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Review

Advances in Watermelon Grafting to Increase Efficiency and Automation

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Abstract: Grafting watermelon (*Citrullus lanatus*) onto resistant rootstocks is an effective technique in the management of biotic and abiotic stresses. Since the first reported grafting of watermelon for disease resistance in 1927, adoption of the practice has been steadily increasing up to 95% in Japan, Korea, Greece, Israel and Turkey. However, for grafting to be further adopted in the United States and other regions of the world with high labor costs and high plant volume demands, the watermelon grafting method must be more time and labor efficient as well as suitable for automation. To accomplish these goals, recent advances have been achieved in splice grafting of watermelon, where both cotyledons are removed from the rootstock. This review provides a summary of the new discoveries regarding watermelon grafting and an overview of the anatomy of cucurbit stems and the physiological processes that occur at the time of grafting and during the healing process in order to enhance the understanding of the complex nature of the cucurbit vascular system, which limits grafting success. This review article further provides insights to guide future research and technology development that will support the expansion of watermelon grafting.

Keywords: abscisic acid; auxin; carbohydrate; cotyledon; grafting method; graft union; efficiency; rootstock regrowth; splice grafting

1. Introduction

Grafting has become a common practice for watermelon (*Citrullus lanatus*) production in many parts of the world, due to its efficacy against biotic and abiotic stressors. The benefits of using grafted plants as a method to improve plant tolerance to soil-borne diseases have been increasingly recognized, in part due to the restrictions or prohibition of chemical fumigation tools throughout the world [1–3]. Grafted watermelon has been reported to resist infection by the pathogens *Verticillium*, *Fusarium*, *Phytophthora*, *Pseudomonas*, *Didymella bryoniae*, and *Monosporascus cannonballus*, as well as nematodes (*Meloidogyne incognita*) [3]. Grafted watermelon can also have increased abiotic tolerance, such as to salinity [4]. There is continued success in the identification of compatible disease-resistant rootstocks with abiotic stress tolerance in many research studies. Further, due to the increased rooting capacity of grafted watermelon plants, they tend to be more vigorous and have greater water and nutrient use efficiency than nongrafted plants. For example, Johnson [5] reported that the productivity of grafted watermelon can be 30–50% higher than nongrafted plants and found that grafted plants can produce optimal yields when planted at two-thirds of the population of nongrafted plants.

The availability of affordable grafted transplants is a prerequisite to attain advantages for their widespread use [6–9]. Medium- and large-scale growers purchase grafted watermelon transplants from professional propagators, and the overall quality is generally very good, but the price can be up

to five times greater than nongrafted plants due to labor inputs [10]. Commercial watermelon grafting currently relies on two methods, the one-cotyledon graft and the hole insertion graft. Both grafting methods are labor intensive due to the time and detail required for grafting, and additional labor is needed to graft up to 20% extra plants due to reduced plant survival during graft healing, and to manage for rootstock regrowth. To reduce costs and expand utilization of watermelon grafting on a large scale as a management tool against biotic or abiotic factors, more efficient practices of grafting are necessary. This review highlights the recent advances in watermelon grafting methods that achieve these goals, specifically advances in splice grafting of watermelon, where both cotyledons are removed from the rootstock. Further, an overview is provided of the complex anatomy of the cucurbit vascular system as well as the physiological processes that occur at the time of grafting and during the healing process, as they limit grafting success. Taken together, these advances and insights provide new recommendations to optimize watermelon grafting for efficiency and automation, as well as provide future research directions to optimize automated production of grafted watermelon transplants on a large scale.

2. Justification

Tateishi [11] was first to report grafting fruit-bearing vegetables as a strategy to manage disease and to increase yield, when watermelon was successfully grafted onto a squash (*Cucurbita moschata*) rootstock by a grower in Japan to overcome fusarium wilt (caused by *Fusarium oxysporum* f. sp. *Lycopersici*). Within the next decade, grafted watermelon seedlings were used as a biological disease management strategy in commercial production in Japan, with bottle gourd (*Lagenaria siceraria*) rootstock [12]. The use of watermelon grafting as a means of resisting impacts of soil-borne disease spread throughout Asia during the following decades, becoming well established in the region, as well as in Israel, by the 1960s [3,13]. In the 1990s, grower recognition of the advantages of grafted plants continued to increase worldwide, in part due to the Montreal Protocol in 1991 that removed or heavily restricted the use of the soil fumigant methyl bromide [14,15]. Today, over 95% of commercial watermelon seedlings are grafted in Japan, Korea, Greece, Israel and Turkey, where farming areas are relatively small and very intensively managed [3,16]. In contrast, North America used less than 100,000 grafted watermelon plants in field production in 2006 [12], and although this increased to approximately 5 million grafted watermelon plants in 2019 [17], this only accounted for approximately 2% of the watermelon grown in the United States [18]. Today, representatives of major seed companies anticipate that 50% of watermelon produced in the United States will be grafted in the near future [19]. To support the expansion of watermelon grafting in the United States and elsewhere where large-scale farms use hundreds of thousands of plants per farm, watermelon grafting methods need to be simplified to reduce costs for the propagator and the grower.

3. Traditional Watermelon Grafting Techniques

The two most common commercial watermelon grafting methods are the hole insertion and the one-cotyledon techniques. Grafting efficiency, especially the speed at which the grafting process occurs, is low with both grafting methods. Furthermore, both grafting methods often leave bud meristem tissue at the base of the rootstock cotyledon, resulting in rootstock regrowth occurring after grafting, which is a major problem inhibiting the use of grafted watermelon plants.

The hole insertion method is the most widely used method for watermelon grafting in China because it tends to have a high success rate with relatively minimal management during the healing period [2,20,21]. At the time of grafting, rootstock seedlings should have one small true leaf, and scion seedlings should have the cotyledons and perhaps the first true leaf just emerging. The diameter of the scion stem must be smaller than the diameter of the rootstock stem so that the scion can be inserted into a hole made between the two cotyledons of the rootstock (Figure 1).

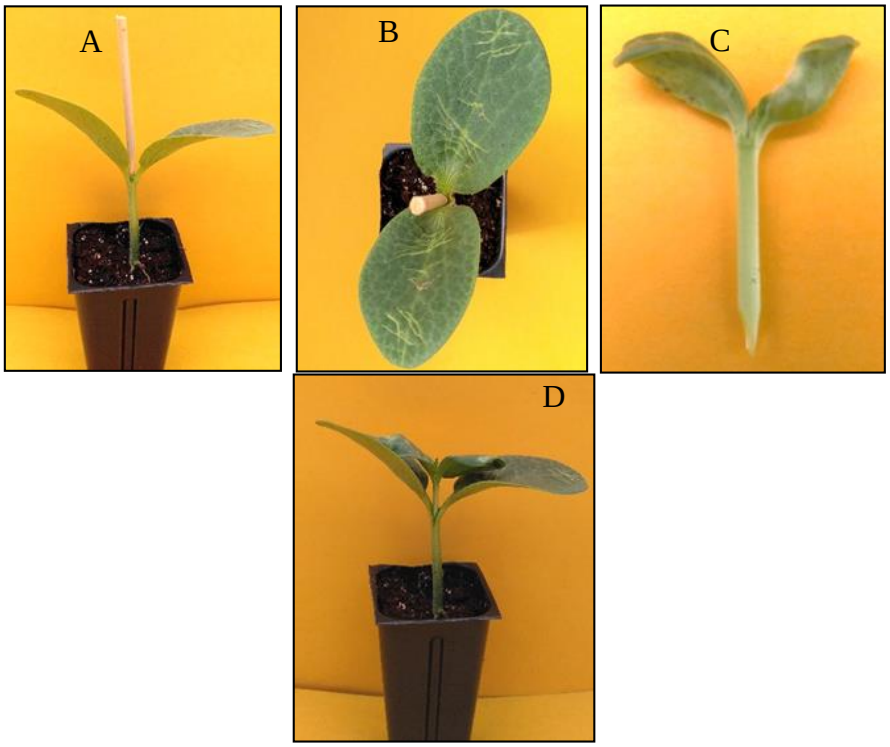

Figure 1. Hole insertion method: (**A**,**B**) create a hole in the rootstock by removing the apical meristem tissue; (**C**) cut the scion at a 45° angle on two sides to form a wedge; and (**D**) insert the scion into the rootstock [22].

The one-cotyledon grafting method was first developed by a Japanese watermelon grower [23,24]. The rootstock is cut at a 60° angle [25], removing one cotyledon while leaving the other cotyledon intact and firmly attached to the rootstock stem. The scion is then cut below the cotyledons at a 60° angle where its stem diameter matches that of the rootstock. The two cut stem surfaces are joined and held together with a grafting clip (Figure 2). Grafting is carried out soon after the rootstock cotyledons unfold to facilitate removal of bud meristem tissue in order to limit rootstock regrowth [26,27]. Due to its simplicity, speed and relatively low rate of rootstock regrowth compared with the hole insertion method, it is the most commonly used grafting method in Japan, Europe and North America [12,22,28]. For example, in Spain, more than 90% of commercial watermelon plants are grafted using the one-cotyledon method [26].

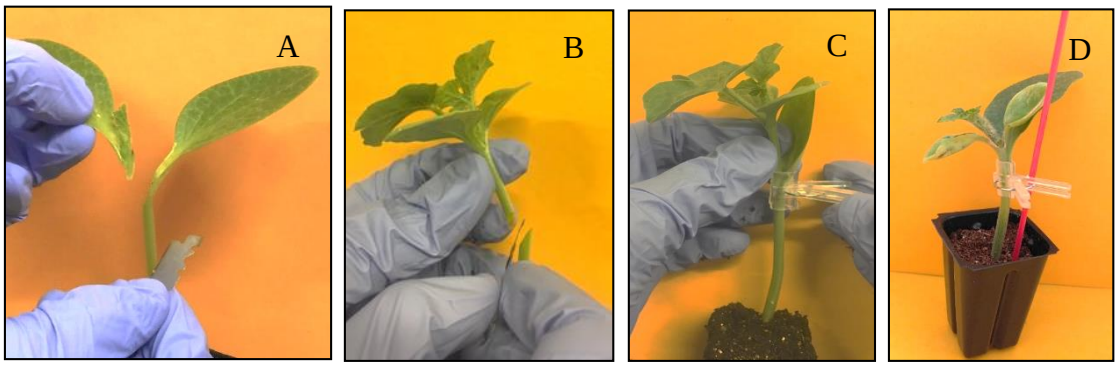

Figure 2. One cotyledon grafting: (**A**) cut the rootstock at a 60° angle with one cotyledon remaining on the seedling; (**B**) cut the scion at a 60° angle below the cotyledons, where its diameter matches that of the rootstock; (**C**) join the two cut stems together; and (**D**) secure with a grafting clip [28].

Rootstock Regrowth

Sometimes referred to as 'suckering', rootstock regrowth is a major concern for watermelon that are grafted with at least one intact rootstock cotyledon, as it is difficult to remove all the rootstock bud meristem tissue (Figure 3). Rootstock regrowth can result in graft failure, or a decrease in yield by competing with the scion for water and nutrients [20]. Many commercial rootstocks are vigorous and will quickly overtake the scion variety if allowed to grow, so scouting for and removal of rootstock regrowth is required in the greenhouse and the field [29]. A study conducted by Daley and Hassell [29] reported that fatty alcohol treatments can be used to eliminate the meristematic regrowth of watermelon grafted with the one-cotyledon method. When the apical meristem of two commonly used rootstock cvs. Emphasis (*Lagenaria sciceraria*) and Carnivor (*Cucurbita moschata* × *C. maxima*) were treated with 6.25% fatty alcohol emulsion (Fair 85®; Fair Products Inc., Cary, NC, USA), cotyledon and hypocotyl size significantly increased 21 days after treatment and total soluble sugars (glucose, sucrose and fructose) and starch content also were increased. This study suggested that the increase in stored energy could greatly increase the success rate of the grafting process. However, labor is required for the application, and damage to seedlings can occur [29]. In addition, the effectiveness of this chemical control on rootstock meristem regrowth has not been documented on a commercial scale.

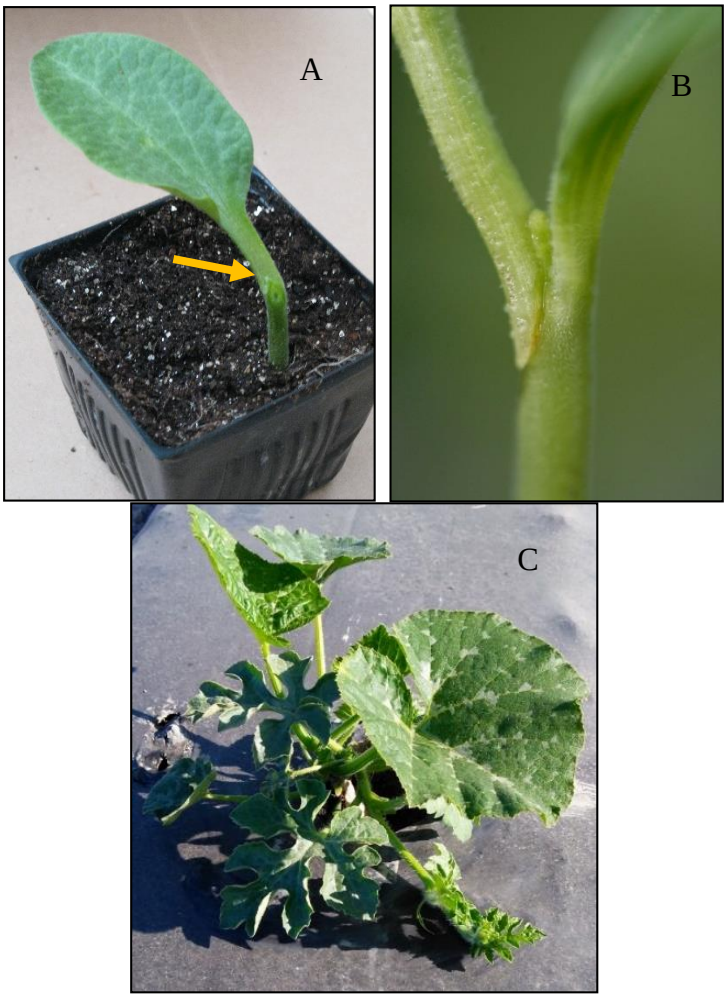

Figure 3. (**A**) The axillary bud is at the base of the cotyledon, and (**B**) if it is not completely removed, the rootstock will regrow, (**C**) resulting in rootstock regrowth (squash leaves) from grafted watermelon [30].

4. Splice Grafting Increases Grafting Efficiency

The splice grafting method (Figure 4), where both cotyledons are removed from the rootstock before grafting (referred to as splice grafting hereafter), is the fastest and most efficient grafting method, and is used to produce large numbers of solanaceous grafted plants [30,31]. This method would significantly increase grafting efficiency in watermelon production as this would eliminate the need for extra attention and time when cutting the watermelon rootstock for grafting and it would eliminate rootstock regrowth [32,33]. However, the success rate of splice-grafted watermelon has historically been low due to limited carbohydrate levels in the rootstock when both the cotyledons are removed [2,27]. Removing both cotyledons from the rootstock delays the time required for callus formation and reduces watermelon grafting success, suggesting a correlation between carbohydrate status in the rootstock stem and seedling survival in grafted watermelon [32]. Dabirian and Miles [32] found that drench applications of sucrose solution to rootstock seedlings before grafting can increase the carbohydrate level in the hypocotyl and increase grafting success when both cotyledons are removed from the rootstock before grafting. Recently, another greenhouse study conducted by Devi et al. [33] evaluated the survival of splice-grafted transplants when sucrose in combination with antitranspirant solution was applied to rootstock seedlings before grafting. This study reported the survival of splice-grafted seedlings 21 days after grafting was 91% for plants that received sucrose and antitranspirant, compared with 67% for plants receiving sucrose alone and 25% for plants that received only water. In addition, a follow-up field study found that fruit yield and quality attributes were similar for watermelon grafted with splice and one-cotyledon methods and for nongrafted watermelon plants.

Figure 4. Splice grafting method: (**A**) cutting watermelon rootstock and (**B**) cutting the scion below the cotyledons, and (**C**) joining of the two cut surfaces with a grafting clip [33].

5. Graft Union Formation of Watermelon

In grafted plants, rootstock/scion compatibility depends on anatomical, physiological and genetic variables, which in turn impact the survival of the grafted transplants. Studies report that in cucurbits, sieve elements are present in the vascular bundles on both sides of the xylem (internal and external fascicular phloem) and also in an extensive system of sieve tubes outside the bundles (extrafascicular phloem) [34,35]. The extrafascicular phloem appear scattered with a complex net-like structure [35]; thus, aligning vascular tissues at grafting can be difficult. A better understanding of cucurbit anatomy and graft union formation would aid overall management and success during grafting procedures, particularly for watermelon, where the probability of successful grafting is lower. Aloni et al. [36] studied a compatible (RS59) and an incompatible (RS62) *Cucurbita* hybrid genotype used as experimental rootstocks with melon (*Cucumis melo* L.) cv. Arava scion to identify physiological and biochemical factors in the scion–rootstock interface that could be associated with graft compatibility. The authors

suggested a physical barrier might be formed between incompatible plants soon after grafting, which may be responsible for degradation of the grafting zone, causing graft failure. Very little information is available regarding the complex anatomy of cucurbit vascular systems and the formation of the graft union. Studies are needed to elucidate the early processes that occur at the interface between grafted watermelon scion and rootstock stems.

5.1. Role of Cotyledons

Traditional commercial watermelon grafting methods depend on retaining at least one rootstock cotyledon to ensure graft success during the healing period [26,27,32,37–41]. Graft healing is dependent on hormonal signaling originating in the cotyledons to successfully heal the wounded region [29,41]. Auxins are one of the signaling hormones found in the cotyledons that promote graft formation by inducing the formation of callus [42–44]. The rootstock cotyledon also functions as the source of the carbohydrates that are used to connect the vascular bundles at the graft interface [27,32,39–41]. Because of the role of the cotyledon in supplying necessary carbohydrates for the developing seedling during the early stages of plant development, a deficit in stored reserves immediately before grafting and after is detrimental to grafting success [29].

5.2. Role of Carbohydrates

The role of carbohydrates in vegetable grafting has not been extensively studied, but at the time of grafting, the growing shoot and roots are the primary carbohydrate sinks. In the case of watermelon, the carbohydrate level in the seedling is low when both the cotyledons are removed as compared to tomato (*Solanum lycopersicum*) and other solanaceous crops [2,27,45]. For healing, newly grafted plants are placed in low light conditions until the graft is healed. The synthesis of new carbohydrates is limited under these conditions, and the grafted seedling is reliant on stored carbohydrates for survival [27]. Carbohydrates from the rootstock are necessary for the callus formation and cell differentiation that creates the connection between vascular bundles at the graft interface, which is essential for successful graft healing [40]. Within the cucurbit family, stachyose and sucrose are the primary carbohydrates and are translocated through the phloem [46].

5.3. Role of Abscisic Acid

Grafted watermelon is extremely susceptible to desiccation following the grafting procedure. Desiccation of the grafted seedling occurs because of water stress imposed on the post-grafted scion during healing [47]. The complete establishment of vascular connection takes approximately 5 to 8 days, during which the scion is unable to uptake water through the rootstock [41,48,49]. Therefore, rootstock-controlled scion transpiration is crucial for grafting survival [33,50,51]. The plant hormone abscisic acid (ABA) is involved in the plant responses to water deficit, especially the control of stomata opening [52,53]. Under severe water stress conditions, antitranspirant products can increase stomatal resistance and mitigate water stress, thereby enhancing plant growth and development [54]. The application of antitranspirants to reduce transpiration has assumed that an increase in resistance at the leaf surface will decrease transpiration more than it will decrease CO_2 uptake [55]. A stomata-coating antitranspirant forms a physical barrier with its thin film coating the surface of the leaf, which inhibits the loss of water vapor from the leaf [56–58]. Stomata closing antitranspirant, made with synthetic ABA or chemicals that elicit an ABA response, can be applied to plants as a foliar spray or soil drench [39,59,60]. These antitranspirants condition the plant to produce additional amounts of ABA, which affect the guard cells around the stomata [53]. Dabirian and Miles [47] reported that by using commercial antitranspirant products that are either film-forming or stomata closing, the survival of the grafted watermelon transplants can be increased. The authors found that when antitranspirant solution was applied to both the scion and rootstock seedlings that were grafted using the one-cotyledon method, plants treated with the stomata-closing antitranspirant had the greatest survival (87% to 97%),

followed by stomata-coating + stomata-closing antitranspirants (84% to 94%), the stomata-coating antitranspirant (50% to 67%), and water (53% to 68%).

5.4. Role of Auxin

Several studies have reported that plant growth regulators such as auxins and cytokinins induce the initiation and proliferation of callus and new vascular tissue by promoting cell division and cell development [61–65]. Cotyledons are an important source of auxin, and cotyledon-derived auxin promotes graft formation in young plants [42–44]. Auxin is transported from leaves to the roots [66], thus, severing the vascular tissues in the stem via grafting impedes its movement and accumulation in the root system. The accumulation of auxin is considered one of the earliest events during vascular differentiation [67,68]. Auxin acts as a common stimulus for the differentiation of both phloem and xylem [69]. Auxin is also involved in the wound response; for example, studies showed that auxin triggers vascular tissue regeneration in *Arabidopsis* stems and pea (*Pisum sativum*) epicotyl [70,71]. In plants, auxin is transported basipetally from leaves to the roots [66] and carbohydrates are transported from photosynthetic sources such as leaves to the roots [72]. Thus, severing the vascular tissues in the stem can deplete the accumulation of auxin and sugars. Sachs [73] reported that xylem formation across the graft was blocked when the majority of the shoot was removed in grafted pea, but exogenous application of auxin in place of the missing shoot allowed xylem to form. Katsumi et al. [74] reported that root formation of cucumber (*Cucumis sativus*) hypocotyl cuttings were inhibited when cotyledons were completely removed, and root growth was inhibited. El-Eslamboly [75] reported that when cuttings of seedless watermelon cv. Yellow Buttercup QV766 (yellow flesh) and SSX7402 (red flesh) were treated with auxin (indole-butyric acid) for vegetative propagation, survival increased along with root number and length. However, it is not known whether auxin applied to grafted watermelon plants can aid in the formation of the graft union or can increase the survival of grafted transplants.

6. Limitations in Watermelon Grafting

6.1. Survival

In contrast to solanaceous crops, it is rare to get 100% graft survival for watermelon, so it is recommended to graft up to 20% more plants than needed, depending on the grafting method and environmental factors [22]. For the graft union to develop a callus bridge (the layer of tissues acting as the interface between the scion and the rootstock) that enables water uptake in grafted plants, optimum healing temperatures are needed. Cucurbits are more sensitive to healing conditions than grafted solanaceous plants, especially tomato. Grafted watermelon survival rates are highest when temperature is maintained between 22 and 28 °C, relative humidity (RH) is above 90%, and light exposure is essentially 0% for several days after grafting. Survival of grafted watermelon can decline significantly if any of these parameters are not maintained. In contrast, tomato graft survival can be greater than 90% when the temperature range is 21–26 °C, RH is 85–100% and light exposure is 0 to 50%, and survival does not greatly decline if any of these parameters vary slightly [26,76,77]. Further, the process of callus bridge formation is reported to take 3 days for tomato and 4–5 days for watermelon under optimum temperatures [78]. Upon cutting both the rootstock and scion, water, phytohormones and carbohydrate transport cease at the graft junction and a wound healing pathway is activated. Grafting success is dependent on the development of vascular tissue (xylem and phloem) and reconnection between rootstock and scion [41,79,80]. Cucurbits have a complex anatomy that inhibits the union between rootstock and scion during healing. Wide sieve tube structure combined with large sieve pores and slow callose (polysaccharide production that is a typical plant response to stress) deposition to seal severed sieve pores makes healing difficult [35,81]. Mullendore et al. [81] reported that sieve tube specific conductivity is 4 to 14 times higher in cucurbits than solanaceous plants, resulting in a low flow velocity of water and nutrient translocation through sieve tubes. This low flow rate may account for the blockage that forms at the cut stem surface during graft healing. Further,

the extrafascicular phloem exudes profusely from cut stems and petioles, indicating that it does not have the normal sealing systems found in other types of phloem tissue [35,82–84]. Furthermore, cell division is inhibited during tissue reunion due to limited carbohydrate levels in the rootstock hypocotyl when both the cotyledons are removed [2,26,85]. Additionally, following successful graft healing, grafted tomato and eggplant seedlings can be stored at low temperatures under dim light for up to 4 to 6 weeks, but storage protocols are not well developed for cucurbits [12,13].

6.2. Cost

The high cost of grafted seedlings is the result of intensive labor inputs for propagation using traditional grafting methods, a longer production period, and the additional costs of the rootstock seed. The cost of grafted watermelon transplants can be up to five times greater than nongrafted plants [10], with labor representing 48% to 60% of the total cost in a manual grafting operation [7,8,86]. Thus, labor-efficient grafting methods have been recognized as a key to success in production of grafted watermelon seedlings on a large scale. The introduction of automation and mechanization technology will help address large-scale production needs.

6.3. Grafting Automation

The first grafting machine prototype to automate cucurbit grafting was developed in the 1980s by Bio-oriented Technology Research Advancement Institution, a national institute of agriculture machinery under the Ministry of Agriculture in Kawasaki, Japan [3,28]. The prototype was developed in 1987 and then adapted in 1989 [12,87]. The machine can make a graft in 4.5 s with 95% success rate. In Japan, the first commercial model of a grafting robot (GR800 series; Iseki & Co. Ltd., Matsuyama, Japan) became available for cucurbits in 1993 [12]. Various semi- and fully automated grafting robots were presented from nine different agricultural machine industries at an international horticultural trade show in Tokyo in 1996 [12,88]. The technologies to create this machine were shared with agriculture machinery companies and a prototype semi-automatic grafting system was developed in Korea in 2004 [12,89]. A grafting machine that utilizes the one-cotyledon method can produce 600 grafts per hour, while a manual grafter can complete approximately 1000 grafts per day [23,26,38,90,91].

The cost of a fully automated grafting operation is approximately US$7.68 million for production capacity of approximately 1.92 million grafted plants weekly or 100 million grafted plants annually [8]. Although automated grafting machines in theory work at the highest capacity specified by the manufacturer, they may not always be operated at that capacity, which results in fluctuating operating costs and returns [8]. The high initial cost of grafting equipment, the strict requirement for uniformity of watermelon and rootstock seedlings, and the complexity of the cutting angle using the one-cotyledon grafting method are obstacles for adoption of automated watermelon grafting, and currently grafting robots are not used outside Japan and Korea [86]. Splice grafting watermelon will streamline and simplify automated grafting, enabling propagators to further explore automation as a cost-effective grafting strategy. For automation to be successful, the scion and rootstock hypocotyls must be of similar size and their vascular systems aligned by grafting. The angle of the grafting cut should be approximately 60° and the rootstock hypocotyl must have adequate level of carbohydrates.

7. Conclusions

Although the utilization of watermelon grafting against biotic and abiotic stresses has been steadily increasing over time such that 95% of commercial watermelon production is grafted in some regions of the world, the higher cost of grafted watermelon transplants is one of the primary factors limiting adoption in regions with medium and large-scale production, such as the United States. If the United States is to achieve the target of 50% grafted watermelon production, grafted seedlings must be available at a reasonable cost both for the propagator and the grower. Recent advances in grafting watermelon using the splice grafting method where both cotyledons are removed significantly decreases the time needed for grafting. In addition, rootstock regrowth is eliminated, thereby reducing

maintenance of grafted watermelon plants. The splice grafting method has significant potential for watermelon grafting with both manual and automated practices. By eliminating the need for rootstock cotyledons, the automated grafting machines would require less time for adjustment than is currently needed to remove the meristematic tissue at the base of the rootstock cotyledon. This will significantly reduce the cost of producing grafted transplants using automation. However, more research is needed to further advance the use of splice grafting as an affordable and effective propagation strategy for watermelon. Primary questions to address through further research include how to optimize the carbohydrate level in the rootstock hypocotyl, the optimum angle of the grafting cut, how to reduce transpiration in the scion, and the role of different plant growth regulators in vascular reconnection. Effective healing and acclimation protocols are also needed to increase the survival of splice-grafted watermelon transplants. In addition, more research is needed to understand the formation of the graft union of watermelon and early processes that occur at the interface between grafted watermelon scion and rootstock stems. Further, it is unknown whether grafting may lead to changes in genetic expression in watermelon that results in mediating the physiological processes of grafted seedlings. Research is needed to better understand the potential roles of gene regulation in diverse biological and metabolic processes in grafted watermelon. Finally, while fruit yield and quality of splice-grafted watermelon in the field were found to be equivalent to one-cotyledon grafted plants [33], more studies are needed to ensure fruit productivity and marketability for numerous scion–rootstock combinations under various environmental conditions.

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