Research Reports

Effects of Grafting Methods and Root Excision on Growth Characteristics of Grafted Muskmelon Plants

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ADDITIONAL INDEX WORDS. *Cucumis melo*, *Cucurbita maxima* × *Cucurbita moschata*, cotyledon, rootstock, scion

SUMMARY. Grafting has been used for controlling certain soilborne diseases and improving abiotic stress tolerance in muskmelon (*Cucumis melo*) production. Grafting methods may vary considerably among geographic regions and nurseries, while excision of rootstock roots before graft healing may also be practiced, which allows root regeneration of the grafted plants. In this greenhouse study, four grafting methods including hole insertion, one-cotyledon, noncotyledon, and tongue approach methods were examined for their impacts on plant growth and root characteristics of 'Athena' muskmelon grafted onto 'Strong Tosa' interspecific hybrid squash rootstock (*Cucurbita maxima* × *C. moschata*). Nongrafted rootstock and scion plants were included as controls. Both the grafted and nongrafted plants were examined with or without root excision. The practice of root excision was unsuccessful with the tongue approach method, while it did not exhibit significant effects on graft quality and growth of plants grafted with the one-cotyledon and hole insertion methods. Grafted plants with root excision started to show active and 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. Plants grafted with the noncotyledon method showed a different root growth pattern with decreased root length and surface area at 16 DAG. As a result, this method reduced the quality of grafted plants. No significant differences in plant growth characteristics were observed among the hole insertion, one-cotyledon, and tongue approach grafted plants.

Grafting, as an effective approach to controlling soilborne diseases and overcoming abiotic stresses, has been practiced in the production of major cucurbitaceous vegetables for decades in many Asian and European countries (Lee and Oda, 2003; Lee et al., 2010). High costs associated with grafted seedlings have limited wide adoption of this technology in the United States (Davis et al., 2008; Memmott and Hassell, 2010). Optimizing grafting procedures and producing high-quality grafted seedlings are of importance in reducing production costs of grafted vegetables.

Methods for cucurbit grafting differ considerably among geographic regions and nurseries, and vary with rootstock and scion combinations (Lee 1994; Lee and Oda, 2003; Lee et al., 2010). Currently, the most commonly used methods are tongue approach, one-cotyledon, and hole insertion methods.

Tongue approach grafting was initiated in the Netherlands and later adopted by propagators in Korea, Japan, and other European countries (Davis et al., 2008). Using this method, rootstock and scion plants are cut halfway through at hypocotyl, respectively; and then they are joined together by aligning the cut surfaces. The rootstock top and the scion root are removed several days after grafting, with the cut near the graft union. Although this method requires additional labor after grafting that could increase the grafting cost, it normally results in a high survival rate and generally has less restrictive requirement for specific postgrafting healing conditions in contrast to other grafting methods (Davis et al., 2008).

One-cotyledon grafting, also called splice grafting, can be easily performed by hand. The graft union is located at the bottom of the remaining rootstock cotyledon, whereas the other cotyledon is removed. One-cotyledon grafting is also the only method that has been automated with grafting machines (Hassell et al., 2008). A grafting machine can produce 600 grafts per hour using the one-cotyledon method as compared with ≈1000 grafts per person per day (Hassell et al., 2008).

Hole insertion grafting is the most popular method used in China

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	$inch^2$	cm^2	0.1550
28.3495	oz	g	0.0353
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

(Davis et al., 2008). With this method, the grafted plants have a strong graft union that is protected by both rootstock cotyledons. In addition, this method does not require grafting clips, which reduces grafting cost. Meristematic regrowth of rootstocks, also known as rootstock suckers, is a common problem of hole insertion and one-cotyledon grafting methods. Additional efforts are required to remove suckers before and/or after field planting.

For large-scale production of grafted plants in the United States, the noncotyledon method with rootstocks cut at the hypocotyl and fatty alcohol treatment of rootstock meristems were recently proposed to eliminate the rootstock sucker problem in grafted watermelon (*Citrullus lanatus*) production (Daley and Hassell, 2014; Memmott, 2010; Memmott and Hassell, 2010).

One major difference between the various grafting methods is the number of rootstock cotyledons that remain after grafting. The noncotyledon method excises both cotyledons from the rootstock, whereas the hole insertion and one-cotyledon methods maintain two and one of the rootstock cotyledons, respectively. The tongue approach method also removes both rootstock cotyledons, but the cotyledons are excised 5 to 10 DAG, when the graft union has healed. Cucurbits have two leaf-like cotyledons. Their photosynthetic activity supports early hypocotyl and root growth (Lovell and Moore, 1971). Before the true leaves are equivalent in size to the cotyledons, cotyledons were essential to maintaining the aerial and root growth of cucumber (*Cucumis sativus*) seedlings (Bisognin et al., 2005). Damage on cucumber cotyledons could also affect sex expression and maturity (Omran, 1981).

In addition to different grafting methods of conjoining rootstock and scion, removing the root from the rootstock before grafting and rerooting the grafted plant during healing has been practiced in producing grafted vegetable seedlings (Lee, 1994). As root excision facilitates the grafting process by preventing growing media from contaminating the grafting machines, excising roots and allowing them to regenerate during the graft healing process is the method currently used by some mechanical grafting operations (Memmott, 2010). As both graft healing and root growth are energy-requiring processes, excising the roots could allow energy reserved in the rootstock hypocotyl to be used for graft healing, which might improve graft success (Lee, 1994; Memmott and Hassell, 2010; Penny et al., 1976).

Although the interest in vegetable grafting has been growing among producers and propagators in the United States, research-based information on grafted melon transplant production is limited. In this study, greenhouse experiments were conducted to examine the effects of different grafting methods and the practice of root excision on seedling quality and growth characteristics of muskmelon plants grafted onto an interspecific hybrid squash rootstock.

Materials and methods

Seedling production. Two experiments were conducted in a greenhouse (air temperature at 20 to 30 °C) on the University of Florida campus in Gainesville. In each experiment, 'Athena' muskmelon (Syngenta Seeds, Gilroy, CA) was grafted onto the 'Strong Tosa' interspecific hybrid squash rootstock (Syngenta Seeds). The scion and rootstock seeds were planted on 23 Nov. 2013 and 11 Jan. 2014 in the first and second experiments, respectively. Seeds were sown into 128-cell Styrofoam flats (Speedling, Sun City, FL) filled with potting mix containing peatmoss, perlite, and vermiculite (Sunshine MVP; Sun Gro Horticulture, Agawam, MA). Seedlings were grown in the greenhouse until both rootstock and scion started to show first true leaves.

Plant grafting and postgraft healing. Grafting was conducted 12 d after sowing the seeds in both experiments. Four grafting methods were performed, including hole insertion, one-cotyledon, noncotyledon, and tongue approach methods. Procedures of hole insertion, one-cotyledon, and tongue approach grafting were followed as previously presented (Guan and Zhao, 2014). Briefly, for the hole insertion grafting, the true leaves and meristem tissue of the rootstock plant were removed. A shaved toothpick was inserted between the rootstock cotyledons, from the bottom of one of the rootstock cotyledons to the other side of the rootstock hypocotyl. The scion hypocotyl was shaved into a V shape, which was then inserted into the slit of rootstock made by the toothpick. When the graft was made using the one-cotyledon method, one of the rootstock cotyledons was cut together with the true leaves and meristem tissue at a 45° angle. The scion hypocotyl was cut at the same angle as the rootstock, and attached to the rootstock using a grafting clip. A similar process was used in the noncotyledon method, except that the rootstock was cut at the hypocotyl, thus resulting in the removal of both rootstock cotyledons. When the tongue approach grafting was conducted, the rootstock and scion were cut halfway through at the same height of hypocotyls with a downward and upward 45° angle, respectively. The cut surfaces of rootstock and scion were then joined together and fixed with a grafting clip. At 9 DAG when the graft union was healed, the scion root was removed by cutting at the scion hypocotyl below the graft union. Meanwhile, the rootstock top including the true leaves and both cotyledons was removed with the cut made adjacent to the top of the graft union.

Nongrafted rootstock and scion plants were included as controls. Both grafted and nongrafted plants were examined with or without root excision. For the root excision treatment, newly grafted plants were cut just above the root zone and replanted in premoistened soil in the 128-cell flat. A randomized complete block design with three replications and 12 plants per treatment per replication was used in both grafting experiments.

Grafted plants were placed in a healing chamber (Guan et al., 2014) immediately following grafting and root excision, except for those grafted with the tongue approach method. The chamber (12 ft × 46 inches × 45 inches) was built on the greenhouse bench with a PVC pipe frame, covered by a clear plastic film, followed by a white on black plastic and a shadecloth. An air conditioner connected to the chamber and two humidifiers placed inside the chamber were used to control temperature and humidity during graft healing. During the first 2 DAG, a dark environment was provided by closing the chamber completely. Temperature was maintained at 27 ± 3 °C and relative humidity was 95% to 100% inside the chamber. On day 3, plants were exposed to light by partially opening the white on black plastic and the shadecloth, while the humidity and temperature were maintained at similar levels as within the first 2 d. Humidity was gradually reduced from day 4 forward by adjusting the humidifier and partially opening the clear plastic. After 7 d, plants were moved out from the healing chamber and grown under normal greenhouse conditions. Plants grafted with the tongue approach method were placed in a shaded area on the greenhouse bench.

GROWTH AND QUALITY OF GRAFTED TRANSPLANTS. All the plants were individually transplanted into 0.1-gal square plastic pots filled with Sunshine® MVP potting mix at 10 DAG. The numbers of healed plants in each grafting treatment were recorded in the first experiment at 16 DAG; while in the second experiment, a 0–10 scale (0 = dead, 1 = almost dead, 2 = moderating between surviving or not, 3 = borderline but will probably die, 4 = severely stunted, 5 = moderately stunted, 6 = somewhat stunted, 7 = fair but not acceptable, 8 = borderline acceptable, 9 = good and acceptable but not the best acceptable, 10 = optimal results) was used to evaluate the healing quality of the grafted plants (Memmott, 2010).

From hole insertion, one-cotyledon, and noncotyledon grafted plants, as well as the nongrafted rootstock and scion controls, three plants with one from each replication, were randomly selected and destructively measured at 0, 4, 8, 12, and 16 DAG. Roots were carefully washed from the potting mix, and total root length and root surface area were evaluated using a root scanning apparatus [color image scanner (LA1600; Epson, Toronto, ON, Canada)] and an image analysis software (WinRhizo 2008a; Regent Instruments, Quebec, QC, Canada). At 16 DAG, aboveground fresh weight and dry weight were also measured. As the healing process of the tongue approach grafted plants was considerably different from the other treatments, root and aboveground growth were not measured on the tongue approach grafted seedlings.

GROWTH CHARACTERISTICS OF GRAFTED PLANTS. Plants from three replications of each treatment were combined after 16 DAG. Six plants of each treatment that showed active growth in the first experiment and received rating scores above eight in the second experiment were individually transplanted at 17 DAG into 1-gal plastic pots filled with potting mix (Sunshine® MVP). Because of the inferior quality of seedlings grafted with the noncotyledon method (with and without root excision) and the tongue approach method (with root excision), plants of the three treatments were not transplanted.

Plants were arranged in a completely randomized design with six plants that served as six replications for each treatment. Plants were grown in the greenhouse, hand watered, and fertilized through watering by using 20N–8.7P–16.6K fertilizer (Peters Professional; United Industries, St. Louis, MO) at the rate of 2.8 g nitrogen per plant per week. The anthesis dates of male and female melon flowers were recorded on each plant. Plants were destructively harvested at 44 and 46 DAG in the first and second experiments, respectively, when at least two female flowers bloomed on each of the melon plants. At harvest, aboveground fresh weight, dry weight, and stem diameter (2 cm above potting mix surface) were recorded. Leaf area was measured with an area meter (LI-300C; LI-COR Environmental, Lincoln, NE). A leaf porometer (SC-1; Decagon Devices, Pullman, WA) and a chlorophyll content meter (CCM-200; Opti-Sciences, Hudson, NH) were used to measure stomatal conductance (g_S) and relative chlorophyll content, respectively, on two fully expanded leaves of the longest vine of each plant. The chlorophyll content was expressed in chlorophyll content index, which is calculated as the ratio of transmittance measurements at 940 and 660 nm. Root length and surface area of each plant were evaluated as previously described.

STATISTICAL ANALYSES. Data analysis was performed using the PROC GLIMMIX procedure of SAS (version 9.2 C for Windows; SAS Institute, Cary, NC). Fisher's least significant difference test (α = 0.05) was conducted for multiple comparisons of different measurements among treatments.

Results and discussion

ROOT GROWTH. Using the hole insertion and the one-cotyledon methods, grafted plants with intact roots (without root excision) exhibited an increase in root length and surface area during the first 4 DAG. In contrast, active root growth in this period was not observed on root-excised plants regardless of grafting methods used (Figs. 1 and 2). As vascular bundles between scion and rootstock started to connect soon after grafting (Aloni et al., 2008), energy reserved in the rootstock hypocotyl might be employed to initiate graft healing instead of root regeneration and growth if the roots were excised (Memmott and Hassell, 2010).

In general, root regeneration was initiated on the root-excised plants at 4 DAG (Figs. 1A, C and 2A, C). The new roots showed more rapid growth starting at 8 DAG, which then developed similar root length and surface area as the root-intact plants at 16 DAG. No significant differences in root length and surface area were observed among the rootstock control, scion control, and hole insertion and one-cotyledon grafted plants at 16 DAG in the first experiment (Fig. 1). In the second experiment, the one-cotyledon grafted plants exhibited less root surface area than that of the rootstock control and hole insertion grafted plants, regardless of root excision. The one-cotyledon grafted plants with intact roots also showed shorter root length compared with the rootstock control, scion control, and hole insertion grafted plants (Fig. 2). As one of the cotyledons was removed from the rootstock hypocotyls in the one-cotyledon grafted plants, root formation and development might be affected (Katsumi et al., 1969), although the remaining cotyledon could partially complement the roles of the removed cotyledon (Mayoral et al., 1985). Some significant differences were also observed at 8 and 12 DAG among the rootstock control, scion control, and hole insertion and one-cotyledon grafted plants; however, inconsistent results were found in the two experiments.

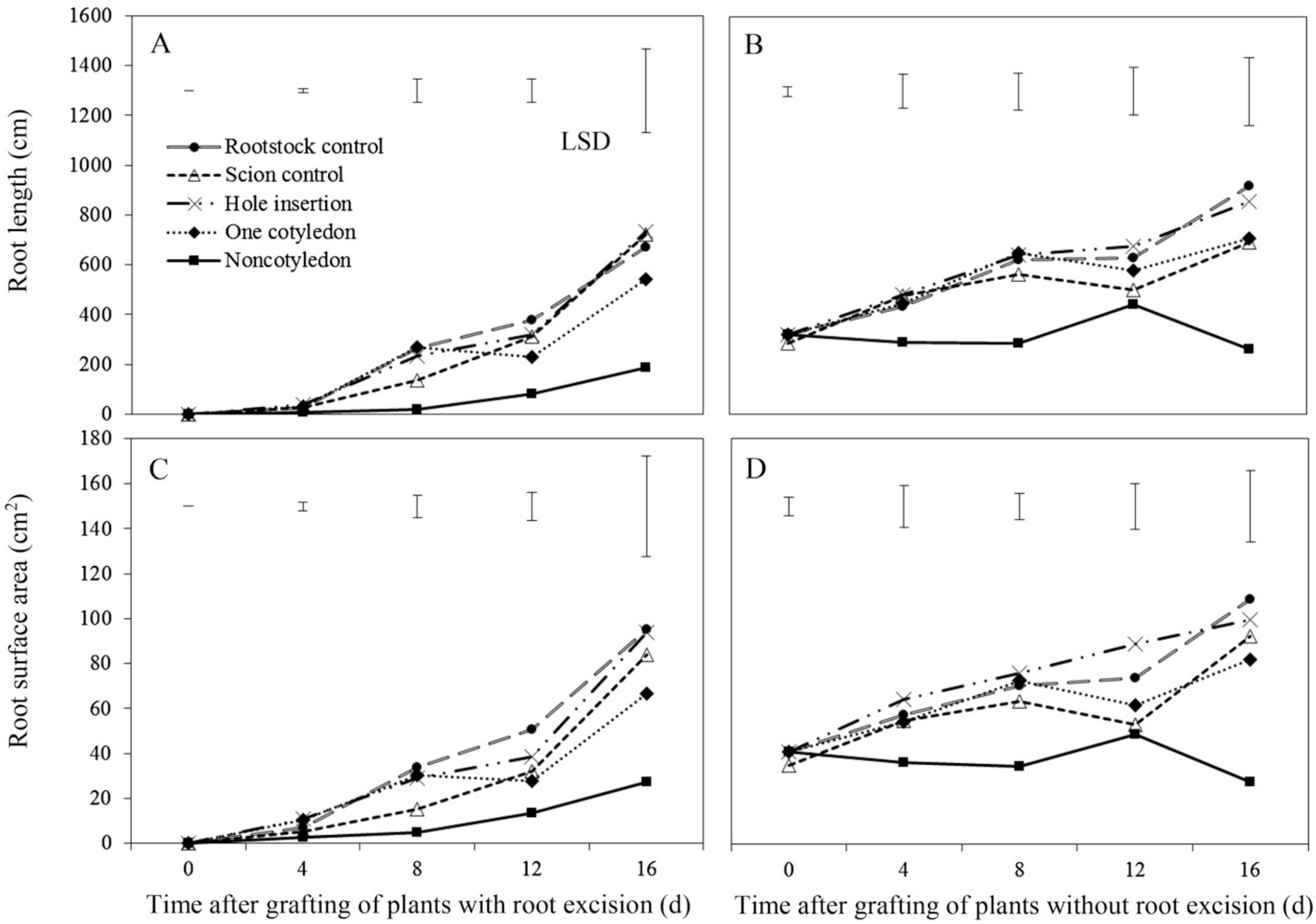

Fig. 1. Root growth of 'Athena' muskmelon grafted onto 'Strong Tosa' interspecific hybrid squash rootstock with hole insertion, one-cotyledon, and noncotyledon methods, and nongrafted 'Athena' and 'Strong Tosa' controls during the 16 d after grafting in the first experiment. (A) Root length of plants with root excision. (B) Root length of plants without root excision. (C) Root surface area of plants with root excision. (D) Root surface area of plants without root excision. Least significant difference (LSD) bars represent the LSD at $\alpha = 0.05$; 1 cm = 0.3937 inch, 1 cm^2 = 0.1550 $inch^2$.

The noncotyledon grafted plants exhibited a root growth pattern different from that of other plants. The new root growth was not initiated in the root-excised plants until after 8 DAG instead of 4 DAG (Figs. 1A, C and 2A, C). In addition, the root length and surface area of the root-intact noncotyledon grafted plants did not exhibit a significant increase during 16 DAG in the first experiment (Fig. 1B and D), whereas it showed some growth during the second experiment (Fig. 2B and D). As a result, the root length and surface area of the noncotyledon grafted plants were significantly lower than other plants at 16 DAG. Katsumi et al. (1969) reported that root formation of cucumber hypocotyl cuttings was inhibited if cotyledons were completely removed. The lack of auxin, which is supplied by cotyledons, was suggested to be the leading factor responsible for root growth inhibition (Elkinawy, 1980).

Interspecific hybrid squash rootstocks are known for their growth vigor (Davis et al., 2008; Lee and Oda, 2003). However, the rootstock control and plants grafted onto the hybrid squash rootstock in this study did not exhibit significantly greater root length and surface area compared with the scion control in the early stage of seedling development.

Survival rate and quality of grafted transplants. Percentages of graft survival in the grafting treatments ranged from 83.3% to 100% except for the low survival rate (45.8%) in grafted plants using the tongue approach method with root excision (Table 1). As grafted plants in this treatment were placed in a shaded area under normal greenhouse conditions, the lack of high humidity environment might not be suitable for root regeneration during the healing of grafted plants. Moreover, the presence of the scion roots together with the excision of the rootstock roots might have caused an adverse impact on graft healing. The tongue approach method works best when scion and rootstock plants have similar stem diameters. Therefore, rootstock and scion seeds are normally planted on different days to meet the requirements (Davis et al., 2008). In this experiment, to accommodate different grafting methods, the rootstock and scion seeds were sown on the same day, which might have affected the survival rates of plants grafted with the tongue approach method. The highest survival rate (100%) was achieved in plants grafted with the

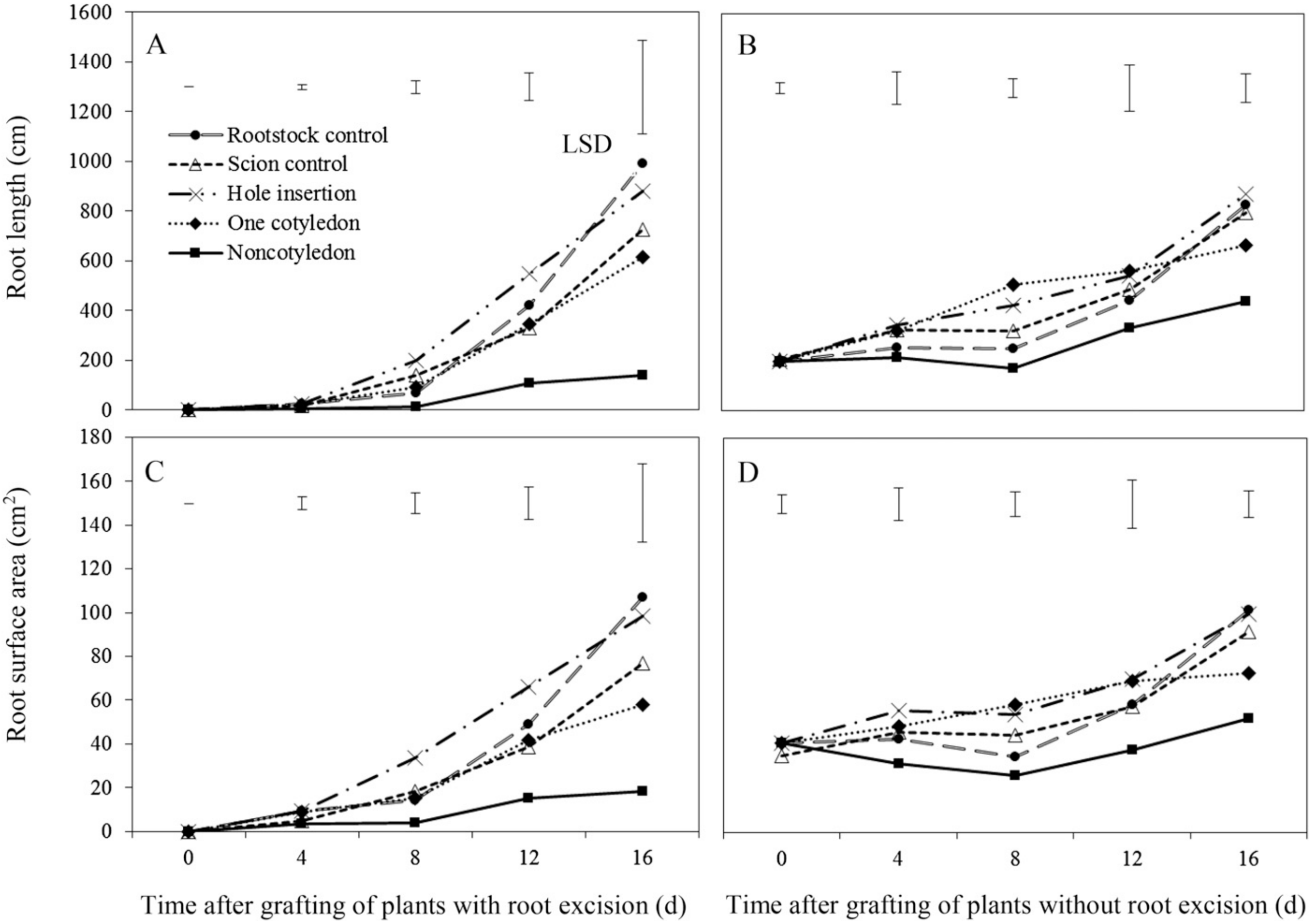

Fig. 2. Root growth of 'Athena' muskmelon grafted onto 'Strong Tosa' interspecific hybrid squash rootstock with hole insertion, one-cotyledon, and noncotyledon methods, and nongrafted 'Athena' and 'Strong Tosa' controls during the 16 d after grafting in the second experiment. (A) Root length of plants with root excision. (B) Root length of plants without root excision. (C) Root surface area of plants with root excision. (D) Root surface area of plants without root excision. Least significant difference (LSD) bars represent the LSD at α = 0.05; 1 cm = 0.3937 inch, 1 cm² = 0.1550 inch².

Table 1. Graft survival rate at 16 d after grafting in 'Athena' muskmelon grafted onto 'Strong Tosa' hybrid squash rootstock with hole insertion, one-cotyledon, noncotyledon, and tongue approach grafting methods in the first experiment.

	Graft survival rate (%)[z]	
Grafting method	Excised root	Intact root
Hole insertion	95.8 aA[y]	91.6 aA
One cotyledon	100 aA	100 aA
Noncotyledon	91.6 aA	87.5 aA
Tongue approach	45.8 bB	83.3 aA

[z]Square root transformation was performed for the statistical analysis, while the actual percentages were presented in the table.

[y]Means followed by the same lowercase letter within a column, and means followed by the same uppercase letter within a row were not significantly different according to Fisher's least significant difference test at $P \leq 0.05$.

one-cotyledon method, indicating this method may have a low requirement for the relative sizes of rootstock and scion plants.

Significant interaction effects between grafting method and root excision on quality of the grafted seedlings were also observed in the second experiment (Table 2). Similar to the results in the first experiment, grafted seedlings with the tongue approach method and root excision had the lowest quality. However, grafted seedlings using the tongue approach method with intact roots had similar plant quality ratings as plants grafted with the hole insertion and one-cotyledon methods.

Interestingly, regardless of root excision, quality of the noncotyledon grafted seedlings was significantly lower than the hole insertion and one-cotyledon grafted plants although the majority of the plants appeared to be alive at 16 DAG (Tables 1 and 2). Despite the incompatibility issue, graft failure is usually attributed to the lack of sufficient vascular connections between scions and rootstocks, which leads to wilt and desiccation of newly grafted plants. In this case, the majority of noncotyledon grafted seedlings healed initially without any evident symptom of wilting when they were taken out of the healing chamber, whereas about one-third of the plants started to exhibit slowed and stunted growth thereafter, then the rootstock hypocotyl declined and eventually died in the following days.

Without the photosynthates from rootstock cotyledons, the insufficient nutrient reservoir for rootstock growth may be one of the factors that lead to rootstock hypocotyl deterioration (Memmott and Hassell, 2010). The decline of noncotyledon grafted plants observed in this study may be caused by inhibited root growth resulting from the removal of both cotyledons from the rootstock (Figs. 1 and 2). Grafted plants with the tongue approach method also had both rootstock cotyledons removed, while those plants did not show the hypocotyl decline of rootstocks. Since rootstock cotyledons were removed after graft healing on tongue approach grafted plants, the critical roles of rootstock cotyledons in supporting graft establishment and growth might be rather lessened when healing was completed.

Using the noncotyledon method, Memmott (2010) reported that root excision significantly enhanced grafting success when watermelon was grafted onto 'Strong Tosa' at the second-true-leaf-unexpanded stage of the rootstock. However, the advantage of root excision was not observed in the present study (Table 2). The plant quality was evaluated at 16 DAG in this study vs. 7 DAG as reported by Memmott (2010), which may partially explain the discrepancies. No significant difference in quality of grafted seedlings was observed between the hole insertion and one-cotyledon grafted plants (Table 2). The aboveground fresh weight and dry weight of the noncotyledon grafted plants at 16 DAG were significantly lower than that of the nongrafted scion controls in the first experiment but not in the second experiment (Table 3). Overall, the rootstock control showed more vigorous growth than the scion control. Compared with the scion control and one-cotyledon grafted plants, the hole insertion grafted plants also showed higher aboveground biomass (Table 3).

Table 2. Transplant quality at 16 d after grafting in 'Athena' muskmelon grafted onto 'Strong Tosa' hybrid squash rootstock with hole insertion, one-cotyledon, noncotyledon, and tongue approach grafting methods in the second experiment.

	Transplant quality (0–10 scale)[z]	
Grafting method	Excised root	Intact root
Hole insertion	9.1 aA[y]	8.6 aA
One cotyledon	9.5 aA	9.4 aA
Noncotyledon	5.7 bA	5.4 bA
Tongue approach	2.7 cB	7.9 aA

[z]0 = dead; 1 = almost dead; 2 = moderating between surviving or not; 3 = borderline but will probably die; 4 = severely stunted; 5 = moderately stunted; 6 = somewhat stunted; 7 = fair but not acceptable; 8 = borderline acceptable; 9 = good and acceptable but not the best acceptable; 10 = optimal results (Memmott and Hassell, 2010).

[y]Means followed by the same lowercase letter within a column, and means followed by the same uppercase letter within a row were not significantly different according to Fisher's least significant difference test at $P \le 0.05$.

Table 3. Aboveground fresh weight and dry weight of 'Athena' muskmelon grafted onto 'Strong Tosa' hybrid squash rootstock with hole insertion, one-cotyledon, and noncotyledon methods, and nongrafted 'Athena' and 'Strong Tosa' controls at 16 d after grafting.

	First Expt.		Second Expt.	
Grafting treatment[z]	Fresh wt (g)[y]	Dry wt (g)	Fresh wt (g)	Dry wt (g)
Rootstock 'Strong Tosa'	2.72 a[x]	0.28 a	2.02 a	0.25 a
Scion 'Athena'	2.07 a	0.18 b	1.23 b	0.10 b
Hole insertion	2.68 a	0.23 ab	1.80 a	0.24 a
One cotyledon	2.23 a	0.18 bc	1.27 b	0.13 b
Noncotyledon	1.26 b	0.12 c	1.10 b	0.10 b

[z]Two-way factorial analysis of variance was conducted in the data analysis. No significant effects of root treatments and root × grafting interaction were observed.

[y]1 g = 0.0353 oz.

[x]Means followed by the same letter within a column were not significantly different according to Fisher's least significant difference test at $P \le 0.05$.

Plant growth. Cucumbers and watermelons grafted onto certain hybrid squash rootstocks could delay bloom of female flowers (Satoh, 1996; Yilmaz et al., 2011). In the present study, no differences in anthesis date between the grafted plants and the scion controls were observed. In addition, grafting methods and

Table 4. Analysis of variance of the effects of grafting and root excision on root and aboveground growth characteristics at 44 and 46 d after grafting in the first and second experiments, respectively.

	Root growth characteristics		Aboveground growth characteristics					
Effect[z]	Length	Surface area	Fresh wt	Dry wt	Leaf area	Stem diam	Chlorophyll content	Stomatal conductance
First experiment								
Grafting[y]	NS[w]	NS	*	***	NS	***	NS	**
Root[x]	NS	NS	NS	NS	NS	NS	NS	NS
Grafting × root	*	NS	NS	NS	NS	NS	NS	NS
Second experiment								
Grafting	NS	NS	*	***	NS	***	**	NS
Root	NS	NS	NS	NS	NS	NS	NS	NS
Grafting × root	NS	NS	NS	NS	NS	NS	NS	NS

[z]Two-factor factorial analysis of variance was conducted in the analysis.

[y]Grafting effect refers to treatments including 'Athena' muskmelon grafted onto 'Strong Tosa' hybrid squash rootstock with one-cotyledon and hole insertion grafting methods, as well as nongrafted 'Athena' and 'Strong Tosa' controls.

[x]Root effect refers to the root excision and intact root treatments.

[w]NS, *, **, ***Nonsignificant or significant at $P \le 0.05$, 0.01, or 0.001, respectively.

root excision treatment did not show any influence on flowering date (data not shown).

A significant interaction between grafting treatments (i.e., one-cotyledon and hole insertion grafted plants, nongrafted rootstock and scion plants) and root excision on root length at 44 DAG was observed in the first experiment (Table 4). The 'Strong Tosa' rootstock controls with root excision significantly increased the total root length by ≈32.1% compared with the plants with intact roots (data not shown). However, such an effect of root excision was not observed on the 'Athena' scion controls and the one-cotyledon and hole insertion grafted plants in either experiment. Overall, root regeneration as a result of root excision reached similar root growth as the root-intact plants at the anthesis stage of plant development. In both experiments, grafting showed significant effects on aboveground fresh weight, dry weight, and stem diameter, whereas significant impacts of grafting on g_S and chlorophyll content were inconsistent in the two experiments (Table 4). Root excision did not exhibit significant effects on any of the aboveground growth characteristics of either grafted or nongrafted plants.

Comparing treatments including the hole insertion, one-cotyledon, and tongue approach grafted plants, and nongrafted rootstock and scion plants without root excision, the nongrafted rootstock plants had significantly higher aboveground dry weight in both experiments (Table 5), but total root length and surface area of 'Strong Tosa' did not differ from that of 'Athena' at 44 DAG in the first experiment or 46 DAG in the second experiment. The rootstock plants also exhibited thicker stems compared with that of the scion plants.

Enhanced vegetative growth was reported on grafted cucurbit plants with hybrid squash rootstocks (Chouka and Jebari, 1999). However, no significant differences in aboveground fresh weight and leaf area were observed between the scion controls and the grafted plants in this study. Stomatal movement is important for plants to adjust carbon dioxide (CO_2) pressure and transpiration rate. Enhanced g_S can increase CO_2 assimilation rate and promote

Table 5. Root and aboveground growth characteristics of root-intact 'Athena' muskmelon grafted onto 'Strong Tosa' hybrid squash rootstock with hole insertion, one-cotyledon, and tongue approach methods, and nongrafted 'Athena' and 'Strong Tosa' controls at 44 and 46 d after grafting in the first and second experiments, respectively.

Treatment	Root growth characteristics		Aboveground growth characteristics					
	Length (cm)[z]	Surface area (cm^2)[z]	Fresh wt (g)[z]	Dry wt (g)	Leaf area (cm^2)	Stem diam (mm)[z]	Chlorophyll content[y]	Stomatal conductance ($mmol\cdot m^{-2}\cdot s^{-1}$)
First experiment								
Rootstock 'Strong Tosa'	2,301.8	714.7	191.1	22.6 a[x]	3,471.7	6.7 a	18.0	2,078.4 a
Scion 'Athena'	2,688.2	761.3	161.6	15.4 c	3,167.2	4.5 b	18.5	1,333.3 ab
Hole insertion	2,988.5	669.4	163.3	17.0 bc	3,197.6	7.2 a	19.4	1,592.1 ab
One cotyledon	2,402.7	732.4	193.6	18.8 b	3,775.9	6.9 a	19.2	915.5 b
Tongue approach	2,368.1	817.8	174.0	17.3 bc	3,444.6	7.5 a	18.0	1,522.5 ab
Significance[w]	NS	NS	NS	**	NS	***	NS	*
Second experiment								
Rootstock 'Strong Tosa'	4,008.5	847.0	284.4 a	41.2 a	4,126.1	9.6 a	27.3 a	1,445.6
Scion 'Athena'	3,562.4	731.7	232.3 b	28.0 b	3,890.3	6.5 b	17.5 b	1,350.2
Hole insertion	3,632.3	692.7	244.6 ab	31.2 b	3,669.0	8.4 a	19.5 b	1,382.8
One cotyledon	3,494.0	787.1	225.5 b	27.7 b	3,591.1	8.7 a	20.1 b	1,264.6
Tongue approach	4,560.6	833.9	214.0 b	25.4 b	2,965.7	8.3 a	22.2 ab	2,145.2
Significance	NS	NS	*	**	NS	***	*	NS

[z] 1 cm = 0.3937 inch, 1 cm^2 = 0.1550 $inch^2$, 1 g = 0.0353 oz, 1 mm = 0.0394 inch.
[y] Chlorophyll content index is calculated as the ratio of transmittance at 940 and 660 nm.
[x] Means followed by the same letter within a column were not significantly different according to Fisher's least significant difference test at $P \leq 0.05$.
[w] NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

photosynthetic activity (Farquhar and Sharkey, 1982). Wei et al. (2006) reported that grafted melons had greater net photosynthetic rate than self-root plants. In the present study, neither g_S nor chlorophyll content of the grafted plants was significantly different from that of the scion controls.

With respect to the grafting methods, plant growth characteristics did not differ significantly among the hole insertion, one-cotyledon, and tongue approach grafted plants. Nevertheless, growing plants for ≈6 weeks in 1-gal pots in the greenhouse might not accurately reflect the treatment differences under field conditions as the fruit yield of grafted plants could differ between the open field and greenhouse systems (Khah, 2011). Future studies are warranted to examine the influence of grafting methods and the root excision practice on yield performance of grafted melons.

Conclusions

Results demonstrated that without root excision, plants grafted with hole insertion, one-cotyledon, and tongue approach methods performed similarly regarding graft quality and growth characteristics; however, the noncotyledon method led to reduced quality of the grafted plants and their ultimate decline. Root excision was unsuccessful with the tongue approach method to produce high-quality grafts. With the one-cotyledon and hole insertion grafting methods, root excision did not exhibit significant impacts on transplant quality and further growth of grafted muskmelon plants. Field studies need to be conducted to evaluate the yield performance of grafted melons as affected by the combination of root excision with different grafting methods. Economic analysis of different grafting practices may also be considered given the various scales of the grafting operations.

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