspirant solutions to rootstock seedlings before grafting could increase the survival of splice-grafted watermelon transplants. Additionally, the study examined if excising the roots from rootstock seedlings prior to grafting and rerooting could increase the survival of splice-grafted watermelon seedlings.

2. Materials and Methods

2.1. Experimental Location and Design

Two greenhouse experiments were carried out in 2020 at the Washington State University Northwestern Washington Research and Extension Center (WSU NWREC) greenhouse facilities in Mount Vernon, WA. Experiment 1 included the external applications of sucrose, antitranspirant, and auxin to rootstock seedlings with intact roots before grafting. The experimental design was a randomized complete block with four replications and 10 treatments and 18 plants per experimental unit. Experiment 2 had a split-plot design with four replications of two main plot treatments and four sub plot treatments, with 18 plants per sub plot treatment. The main plot treatments were rootstock (roots either intact or excised during grafting), and the sub plot treatments were the three most successful exogenous treatments from experiment 1. Both experiments were carried out three times. Experiment 1 was carried out between March and April, and experiment 2 was carried out between August and November. The greenhouse temperature was set with an environmental control system (ARGUS Control Systems, Surrey, BC, Canada) at 24/18 °C for day/night. Inside the greenhouse, ambient sunlight was supplemented with 600-W high-pressure sodium bulbs (PL Light Systems, Beamsville, ON, Canada) for 14 h (6.00 a.m. to 8.00 p.m.) in trial 1 and 2, and 12 h (6.00 a.m. to 6.00 p.m.) in trial 3 of experiment 1 and all three trials of experiment 2. The differences in the amount of time of supplemental lighting were due to the different daylight durations during the times of year that the trials were carried out.

2.2. Plant Material

For both the experiments, seedless watermelon cv. Secretariat (Sakata Seeds America, Inc., Morgan Hill, CA, USA) was selected for the scion and interspecific squash hybrid cv. Super Shintosa (*C. maxima* × *C. moschata/Calabacita* Hyb) (Syngenta Seeds, Minneapolis, MN, USA) was used as the rootstock. The scion and rootstock seeds were sown into 72-cell trays filled with potting mix (Sunshine #3 N&O; Sun Gro Horticulture, Agawam, MA, USA), with rootstock seeded in every other row to allow for air circulation around the seedlings during graft healing. The planting dates were staggered for the scion and the rootstock so that seedlings had similar stem diameters (3.5–4.0 mm) at the time of grafting. For experiment 1, 'Secretariat' was sown on 10 February, 20 February, and 2 April, while 'Super Shintosa' was seeded on 24 February, 9 March, and 14 April for trials 1, 2, and 3, respectively. For experiment 2, 'Secretariat' was sown on 5 August, 30 September, and 19 October, while 'Super Shintosa' was seeded on 12 August, 7 October, and 30 October for trials 1, 2, and 3, respectively. In addition, for experiment 2, 72-cell trays were filled

with the same potting mix one day before grafting for the insertion of root excised grafted seedlings on the day of grafting.

2.3. Exogenous Treatments

In experiment 1, the products applied as treatments are presented in Table 1. Sucrose (2% *w/v* in water) and antitranspirant A (root drench, 2% *v/v* in water) and B (Glycerin, 4% *v/v* in water) were selected based on Devi et al. [8]. Both of these antitranspirant products are labeled for use on vegetable transplants, and are the stomata-closing type; that is, they condition the plant to produce additional amounts of ABA, which causes the guard cells around the stomata to close [26,40,41]. The auxin (IBA) selected for this experiment is used as a plant growth regulator for vegetable propagation and can be used as drench, and the application rates (10 and 20 mg·L^{-1}) are recommended on the label for vegetable seedlings.

Table 1. List of commercial products that were applied to rootstock cv. Super Shintosa before splice-grafting.

Treatment Product [x]	Product and Manufacturer
Sucrose 2% (*w/v*)	IB37160 Sucrose; IBI Scientific, Peosta, IA, USA
Antitranspirant A 2% (*v/v*)	Root-Drench; Zorro Technology Inc., Clackamas, OR, USA
Antitranspirant B 2% (*v/v*)	Glycerin 99.7% USP/BP grade; Deepthi Organics LLC, Greensboro, NC, USA
Auxin	Indole-3-butyric acid (IBA), 98%, Alfa Aesar™; Fisher Scientific, Waltham, MA, USA
Tap water	

[x] The solutions of sucrose, antitranspirant, and auxin were made by dissolving in tap water.

All treatments were applied as soil drenches to each rootstock seedling following Devi et al. [8] and are summarized here. The sucrose treatment was split into three applications, and applied every other day starting 6 days prior to grafting. The first and second applications were 20 mL, and the third application was 10 mL 2 days prior to grafting. Both antitranspirant treatments A and B were applied at 20 mL and auxin (both application rates) was applied at 10 mL in combination with the third application of sucrose. The control consisted of 20 mL of tap water for the first and second application, and 40 mL for the third application. On each treatment application day, tap water was collected at the same time to dilute all of the chemical treatments and to apply to the control treatment. Seedlings were watered following common greenhouse practices on days when treatments were not applied.

Experiment 2 included the three treatments that had more than 80% survival of the grafted watermelon plants in experiment 1: sucrose and antitranspirant A; sucrose and antitranspirant A with 10 mg·L^{-1} auxin; and sucrose and 10 mg·L^{-1} auxin. In addition, a water control treatment was included, and the method of applying treatments was the same as described in experiment 1.

2.4. Grafting Methods and Healing

For experiment 1, splice grafting occurred on 5 March, 15 March, and 23 April for trials 1, 2, and 3, respectively. For experiment 2, splice grafting occurred on 19 August, 14 October, and 10 November for trials 1, 2, and 3, respectively. For the root-excised treatment in experiment 2, the rootstock was cut at the base of the hypocotyl just above the soil line, inserted about 2–3 cm deep in the seedling trays filled with potting mix, and then grafted. The grafting and healing techniques for both experiments followed protocols developed by Devi et al. [8] and are summarized here. Both the rootstock and the scion seedlings were cut at a 60° angle below the two cotyledons. The rootstock seedlings were cut 0.5 cm below the cotyledons, whereas the scion was cut about 2 cm below the cotyledons to match stem diameters. The two cut stem surfaces were placed together and a watermelon grafting clip (3 mm; Johnny's Selected Seeds, Fairfield, ME, USA) held the graft union together. Immediately after grafting, the plants were placed in healing chambers on a bench in the greenhouse and followed a 9 day, site-specific protocol [42]. The healing chamber was covered with clear plastic (0.15 mm polythene; Ginegar Plastic Products,

Ginegar, Israel) to maintain relative humidity (RH), and a layer of black polyethylene woven fabric (Contractor Landscape Fabric; American Nettings & Fabric, Ferndale, WA, USA) was placed over the chamber to limit light penetration to the plants. The plants were maintained in complete darkness on days 1 and 2, and the chamber was opened for 5 min on day 3, 15 min on day 4, 30–45 min on day 5, 1.5 h on day 6, 4 h on day 7, and 6 h on day 8. Starting on day 4 and each day thereafter, the black fabric was removed from one side of the chamber, and on day 7 it was completely removed. On day 1 and each day thereafter, the chamber was opened, a thin film of water was added to the floor of the chamber to attain 100% RH, and on days 7 and 8, the grafted plants were misted with water. The plants were removed from the chamber on day 9 and placed on the greenhouse benches and watered slowly as needed, following common greenhouse practice.

2.5. Environmental Conditions

The temperature, RH (%), and light intensity were recorded every 15 min with data loggers (H21-002; Hobo Onset, Bourne, MA, USA). The measurements were recorded throughout the three trials of both experiments in the healing chamber and on the bench in the greenhouse next to the healing chamber, where plants were placed when they were taken out of the chamber.

2.6. Grafted Plant Survival

The survival of the grafted plants in both experiments was assessed on 4, 9, 16, and 21 DAG. The grafted plants were considered alive and 'survived' if the scion leaves and the rootstock stems were turgid, whereas severely wilted scion leaves and stems of both the scion and the rootstock were considered as graft failure.

2.7. Data Analyses

All data were analyzed using JMP software (Version 14.0.0 for Windows; SAS Institute, Cary, NC, USA). The data for all parameters were tested for normality using the Shapiro-Wilk test and were analyzed using an analysis of variance (ANOVA). The sucrose, antitranspirant, auxin, water, and the root treatments of the rootstock (root-intact and root-excised) were explanatory variables, whereas grafting survival was a response variable. When significant effects were detected, means were separated using Tukey's honestly significant difference test at a significance level of $p < 0.05$. When there were no significant interactions between the factors of the treatment and a trial, treatment means were pooled over the trial.

3. Results

3.1. Environmental Conditions in the Greenhouse

In both the experiments, the daily average temperature and RH in the healing chamber and in the greenhouse were similar for all three trials. The daily average temperature inside the healing chamber was 22 to 27 °C and the daily average temperature in the greenhouse was 21 to 26 °C. The greenhouse temperature range was due to the age and construction of the greenhouse, where it was not possible to precisely control environmental conditions. During days 1 to 4 after grafting, when the healing chamber was closed and entirely covered with black fabric, the daily average temperature inside the chamber was the same as the temperature inside the greenhouse, 22 to 26 °C (Figure 1). The daily average RH during this time was 92% to 98% inside the chamber and 53% to 70% in the greenhouse (Figure 2), and the daily average light intensity in the chamber was 1.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 3), compared to 258 to 305 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the greenhouse. From 5 to 6 DAG, when plants in the chamber were exposed to the greenhouse environment for 1.5 h or less each day, and the chamber was partially covered with black fabric, the daily average temperature inside the chamber was 23 to 27 °C (Figure 1). The daily average RH during this time was 90% to 93% inside the chamber and 42% to 55% in the greenhouse (Figure 2). The daily average light intensity in the chamber was 7.0 to 7.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$, whereas the light intensity in the greenhouse

was 280 to 400 µmol·m^{-2}·s^{-1}. For 7 to 9 DAG, when plants in the chamber were exposed 5 h on average each day to the greenhouse environment, the temperature inside the chamber and the temperature in the greenhouse was 22 to 26 °C (Figure 1). The daily average RH during this time was 72% to 88% inside the chamber and 44% to 55% in the greenhouse (Figure 2). The daily average light intensity in the chamber was 155 to 250 µmol·m^{-2}·s^{-1} (Figure 3), and the light intensity in the greenhouse was 320 to 440 µmol·m^{-2}·s^{-1}. For 10 to 21 DAG, when the plants were on the greenhouse bench, the daily average temperature in the greenhouse was 24 °C (Figure 1), the RH was 60% (Figure 2), and the daily average light intensity was 300 to 480 µmol·m^{-2}·s^{-1} (Figure 3).

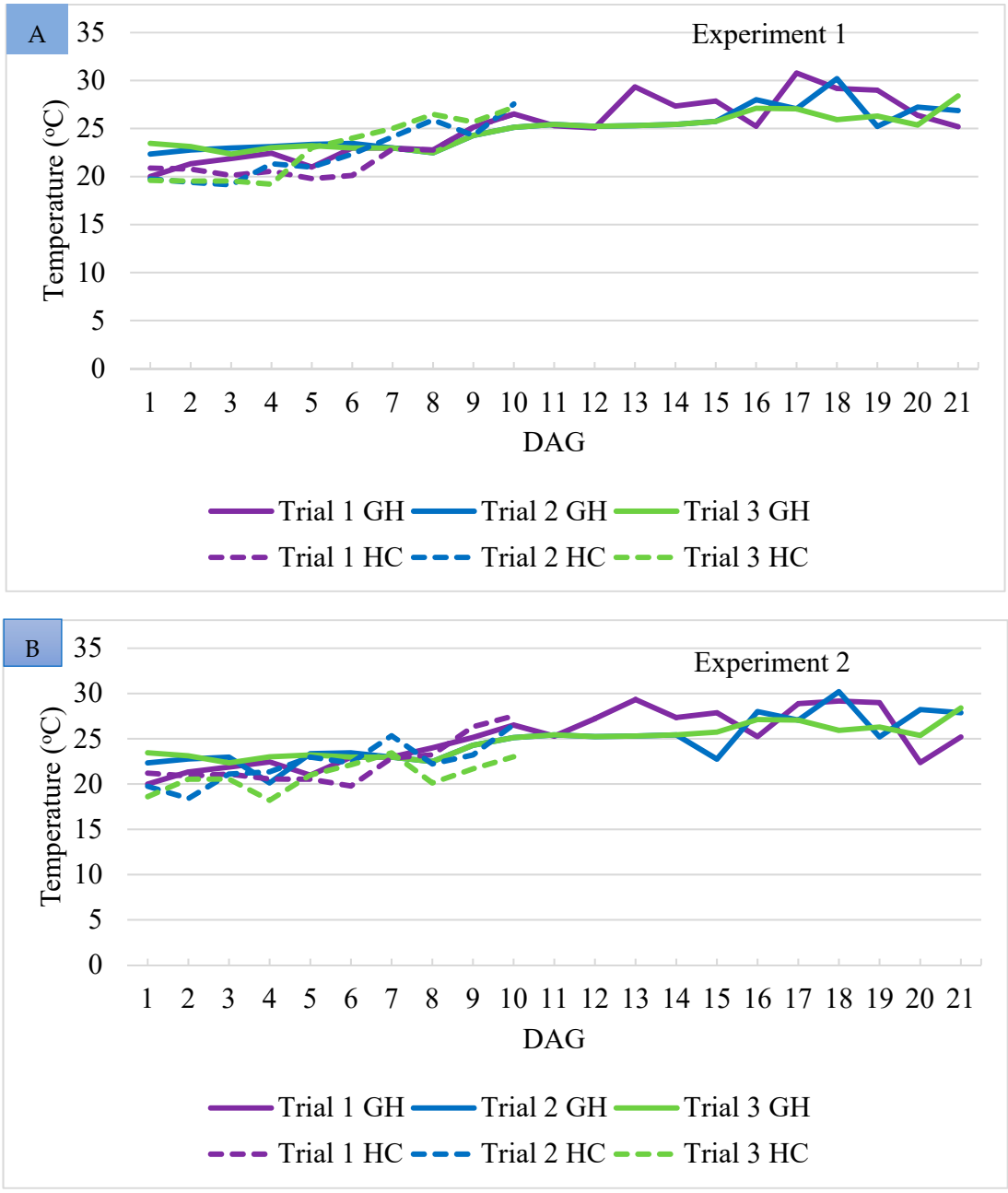

Figure 1. Temperature (°C) in the healing chamber (HC) for 10 days after grafting (DAG) and in the greenhouse (GH) for 21 DAG for trials 1, 2, and 3 on 5 March, 15 March, and 23 April 2020, respectively, for experiment 1 (**A**), and for trials 1, 2, and 3 on 19 August, 14 October, and 10 November, 2020, respectively, for experiment 2 (**B**) at Mount Vernon, WA, USA. Data were recorded every 15 min (Onset HOBO, Bourne, MA, USA).

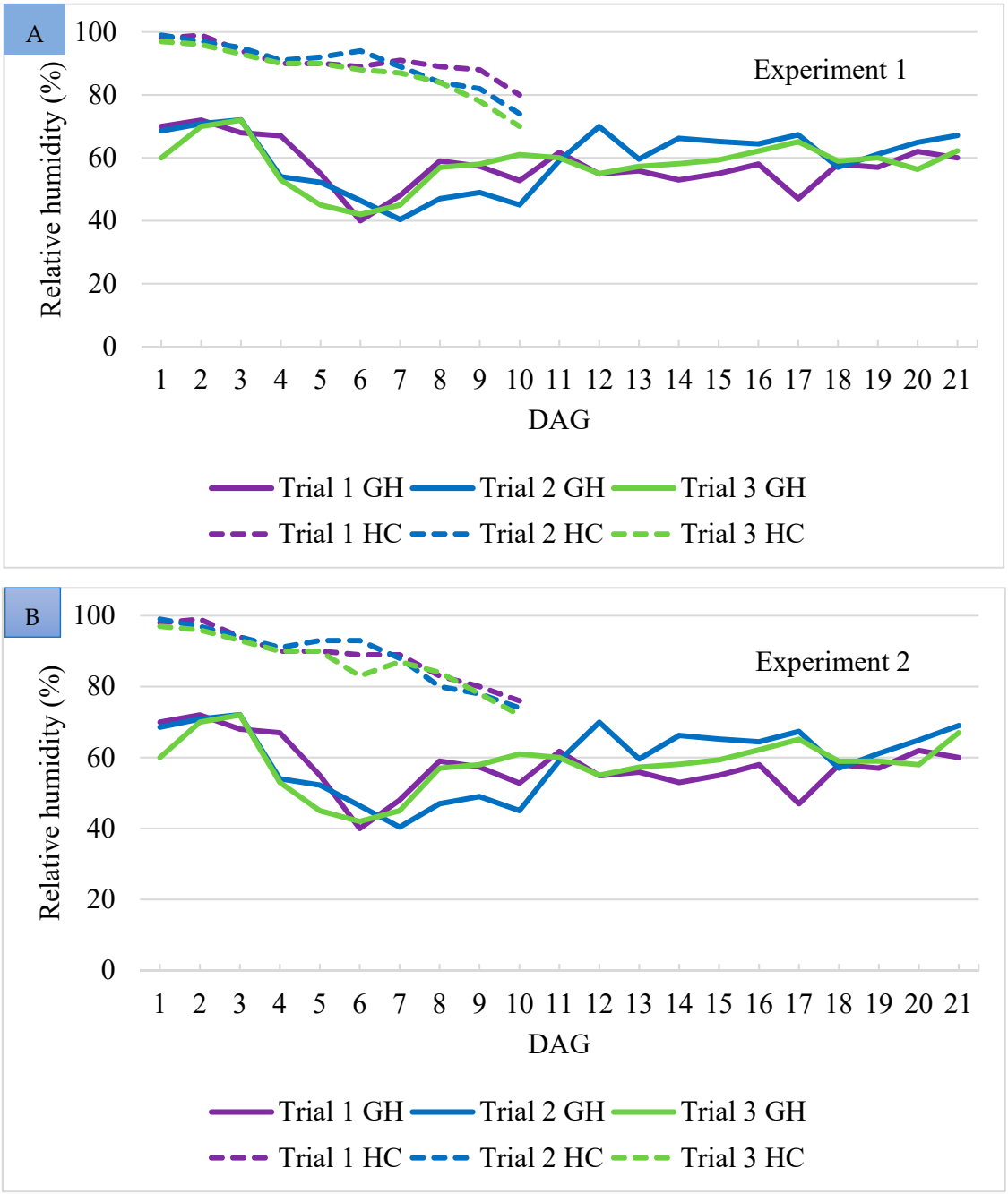

Figure 2. Relative humidity (%) in the healing chamber (HC) for 10 days after grafting (DAG) and in the greenhouse (GH) for 21 DAG for trials 1, 2, and 3 on 5 March, 15 March, and 23 April 2020, respectively, for experiment 1 (**A**), and for trials 1, 2, and 3 on 19 August, 14 October, and 10 November 2020, respectively, for experiment 2 (**B**) at Mount Vernon, WA, USA. Data were recorded every 15 min (Onset HOBO, Bourne, MA, USA).

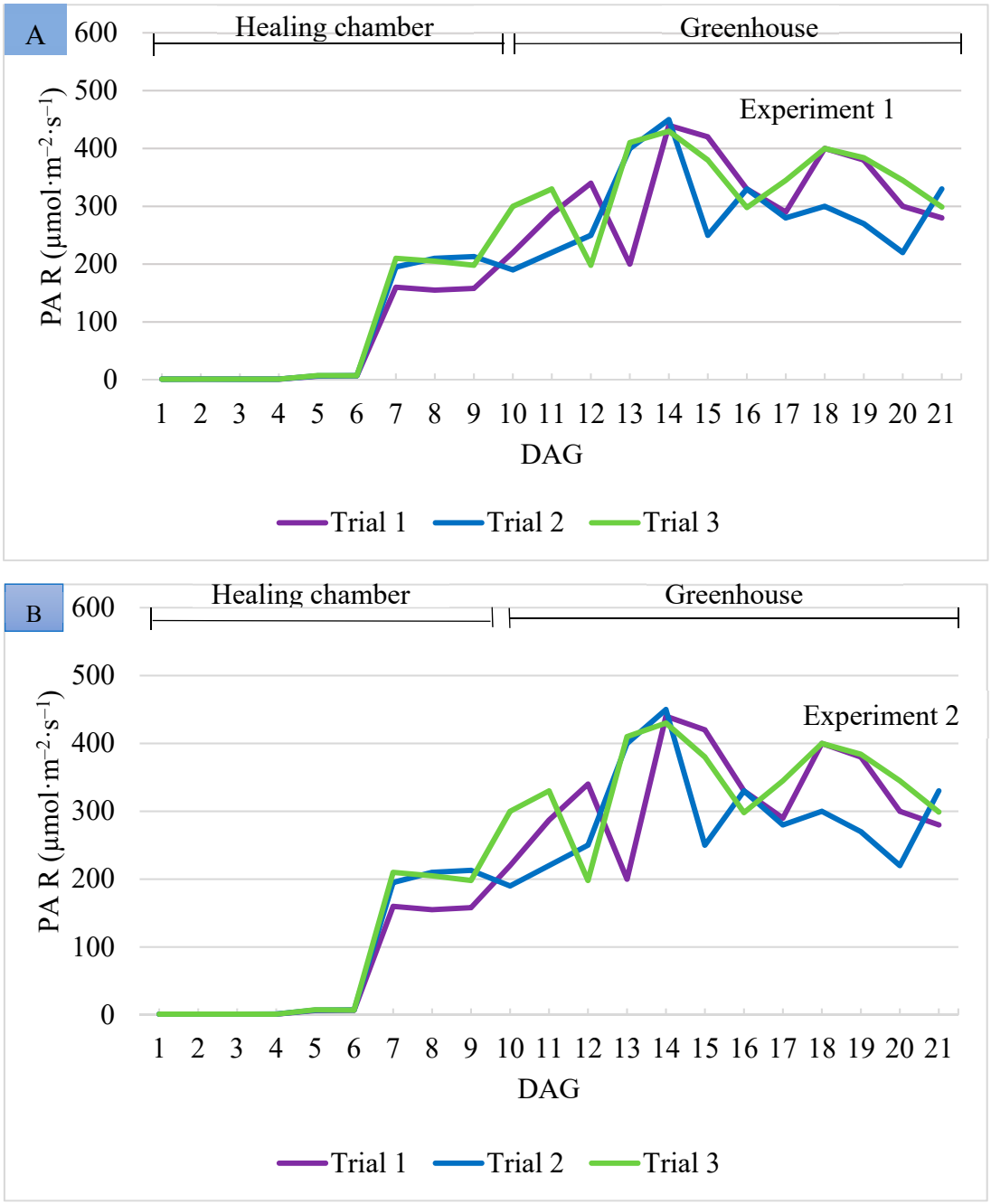

Figure 3. Light intensity of photosynthetically active radiation (PAR, µmol·m^{-2}·s^{-1}) in the healing chamber for 10 days after grafting (DAG) and in the greenhouse for 21 DAG for trials 1, 2, and 3 on 5 March, 15 March, and 23 April 2020, respectively, for experiment 1 (**A**), and for trials 1, 2, and 3 on 19 August, 14 October, and 10 November 2020, respectively, for experiment 2 (**B**) at Mount Vernon, WA, USA. Data were recorded every 15 min (Onset HOBO, Bourne, MA, USA).

3.2. Grafted Plant Survival

In experiment 1, the survival (%) of splice-grafted watermelon plants differed due to DAG and treatment ($p < 0.0001$, for both), but there was no difference due to trial ($p = 0.33$) (data not shown). On average, the survival of grafted plants was near 99% for all 10 treatments at 4 DAG ($p = 0.68$), 89% at 9 DAG ($p = 0.07$), 73% at 16 DAG ($p = 0.003$), and 65% at 21 DAG ($p < 0.0001$) (Table 2). The plants treated with water tended to have the lowest survival on all dates, which was 70% at 9 DAG, 40% at 16 DAG, and further declined to 18% at 21 DAG. In contrast, at 21 DAG, plants treated with sucrose and

antitranspirant A, and sucrose and antitranspirant A with 10 mg·L^{-1} auxin had 90% and 88% survival, respectively, and the treatments of sucrose and 20 mg·L^{-1} auxin, sucrose and antitranspirant B, and sucrose and 10 mg·L^{-1} auxin, were statistically similar (70 to 80% survival). The treatments of sucrose and antitranspirant B with 20 mg·L^{-1} auxin, sucrose and antitranspirant B with auxin 10 mg·L^{-1}, sucrose and antitranspirant A with 20 mg·L^{-1} auxin, and sucrose were intermediate (48 to 61% survival).

Table 2. Survival (%) of grafted watermelon transplants measured at 4, 9, 16, and 21 days after grafting (DAG) at Mount Vernon, WA, USA. Prior to grafting, rootstock seedlings received applications of sucrose, antitranspirant, and auxin treatments. Grafting was carried out for trials 1, 2, and 3 on 5 March, 15 March, and 23 April 2020, respectively, and data were combined (p = 0.33).

	Survival (%)			
Treatment [z]	4 DAG	9 DAG	16 DAG	21 DAG
Sucrose	99 (1.02) [y]	88 (2.18)	63 b [x] (4.82)	60 b (4.13)
Sucrose + antitranspirant A	100 (0.00)	98 (1.05)	92 a (1.65)	90 a (1.87)
Sucrose + antitranspirant B	99 (1.02)	92 (1.62)	80 a (2.34)	75 a (3.52)
Sucrose + 10 mg·L^{-1} auxin	100 (0.00)	93 (1.71)	85 a (2.07)	80 a (2.30)
Sucrose + 20 mg·L^{-1} auxin	100 (0.00)	89 (2.15)	72 ab (3.22)	70 ab (3.77)
Sucrose + antitranspirant A+ 10 mg·L^{-1} auxin	99 (1.02)	92 (1.62)	90 a (1.87)	88 a (2.08)
Sucrose + antitranspirant A+ 20 mg·L^{-1} auxin	100 (0.00)	90 (1.82)	70 ab (3.47)	61 b (4.01)
Sucrose + antitranspirant B+ 10 mg·L^{-1} auxin	99 (1.02)	85 (2.32)	71 ab (3.57)	58 b (5.30)
Sucrose + antitranspirant B+ 20 mg·L^{-1} auxin	99 (1.02)	88 (2.18)	65 b (4.71)	48 bc (5.92)
Tap water (control)	98 (1.23)	70 (3.67)	40 c (5.12)	18 c (8.17)
p-value	0.68	0.07	0.003	<0.0001

[z] Sucrose (2% solution; IB37160 Sucrose; IBI Scientific, Peosta, IA, USA), antitranspirant A (2% solution; Root-Drench; Zorro Technology Inc., Clackamas, OR, USA), antitranspirant B (4% solution; Glycerin; Deepthi Organics, Greensboro, NC, USA), 10 mg·L^{-1} auxin (indole-3-butyric acid (IBA), Waltham, MA, USA), and 20 mg·L^{-1} auxin. Scion is 'Secretariat' and rootstock is 'Super Shintosa'. [y] The values in parenthesis are the standard deviation of the mean. [x] Mean separation letters generated using Tukey's honestly significant difference test at a significance level of $p < 0.05$ in JMP (version 14.0 for Windows; SAS Institute) at $p \leq 0.05$. Values followed by the same letter within a column are not significantly different.

In experiment 2, there was a significant difference in the survival (%) of splice-grafted watermelon due to root treatments, exogenous treatments, and DAG ($p < 0.0001$, for all), but there was no difference due to trial ($p \geq 0.18$) (data not shown). There was an interaction between root treatments and DAG ($p = 0.007$), and between exogenous treatments and DAG ($p < 0.0001$), but there was no interaction between root excision and exogenous treatments ($p = 0.38$), nor was there an interaction between root excision, exogenous treatments, and DAG ($p = 0.87$) (data not shown). For both root-intact and root-excised treatments, the survival of splice-grafted plants was near 99% at 4 DAG ($p = 0.24$) and declined to 71% on average at 16 DAG ($p = 0.17$). However, at 21 DAG, survival declined to 58% for the root-excised treatments, whereas the survival was 65% for the root-intact treatment ($p = 0.02$) (Table 3). For the exogenous treatments, plants treated with water tended to have the lowest survival on all dates and declined to 14% at 21 DAG ($p = 0.0002$) (Table 3). In contrast, at 21 DAG, plants treated with sucrose and antitranspirant A, and sucrose and antitranspirant A with 10 mg·L^{-1} auxin had 87% and 86% survival, respectively. The survival of plants treated with sucrose and auxin 10 mg·L^{-1} at 21 DAG was intermediate (59%).

Table 3. Survival (%) of grafted watermelon transplants measured at 4, 9, 16, and 21 days after grafting (DAG) at Mount Vernon, WA. Prior to grafting, rootstock seedlings received applications of sucrose, antitranspirant, and auxin treatments and root-excision from rootstock occurred immediately prior to grafting. Grafting was carried out for trials 1, 2, and 3 on 19 August, 14 October, and 10 November 2020, respectively, and data were combined ($p = 0.18$).

	Survival (%)			
Treatment [z]	4 DAG	9 DAG	16 DAG	21 DAG
Root-intact	99 (1.07) [y]	86 (1.88)	73 (2.87)	65 a [x] (3.75)
Root-excised	99 (1.07)	80 (1.71)	68 (2.56)	58 b (4.54)
p-value	0.24	0.29	0.17	0.02
Sucrose + antitranspirant A	99 (1.02)	91 (1.67)	89 a (2.14)	87 a (2.53)
Sucrose + auxin 10 mg·L^{-1}	98 (1.22)	85 (2.37)	69 b (4.33)	59 b (5.43)
Sucrose + antitranspirant A+ auxin 10 mg·L^{-1}	98 (1.22)	92 (1.59)	89 a (2.14)	86 a (2.71)
Tap water (control)	98 (1.22)	64 (3.07)	37 c (6.89)	14 c (8.32)
p-value	0.09	0.06	0.002	0.0002

[z] Root-intact and root-excised rootstocks were used and all grafted plants had watermelon cv. Secretariat as the scion and rootstock cv. 'Super Shintosa' as the rootstock. Sucrose (2% solution; IB37160 Sucrose; IBI Scientific, Peosta, IA, USA), antitranspirant A (2% solution; Root-Drench; Zorro Technology Inc., Clackamas, OR, USA), antitranspirant B (4% solution; Glycerin; Deepthi Organics, Greensboro, NC, USA), 10 mg·L^{-1} auxin (indole-3-butyric acid (IBA), Waltham, MA, USA), and 20 mg·L^{-1} auxin. [y] The values in parenthesis are the standard deviation of the mean. [x] Mean separation letters generated using Tukey's honestly significant difference test at a significance level of $p < 0.05$ in JMP (version 14.0 for Windows; SAS Institute) at $p \leq 0.05$. Values followed by the same letter within a column are not significantly different.

4. Discussion and Conclusions

In both experiments in this study, plants treated with sucrose and antitranspirant A, and sucrose and antitranspirant A with auxin at 10 mg·L^{-1} had the greatest graft survival. Additionally, in both experiments, plants treated with sucrose and auxin at 10 mg·L^{-1} had lower plant survival. Increased graft survival with the addition of antitranspirant A could potentially be due to the ABA present in the antitranspirant. The ABA reduces scion transpiration by inducing stomatal closure, thereby limiting the severity of water stress during graft healing [43]. Free ABA is readily taken up by the roots and loaded into the xylem vessels. Borel et al. [44] reported that in water-stressed grafted tobacco (*Nicotiana tabacum*) plants (grafted onto tobacco rootstock), ABA moved from roots into the xylem sap and then into the ABA-deficient shoots where it closed stomata. Niu et al. [45] reported that cucumber cv. Jinchun No. 2 grafted onto pumpkin cv. Chaojiquanwang had increased ABA when exposed to salt stress (75 mM NaCl), compared to self-grafted plants. The authors reported that ABA served as a key signal to mediate rapid stomatal closure and that the cucumber grafted onto pumpkin had enhanced salt tolerance due to reduced water loss compared to self-grafted plants. In contrast, auxin induces ethylene production, and the decrease in grafting survival with exogenously applied auxin could be due to the inhibition of cell elongation and root growth produced by the auxin-ethylene interaction [46–48]. Alternatively, the exogenous application of auxin may increase the production of reactive oxygen species, for example superoxide radicals, hydrogen peroxide, and hydroxyl radicals, which can induce oxidative stress activities that result in the cell death of plant tissue [10,49]. Based on the results of the current study, the addition of auxin did not result in greater graft survival in either experiment, and so would not be cost effective.

Additionally, in experiment 2, the survival for root-excised grafted plants was 11% lower compared to root-intact plants. Root excision has the advantage in mechanized grafting of preventing the growing media from contaminating the grafting machines. Memmott [19] reported that root excision can be useful for conserving carbohydrates in the rootstock hypocotyl and for encouraging healing. However, the advantage of root excision was not observed in the current study. The splice-grafting method used in the current study might be the reason for the low survival of watermelon grafted onto rootstocks with excised roots, as root growth may have been inhibited by the removal of both cotyledons from the rootstock. A similar result was reported by Guan and Zhao [18] for muskmelon cv. Athena grafted onto Strong Tosa (*C. maxima* × *C. moschata*) with rootstock roots removed

prior to grafting. At 16 DAG, the survival of one-cotyledon grafted plants was near 100%, whereas the survival of splice-grafted plants was 90%. The survival of splice-grafted muskmelon plants further declined in the following days, when one-third of the plants died. In the current study, low survival may have been caused by the range in daily average temperature inside the healing chamber (22 to 27 °C) and the greenhouse (21 to 26 °C), which may have been outside the optimal range for healing splice-grafted watermelon. Additionally, the lack of precise environmental conditions may not be suitable for root regeneration during the healing of grafted plants. This lack of precise environmental control could also be the reason plants treated with water had lower survival in the present study (<20%) compared to previous studies (25% survival in Devi et al. [8] and 58% survival in Dabirian and Miles [7]). These results suggest that healing splice-grafted watermelon, especially with excised roots, may not be suitable for healing chambers within a greenhouse. It may be necessary to heal these plants in a controlled environment.

In conclusion, the current study did not show any difference in the survival of splice-grafted watermelon plants when the rootstocks were treated with sucrose and antitranspirant A with or without auxin. Thus, the external application of auxin to the rootstock seedlings does not appear to be cost-effective. However, other products and combinations of carbohydrate sources, antitranspirants, and growth regulators should be evaluated. Once a splice grafting method with a more than 90% success rate has been achieved, then the cost analysis should be addressed. Although in the current study root excision resulted in decreased graft survival compared to root-intact plants, more research is needed to find treatments that can increase survival if roots need to be removed for grafting efficiency, for example in mechanical grafting. Future research should focus on increasing carbohydrate reserves and ABA in the rootstock hypocotyl, as well as evaluating the optimum environmental conditions, such as temperature, relative humidity, and light, in the healing chamber for splice-grafted watermelon and rerooting grafted plants with excised roots. Finally, Devi et al. [8] assessed the field performance of watermelon splice-grafted onto rootstock treated with sucrose and antitranspirant prior to grafting and found plant survival, fruit yield, and quality were similar to one-cotyledon grafted and nongrafted watermelon plants. Other treatments applied to splice-grafted watermelon seedlings should be similarly evaluated in field studies to ensure plants are vigorous, with acceptable yield and fruit quality.

Author Contributions: Conceptualization, C.M. and P.D.; writing—original draft preparation, P.D.; writing—review and editing, C.M., P.D., L.W.D. and S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the U.S. Department of Agriculture National Institute of Food and Agriculture (NIFA) Specialty Crop Research Initiative Grant 2016-51181-25404, and NIFA Hatch project 1017286.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This study doesn't contain any data. Therefore, data sharing is not applicable to this article.

Acknowledgments: The authors would like to acknowledge Patricia Kreider, and Edward Scheenstra is gratefully acknowledged for invaluable technical support.

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

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