Increasing Survival of Splice-grafted Watermelon Seedlings Using a Sucrose Application

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Abstract. Rootstock regrowth can prevent effective healing of grafted vegetable seedlings and outcompete the scion for light, space, and nutrients later in production. Rootstock regrowth is especially problematic for watermelon (*Citrullus lanatus*) because the crop is most commonly grafted using methods where meristematic tissue remains on the rootstock. The objective of this study was to test whether sucrose solutions [0% (water control), 1%, 2%, and 3%] applied as a drench to rootstock seedlings before grafting would increase the survival of watermelon grafted using the splice method where both rootstock cotyledons were removed to eliminate meristem tissue and rootstock regrowth. Starch accumulation in rootstock seedlings was the highest for plants that received 3% sucrose solution (71%), followed by plants that received 2% sucrose solution (52%), 1% sucrose solution (29%), and water (6%) ($P < 0.0001$). Survival (%) of splice-grafted watermelon seedlings 21 days after grafting was the greatest for plants that received 2% and 3% sucrose solution (89% and 82%, respectively), followed by plants that received 1% sucrose solution (78%), and was the lowest for plants that received water (58%) ($P < 0.0001$). There was a significant interaction due to repeat for both starch accumulation and grafted transplant survival; however, environmental conditions were similar for both repeats: the daily average temperature was 23 °C, the relative humidity (RH) was 64% to 67%, and the daily average light intensity was 224–243 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Furthermore, while the vapor pressure deficit from 1:00 to 6:00 PM was 2.49 kPa for repeat 1 and 1.42 kPa for repeat 2, plant survival was greater in repeat 1 than repeat 2. These results indicate that drench applications of sucrose solution to rootstock seedlings before grafting can increase grafting success when both cotyledons are removed from the rootstock before grafting, but further research is needed to optimize the environmental conditions for the survival of grafted plants.

Watermelon (*C. lanatus*) seedling grafting is a means of managing abiotic stresses (e.g., high soil salinity, drought, and high temperature), reducing the reliance on chemical and fertilizer inputs, and enhancing fruit quality (Colla et al., 2010; Proietti et al., 2008). The two most commonly used grafting methods for commercial production of grafted watermelon transplants are the hole-insertion and the one-cotyledon methods (Hassell et al., 2008). Both of these methods require at least one cotyledon to remain on the rootstock to ensure graft success (Bisognin et al., 2005; Hassell et al., 2008). However, to prevent rootstock growth with these methods, the meristem tissue at the

base of the rootstock cotyledon must be completely removed. Extra attention is required when cutting the watermelon rootstock for grafting, and additional time is needed to remove any residual meristem tissue. Owing to the difficulty in detecting the meristem bud at the base of the cotyledon, the meristem is often removed only partially, and thus, meristem regrowth occurs. Even in the most sophisticated grafting operations today, 2% to 3% of grafted plants will have rootstock regrowth (G. Causarano, personal communication). Additionally, if the regrowth is removed at the transplant stage, labor is still required to scout and remove further regrowth in the field. Much of the additional cost of producing grafted watermelon transplants as compared with tomato (*S. lycopersicum*) and other solanaceous crops is associated with the labor involved in the removal of rootstock regrowth both in the greenhouse and the field (Choi et al., 2002; Davis et al., 2008; Memmott and Hassell, 2010).

Rootstock meristem regrowth additionally decreases grafting success as it can prevent effective healing, and the rootstock shoot will outcompete the scion for light, space, and nutrients later in production. A labor-free method of eliminating meristematic rootstock

regrowth, such as splice grafting below both cotyledons, could significantly reduce the overall cost of producing grafted watermelon transplants and could help increase the adoption of grafted cucurbit transplants in the United States. Splice grafting, also known as top grafting or tube grafting, involves cutting the rootstock at a 45° or greater angle below the cotyledons (Bausher, 2011; Oda, 1999, 2007; Rivard and Louws, 2006; Sakata et al., 2007). By cutting the rootstock below the cotyledons, meristematic tissue is completely removed, and the potential for rootstock regrowth is essentially eliminated in grafted watermelon plants. Splice grafting is the fastest, most efficient, and easiest grafting method to learn and use and therefore is the most commonly used technique for producing large numbers of solanaceous grafted plants as it is possible to graft plants 2–3 times faster than other methods (Oda, 1999, 2007; Rivard et al., 2010). Furthermore, the steps for splice grafting can easily be divided among multiple workers, further increasing the efficiency (Hartmann et al., 2002; Oda, 2007).

When both rootstock cotyledons are removed for watermelon grafting, splice-grafted plants have a significantly lower survival rate in comparison with grafting methods where at least one cotyledon remains intact on the rootstock. The remaining cotyledon functions to provide the required carbohydrates for grafting success (Memmott, 2010). Carbohydrates from the rootstock appear to be necessary for the callus formation and cell differentiation that forms the connection of vascular bundles at the graft interface, which are essential for successful graft healing (Bartolini et al., 1996; Hunter et al., 2004; Ogata et al., 2005; Rapaka et al., 2007; Schmid and Feucht, 1981). In general, carbohydrates play an important role in the construction of carbon skeletons and overall seedling metabolism by serving as a source of energy for plant cellular activity; they also influence osmotic effects (Rapaka et al., 2007). Soluble sugars are the primary form of carbohydrates in watermelon seedlings, with fructose and glucose dominating in petiole tissue, and sucrose, raffinose, and stachyose dominating in leaf tissue (Ranwala et al., 2002). Within the Cucurbit family, stachyose and sucrose are the primary carbohydrates that are translocated (Bruton et al., 1998). At the watermelon seedling stage, the cotyledons are the primary source of carbohydrates, supplying energy for metabolic processes that are encompassed in grafting (Memmott, 2010). At grafting, the growing shoot and roots become the primary carbohydrate sinks. During healing, grafted plants are placed in low light levels until the graft is healed, so the synthesis of new carbohydrates is limited and seedlings rely on stored carbohydrates for subsequent growth and development (Memmott, 2010).

Carbohydrate reserves in scion wood and rootstocks of perennial macadamia (*Macadamia* spp.) and litchi (*Litchi chinensis*) at the time of grafting have been found to be the likely cause of seasonal variation in the grafting

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success of these plants (Jones and Beaumont, 1937). Beaumont and Moltzau (1937) found that an increase in starch content of the scion wood was positively correlated with an increase in grafting success. Similarly, Rodrigues et al. (1960) found that a lack of adequate carbohydrate supply limited the grafting success of avocado (*Persea americana*) plants. Likewise, carbohydrate levels in rootstocks and scion plant tissue can impact callus formation and vegetative growth of newly grafted grape seedlings (Hunter et al., 2004). The role of carbohydrates in vegetable grafting has not been extensively reviewed. The development of the graft union may largely depend on the amount of carbohydrates present in the rootstock and the scion at the time of grafting. High levels of carbohydrates, as well as auxin and cytokinin, are required for successful callus formation, and there can be an interaction between rootstock and scion cultivars in carbohydrate utilization, which can affect the level of starch depletion that occurs during callus development. This phenomenon, in turn, can impact the time required for callus formation and grafting success. Bartolini et al. (1996) suggested that there is a positive relationship between carbohydrate level and callus formation in grafted grape where increased carbohydrate content of rootstock seedlings led to an increase in the survival rate of grafted transplants (Phillips et al., 2015).

Currently, commercial watermelon grafting uses relatively slow grafting methods that are labor-intensive, and for which transplants have relatively low and variable survival rates, and additionally, there is a need to manage rootstock regrowth. These factors affect the cost and adoption of grafted watermelon transplants. More research is needed to address these issues to advance the use of grafting as an affordable and effective propagation strategy for watermelon. Research with perennial crops suggests that increasing carbohydrate concentrations in grafted tissues may increase grafting success. The objective of this study was to test whether increasing carbohydrate levels through application of a sucrose solution to rootstock seedlings before grafting can increase the survival of splice-grafted watermelon transplants where both cotyledons are removed from the rootstock.

Materials and Methods

Experimental design. Two experiments were carried out at the Washington State University Mount Vernon Northwest Washington Research and Extension Center (NWREC): one to evaluate starch accumulation in watermelon rootstock stems and the other to evaluate grafting success, after four sucrose treatments, 0% (water control), 1% (1/99 g/mL), 2% (2/98 g/mL), and 3% (3/97 g/mL) of sucrose in water. Both experiments were seeded at the same time, and sucrose treatments were also applied to seedlings in both experiments at the same time so as to have the same environmental conditions for plants in both experiments. For the

starch-detection experiment, there were three replicates of four plants per treatment, and the experiment was repeated two times. The first repeat was carried out from 4 June to 17 June 2016, and the second repeat was carried out from 11 June to 24 June 2016. For the grafting success experiment, the design was a randomized complete block, with five replications and 12 plants per experimental unit, and the experiment was repeated two times. The first repeat was carried out from 27 May to 8 July 2016, and the second repeat was carried out from 2 June to 15 July 2016.

Plant material. For both experiments, rootstock cv. Tetsukabuto (*Cucurbita maxima* × *Cucurbita moschata*) was seeded in every other row of 72-cell trays containing a commercial potting medium (Sunshine #3 N&O; Sun Gro Horticulture, Agawam, MA) on 4 June (repeat 1) and 11 June (repeat 2). For the second experiment, watermelon cv. Tri-X Palomar was sown into 72-cell trays filled with the same potting medium on 27 May for repeat 1 and 2 June for repeat 2.

Sucrose application. A total of 50 mL sucrose solution (IB37160 Sucrose; IBI Scientific, Peosta, IA) or water was applied as a soil drench to rootstock seedlings in both experiments. The application was split into three applications, with 20 mL for the first and second application, and 10 mL for the third application. For each application, water or sucrose solution was applied between 8:00 AM and 10:00 AM to each cell every other day, with the first application 2 d after germination and the third application 2 d before grafting of seedlings in experiment 2. Seedlings were watered following common greenhouse practices on days when sucrose treatments were not applied.

Grafting methods and healing. Grafting for seedlings in experiment 2 was carried out on 17 June and 24 June for repeat 1 and 2, respectively. The splice grafting method was used for grafting, with rootstock and scion material both cut at about a 60° angle below the two cotyledons. A 60° angle cut provided a larger area for the connection of vascular bundles at the graft interface (Ogata et al., 2005). The two cut stem surfaces were then placed together, and a watermelon grafting clip (3 mm; Johnny's Selected Seeds, Fairfield, ME) held the grafted transplant together. Immediately after grafting, each tray was placed in a healing chamber (0.9 m wide × 1.7 m long × 0.5 m tall) on a bench in the greenhouse, and the healing regimen described by Miles et al. (2016) was followed and is summarized here. The chamber was covered with a clear plastic (0.15 mm polythene; Ginagar Plastic Products, Ginagar, Israel), a thin film of water was added to the floor of the chamber to provide 100% RH inside the chamber, and the target temperature inside the chamber was 25 °C. The chamber was completely covered with black fabric (Geotex 200st; Propex operating company, LLC, Chattanooga, TN) to prevent light penetration into the plants. For days 1 and 2 after grafting, the plastic of the healing chamber was kept closed, and the chamber was covered

with the black fabric. The chamber was opened on day 3, and water was added to the chamber floor so that it was barely wet, then the chamber was closed. On day 4, the chamber was opened for 15 min, water was added to the floor of the chamber as needed, the chamber was closed, and the black fabric was folded up to expose the front side of the chamber. On day 5, the chamber was opened for 30 min, water was added to floor of the chamber as needed, the chamber was closed, and the black fabric was folded up to expose the sides of the chamber but the top of the chamber was covered. On day 6, the chamber was opened for 1 h, water was added to the floor of the chamber as needed, the chamber was closed, and the black fabric

Table 1. Starch accumulation in rootstocks after the application of 0% (water), 1%, 2%, or 3% sucrose solution, from 4 to 17 June (repeat 1) and 11 to 24 June (repeat 2) in 2016 at Mount Vernon, WA.

Sucrose treatment	Starch concn ^a	
	Repeat 1	Repeat 2
1% Sucrose	3.0 c ^b	2.9 b
2% Sucrose	4.4 b	4.5 a
3% Sucrose	6.4 a	5.0 a
Water (control)	1.5 d	1.3 c
P value	<0.0001	<0.0001

^aThe presence of starch was rated using a modified version of the Cornell starch-iodine starch staining test, where 1 is 0%, 2 is 15%, 3 is 30%, 4 is 45%, 5 is 60%, 6 is 75%, 7 is 90%, and 8 is 100% starch [adapted from Blanpied and Silsby (1997)].

^bMean separation letters were generated using the least squares means statement in JMP (Version 11.0 for Windows; SAS Institute, Cary, NC) with $\alpha = 0.05$. Treatments followed by the same letter within a column are not significantly different.

Table 2. Survival (%) of grafted watermelon after drench application of sucrose treatments [1%, 2%, 3% sucrose, and water (control)] measured 7, 10, 14, and 21 d after grafting at Mount Vernon, WA; repeat 1 was from 27 May to 8 July and repeat 2 was from 2 June to 15 July 2016.

Sucrose treatment	Survival ^a (%)			
	7 d	10 d	14 d	21 d
Repeat 1				
1% Sucrose	100	100	85	78
2% Sucrose	100	100	95	90
3% Sucrose	100	97	93	87
Water (control)	100	97	77	68
P value		0.19	0.11	0.09
Repeat 2				
1% Sucrose	99	93	88 a ^b	77 a
2% Sucrose	98	93	90 a	88 a
3% Sucrose	98	90	81 a	76 a
Water (control)	99	77	62 b	48 b
P value	0.85	0.06	0.005	0.004

^aSurvival data for the first repeat were transformed for statistical analysis (arcsin of square root transformation was the most appropriate); means are presented in their original units.

^bMean separation letters were generated using the least squares means statement in JMP (Version 11.0 for Windows; SAS Institute, Cary, NC) with $\alpha = 0.05$. Treatments followed by the same letter within a column are not significantly different. Means without letters indicate that no statistical differences were found.

Fig. 1. Temperature (°C) in the healing chamber (top) for 10 d and in the greenhouse (bottom) for 21 d after grafting, from 17 June to 8 July (repeat 1) and 24 June to 15 July (repeat 2) in 2016 at Mount Vernon, WA. Data were recorded every 15 min (Onset HOB0, Bourne, MA).

Fig. 2. Relative humidity (%) in the healing chamber (top) for 10 d and in the greenhouse (bottom) for 21 d after grafting, from 17 June to 8 July (repeat 1) and 24 June to 15 July (repeat 2) in 2016 at Mount Vernon, WA. Data were recorded every 15 min (Onset HOB0, Bourne, MA).

was removed entirely. On days 7 and 8, the chamber was opened for 3 and 6 h, respectively, water was added to the floor of the chamber as needed, and the chamber was closed. Plants were removed from the chamber on day 9 and placed on the greenhouse bench and watered.

Plant starch detection and grafting survival. For each treatment in experiment 1, rootstock seedlings were destructively sampled 1 d before seedlings in experiment 2 were grafted, and starch accumulation was measured. A 2-cm segment of hypocotyl of each seedling was cut midway between the soil surface and cotyledons, and then each segment was cut in half longitudinally. The 2-cm longitudinal segments were stained with I₂/KI solution (5 g KI, 0.5 g I₂, 500 mL H₂O) for 2 min, followed by an 80% ethanol wash (20/80) (Alvarado et al., 2012). A dark blue precipitate was observed in the tissue where the starch was present. The presence of starch was rated using a modified version of the Cornell starch-iodine starch staining test, such that 1 is 0%, 2 is 15%, 3 is 30%, 4 is 45%, 5 is 60%, 6 is 75%, 7 is 90%, and 8 is 100% starch [adapted from Blanpied and Silsby (1997)]. In experiment 2, survival was assessed for grafted plants in each replicate treatment 7, 10, 14, and 21 d after grafting. Graft survival was defined as turgidity of scion leaves and stems; failed grafted plants had entirely wilted scion leaves and stems.

Environmental conditions. The temperature, RH, and light 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) were measured every 15 min (Onset HOB0, Bourne, MA). To better understand the impact of temperature and RH on graft survival, the vapor pressure deficit (VPD) was measured in the greenhouse for each repeat using the equation: $[1 - (RH/100)] \times \text{saturated vapor pressure (SVP)}$ (Campbell and Norman, 1998).

Data analysis. Data were subjected to analysis of variance using JMP software (version 11.0 for Windows; SAS Institute, Cary, NC). The sucrose treatments and water control were explanatory variables, whereas starch accumulation and grafting survival were response variables. The survival data for grafted transplants in the first repeat were transformed using an arcsine of square root transformation to meet assumptions of normality and equality of the variance. Microsoft Excel (Microsoft Office 2013 for Windows; Microsoft Corporation, Redmond, WA) was used to calculate daily averages of measured environmental factors.

Results and Discussion

Starch detection. Starch accumulation in the rootstock differed because of sucrose treatment in both repeats ($P < 0.0001$), and there was also a difference due to repeat ($P = 0.02$) and an interaction between treatment and repeat ($P = 0.02$). Overall, starch accumulation

in rootstock seedlings was the highest for plants that received 3% sucrose solution (71%), followed by plants that received 2% sucrose solution (52%), 1% sucrose solution (29%), and water (6%). Starch accumulation was similar in the two repeats for 2% sucrose (4.4–4.5 rating, $\approx 51\%$ to 53% starch), 1% sucrose (2.9–3.0 rating, $\approx 29\%$ to 30% starch), and water (1.3–1.5 rating, $\approx 5\%$ to 8% starch), but was greater for 3% sucrose in repeat 1 (6.4 rating, $\approx 81\%$ starch) than in repeat 2 (5.0 rating, $\approx 60\%$ starch) (Table 1). In repeat 1, starch accumulation was greater for plants treated with 3% sucrose and lesser for plants treated with water, and was intermediate for plants treated with 2% or 1% sucrose. In repeat 2, starch accumulation was the greatest for plants treated with 3% or 2% sucrose solution, intermediate for plants treated with 1% sucrose, and the lowest for plants treated with water. Thus, overall starch accumulation in rootstock seedlings after drench application of sucrose solution was 30% to 80%, whereas starch accumulation in seedlings treated with water was 5% to 8%. These results indicate that sucrose solution applied as a soil drench was taken up by the rootstock seedlings and increased seedling starch concentrations.

Grafted plant survival. Survival (%) of splice-grafted watermelon differed because of sucrose treatment, repeat, and days after grafting ($P < 0.0001$ for all). There was also a significant interaction between sucrose treatment and days after grafting ($P = 0.0005$), and between sucrose treatment and repeat ($P = 0.02$). Survival of splice-grafted watermelon seedlings 21 d after grafting was the greatest for plants that received 2% and 3% sucrose solution (89% and 82%, respectively), followed by plants that received 1% sucrose solution (78%), and was the lowest for plants that received water (58%) ($P < 0.0001$). Survival of grafted plants that received sucrose solution was 85% in repeat 1 and 80% in repeat 2, whereas survival of grafted plants that received water was 68% in repeat 1 and 48% in repeat 2. In repeat 1, due to variation among replicates, there was no significant difference in plant survival because of treatment at each measurement date, even though final plant survival was 22% greater with 2% sucrose solution as compared with water (Table 2). Plant survival declined over the course of the study; there was no loss in grafted plants at 7 d (100% survival on average), but plant survival declined to 98% on average at 10 d, 88% on average at 14 d, and 62% on average at 21 d after grafting. In repeat 2, there was no significant difference in plant survival because of sucrose treatments at 7 d (99% on average) or 10 d (88% on average) after grafting; but at 14 d after grafting, survival of plants treated with water (61%) was lower than that of plants treated with sucrose solution (87% on average). Survival declined even further at 21 d after grafting for plants treated with water (48%) compared with plants treated with sucrose (80% on average).

These results indicate that a drench application of a sucrose solution to rootstock

seedlings before grafting can increase grafting success when both cotyledons are removed from the rootstock before grafting. Sucrose solutions can provide a carbohydrate source for the support of plant growth (Dormer and Street, 1949; Ferguson et al., 1958; Thomas and Weir, 1967; White, 1934, 1940), and in the current study, increased starch levels in rootstock tissue appeared to enhance watermelon graft survival when both cotyledons were removed from the rootstock. Sucrose is commonly used in the medium for plant tissue culture to enhance plant growth (Fuentes et al., 2000; Ferguson, 1958; Li et al., 2015) and has been reported to serve as an osmotic regulator in addition to a carbohydrate source (Biahoua and Bonneau, 1999). Sucrose may improve callus formation and connectivity of vascular bundles at the graft interface (Hunter et al., 2004; Ogata et al., 2005). Phillips et al. (2015) suggested that in grafted grapes, an increased concentration of soluble sugars, which consisted primarily of sucrose in rootstock seedlings, could increase the survival of grafted plants. More studies are needed to evaluate different levels of sucrose concentration to optimize watermelon grafting success. Additionally, larger studies that include more plants per replicate, more replicates, and more repeats will likely provide more statistically significant results.

Environmental conditions. Daily average temperature and RH in the healing chamber and in the greenhouse were similar for both repeats (Figs. 1 and 2). Light intensity measured inside the chamber was also similar for both repeats, but differed after plants were placed on the bench in the greenhouse from days 9 to 21 (Fig. 3). Overall, the daily average temperature inside the chamber was 24–26 °C, whereas the daily average temperature in the greenhouse was 21–24 °C. In the first 3 d after grafting, when the plastic of the chamber was closed and the chamber was entirely covered with black fabric, the daily average temperature inside the chamber for repeats 1 and 2 was 25–26 °C, and the temperature in the greenhouse was 21–24 °C. The daily average RH during this time for both repeats was 97% inside the chamber and 63% to 74% in the greenhouse. The daily average light intensity in the chamber for both repeats was $1.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For days 4 to 6, when plants in the chamber were exposed to the greenhouse environment for 1 h or less each day and the chamber was partially covered with black fabric, the daily average temperature inside the chamber was 24–25 °C, and the temperature in the greenhouse was 22–23 °C. The daily average RH during this time was 96% to 98% inside the chamber and 62% to 74% in the greenhouse. The daily average light intensity inside the chamber was $6.6\text{--}7.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. For 7 to 9 d after grafting, when plants in the chamber were exposed for an average of 5 h each day to the greenhouse environment and the black fabric was removed, the temperature inside the chamber was 23–25 °C, and the temperature in the greenhouse was 23–21 °C. The

Fig. 3. Light intensity of photosynthetically active radiation ($PAR, \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the healing chamber for 9 d and in the greenhouse for 10 to 21 d after grafting, from 17 June to 8 July (repeat 1) and 24 June to 15 July (repeat 2) in 2016 at Mount Vernon, WA. Data were recorded every 15 min (Onset HOB0, Bourne, MA).

Fig. 4. Average daily temperature (top) and relative humidity (%) (bottom) each hour in the greenhouse for days 10–21 (when plants were on the greenhouse bench), from 27 June to 8 July (repeat 1) and 4 to 15 July (repeat 2) in 2016 at Mount Vernon, WA. Data were recorded every 15 min (Onset HOB0, Bourne, MA).

daily average RH during this time for both repeats was 92% inside the chamber and 65% to 72% in the greenhouse. The daily average light intensity in the chamber was similar for both repeats, and was $147 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in repeat 1 and $152 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in repeat 2. For 10 to 21 d, when the plants were on the greenhouse bench, the daily average temperature in the greenhouse for both repeats was 23 °C and the RH was 64% to 67%. The daily average light intensity in the greenhouse was $243 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in repeat 1 and $224 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in repeat 2. During this period, the average temperature and RH each hour were similar for both repeats from 10:00 PM to 7:00 AM but differed from 1:00 to 6:00 PM (Fig. 4). For repeat 1, the temperature reached a maximum of 32 °C and RH reached a minimum of 43% during the afternoon, whereas for repeat 2, maximum temperature was 27 °C and minimum RH was 60%. The VPD from 10:00 PM to 7:00 AM was 0.60 kPa for repeat 1 and 0.57 kPa for repeat 2, whereas from 1:00 to 6:00 PM the VPD was 2.49 kPa for repeat 1 and 1.42 kPa for repeat 2. In general, the ideal range for VPD is 0.40 to 1.34, but can vary based on crop species and the stage of growth (Argus Control Systems LTD, 2009). In the current study, the VPD and plant survival were the greatest for repeat 1; thus, VPD did not account for the lower plant survival in repeat 2.

Conclusions

The cotyledon is a carbohydrate source and plays a key role in grafting success for watermelon and other cucurbit crops (Memmott, 2010). When both cotyledons are removed, as in the splice grafting method, this can reduce watermelon grafting success, suggesting a correlation between carbohydrate status and seedling survival in grafted watermelon. In the current study, when sucrose solution was applied to rootstock seedlings as a soil drench before grafting, starch accumulated in the rootstock stem, and the survival of plants grafted with the splice technique was 83% on average, as compared with 58% survival for grafted plants that received water.

Grafting watermelon, and other cucurbit crops, using the splice method where both cotyledons are removed, could significantly decrease costs of grafting as this would eliminate the need for extra attention and time when cutting the watermelon rootstock for grafting. In addition, rootstock regrowth would essentially be eliminated as the rootstock meristem tissue would be removed when both cotyledons are removed. This could help increase the adoption of grafted watermelon plants as there would be no need to scout fields and remove rootstock regrowth. Additional studies are needed to investigate the optimum sucrose solution concentration, timing of application, and whether sucrose solution should be applied to both rootstock and scion seedlings to increase success in splice-grafting watermelon seedlings. Furthermore, research is needed to determine the optimal environmental conditions for the survival of splice grafted watermelon plants. It may also be useful to test different angles of cutting the seedling stems as well as the distance of the cut below the cotyledons. Moreover, splice-grafted seedlings should be evaluated in field studies to ensure they are vigorous with good yield and high fruit quality. In the current study, a standard healing regimen was followed based on the one-cotyledon grafting method. More studies are needed to determine the optimum temperature, RH, and light levels for each day of the healing regimen for watermelon grafted with the splice method.

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