Continuation of Improved Grafting Method for Watermelons Transplants

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INTRODUCTION

Watermelon grafting is an important part of watermelon production to avoid soil-borne diseases and/or chemical fumigation in areas where land rotation is not feasible. Current commercial grafting practices depend on maintaining at least one rootstock cotyledon during the healing period following grafting for high survival (Cushman, 2006; Lee, 1994; Lee and Oda, 2003; Oda, 1995). Rootstock re-growth originating from meristematic tissue next to the remaining cotyledon is one main contributing barrier preventing affordable costs which prevent its introduction into the United States agricultural system (Edelstein, 2004). With the phase-out of methyl bromide fumigant, new interests are being reviewed for potential alternatives (Cohen et al., 2007; Davis et al., 2008). For many years grafting in watermelons has been viewed as an option solely in areas where labor costs are minimal. This benefit has great potential to have a very positive effect for commercial production in the United States by improving the plants overall environmental efficiency and overcoming soil-borne pathogens. An alternative grafting method which eliminates potential re-growth is needed in order for grafting technology and benefits to progress into the United States. Removal of both cotyledons in a one step fashion at time of grafting eliminates all potential re-growth and greatly reduces overall grafting costs. Observations indicate however that the rootstock hypocotyl begins to yellow and declines until death when grafted at the 1^{st} leaf stage or younger which is customary for current commercial grafting techniques. The yellowing and steady decline of the hypocotyl, which results in rootstock death, simulates leaf senescence and suggests that insufficient nutrient reserves were available to the hypocotyl prior to grafting. Without sufficiently stored carbohydrates, the hypocotyl cannot sustain itself long enough before receiving photosynthates from the newly grafted vegetative tissue. When plants are allowed to mature to the appearance of the 2^{nd} or 3^{rd} leaf, hypocotyl deterioration is not observed, which suggest that more reserves are available with maturity to maintain the rootstock until graft healing is complete.

MATERIALS AND METHODS

Material for growing seedlings

For this experiment four rootstocks were tested: *Lagenaria siceraria* '*Emphasis'* (bottle gourd), *Citrulus lanatus* var. *citroides* '*Ojakkyo'* (wild watermelon), *Cucurbita moschata* x *Cucurbita maxima 'Strongtosa'* (inter-specific squash hybrid), and *Citrullus lanatus* var. *lanatus* 'Tri-X 313' (triploid seedless watermelon). Scion material was *Citrullus lanatus* var. *lanatus* 'Tri-X 313'. All seeds were obtained through Syngenta Seeds, Inc. (Boise, Idaho). The soilless mix used for this research has the following composition: 75% NB nursery peat, 25% coarse perlite, 198 grams/square meter of dolomitic limestone, and 454 grams/square meter of gypsum, (Conrad Fafard, Inc., Agawam, MA). No premix (nutrient charge) was added to the soilless mix. Rootstocks were grown in 72 square vented plug trays with cell depths of 5.71 cm and top and bottom cell diameters of 3.96 cm and 2.54 cm respectively (TLC Polyform, Inc. Morrow, GA). Scion was seeded in 288 square plug trays with cell depths of 3.81 cm inches and top and bottom cell diameters of 2.05 cm and 1.14 cm respectively (TLC Polyform, Inc. Morrow, GA). All fertilizer applications consisted of 100 parts per million (ppm) of 15-5-15 Peters Excel water soluble fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) using the Anderson Injector Series S (H.E. Anderson Company, Muskogee, OK).

Material for grafting

Rootstocks species were seeded in 72 cell flats and divided into ten plant subsamples replicated five times for grafting and tissue analysis. Rootstocks were grafted at separate times starting with interspecific squash hybrid, followed by the bottle gourd, wild watermelon, and the seedless hybrid watermelon. The 1st leaf stage in this study is defined as visibly seeing the first unexpanded true leaf. The 2nd leaf stage is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd leaf stage is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf. Prior to grafting separate plants samples were then severed from the roots at the cell line and divided into cotyledons, leaves, and hypocotyls for area measurements of the vegetative tissue which was calculated using the LI-3100 area meter (Li-Cor, Inc., Lincoln, Nebraska). At each leaf stage all the rootstock plants were grafted using the cotyledon devoid grafting method (Figure 1). The “cotyledon devoid” grafting technique is a new method aimed at eliminating rootstock re-growth and is the method under investigation. The cotyledon devoid graft is described as follows: using sterile single edge kobalt blade (Warner Manufacturing Company, Minneapolis, MN) rootstocks were first cut just below both the cotyledons at a 90º angle. This was performed to increase accessibility and precision for the grafting slant cut. An approximate 65º slant cut was then made at the tip of the hypocotyl. The scion was cut at the base from the roots in large quantities and set on sterile paper towels. It was then individually cut at approximately 1.9 cm below the cotyledons with an opposing but complimentary 65^{o} angle to the rootstock slice and preserved in a 3.8 liter size zip-lock bag to help prevent wilting until it was used. Finally the scion was joined together to align the vascular bundles with the rootstock and secured with a spring loaded clip (Syngenta Seeds Inc., Boise, Idaho) (Figure 1). Grafting treatments consisted of ten plants replicated five times. Following grafting, the newly grafted plants were immediately placed randomly inside a custom made healing chamber for seven days which was located inside the greenhouse. The healing chamber was constructed with a top wire hooped rectangular wooden box with the following dimensions: width of 86 cm, a length of 300 cm, and a depth of 14 cm and covered with clear polyethylene sheet. The humidity was maintained using the 707U-duct mount centrifugal atomizer humidifier (Herrmidifier, Effingham, Illinois).

RESULTS AND DISCUSSION

There was a significant rootstock by leaf stage interaction suggesting that the four rootstocks responded differently at each of the leaf stages (Table 1). The seedless watermelon type did not significantly increase in hypocotyl length with each leaf stage but the diameter did increase significant with growing time. This then was reflected on the overall hypocotyl area with a decrease at the third leaf stage. However an increase in grafting success was not seen until the rootstock had reaches the third leaf stage. The wild watermelon type did significantly increase at both the hypocotyl length and diameter at each of the leaf stages which resulted in an overall increase in the hypocotyls area. However grafting success was fully achieved at the second leaf stage of plant development. Both the interspecific hybrid and the bottled gourd rootstock significantly increased in both hypocotyl diameter and length with each increase of leaf stage resulting in a significant increase in hypocotyl area and grafting success. There was a strong correlation with grafting success and hypocotyl growth (Table 2). As hypocotyls increased in both length, diameter and area grafting success also increased. The weakest correlations seemed to be with the wild and seedless watermelon rootstock types in the hypocotyl length. However the strongest correlation came with the interspecific hybrid rootstock with the hypocotyl length. These results suggest that as the rootstock increases in size and development the greater grafting success can be seen in the absence of the cotyledon leaf.

CONCLUSION

Larger hypocotyls suggest an increase in storage capacity or an overall increased carbohydrate reserves from the 1st leaf stage to the 2nd and 3rd due to larger hypocotyl size. Results indicate that this new method could be used to reduce costs by eliminated rootstock side shoots when performed at the developed of the 2nd or 3rd true leaf stage for these rootstocks. We also found that using scion material at the 3rd leaf stage may be another contributing factor to increase grafting success.

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Figures

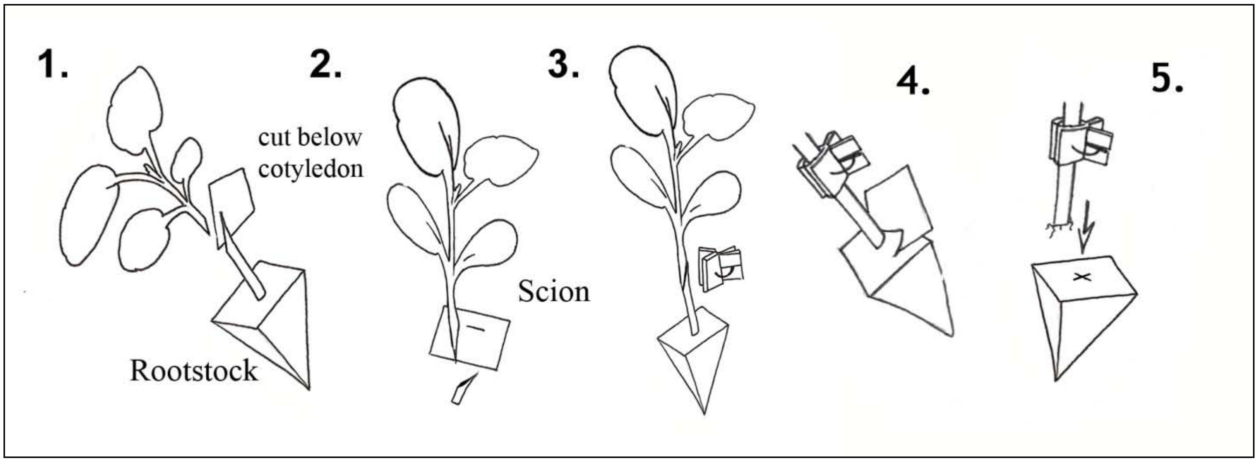

Fig. 1. Cotyledon devoid method described as a five step process.

Tables

Table 1. Grafting success and hypocotyl growth of seedless watermelon grafted on various rootstocks at different leaf stages of development.

Rootstock			Hypocotyl[w]			Grafting
Cultivar	Type	Leaf Stage[x]	Area (cm^2)	Diameter (mm)	Length (cm)	Success (%)[y]
Ojakkyo	Wild Watermelon	1	0.56 g[z]	2.53 f[z]	28.98 e[z]	57.60 d[z]
		2	1.10 e	2.70 e	37.85 d	100.00 a
		3	1.80 b	3.25 cd	55.00 b	100.00 a
Strong Tosa	Interspecific Hyb.	1	1.30 d	3.59 bc	39.00 d	15.00 f
		2	1.50 c	3.30 c	48.61 c	60.00 d
		3	4.30 a	5.30 a	73.72 a	84.00 bc
Emphasis	Bottled Gourd	1	0.72 f	2.72 ef	27.40 e	39.12 e
		2	1.10 e	3.30 c	30.15 e	84.70 bc
		3	1.80 b	3.09 d	57.29 b	98.33 a
Tri-X 313	Seedless Watermelon	1	0.66 fg	2.25 g	28.18 e	75.00 c
		2	1.76 f	2.80 e	31.63 e	83.18 c
		3	1.00 e	3.22 cd	30.16 e	95.00 ab

[z]Means within columns followed by the same lowercase letter are not significant at the $P \leq 0.05$.
[y]Analysis was performed after arcsin transformation of the percentage data.
[x]The 1st leaf stage in this study is defined as visibly seeing the first unexpanded true leaf. The 2nd leaf stage is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd leaf stage is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[w]Hypocotyl measurements represent a total of a five plant sample replicated five times.

Table 2. Pearson correlation coefficients between rootstock hypocotyl area, diameter or length and grafting success.

Rootstock		Hypocotyl[y]		
Cultivar	Type	Area (cm^2)	Diameter (mm)	Length (cm)
Ojakkyo	Wild Watermelon	0.781[z]	0.681	0.565
Strong Tosa	Interspecific Hyb.	0.784	0.655	0.828
Emphasis	Bottled Gourd	0.825	0.655	0.676
Tri-X 313	Seedless Watermelon	0.721	0.874	0.630

[y]Hypocotyl measurements represent a total of a five plant sample replicated five times.
[z]Values are significant at P = 0.05.