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REFINEMENT OF INNOVATIVE WATERMELON GRAFTING METHODS WITH APPROPRIATE CHOICE OF DEVELOPMENTAL STAGE, ROOTSTOCK TYPE, AND ROOT TREATMENT TO INCREASE GRAFTING SUCCESS

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REFINEMENT OF INNOVATIVE WATERMELON GRAFTING METHODS WITH APPROPRIATE CHOICE OF DEVELOPMENTAL STAGE, ROOTSTOCK GENOTYPE, AND ROOT TREATMENT TO INCREASE GRAFTING SUCCESS

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ABSTRACT

Watermelon grafting methods used in Europe and Asia vary, but are based on efficiency, skill and needs. China mainly practices the whole insertion grafting method, whereas, Europe and Japan employ the one cotyledon (splice/slant-cut) grafting method. These methods are not suitable for grafting production in the U.S. due to the labor intensive and high labor cost necessary to successfully produce grafted transplants. This thesis introduced a modified grafting technique called the "Cotyledon Devoid Method" and in three experiments determined; 1) the rootstock leaf number stage (RLNS) at which the greatest grafting success is achieved; 2) the relationship between total soluble carbohydrates in rootstock hypocotyl seedlings and grafting success; and 3) the effects of root excision performed after grafting but prior to healing on grafting success and hypocotyl carbohydrate depletion. Grafting was performed on ten plants in five replications using four different rootstocks: *Lagenaria siceraria* 'Emphasis', *Citrullus lanatus* var. *citroides* 'Ojakkyo', *Cucurbita moschata* x *Cucurbita maxima* 'Strong Tosa', and *Citrullus lanatus* var. *lanatus* 'Tri-X 313'. All scion material was *Citrullus lanatus* var. *lanatus* 'Tri-X 313'. Rootstocks and scion material were developed in synchrony to the appearance of the first (9-15 days), second (13-18 days), and third (19-24 days) leaf number stage. Aerial measurements were taken on both the rootstocks and scion material before grafting. Both rootstock cotyledons were removed at time of grafting to eliminate any potential rootstock regeneration. Furthermore, roots were excised from the hypocotyl of one set of grafted seedlings to reduce the need to maintain an active root system during healing which allowed the hypocotyl energy reserves to be conserved to initially heal the graft union and then generate new roots (Excision treatment). Grafts were randomly placed inside a healing chamber for 7 days and evaluated 14 days later for grafting success. The second and third experiments were designed

to analyze total soluble carbohydrates accumulated in the rootstock plant tissues before and after grafting at each of the three RLNS with and without roots present. Plants were carefully dissected on the day of grafting and 7 days after grafting to measure individual plant organs including root, hypocotyl, cotyledon, and leaf or scion hypocotyl, scion cotyledon, and scion leaf area. All individual plant organs measurements consisted of ten plants per samples replicated five times. Carbohydrates were extracted using the methanol-chloroform-water method. The carbohydrate concentrations were determined using the phenol sulfuric acid assay and read by the micro plate spectrophotometer. Measured samples for carbohydrate analysis consisted of a subsample taken from ten plants ground samples replicated five times. Each ten-plant sub sample was determined by the mean of two read replications on the micro plate with the coefficient of variation values generally less than 10. Grafting success increased with each increase in RLNS. Aerial dimensions taken before grafting revealed that the rootstock hypocotyl diameter, length, and area increased from the first to the third RLNS and were related to grafting success. Total carbohydrate measurements taken from each rootstock hypocotyl organ before grafting increased from the first to the third RLNS suggesting a relationship between grafting success and hypocotyl carbohydrates. The overall carbohydrate concentration remained the same among RLNS, but the increase in dry weight from the first to the third RLNS accounted for the vast increase in total carbohydrates per hypocotyl and thereby increased grafting success. Rootstock hypocotyl total carbohydrates greatly decreased when roots were left intact versus excised, indicating root excision can be employed to conserve hypocotyl carbohydrate to encourage healing which is also essential for mechanical grafting. Excising the rootstock root prior to healing but after grafting did not decrease grafting success at the second or third RLNS on three of the rootstocks tested. The “Cotyledon Devoid Method” provides a successful option that may have potential to reduce

grafting cost by successfully removing rootstock regeneration; however, precise seed germination and seedling development guidelines must be followed in order to achieve acceptable grafting success.

DEDICATION

I would like to dedicate this page to the committed teachers and good friends that have inspire me during my early years and throughout my life to seek for learning and greater education. To the professors that have encouraged me along the way and have helped make this dream a reality. To my parents who have given their love, encouragement, and sacrificed time and pleasure to teach me the great value of persistence in hard worthwhile work and study. To my Heavenly Father who has given me the intelligence, faith, strength and support necessary to succeed. I have learned that achieving requires first the desire and then faith to move ahead with vision, dedication, and determination. One must have self confidence, be open-minded, willing to work and endure until the end.

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PREFACE

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 (Cohen et al., 2007; Oda, 1995; Yetişir and Sari, 2003). For many years grafting in watermelons has been viewed as an option solely in areas where labor costs are minimal. With the ongoing search for alternatives from band fumigants such as methyl bromide, grafting in watermelons has come under the spot light as a possible alternative (Cohen et al., 2004; Cohen et al., 2007; Koren and Edelstein, 2004). Grafting has great potential to have a very positive effect for commercial production in the United States by overcoming soil-borne pathogen impediments (Cohen et al., 2007; Kurata, 1994; Lee, 1994; Lee and Oda, 2003; Oda, 1995; Yetişir et al., 2003), increasing fruit quality (Cohen et al., 2007; Core, 2005; Davis and Perkins- Veazie, 2005-2006), and improving the plants overall environmental efficiency (Cohen et al., 2007; Koren and Edelstein, 2004; Lee, 1994; Oda, 1995; Pulgar et al., 2000; Venema et al., 2008; Yetişir and Sari, 2003).

A major problem inhibiting the use of grafting is rootstock re-growth occurring after grafting. Rootstock re-growth occurs in the current commercial grafting practices and has prevented introduction to the United States agriculture market because of increased cost during transplant production. Re-growth initiates at the base of the cotyledon and will cause the graft to weaken, abort, or delay production if left intact. Re-growth removal is labor intensive, and very costly. An alternative grafting method which eliminates potential re-growth is needed in order for grafting technology and benefits to successfully increase into the United States. Current commercial grafting practices depend on maintaining at least one rootstock cotyledon during the healing period following grafting for survival (Cushman, 2006; Hassell et al., 2008; Oda, 1995). Removal of both cotyledons in a one step fashion at

time of grafting, eliminates all potential re-growth and potentially reduces overall grafting cost. I have observed that the rootstock hypocotyl begins to yellow, decline and senesce when grafted at the 1st true rootstock leaf number stage (RLNS) which is customary for current commercial grafting techniques. The removal of both cotyledons during grafting initiates a steady decline of the hypocotyl resulting in rootstock death suggesting the hypocotyl had insufficient nutrient reserves prior to grafting. Without this stored supply of carbohydrates, the hypocotyl cannot live long enough to benefit from photosynthates elaborated by the newly grafted vegetative tissue (Bisognin et al., 2005; Lovell and Moore, 1971; Lovell and Moore, 1970). When plants are allowed to mature to the appearance of the 2nd or 3rd true leaf, hypocotyl deterioration does not occur, suggesting perhaps that more reserves were available with maturity to maintain the rootstock until graft healing takes place. The objectives of this research study were: 1) to determine the developmental stage at which grafting success is achieved while removing both cotyledons during the grafting procedure; 2) to determine plant tissue carbohydrate concentration in four different rootstocks at each 1st, 2nd and 3rd true leaf developmental stages before grafting; and 3) to determine whether rootstock hypocotyl carbohydrate levels relate to grafting success at the three developmental stages for each rootstock. Specific research data to achieve my objectives include determining: 1) organ carbohydrate concentration in rootstock seedling leaves, cotyledon, hypocotyl, and roots tissues at time of grafting for three developmental stages; 2) the carbohydrate concentration of scion material at three developmental stages; 3) the carbohydrate concentrations in grafted seedling tissues after healing takes place with and without roots present: hypocotyl, scion hypocotyl, scion cotyledons, and scion leaves; 4) hypocotyl length, diameter and area before grafting; 5) leaf and cotyledon area before and after grafting; 6) leaf and cotyledon chlorophyll content before and after grafting; and 7) the relationship of carbohydrate

accumulation in rootstock hypocotyls with grafting success with and without roots present. My research goal is to enable transplant producers in the United States to successfully produce grafted watermelon transplants as an alternate to methyl bromide fumigation at a potentially lower cost to the grower.

LITERATURE REVIEW

United States Watermelon Production History

Since the introduction of watermelon into the Americas from Africa (Mallick and Masui, 1986), its production has become a significant crop in the United States, reaching as high as 4.3 billion lbs in 2007 and revenues surpassing $475.8 million (USDA, 2008). Watermelons are produced on crop rotation fields once every 5-6 years due to the accumulation of soil borne pathogens that severely reduce and limit crop yield (Bruton, 1998; Yetişir and Sari, 2003). Inadequate rotation has perhaps contributed the greatest to increased incidence and severity of soil borne diseases (Bruton et al., 1998).

In some areas where land rotation is not feasible, such as Asia, watermelon grafting is an important part of production to avoid soil-borne diseases and/or chemical fumigation (Cohen et al., 2007; Oda, 1995; Yetişir and Sari, 2003). Growers in the United States have used fumigants such as methyl bromide, to overcome soil borne diseases, and successfully harvest their crop. Beginning in 1995, a partial ban and now a full ban, was placed on the use of methyl bromide according to the Montreal Protocol to prevent the depletion of the ozone layer, and to conserve other non-targeted organisms (Ristaino and Thomas, 1997). Since the ongoing limiting use of fumigants, grafting has become of greater interest as an alternative to methyl bromide fumigation for disease avoidance (Cohen et al., 2004; Cohen et al., 2007; Koren and Edelstein, 2004).

Grafting History

Grafting is the union of two or more plant tissues that subsequently grow as a single plant (Andrews and Marquez, 1993). Plant grafting has been performed in China before 1500 B.C. (Lee and Oda, 2003; Oda, 1995). The first vegetable crops to be grafted date back to the seventeenth century; however, it did not become popular until the late 1920’s. Farmers in

Korea and Japan grafted watermelon plant onto a gourd rootstock (*Lagenaria siceraria*) to provide resistance to soil borne diseases caused by successive cropping (Ashita, 1927). Many areas with intense watermelon production and/or little land availability such as Turkey, China, Korea, Japan, and Israel have had to overcome infestations of soil borne pathogens in watermelon that arise from the inability to rotate crops (Cohen et al., 2007; Oda, 1995; Yetişir and Sari, 2003). Current uses in other countries confirm the feasibility of grafting the horticultural designed cultivars on a resistant cucurbit rootstock as an alternative method to crop rotation and disease avoidance.

Watermelon breeding programs have attempted to increase the resistance to soil borne diseases by cross breeding lines exhibiting resistance (Bruton, 1998). Successful breeding advances continue to allow watermelon cultivation in the U.S. at high costs. Attempts to breed for genetic resistance are very time consuming and costly due to the nature of introducing wild type resistance with unacceptable morphological characteristics into highly selected cultivars ready for market consumption. These unacceptable characteristics must be bred out while maintaining the resistance and increasing the fruit quality. These costs are further amplified when the resistance is overcome by mutating diseases and then new additional disease resistance genes must be introduced (Bruton, 1998). New ways of incorporating and maintaining resistance is continuously sought by breeding researchers. Countries such as: Japan, Korea, China, Turkey, and Israel, began grafting watermelon cultivars onto resistant rootstocks to overcome crop loss from disease infection (Cohen et al., 2007; Kurata, 1994; Lee, 1994; Lee and Oda, 2003; Oda, 1995; Yetişir et al., 2003). Today a watermelon graft consists of a vegetative horticultural designed cultivar portion called a scion that is united with a desired cucurbit hypocotyl and root hypocotyl termed the rootstock.

By breeding resistance into the rootstock, breeding time is reduced significantly because the screening traits are fewer. In watermelons, there are at least three species available to find different plausible rootstocks suited for grafting and disease resistance. Watermelon is currently grafted on *Lagenaria siceraria* (bottle gourd), *Citrullus lanatus* (wild watermelon), *Cucurbita moschata* x *Cucurbita maxima* (inter-specific squash hybrid), squash hybrids, (*Cucurbita moschata* x *Cucurbita maxima*). *Lagenaria siceraria* can be used to control Fusarium wilt (Yetişir and Sari, 2003). Over 95% of the commercial watermelon seedlings are grafted in Japan, Korea and Greece where farming areas are small, very intensive and crop rotation is an uncommon practice to overcome soil-borne pathogens (Kurata, 1994; Lee, 1994; Traka-Mavrona et al., 2000).

Current Grafting Methods in Watermelon

Many different watermelon grafting techniques are available today namely "the tongue approach graft", "one cotyledon graft", "hole insertion graft", and the "side insertion graft" (Cushman, 2006; Hassell et al., 2008; Lee, 1994; Lee and Oda, 2003; Oda, 1995). The approach graft is one of the original grafting methods performed (Lee and Oda, 2003);however, the one cotyledon and hole insertion grafts are most commonly used today in commercial production. Preferences to grafting techniques are a compromise among a number of influential factors to maximize the benefit to fit the individual's needs and available resources. These contributing factors include the ease and technicality of grafting, success rate, and overall cost (Davis et al., 2008; Hassell et al., 2008; Lee, 1994).

1- Tongue Approach Graft

The "tongue approach graft", or simply known as the "approach graft", is relatively simple to graft (Fig. 1) (Hassell et al., 2008). It is the oldest grafting technique, which became widely used in the 1920's in Asia due to its higher success rate (Lee and Oda, 2003) and the

growth uniformity (Hassell et al., 2008). This method continues to be preferred by inexperienced growers because of its simplicity, high success rate, and little care since it does not require healing chambers (Lee and Oda, 2003). Referring to figure 1 at the first true RLNS and older RLNS a diagonal slice is made below the cotyledons, in both hypocotyls of 1)the scion and 2), rootstock; slices should be opposite to one another, upward and downward, respectively (Cushman, 2006; Oda, 1995). Each cut should be comparable in length so they can match up together, 3). Each slit acts like a tongue and both are fitted together and sealed with an aluminum wrap to allow healing to take place. The rootstock meristem and cotyledons are 4) completely removed three days after grafting and 5) the scion rootstock is removed at seven days after grafting. The scion is now solely dependent on the new rootstock (Oda, 1995). The plants must be individually handled manually at the time of grafting, again at three days after grafting to remove the meristem from the rootstock, and once more at day seven to remove the root portion from the scion. This makes it a very labor intensive and time consuming grafting method. Both rootstocks are then replanted together during the grafting procedure to increase the proximity during the healing time. This is a significant drawback if it's being done in a greenhouse as it occupies twice the amount of space and is costly to maintain (Cushman, 2006). Because all meristematic tissue from the rootstock is removed during the grafting procedure, rootstock re-growth can no longer occur.

2- One Cotyledon Graft

The "one cotyledon graft" is also known as "splice", "slant" or "tube" graft. This graft is moderately simple being less labor intensive than the approach graft (Fig. 2) (Hassell et al., 2008). The one cotyledon graft can be completed at one time and minimizes greenhouse occupancy making this method the most popular grafts among experienced growers and commercial nurseries in Korea. It is performed by either by hand, semi-

automatic, and with automatic robots (Kobayashi et al., 2006; Kurata, 1994; Lee and Oda, 2003). Plants are ready for grafting when the first true leaf is present on the rootstock or as young as the scion cotyledon stage (Cushman, 2006; Oda, 1995). The meristematic region becomes increasingly difficult to completely remove when the rootstock plant material ages past the first true RLNS. The procedure is as follows: 1) the scion is cut at an opposing 45º to 65º angle to the rootstock, approximately one inch below the cotyledons to facilitate clamping; 2) the rootstock meristem and one of the cotyledons are cut simultaneously from the plant at a 45º to 65º angle to maximize the grafting surface area; 3) the sliced portion of the scion and rootstock hypocotyl is then joined together to ensure the vascular tissues are contacting each other: and 4) the graft secured with a spring clamp that is placed around the outside region of the splice. Immediately following grafting, plants require special environmental conditions for healing. This includes: high levels of shade and humidity, and healed at approximately 25 ºC in a healing chamber. The healing chamber minimizes environmental stresses to allow newly grafted plants to heal without undue environmental stress rather than continue with photosynthetic activity until healing is complete. Under low light conditions, the stomata on the leaf close forcing gas exchange and photosynthetic activity to cease which slow wilting to maintain the plant vascular system at optimal survivability. The high humidity prevents the plant from excessive wilting and assists in maintain high tugor pressure which aids in graft healing. Newly grafted seedlings should be kept in the healing chamber for the duration of the graft healing lasting approximately seven days. Three days into graft healing, light intensity is increased, and humidity is gradually decreased in the healing chamber to prepare the seedlings for ambient environmental conditions outside the chamber.

The overhead cost of the humidity chamber increases the overall cost to produce a quality grafted transplant. The unique spring loaded clips which are used require labor costs for placement and removal. Finally, removal of meristematic re-growth which occurs using this graft method increases overall cost. Costs can be further increased using this method if grafting is performed on older plants. Rootstock re-growth occurs at even higher rates because it is more difficult to remove all meristematic tissue during grafting which adds to the cost of labor even once the seedlings are planted in the field.

3- Hole Insertion Graft

The "hole insertion graft", which is also called "terminal", "cut" or "top insertion" graft (Fig. 3) (Hassell et al., 2008), is favored by watermelon growers in Japan because of the shorter growing time required for scion material compared to the rootstock (Lee and Oda, 2003). Grafting can begin once the first leaf emerges from the rootstock. The scion is ready for grafting during the cotyledon stage and up to the first true leaf. Some experts report that it can be used even as soon as the shoot emerges from the soil (Lee and Oda, 2003).

The procedure for this method is outlined in figure 3 as follows: 1) the scion hypocotyl is cut 2 cm below the cotyledons at a slant on opposing sides to expose the vascular tissue; 2) During this step as much of the meristematic tissue should be removed as possible; 3)A specialized tool, such as a bamboo stick or small drill bit, is used to make a hole that is slant to the longitudinal direction between the cotyledons and into the hypocotyls which should slightly pass through the hypocotyl on one side for the scion hypocotyl to be inserted allowing the vascular system of both hypocotyls to come into contact with each other; 4) The pointed region on the scion is then snuggly inserted through the slanted hole in the hypocotyl to complete the graft ; and 5) This method does not require the same scion/rootstock hypocotyl slant cut matchup, does not require clips, and the newly grafted

plant is then placed inside a healing chamber for seven days as described previously. There is a high success rate on rootstocks that are compatible with Lagenaria; however, a great concern lies within the high rate of remaining meristematic tissue since which will necessitate future re-growth removal and increasing grafting cost. Rootstock plants that have a pronounced hollow stem, such as inter-specific squash hybrids, are less likely to work because of hollow stem creates a gap which prevents the scion from adhering to the rootstock and/or inserting the seedling into the pith cavity of the rootstock. By doing so allows adventitious roots from the scion to elongate downward through the pith center and into the soil which will void the resistance and lead to complete rootstock decline (Lee and Oda, 2003). This technique has not been successfully automated because of the technicalities of performing this graft.

4- Side Insertion Graft

The “side insertion graft”, also known as the “cleft” or “splice” graft (Fig. 4) (Hassell et al., 2008), is a modified whole insertion graft (Lee and Oda, 2003). Seedlings are ready to be grafted at the first true RLNS. The graft is as follows: 1) using a sharp blade, the scion is cut at an angle on both sides of the hypocotyl below the cotyledons to form a v-shape; 2) cut a small vertical slit through the middle of the rootstock stem instead of at the top of the meristem; 3) The slit is propped open with a toothpick; 4) The scion is then inserted into the slit at an approximate 30º to the rootstock tip and a clip is placed over the union to secure the graft during the healing process, but its removal will be required once healing is complete; and 5) Three days after grafting carefully cut off the rootstock vegetative tissue just below its cotyledons. This grafting technique seems very simple, but inserting the scion into the rootstock can be somewhat difficult. The involvment of toothpick, makes it more time consuming and cumbersome. Once grafting is complete, the seedlings must be placed inside a

healing chamber for three days after grafting, but an intense amount of labor is required to remove the rootstock shoot above the graft once the embedded scion has healed. Because of this step, this procedure cannot be automated; however, meristematic re-growth is no longer a problem. A further reason why this grafting technique is unpopular is the failure of vascular bundles to align sufficiently for a strong healing to take place to secure the graft.

Watermelon Grafting Benefits and Disadvantages

Advantages

Valuable benefits can also be introduced from grafting watermelons on intra- and interspecific rootstocks (Cohen et al., 2007). Resistant rootstocks can be alternated to overcome disease to maintain high watermelon production yields (Edelstein, 2004a). *Fusarium oxysporum* f. sp. *melonis* can be avoided by using interspecific rootstocks (Cohen et al., 2007). Some rootstocks from *Lagenaria* are able to confer resistance in Cucurbitaceae against carmine spider mite, *Tetranychus cinnabarinus*, (Edelstein et al., 2000). Other rootstocks display tolerance for other soil-borne pathogens such as *Monoaporascus* and *Macrophomia* (Koren and Edelstein, 2004). Another highly positive benefit is that some rootstocks have been known to effect fruit quality (Core, 2005; Davis and Perkins- Veazie, 2005-2006). By grafting watermelons on to different rootstocks, the quality of the fruit has been known to increase fruit firmness and thus increase shelf life. These results have added to the quality of the fruit, in other countries, when shipping to foreign lands. This is a valuable potential preservation characteristic for this country in the fact that this may extend fruit longevity for both a harvest window for growers and on the shelf storage for produce buyers. It could also open new markets for the fresh cut industry. One benefit is that some grafts increase nutrient and water uptake due to a higher capacity for nitrogen uptake and transport to the scion, which greatly increases its growth (Pulgar et al., 2000). This advantage allows

the plants to better use fertilizers and other nutrients that would have been left in the soil. The absorption efficiency of water is increased by vigorous rootstocks (Lee, 1994). These benefits have the potential to lower nutrient costs and amount of required water per plant to harvest the same yield.

Grafted plants show a greater cold tolerance which is a great benefit since non-grafted watermelon plants have such little tolerance for low temperatures (Oda, 1995; Venema et al., 2008). Water logging is another watermelon production problem which causes the root to suffocate and crop production to halt. Studies show an increase in water logging tolerance with grafted plants (Yetişir and Sari, 2003). In another study, grafted watermelons had a greater tolerance when watered with saline water than did the non-grafted plants (Cohen et al., 2007) which implied the increase in drought tolerance in grafted plants as well (Koren and Edelstein, 2004).

Disadvantages

Although there are many impressive advantages to grafting, some disadvantages have discouraged this technology from use in the U.S. These disadvantages are distributed between incompatibility, fruit quality, and cost. Incompatibility is the failure of the scion to unite and adhere to the rootstock. Lesser but still problematic incompatibilities occur when the plant is unable to grow in a healthy manner, or exhibits premature death (Garner, 1979). Other incompatibilities can cause poor fruit quality, yield reduction, and possibly plant collapse. This may be due to the reduction in or blocking of photosynthate transport. Vascular bundles must come in contact with each other in order for grafting to be successful and to avoid incompatibility (Oda et al., 1993). In order for healing to take place, vascular bundles from the scion and rootstock, severed during grafting, must come into intimate contact with one another for correct healing to take place. Vascular tissue differentiation from the callusing

cells occurs in compatible grafts only (Andrews and Marquez, 1993). Grafting success can be increased by increasing the surface area and contact region between the scion and rootstock by increasing the sliced region allowing the vascular bundle on the whole to increase contact. Different plant species have a varying number of vascular bundles. This may increase the difficulty to adequately align vascular bundles from the rootstock and scion if they are unequal to achieve a successful graft (Oda et al., 1993). Some studies also shown that rootstocks can adversely affect the taste and shape of watermelon fruits (Edelstein, 2004a). Plant proteins, either structural or nonstructural that are synthesized in the root, are translocated to the scion can give the fruit an off flavor that has been reported. These discrepancies are not reported in all rootstocks and can be overcome through screening procedures to evaluate for rootstock performance.

Overall cost versus benefit becomes the bottom line when growers think about production within the United States: A grafted seedling in the U.S. is estimated to cost more than $ 0.75, as suggested by Taylor et al. (2008) being far more than $ 0.28 for a non-grafted seedling. There is an additional cost for growing the rootstock seedlings in comparison with a non-graft seedling transplant. This cost can be broken down into twice the amount of growing material, space, and time. Additionally equipment is needed for grafting such as a sharp blade, clips and a healing chamber. Labor is necessary to carefully handle the seedlings while performing the grafting procedure and with removing rootstock re-growth and this removal can be very expensive and of major concern due to overall cost. Rootstock re-growth occurs at the base of the rootstock cotyledons where meristematic tissue is present. Current grafting techniques attempt to remove all meristematic tissue during the grafting procedure. When the meristematic tissue is not removed, re-growth occurs at high rates. Even when grafting experience is increased and rootstock re-growth minimized, the remaining re-growth is yet

too costly to remove at a reasonable cost. Overall cost must be decreased in order for grafting technology to be considered for commercial practice within the United States. This problem can be reduced by completely removing the cotyledon during grafting which eliminates the meristematic region; however, some attempts to successfully graft by removing both rootstock cotyledons in a one step fashion has not been successful (Oda et al., 2002).

Plant Physiology: Role of Cotyledons

The cotyledon leaf appears to play an important role in successful grafting. Although it is ultimately the ability of the vascular bundles to come into alignment and interact with one other that determines success, the cotyledons play an initial role that is not fully understood. Graft healing appears to be dependent on hormonal signaling manufactured in the cotyledons that successfully heal the wounded region which will be explained below.

The cotyledons are the initial energy source for the developing seedling, and are responsible for 80% of the CO_2 fixation (Lasley and Garber, 1978). After emerging, the cotyledons continue to expand from 14-(Bisognin et al., 2005) to 50-fold and become leaf-like to photosynthesize the needed carbohydrates for the plant's developing organs (Lovell and Moore, 1970). Bisognin et al. (2005) suggested that cotyledons should not be damaged until leaf surface area is equivalent to cotyledon surface to prevent a large decrease in CO_2 exchange and possible plant death. In cucurbits, cotyledons undergo a high rate of expansion growth involving an increase in cell number and size with the development of functional stomata on both sides of the leaf after emergence (Bisognin et al., 2005; Lovell and Moore, 1970). The overall CO_2 exchange rate is much higher per area than those of leaves (Lasley and Garber, 1978)

Tissue below ground can display an even stronger dependence on cotyledons (Bisognin et al., 2005). If the cotyledons are removed within the first days of germination, the

seedlings growth and development will be delayed and may result in death. The young seedling development is dependent on cotyledon photosynthetic activity (Penny et al., 1976) as the tissue below ground is still maturing and requires a vast amount of energy. The establishment of cucumbers is highly dependent on cotyledons (Bisognin et al., 2005). Because of the role of the cotyledon in supplying necessary energy for the developing seedling during the young stages of development, a deficit in stored reserves during grafting at early stages would be detrimental to grafting success. Removing cotyledons to prevent rootstock re-growth immediately after germination would prevent cell tissue from maturing resulting in graft failure.

Plant Physiology: Graft Healing

Graft healing and survival greatly depend on the compatibility of scion and rootstock combinations which can be anatomical, physiological, and genetic variables (Edelstein, 2004a; Edelstein, 2004b). A low survival rate in grafted plants can be due to two main characteristics: 1) the removal of the cotyledons from the rootstock; and 2) limited number of the vascular bundles that contact the scion to the rootstock (Oda et al., 1994).

Hormonal interactions such as gibberellins, auxins, and cytokinins have also been shown to affect graft healing. Gibberellic acid is a product produced in the cotyledons that is essential to the cell division in reuniting the cortex of the graft union (Asahina et al., 2002). To better understand the involvement of the cotyledons in the healing process, the cotyledons were removed and cell division was inhibition during tissue reunion (Asahina et al., 2002). This inhibition was further reversed upon the application of gibberellins to the apical tip of the cotyledon-less plant. Reports showed that this inhibition was also present in a GA-deficient *gib-1* mutant of tomato (*Lycopersicon esculentum)*. These results conflicted with a previous study on tomato which showed that the addition of gibberellic acid in a culture

medium was inhibitory to the graft development (Parkinson and Yeoman, 1982), which suggested they could be specific to a species, or they did not get the rate right.

Cytokinins and auxins are also considered very important in grafting. A deficit in cytokinins is associated with incompatible grafting combinations (Andrews and Marquez, 1993). Further investigations showed that the application of kinetin to a culture medium stimulated the graft development (Parkinson and Yeoman, 1982). In regards to auxin, the application of one indole-3-acetic acid to the apical end was suggested to be an absolute requirement for healing success (Parkinson and Yeoman, 1982). Furthermore in a preliminary study by Shan-fa et al. (1996), an optimal level of plant hormone including the auxins, IBA and cytokinin 6-BA, was found to control the formation of graft unions by influencing the number of vascular bridges formed between rootstock and scion.

There are differing opinions on whether a difference in hypocotyl diameters between scion and rootstock affect grafting incompatibility. Both Oda et al. (1993) and Traka-Mavrona et al. (2000) reported that the smaller differences in the hypocotyl diameter between the cucumber scion and squash rootstock may increase compatibility and the quantity of vascular bundles has no effect. Edelstein et al. (2004b) found no correlation with the difference between scion and rootstock hypocotyl diameters or vascular bundles and the survival rate of the grafts and concluded that the difference was attributed to different grafting techniques being.

Role of Carbohydrates and Sink-Source Relationship

The role of the cotyledon in graft success and seedling survival, suggest a correlation between the two, and merits additional research to understand the sink/source relationship in relation to grafting success. During the developmental process and seedling establishment, plant tissues can be classified as either a source or sink to define the patterns of carbohydrate

translocation. Areas that produce more photosynthate than is consumed become a source. Photosynthate is translocated from the "source" (the sight of photosynthesis) to a "sink" (another plant organ that is consuming photosynthate at a higher rate than it is producing for development or storage). Sink areas may shift during plant development. The major sinks during vegetative growth are the shoot and root tips. The seeds and fruits become dominant sinks for the duration of reproductive development (Taiz and Zeiger, 2006).

In a study to better understand the distribution and effect of the cotyledons on carbohydrates, Mayoral et al. (1985) found that with a 12-day-old seedling, the sucrose and starch contents of the cotyledon increased upon the removal of the primary leaf. With the primary leaf still intact, the removal of one cotyledon decreased the carbohydrate content of the remaining cotyledon. This redistribution and fluctuations of carbohydrates appears to coincide with the source/sink relationship of the developing organs; the cotyledons being the source, and leaf being the sink. The removal of either cotyledon will increase the dependence for the demand in carbohydrates for the developing leaf resulting from the remaining cotyledon. By removing the developing leaf, the high demand for carbohydrates will cease thereby decreasing the translocation of photosynthates from the cotyledons.

Carbohydrates play an important role in the survival of the seedling including construction of the carbon skeletons, energy source, osmotic effects, induce signal transduction, and modulating gene expression (Rapaka et al., 2007a). Sampling time affects carbohydrate levels in leaves and stem tissue. *Portulaca grandiflora* cuttings harvested earlier in the day have fewer carbohydrates than those harvested later (Rapaka et al., 2007b). Total carbohydrate concentration in the plant is dependent conjointly on sunlight intensity and overall accumulated carbohydrates during the day. By the end of the dark period the carbohydrates are completely remobilized (Rapaka et al., 2007a). Further studies have

demonstrated that changes in carbohydrate levels affect postharvest shelf life of leafy green vegetables with lower carbohydrate concentrations having a shorter storage life (Rapaka et al., 2007a). Additionally adventitious rooting intensity was also correlated with carbohydrate concentration. Cuttings with higher carbohydrate concentrations had greater rooting intensity than those with lower concentrations (Rapaka et al., 2005).

Watermelon seedlings contain various carbohydrates in petiole and leaf tissue. These carbohydrates are fructose and glucose, found mainly in the petiole and sucrose, raffinose and stachyose found in the leaf (Ranwala et al., 2002). Within Cucurbitaceae, stachyose and sucrose appear to be the major translocated carbohydrates with stachyose the predominant carbohydrate within the cantaloupe leaf, and monosaccharides are the most abundant carbohydrates present in young fruit and stem tissue (Bruton et al., 1998).

The involvement of carbohydrates in grafting has not been previously reviewed. The interaction and redevelopment of the graft union in large extent should be dependent on the amount of carbohydrates present in the plant at the time of grafting due to the role of the cotyledons in supplying energy, and the complex ways in which carbohydrates are used within a plant. At grafting, the growing shoots and roots will be the main sinks. During healing, grafts are placed in low light levels until the graft is healed, the synthesis of new carbohydrates would be prevented and the seedling would be completely reliant on stored carbohydrates for survival.

Grafting success appears to be dependent on a variety of characteristics which are not completely understood including environmental conditions, plant vigor, carbohydrate content, and the proper alignment of vascular bundles (Bisognin et al., 2005). According to Oda (1995) newly grafted plants must be placed in a humidity chamber with low light intensity for healing to take place. The ability of the rootstock and scion to heal the wound created through

grafting may be dependent on the total energy available. Studies showed that grafting success is determined greatly to the extent that vascular bundles for the scion and rootstock are aligned (Oda et al., 1994). Other reports showed that auxin, gibberellic acid and cytokinin promote vascular cambium formation as discussed previously. Additionally, the cut regions of both seedlings should not be allowed to dry. After grafting, the grafted seedlings should be kept in 100% humidity for three days followed by a gradual drying until day seven. The light intensity should be at 3-5 klx (Oda, 1995) and the temperature should be maintained at 25^{o}C (Cushman, 2006).

Grafting costs increase due to meristematic re-growth which occurs at high frequencies as long as active meristematic regions remain on the rootstock after grafting with current commercial grafting procedures. The splice graft method is also not cost effective in the United States because of the intense manual labor involved. General re-growth does not occur at the same time which necessitates removal at different times to ensure complete removal. Cost for removal are further escalated when the workers are required to walk the field to individually remove the re-growth once the grafted plants are planted out in the field. (Cushman, 2006; Lee and Oda, 2003; Oda, 1995).

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MATERIALS AND METHODS

Seedling Development

Four rootstocks were tested: *Lagenaria siceraria* cv. Emphasis (bottle gourd), *Citrullus lanatus* var. *citroides* cv. Ojakkyo (wild watermelon), *Cucurbita moschata* x *Cucurbita maxima* cv. Strong Tosa (inter-specific squash hybrid), and *Citrullus lanatus* var. *lanatus* cv. Tri-X 313 (triploid seedless watermelon). Scion material was *Citrullus lanatus* var. *lanatus* cv. Tri-X 313(triploid seedless watermelon). All seeds were provided by Syngenta Seeds, Inc., Boise, Idaho. The soilless mix was a custom mix prepared by Conrad Fafard Inc., Anderson, SC with the following composition: 75% NB (New Brunswick) nursery peat, 25% coarse perlite, 2.04g/m of dolomitic limestone, and 453.6g/ m of gypsum. This mix is similar to the 3B mix (Conrad Fafard Inc.) but without a nutrient charge. Rootstocks were grown in 72 square vented plug trays (cell depth of 5.7 cm with top and bottom cell diameters tapering from 4.0cm to 2.5 cm TLC Polyform, Inc. Morrow, GA). The scions were seeded in 288 square plug trays (cell depths of 3.8 cm with top and bottom cell diameters tapering from of 2.1 cm. to 1.1 cm TLC Polyform, Inc. Morrow, GA). Rootstock and scion seeds were sown in a truss built glass greenhouse at the United States Department of Agriculture Vegetable Laboratory in Charleston, SC during the fall 2008 and winter 2009. The greenhouse was one compartment from the multi-greenhouse structure. The greenhouse area and specifications were as follows: 289.6 m^2. The environmental conditions were controlled using a step 50 alpha control system (Wadsworth Co., Arvada, CO). This control system controlled the TF-75 gas fired heater (Sterling Co., Westfield, MA), the evaporative (6.7 m long) cooling system (Aerotech Amunters Co., Madison, MI), circular vent fan 50.8 cm patented plant-air VS20PA circulation and the 40.6 in direct drive flush mount style

variable speed exhaust fan placed in the gable (Schaefer, Souk Rapids, MN), and two 76.2 cm fans (Acme Engineering and Manufacturing Corp., Muskogee, OK).

Each rootstock and scion seeds were sown (30 trays of each) at different dates based on a preliminary study (data not shown) to coincide with the development of each respective 1st, 2nd, and 3rd rootstock leaf number stage (RLNS) (see Table I & Appendix-A). The RLNS development in this study is defined as follows: The 1st RLNS is when the cotyledons are fully expanded and the appearance of the 1st leaf is visible to the eye; the 2nd RLNS is when cotyledons and the 1st true leaf are fully expanded and the appearance of the 2nd leaf is visible to the eye; and the 3rd RLNS is when the cotyledons, 1st, and 2nd true leaf are fully expanded and the appearance of the 3rd leaf is visible to the eye. Rootstock seeds were sown at approximately 1.5 cm depth in the soilless mix and maintained moist until germination was complete. Scion 'Tri-X 313' seeds were sown at approximately 1 cm depth in soilless mix using germination methods developed by Hassell and Schulthies (2002). All seedlings were fertilized with 100 ppm with 15-5-15 fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) using the Anderson Injector Series S (H.E. Anderson Co., Muskogee, OK) once cotyledons were fully extended and as needed to prevent excessive etiolating and to maintain healthy plants.

New Grafting Method and Analysis

Rootstocks species were grafted at separate times starting with interspecific squash hybrid, followed by the bottle gourd, wild watermelon, and the seedless hybrid watermelon at three different RLNS each. All rootstock plants were grafted using the cotyledon devoid grafting method.

Cotyledon Devoid Graft

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: 1) using a sterile single edge Kobalt blade (Warner Manufacturing Company, Minneapolis, MN) rootstocks were first cut just below both the cotyledons at a 180º angle to remove all possible meristematic regions (Fig. 5 & Appendix-A). 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. 2) 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 2 cm below the cotyledons with an opposing 65 º angle to the rootstock slice and preserved in a 3.8 L zip-lock bag to help prevent wilting until it was used. 3) Exposed vascular tissue in the scion and rootstock hypocotyl was then joined together as precisely as possible to maximize the contact region between the two and immediately secured with a spring loaded clip to finalize the grafting procedure (Syngenta Seeds Inc., Boise, ID). 4) Using a sterile blade, the rootstock was then excised below the soil line, and 5) stuck in new soil media for re-rooting.

Grafting Experiment

The night prior to grafting, 10 flats (at the first true leaf) of the original 30 of both the rootstock (72 cell count) and scion (128 cell count) material were placed inside the head house with approx. room temperature at 23ºC. This was done to promote the closure of the stomata prior to grafting to minimize wilting. One flat from each (scion and rootstock) was randomly selected and set aside for plant growth analysis. Within this flat, plants were randomly divided into ten plant subsamples with five replications. While keeping the plants intact, relative chlorophyll content for each of the 10 plant subsamples was individually

measured of the cotyledon and leaf (if present) using the Chlorophyll Meter SPAD-502 (Minolta Inc., Ramsey, NJ). Each value measured by the SPAD meter corresponds to the amount of chlorophyll present in the plant tissue being measured. One reading was taken from each tissue of interest. These values are calculated based on the amount of light transmitted by the leaf in two wavelength (red and infrared) regions in which the absorbance of chlorophyll is different.

These same subsamples were then severed from the roots at the soil line and then further divided into cotyledons, leaves, and hypocotyls for leaf area measurements of the vegetative tissue using a LI-3100 area meter (Li-Cor, Inc., Lincoln, NB). Hypocotyl diameter and length were recorded using a digimatic caliper (Mitutoyo Corp., Aurora, IL).

A second flat of pre-grafting plants were used for carbohydrate analysis. Each sample consisted of a subsample of ten plants that was replicated five times. Samples were taken at random within each subsample and were partitioned according to the leaves, cotyledons, hypocotyl, and roots. The hypocotyls were severed from the roots at the media surface line. The hypocotyl, cotyledons and leaf were partitioned and placed in plastic 0.5 L size freezer bags and immediately stored in the -80ºC ULT 1786 Revco freezer (Kendro Laboratory Products, Asheville, NC) for preservation. Roots were then hand washed by first gently rinsing of the bulk soil in a sink. The roots were then placed on a custom made box sieve, made from 3.2 mm stainless steel hardware cloth on a 60cm x 47cm wood frame, which would allow small particles of soilless mix to wash through while keeping the roots intact. They were then sprayed using a fine mist spray nozzle (low-flow spray valve asm) (T&S Brass, Simi Valley, CA) to remove the remaining debris and then stored in a zip-lock bag in the -80ºC freezer. The scion material tissue samples consisted of the complete scion portion

of the leaves, cotyledon and partial hypocotyl (used in grafting) all still intact. These samples were also placed in the -80ºC freezer at the same time as the rootstock samples.

The remaining eight flats were grafted using the "Cotyledon Devoid Method". Within those eight flats, four flats were grafted as explained previously (excluding steps 4 and 5) and randomly placed in the healing chamber. The other four flats were also grafted but had the roots excised and repotted as a final step (Fig.5, step 5). This was done by cutting the hypocotyl just below the soil baseline using a sharp blade. Cutting below the baseline ensured minimal root primordia would remain to help speed the rooting process. The amount of root primordia left varied with each excision. Seedlings were then replanted in pre-moistened soilless mix within a 72 cell tray and were randomly-placed inside the same healing chamber as the other four. The custom made healing chamber was located inside the greenhouse and was tunnel shaped to keep humidity in, while allowing sun light to reach the plant leaves. It was constructed using wire hoops on top of a rectangular wood box with the following dimensions: width of 86 cm, a length of 300 cm, and a depth of 14 cm. The covering consisted of 6mm thick clear polyethylene sheeting. The hoops over the box top increased the height 28 cm above the wood frame box. The humidity was maintained using the 707U-duct mount centrifugal atomizer humidifier (Herrmidifier, Effingham, IL) located at one end of the chamber, and was recorded in conjunction with the temperature using the Watchdog model 100 water resistant button loggers (Spectrum Technologies, Inc., Plainfield, IL). Photosynthetic light was measured using the quantum light sensor (Spectrum Technologies, Inc., Plainfield, IL). Temperatures inside the chamber varied from 21.1 ^{o}C to 35.6 ^{o}C during the night and day respectively. The relative humidity was maintained close to 100%. Seedlings were grown under low light intensity, with photosynthetic active radiation (PAR) at 286 $\mu M/m^2s$ at noon. Low light reduced phototranspiration to prevent plant wilting. Forty-

eight hours after grafting, light intensity was increased to approximately 900 μM/ m^2s PAR by removing shade cloths. Humidity was gradually reduced after day three in the humidity chamber

One day prior to healing completion and seven days post grafting, four flats were removed from the healing chamber, two from each treatment (with roots or without roots). From each of these treatments, one flat was used to take additional subsamples from the post-grafted plants in preparation for carbohydrate analysis as described before and the second was used for plant growth analysis. This time plant tissue samples consisted of the leaves, cotyledons, and hypocotyl from the scion, and the hypocotyl and root (if present) from the rootstock. Subsamples were taken in a destructive manner as before, consisting of ten plants, with five replications. The scion portion was severed from the grafted plants, and the hypocotyl and vegetative portions were then separated and immediately stored in the -80°C freezer. The rootstock hypocotyl was cut off of the roots (if present) for sub-sampling and also stored in the -80°C freezer. Available roots were then washed to remove soilless media as the same manner as described before and then stored in the -80°C freezer. The second tray was used to measure leaf/organ area and chlorophyll measurements from the cotyledons and leaves.

Eight days after grafting, the remaining four trays of transplants, were then removed first thing in the morning from the healing chamber and placed randomly on the greenhouse benches and watered to saturation with 100 ppm fertilizer treatment of 15 (N)-5 (P_2O_2)-15 (K_2O). Grafting clips were removed nine days after grafting. Graft survival was then evaluated and recorded using the subjective rating system outlined in Table 1, eleven days after healing completion. Plants were evaluated and scored depending on the degree of survival of each plant. Rating score ranged between 1-10, with one being completely dead,

and 10 being very alive. Values in between the range indicated relative survival or desiccation.

Grafting and plant analysis for the second and third RLNS for 'Strong Tosa' rootstock took place at a later date (Table 2) and was performed in the same manner as described for the first leaf. Additionally, 'Emphasis', 'Ojakkyo', and 'Tri-X 313'were subsequently individually grafted, evaluated, and prepared for carbohydrate analysis at each RLNS (1^{st}, 2^{nd}, and 3^{rd}) using a different time table but using the same method as was described for the 'Strong Tosa' rootstock (Table 2).

Carbohydrate Analysis

Plant subsamples, consisting of plant tissue from 10 plants each were removed from the -80ºC freezer and immediately freeze dried using a Vertis-Genesis 25EL freeze dryer (FP Industries, Gardiner, NY) for approximately 7 days until completely dry. All subsamples were then ground, before proceeding to the carbohydrate extraction, using the A11 Basic S1 Analytical Mill with the A 11.1 SS grinding blade (IKA Works, Inc., Wilmington, NC) and placed into 20 ml vials and re-stored in a -20 ºC (8.8 cu. Ft Chest Freezer Frigidaire, Martinez, GA) to prevent carbohydrate and tissue disintegration. The dry weight for each composited 10 plant sample was recorded.

For each sample, fifty mg ($\pm$.03mg) of dried plant tissue was weighed using the Analytic Sartorius Weigh Balance (Brinkmann Instruments, Inc., Westbury, NY). All extractions followed a methanol-chloroform-water extraction protocol (Miller and Langhans, 1989). Once carbohydrate extraction was complete, five ml of the final volume of extracted carbohydrates was dispensed into five 1 ml micro-tubes in preparation to remove methanol from the extract. Samples were dehydrated for approx. four hours using the Thermo Savant SC100 SpeedVac Centrifugal Vacuum (Thermal Scientific, Waltham, MA). Samples were

then prepared for quantification by suspending the dried sample in 1 ml pure H_2O: 50ul of the carbohydrate-H_2O solution was transferred into two wells each on a 96-well flat bottom bacterial micro-plate (VWR International, LLC, Suwanee, GA). Total carbohydrates were then further prepared using the phenol-sulfuric acid method (Masuko, 2005) and immediately analyzed in the SpectraMax Plus 384, a high throughput micro plate spectrophotometer (Molecular Devices, Sunnyvale, CA) for total carbohydrates. Each micro-plate contained a standard prepared from D- (-) Fructose (Life Sciences and Biochemicals, St. Louis, MO). The standard consisted of the following concentrations: 31μg/ml, 62μg/ml, 100 μg/ml, 150μg/ml, 200 μg/ml, and 250 μg /ml. This protocol gave us total carbohydrate concentration within the sample based off of calorimetric reaction. Carbohydrates per plant organ were then calculated based of the total dry weight of the10 plant composite sample and total carbohydrate concentration measured.

Statistical Analysis

Data were analyzed using PROC GLM procedure of PC SAS (SAS v.8, Cary, N.C.) to determine the effects of rootstock, RLNS and root treatment and their interactions. If the F test was significant at P=0.05 and 0.01, the means were separated by LSD at P = 0.05 and 0.01. The relative importance of the rootstock, RLNS and root treatment factors and uncontrolled error were determined by partitioning of the total sum of squares in the analysis of variances (ANOVA) into main and interaction effects and expressing these individual contributions to variation as a percentage of the total sum of squares for the model. The value of these percentages is that they become very useful indicators to compare which factors contributed most variation to growth, quality and yield variables relative to the other factors. Significant differences will be referred in this thesis simply as a decrease or increase if

significant. Insignificant increases or decreases will not be mentioned unless stated as not significant.

Once plants were grafted and put in the healing chamber, the experimental design was a complete randomized design. Data was then analyzed as a three factor design. The third factor was rootstock treatments where roots were left intact or excised and re-rooted in fresh media. ANOVA was performed on main effects (rootstock scion, leaf stage and root treatment) and interactions using the GLIMMIX procedure (Table 11).

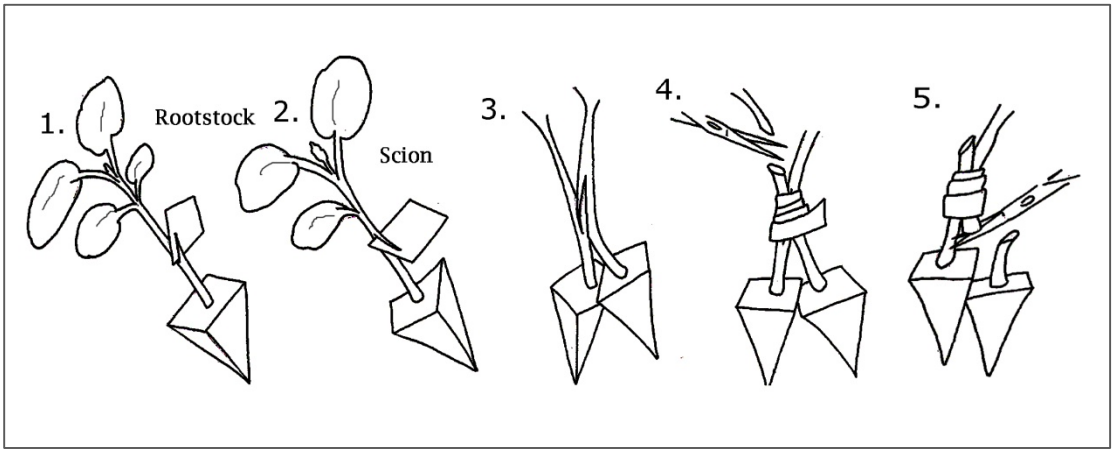

Figure 1. Tongue approach graft 1) the rootstock and 2) scion being cut; 3) union of scion and rootstock; 4) complete removal of rootstock meristem; and 5) complete removal of scion root. Picture provided by (Hassell et al., 2008).

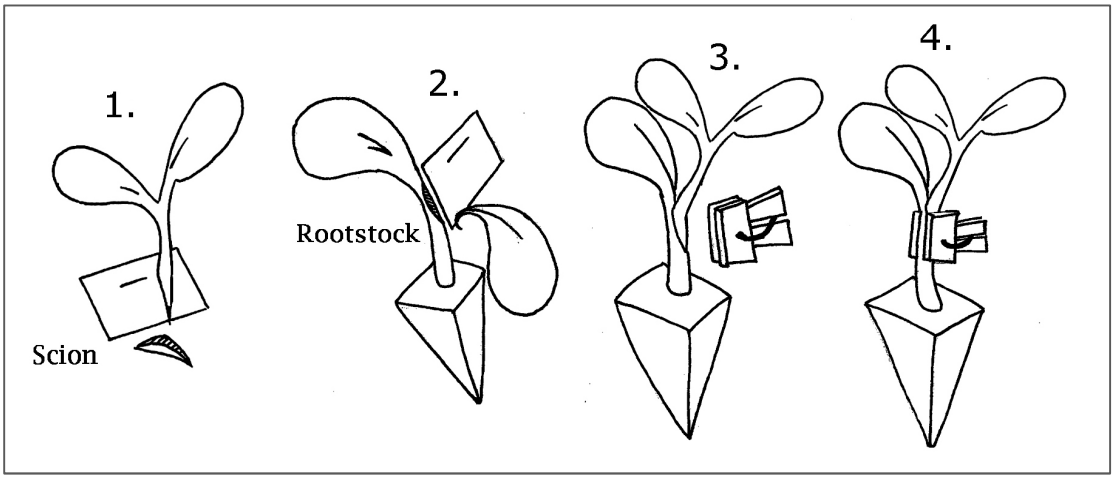

Figure 2. One cotyledon graft 1) cut scion at an approximate 65^{o} angle; 2) remove apical meristem and one cotyledon; 3) cut off cotyledon at an approximate 65^{o} angle; 4) attach scion onto rootstock; and 5) secure the graft with a clip. Picture provided by (Hassell et al., 2008).

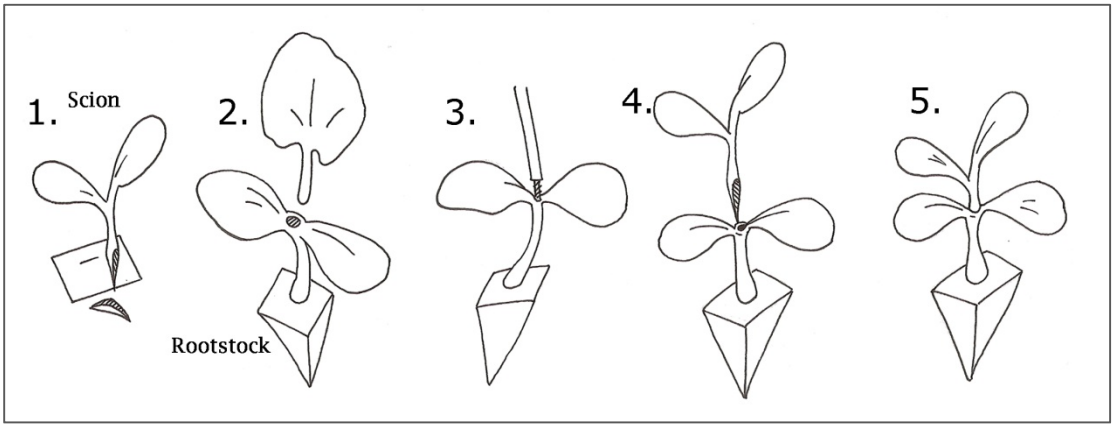

Figure 3. Hole insertion grafting method 1) the scion is cut at approximately 65º on two sides forming a point; 2) meristematic tissue is removed; 3) a hole for the scion to be fitted in is drilled at a slant between the cotyledons and just through the hypocotyl of rootstock; 4) the scion is aligned to fit snugly in the rootstock; and 5) it is then securely inserted into the rootstock. Picture provided by (Hassell et al., 2008).

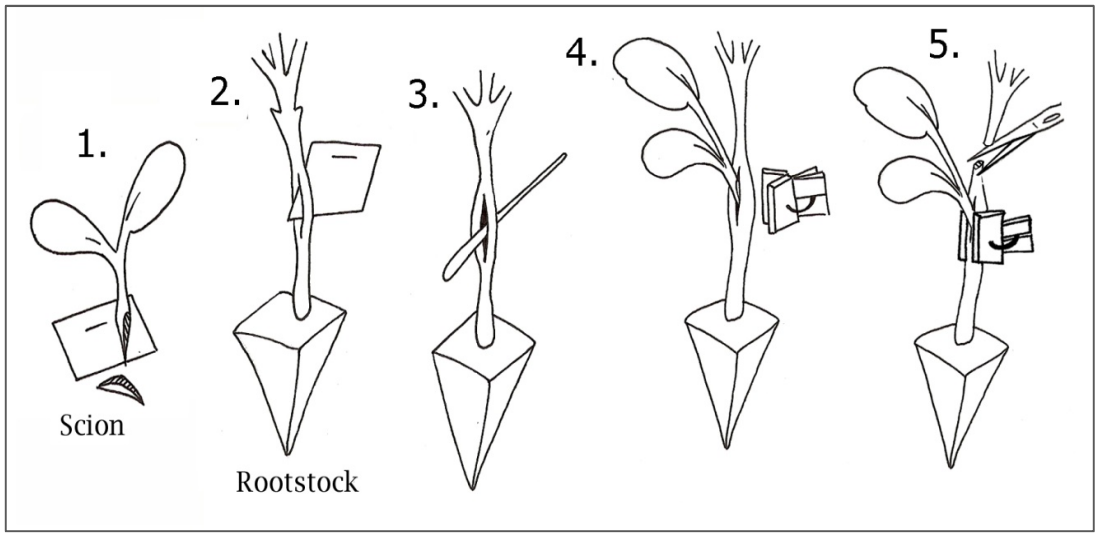

Figure 4. Side graft 1) the scion is cut at approximately 65º on two sides forming a point; 2) a simple slice is made through the rootstock hypocotyl; 3) the splice is then prop open using a toothpick or stick; 4) the scion is inserted into the rootstock, and secured with a graft clip; and 5) the vegetative portion from the rootstock is cut just below the cotyledons**.** Picture provided by (Hassell et al., 2008).

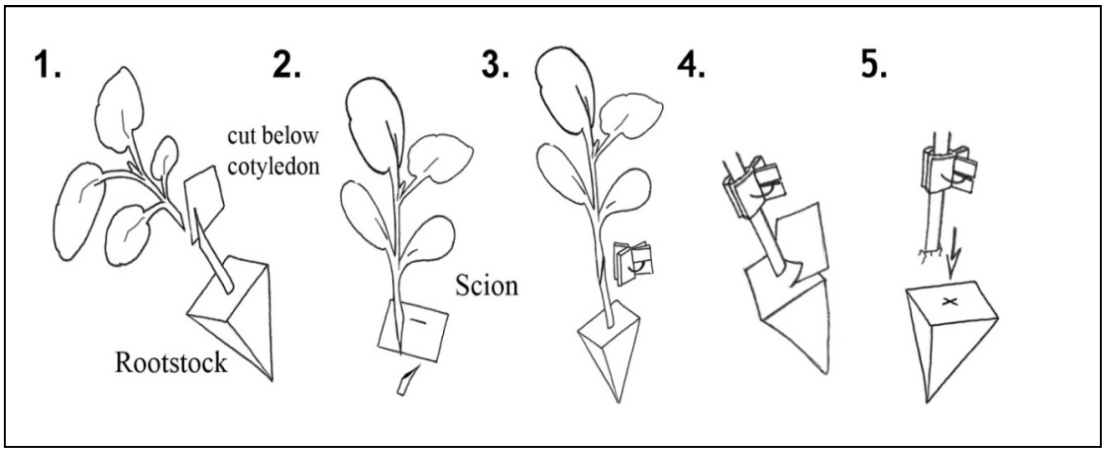

Figure 5. Cotyledon devoid grafting method 1) both cotyledons are cut from the rootstock removing all meristematic tissue at an approximate 65^{o} angle; 2) the scion is cut at an approximate 65^{o} opposing slant to the rootstock; 3) the scion and rootstock wounded regions are joined and secured with a clip; 4) the rootstock hypocotyl is cut just below the baseline; and 5) the grafted seedling is then planted in a new cell with soil media.

Table 1. Subjective qualitative rating scale to describe the condition of the grafted transplants after healing and hardening occurred.

Rating	Degree of condition	Description	Notes
Very poor	0	Dead	Dead
poor	1	Alive but survival highly unlikely	Almost dead
	2		Moderating between surviving or not
	3		Borderline but will probably die
Poor to fair	4	Will survive but be slowed and stunted	Severely stunted
	5		moderately stunted
	6		Somewhat stunted
Fair	7	Survive but growth less than optimal	Fair but not acceptable
	8		Borderline acceptable
Good	9	Satisfactory or acceptable, survival, growth and vigor	Good and acceptable but not the best Acceptable
Superb	10	Impressive and optimal growth, vigor	Optimal results

Table 2. Scheduled dates when each rootstock, scion and leaf number (RLNS) were seeded and treatment data recorded.

Rootstock	Rootstock type[z]	RLNS	Rootstock planting dates	Scion planting dates	Data Collection A[y]	Data Collection B[x]
Strong Tosa	C.mo. x C.ma.	1	20-Oct	17-Oct	30-Oct	8-Nov
		2	20-Oct	17-Oct	4-Nov	13-Nov
		3	20-Oct	17-Oct	10-Nov	19-Nov
Emphasis	L.s.	1	17-Nov	18-Nov	2-Dec	11-Dec
		2	17-Nov	18-Nov	5-Dec	14-Dec
		3	17-Nov	18-Nov	11-Dec	20-Dec
Ojakkyo	C.l Var.c.	1	8-Feb	6-Feb	17-Feb	24-Feb
		2	6-Feb	3-Feb	19-Feb	26-Feb
		3	8-Feb	6-Feb	27-Feb	6-Mar
Tri-X 313	C.l. Var.l(3x)	1	4-Mar	4-Mar	16-Mar	23-Mar
		2	4-Mar	4-Mar	19-Mar	28-Mar
		3	4-Mar	4-Mar	25-Mar	1-Apr

[z]Type is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima*, L.s. = *Lagenaria siceraria*, C.l Var.c.=*Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).

[y]A includes dates consist of when area measurements and carbohydrate preparation prior to grafting.

[x]B includes dates consist of when area measurements and carbohydrate preparation were recorded after seven days in the healing chamber.

RESULTS AND DISCUSSION

Aerial Growth Results Prior to Grafting

The ANOVA for aerial growth indicated that rootstock genotype interacted with RLNS for hypocotyl, cotyledon and leaf variables (Table 3). Even though there were interactions, the amount of variation assigned to the main effects varied among each aerial growth factor. The main effects of rootstock and RLNS were similar with the hypocotyl length, diameter and area. Rootstock main effect accounted for most of the variation in cotyledon area yet RLNS main effect accounted for most of variation in color. RLNS accounted for most of variation in leaf area and leaf color. The hypocotyl length and area, cotyledon area and color, and leaf area and color had low error values indicating the model accounted for most of uncontrolled error. Hypocotyl diameter had the greatest error value; even though the coefficient of variation was low. The hypocotyl length and area, and leaf area had the greatest coefficients of variation then other variables. The hypocotyl diameter, cotyledon area and color and leaf color all had low coefficients of variances, indicating a better degree of precision.

Rootstock genotype interacted with RLNS affecting all pre-grafting aerial growth variables (Table 4). Rootstocks will be referred to simply by its cultivar name. Hypocotyl length, total area, and leaf area of ‘Strong Tosa’ increased as each RLNS increased. Hypocotyl diameter as well as the cotyledon area remained similar at the first and second RLNS, but increased at the third RLNS. Rootstock cotyledon color decreased as RLNS increased from first to third RLNS. The leaf color decreased from the second RLNS to the third RLNS. ‘Emphasis’ hypocotyl diameter, hypocotyl area and leaf area increased at each RLNS. Hypocotyl length and cotyledon area were similar at the first and second RLNS, but increased at the third RLNS; however, the cotyledon color remained unchanged for the first

and second RLNS and decreased at the third RLNS. The leaf color was unaffected by RLNS. Hypocotyl length, diameter, and total area, and leaf area of 'Ojakkyo' increased as RLNS increased. The cotyledon color decreased as each RLNS increased. The cotyledon area increased from the first to the second RLNS and then leveled, and remained unchanged at the third RLNS. Leaf color increased from the second to the third RLNS. With the 'Tri-X 313', the hypocotyl diameter, leaf area and leaf color increased as RLNS increased, however, cotyledon color decreased. The hypocotyl length remained unaffected by RLNS. Subsequently, the total area of the hypocotyl remained the same at the first and second RLNS but increased at the third. The cotyledon area were fully developed once they reached the second RLNS and leveled without any further increases at the third RLNS. The leaf color increased from the second to the third RLNS. Not only did RLNS affect rootstock genotype, but scion hypocotyl, cotyledon and leaf also showed similar effects.

With scion 'Tri-X 313', RLNS main effect accounted for the majority of variation in the hypocotyl, cotyledon and leaf aerial growth and cotyledon and leaf color (Table 5). The hypocotyl diameter, hypocotyl area and cotyledon area had the greatest unexplained error values. Scion plant growth factors, hypocotyl area and leaf area had the greatest coefficients of variation values. All other error and coefficients of variation values were considered minimal. The scion 'Tri-313', which was also a rootstock cultivar, developed in a similar manner (Table 6). The hypocotyl length, hypocotyl area, and leaf area increased as each RLNS increased however, this did not occur when used as a rootstock. The explanation for this difference is as follows: these plants were grown in a smaller tray size with half the surface area; therefore, the hypocotyl continued to stretch to compete for enough sunlight for growth. The cotyledon color decreased with each RLNS increase. The cotyledon area increased up to the second RLNS and remained unchanged at the third RLNS. There was no

difference in the leaf color at the second and third RLNS. Leaf and cotyledon chlorophyll color readings varied among the first, second and third RLNS (all scion material appeared to be healthy at the time of grafting). The average SPAD values recorded represents a healthy value at each respective first, second, and third RLNS. Readings below 20 SPAD units can be considered very low in chlorophyll color and in poor health.

Carbohydrate Tissue Concentration Prior to Grafting

The carbohydrate ANOVA indicated that rootstock genotype interacted with RLNS for rootstock cotyledon, hypocotyl, leaf and root tissues, but not with scion 'Tri-X 313' tissue (Table 7). Additionally, the amount of variation accounted by the main effects varied among the carbohydrate growth variables. The majority of variation on hypocotyl and root carbohydrates was attributed to the main effect of rootstock; however, RLNS greatly affected leaf carbohydrates. Scion carbohydrates were only affected by both main effects of rootstock and RLNS. The rootstock cotyledon, hypocotyl, and roots possessed great levels of unexplained error; however, the hypocotyl coefficients of variance were quite low. Rootstock leaf carbohydrates had the greatest level of unexplained error of all. Rootstock leaf error was low, with a high coefficient of variation.

Rootstock genotype interacted with rootstock RLNS treatment indicating that rootstock genotype developed differently at each of the pre-grafted RLNS (Table 8). The carbohydrate concentrations in 'Strong Tosa' was unaffected by the change in RLNS in the cotyledon, hypocotyl, leaf or root tissues. 'Emphasis' cotyledon carbohydrates decreased at the third RLNS only. The carbohydrate concentration within the hypocotyl, leaf, and root were similar by RLNS, identical effect as with 'Strong Tosa'. 'Ojakkyo' carbohydrate concentrations decreased in the cotyledon from the first to the second RLNS and stayed stationary at the third RLNS. The hypocotyl and root carbohydrate concentrations decreased

from the first to the second RLNS, but returned to the same levels as the first RLNS. Leaf tissue carbohydrate concentration increased from the second to the third RLNS. 'Tri-X 313' carbohydrate concentration in the cotyledon progressively decreased from the first through the third RLNS but the only significant decrease difference was from the first to the third RLNS. The hypocotyl carbohydrate concentration was similar as the first and second RLNS and then increased at the third RLNS. Leaf carbohydrate concentrations were unaffected by the change in RLNS. Root carbohydrate concentrations were similar at the first and third RLNS, but decreased at the second RLNS. The scion 'Tri-313' total plant carbohydrates were similar and greater at the first and third RLNS, but reduced at the second RLNS (Table 6).

Total Plant Organ Carbohydrates Prior to Grafting

The ANOVA for total carbohydrate per plant organ revealed that rootstock interacted with RLNS for each of the plant organs (Table 9). Amount of variation attributed to the main effects varied among the plant organs. Both rootstock and RLNS accounted for the main source of variation in the cotyledon, but the change in RLNS accounted for the main source of variation in the hypocotyl, leaf and scion tissue. The rootstock, however, was the main source of variation in the root. The amount of unexplained error was very low, but the coefficient of variation was slightly high for each of the response variables. Scion tissue showed the greatest coefficient of variation.

Rootstock genotype interacted with rootstock RLNS treatment indicating that rootstock genotype developed differently at each RLNS (Table 10). 'Strong Tosa' increased in total carbohydrates per sample at each increase in RLNS in the cotyledon, hypocotyl, leaf and root organs. 'Emphasis' increased in carbohydrates at each increase in RLNS in the hypocotyl and roots, but increased only at the second RLNS and remaining unchanged at the third RLNS in the cotyledon organ. The total carbohydrates in the leaf increased from the

second to third RLNS. ‘Ojakkyo’ carbohydrates did not increase at any RLNS in the cotyledon organ; however, a non-significant rise was observed at each RLNS. The hypocotyl carbohydrates did increase at the second RLNS from the first RLNS and remained unchanged through the third RLNS. The leaf carbohydrates increased from the second to the third RLNS. The roots carbohydrates increased from the first RLNS through the third RLNS. ‘Tri-X 313’ cotyledon carbohydrates increased at the third RLNS compared to the first, while the second RLNS did not differ from either of the first or third RLNS. The carbohydrates for the hypocotyl increased at the third RLNS. The leaf carbohydrates did not increase at either RLNS while the total carbohydrates in the root increased at the first through the third RLNS. When using the ‘Tri-X 313’ as the scion material, total plant organ carbohydrates increased at the third RLNS (Table 6).

Aerial Growth and Carbohydrate Discussion Prior to Grafting

Each of the four rootstock’s aerial growth variables increased from the first to the third RLNS. The rootstock hypocotyl is of greatest interest because it’s the organ specifically used in grafting. As the hypocotyl increased from the first to the third RLNS, the length, diameter, and area also increased. The carbohydrate analysis revealed that hypocotyl carbohydrates per gram of tissue did not increase directly with any increase in RLNS. However, calculations of sample dry weight with its respective carbohydrate concentration suggested that total carbohydrates within the plant organ greatly increased from the first to the third RLNS, due to the fact that the organ was larger. ‘Tri-X 313’ hypocotyl area increased only at the third RLNS even though the diameter increased with each increasing RLNS. Apparently, indicating grafting should be delayed until the appearance of the third RLNS in order to allow the hypocotyl to fully develop before it is excised for grafting.

The rootstock hypocotyl may not be fully developed at the first RLNS. At the appearance of the first RLNS, the rapid developing seedling appeared to be very tender more than at the second or third RLNS suggesting the inferior structural development and a greater dependency for photosynthates at this first RLNS stage. At the third RLNS, leaf area greatly increased, the hypocotyl should have decreased its need to grow at this point to not compete with the true leaves as a sink, during this critical growth change. The strength of the sink dictates where the photosynthates accumulate Taiz and Zeiger (Taiz and Zeiger, 2006). If the true leaf should grow rapidly before the hypocotyl is fully developed, the hypocotyl may not be able to compete for photosynthates, which will impede its ability to grow and support the aerial tissues' demand.

Aerial Growth After Grafting

Variation assigned to interactions and main effects differed among the scion aerial growth variables (Table 11). Leaf area interacted with rootstock, RLNS and root treatment. Of the three factors, RLNS accounted for most of the variation in all factors. Leaf color also exhibited a three way interaction with the source of variation almost equivalent among the rootstock, RLNS. The scion cotyledon area displayed three, two way interactions; root treatment by RLNS, rootstock by RLNS, rootstock by root treatment with RLNS contributing the greatest amount of variation. The scion cotyledon color also had a three way interaction with RLNS contributing for most of the variation than the other factors. Grafting success, like other variables, also had a three way interaction with RLNS assigned the majority of variation. In contrast to all variables, the scion leaf color, cotyledon area and color had the greatest levels of unexplained error. The leaf color and grafting success coefficient of variation were low but, scion leaf area, cotyledon area and color in contrast had greater coefficient of variation.

Rootstock, RLNS and root treatment interacted, affecting scion cotyledon, scion leaf and overall success of the graft (Table 12). Evaluating only the roots treatment intact, rootstock cultivars interacted with RLNS on scion cotyledon color, scion leaf color, scion leaf area and grafting success. When grafted on 'Strong Tosa', scion cotyledon and true leaf color decreased at each RLNS while the scion leaf area increased at each RLNS. Grafting success score increased with each RLNS and reached 8.4 by the third RLNS (highest level reached with roots intact) judged by the criteria on Table 1. With 'Emphasis', the scion cotyledon color decreased at each RLNS; however, the scion leaf color decreased only at the third RLNS. The scion leaf area increased as RLNS increased. Grafting success score increased at each RLNS and reached 9.8 by the third RLNS. The scion cotyledon color of 'Ojakkyo' decreased at each RLNS; however, the scion leaf color increased from the first to the second RLNS and then slightly decreased at the third RLNS. The scion leaf area increased with each RLNS. The grafting success score increased from the first to the second RLNS achieving 10, and remained the same through the third RLNS. When grafted on 'Tri-X 313', the scion cotyledon chlorophyll color decreased at each RLNS; however, the scion leaf chlorophyll color decreased from the first to the second RLNS and remained unchanged at the third RLNS similar to the first RLNS. The scion leaf area increased from the first to the second RLNS and remained unchanged at the third RLNS. Grafting success score increased only from the second to the third RLNS and reached a final score of 9.5.

In evaluation of only root treatment excised, rootstock cultivars interacted with RLNS affecting the scion cotyledon, scion leaf and overall success of the graft (Table 12). When grafted on 'Strong Tosa', scion cotyledon color remained unchanged from the first to the second RLNS and decreased at the third RLNS while leaf area increased at each RLNS. The scion leaf color increased from the first to the second RLNS, but decreased from the

second to the third RLNS. Grafting success score increased from the first to the second RLNS and remained unchanged at the third RLNS reaching its highest score of 8.9. With ‘Emphasis, the scion cotyledon color decreased at each leaf stage while the scion leaf area increased from the first to the second RLNS and remained unchanged at the third RLNS. The scion leaf color remained unchanged from the first to the second RLNS but decreased at the third RLNS compared to the first RLNS. Grafting success score increased at each of the three RLNS and peaked at 98. With ‘Ojakkyo’, the scion cotyledon color decreased at each leaf stage while the scion leaf area increased at each RLNS. The scion leaf color however, decreased from the first to the second RLNS and remained unchanged at the third RLNS. The grafting success score increased from the first to the second RLNS reaching 10 and then remained unchanged through the third RLNS. When grafted on ‘Tri-X 313’ the cotyledon color remained unchanged from the first to the second RLNS and decreased at the third RLNS while the scion leaf area increased from the first to the second RLNS, but decreased at the third RLNS; however, this decrease remained greater than the first RLNS. Scion leaf color decreased at the third RLNS only. Grafting success score increased with each RLNS reaching 88 by the third RLNS.

Rootstock cultivars interacted with RLNS, regardless of root treatment, affecting scion cotyledon area (Table 13). When grafted on rootstock, ‘Strong Tosa’, ‘Emphasis’ or ‘Ojakkyo’ scion cotyledon area increased at the second RLNS and remained the same at the third RLNS. ‘Tri-X 313’ also increased at the second RLNS, similar to ‘Strong Tosa’, ‘Emphasis’ and ‘Ojakkyo’, but decreased at the third RLNS. ‘Strong Tosa’ and ‘Emphasis’ had the greatest cotyledon area at both the second and third RLNS compared to ‘Ojakkyo’ and ‘Tri-X 313’.

Rootstock interacted with root treatment regardless of RLNS affecting scion cotyledon area (Table 14). ‘Emphasis’ had the greatest scion cotyledon area with roots left intact. ‘Strong Tosa’ and ‘Tri-X 313’ equally followed with ‘Ojakkyo’ having the smallest cotyledon area of all rootstocks. ‘Strong Tosa’ and ‘Emphasis’ scion cotyledon had the largest scion cotyledon area when roots were excised. When grafted on ‘Ojakkyo’ and ‘Tri-X 313’, the scion cotyledon area decreased equally having the smallest area of the four rootstocks. ‘Emphasis’ and ‘Tri-X 313’ decreased in scion cotyledon area with roots excised. ‘Strong Tosa’ and ‘Ojakkyo’ remained unchanged regardless to root treatment.

RLNS interacted with root treatment regardless of rootstock affecting scion cotyledon area (Table 15). After grafting, the scion cotyledon area increased at the second RLNS without further increase at the third RLNS with roots left intact. With roots excised, the scion cotyledon area also increased at the second RLNS with no further increase at the third RLNS. The third RLNS decreased in area when roots were excised versus intact. The scion cotyledon area appeared greatest at second RLNS with roots left intact or at the third RLNS with roots excised.

Carbohydrate Tissue Concentration After Grafting

The ANOVA from the carbohydrate concentrations confirmed that a three way rootstock by RLNS by root treatment interaction existed for the rootstock hypocotyl and in the scion hypocotyl (Table 11). With the rootstock hypocotyl interaction, root treatment and RLNS main effects were similar in amount of variation each contributed. The root treatment effect contributed the majority of variation in the scion hypocotyl. The rootstock by RLNS interaction affected rootstock roots, with RLNS contributing most of the variation. The scion cotyledon and leaf had three two-way interactions of rootstock by RLNS, rootstock by root treatment and RLNS by root treatment. The variation in scion cotyledon rootstock by RLNS

interaction dominated over the other two interactions with the rootstock as the major source of variation. The rootstock by root treatment interaction contributed most of the variation to scion leaf carbohydrates with rootstock being the more dominant effect. The rootstock roots, rootstock hypocotyl, scion cotyledon, scion hypocotyl and scion leaf variables, all possessed very large amount of unexplained error. The rootstock roots and hypocotyl both had larger coefficient of variation but scion cotyledon, hypocotyl and leaf had smaller coefficient of variation, indicating greater precision in predicting a response.

Carbohydrate analysis from the post-graft seedling material displayed a three way rootstock by RLNS by root treatment interaction with rootstock roots, rootstock hypocotyl, and scion hypocotyl (Table 16). With rootstock roots left intact, rootstock interacted with RLNS development for rootstock roots, rootstock hypocotyl, and scion hypocotyl. The rootstock roots and rootstock hypocotyl from grafted 'Strong Tosa' increased in carbohydrate concentration from the second to the third RLNS. Carbohydrates in the grafted scion hypocotyl portion decreased from the first RLNS to the second, but then returned to the same level at the third RLNS as in the first RLNS. Similar to 'Strong Tosa' and 'Emphasis' rootstock roots and hypocotyl carbohydrates increased only at the third RLNS. The carbohydrate concentration in the 'Emphasis' rootstock grafted scion hypocotyl remained unchanged at any of the three RLNS. Carbohydrate concentrations in 'Ojakkyo' roots and scion hypocotyl increased at the second RLNS, and remained unchanged at the third RLNS. 'Ojakkyo' root carbohydrate levels were lower at the third RLNS compared to 'Strong Tosa' and 'Emphasis' root carbohydrate levels. 'Ojakkyo' rootstock hypocotyl carbohydrates increased at the third RLNS compared to the first, but was not different from the second RLNS. The roots from grafted 'Tri-X 313' progressively increased in carbohydrates at each of the three RLNS. At the first and second RLNS, 'Tri-X 313' rootstock roots had the

greatest carbohydrate levels than the other rootstocks at the same RLNS. The third RLNS root carbohydrate levels were similar to levels found in ‘Strong Tosa’ and ‘Emphasis’ third RLNS roots. The rootstock hypocotyl and scion hypocotyl remained unchanged through the all three RLNS for ‘Tri-X 313’.

With rootstock roots excised, RLNS and rootstocks interacted, affecting rootstock and scion hypocotyl carbohydrates (Table 16). Rootstock roots were absent after having been excised prior to healing. ‘Strong Tosa’ rootstock and scion hypocotyl soluble carbohydrate concentration did not differ at any of the RLNS. ‘Emphasis’ hypocotyl increased in carbohydrates at the third leaf only compared to the first and second RLNS. The grafted scion cotyledon displayed no increase at any RLNS but remained large and unchanged through the third RLNS. For ‘Ojakkyo’, the carbohydrates in the rootstock and scion hypocotyl did not differ at any of the three RLNS. ‘Tri-X 313’ hypocotyl increased in carbohydrates at the third RLNS in contrast to the first and second RLNS. The scion hypocotyl was not at the three RLNS.

Rootstock hypocotyls maintained greater levels of carbohydrate concentrations when roots were excised versus left intact during healing (Table 16). ‘Strong Tosa’ hypocotyl decreased over seven-fold in carbohydrate concentration with roots intact at the first RLNS. At both the second and third RLNS, hypocotyl carbohydrate concentrations decreased over three-fold with roots intact. ‘Emphasis’ decreased over three-fold in carbohydrate concentration at the first and second leaf with roots intact, but had comparable concentrations at the third leaf with roots intact versus excised. With roots excised, ‘Ojakkyo’ hypocotyl maintained over three-fold greater carbohydrate concentrations at the first RLNS. At both the second and third RLNS, carbohydrate concentrations decreased over two-fold with roots intact. ‘Tri-X 313’ hypocotyl did not differ in carbohydrate concentration at the first and

second RLNS with roots excised over left intact. At the third RLNS, the rootstock hypocotyl decreased slightly in carbohydrate concentration at the third RLNS with roots intact. The scion hypocotyl had similar carbohydrate concentration with roots left intact or excised for all rootstocks.

Rootstock interacted with RLNS (pooled over root treatment) affecting the grafted scion cotyledon and leaf soluble carbohydrate concentration (Table 13). 'Strong Tosa' rootstock's scion cotyledon carbohydrate concentration decreased at the second RLNS only, but increased at the third RLNS similar to the first RLNS. 'Emphasis' rootstock's scion cotyledon carbohydrate concentration increased at the third RLNS compared to the first. The second RLNS did not differ from the first or third RLNS. 'Ojakkyo' rootstock's scion cotyledon carbohydrate concentration was similar at the first and second RLNS, but decreased at the third RLNS. 'Tri-X 313' rootstock's cotyledon carbohydrates did not differ at any of the three RLNS. The scion leaf carbohydrate concentration remained unchanged for 'Strong Tosa', 'Emphasis', and 'Tri-X 313'; however, 'Ojakkyo' rootstock's scion leaf carbohydrate concentration increased from the first to the second without further increase in the third RLNS.

Rootstock also interacted with root treatment affecting the scion cotyledon and leaf carbohydrate concentration regardless of RLNS (Table 17). 'Strong Tosa', 'Emphasis', and 'Tri-X 313' rootstock's scion leaf carbohydrate concentration did not differ whether roots left intact or excised. 'Ojakkyo' rootstock's scion leaf concentration was lower in contrast to the three other rootstock cultivars when roots were left intact. Excising the roots, though, showed greater carbohydrates present that were equivalent to the other rootstocks concentration. With roots left intact, the scion cotyledon carbohydrate concentration appeared to be greatest with 'Emphasis'. 'Strong Tosa' and 'Tri-X 313' cotyledon carbohydrates were lower compared to

‘Emphasis’ when roots were left intact. ‘Ojakkyo’ had the lowest scion cotyledon carbohydrate concentration among the rootstocks with roots left intact. However, with roots excised, both ‘Emphasis’ and ‘Tri-X 313’ had the greatest amount of scion cotyledon carbohydrate concentration. ‘Strong Tosa’ and ‘Ojakkyo’ also had great scion cotyledon concentration values, but both had lower carbohydrate concentration compared to ‘Emphasis’ and ‘Tri-X 313’.

RLNS also interacted with root treatment in the scion cotyledon and leaf carbohydrate concentration regardless of rootstock (Table 18). The scion leaf carbohydrates had the lowest concentration at the first RLNS with roots intact. The second and third RLNS increased in carbohydrate concentration compared to the first RLNS, but did not differ from one another. When roots were excised both the first and third RLNS had the lowest concentration; however, these concentrations were greater than when roots were left intact. The second RLNS had the greatest amount of carbohydrate concentration, but did not differ from the third RLNS. The scion cotyledon carbohydrate concentration did not differ at any of the three RLNS with roots left intact. With roots excised, the first RLNS had the greatest amount of carbohydrates and was also greater than when roots remained intact. At the second RLNS the carbohydrate concentration decreased, and did not differ from the third RLNS which also did not differ from the first RLNS.

Total Plant Organ Carbohydrate After Grafting

The interaction of greatest interest in the ANOVA for rootstock hypocotyl plant organ carbohydrates after grafting was a three-way interaction of rootstock by RLNS by root treatment (Table 19). The RLNS main affect contributed the greatest portion of variation for the hypocotyl carbohydrates. Rootstock interacted with RLNS affecting carbohydrates in the rootstock roots, scion cotyledon, scion hypocotyl and scion leaf; root treatment had no effect

on these variables. The RLNS effect contributed the most variation to carbohydrates in rootstock roots and scion hypocotyl. Rootstock and RLNS both affected the scion cotyledon and scion leaf variation apparently similarly. All variables, rootstock and scion organ types have small uncontrolled and unexplained errors, and small coefficients of variance, indicating precision.

Root and hypocotyl total organ carbohydrate levels varied with rootstock genotype, RLNS and root treatment (Table 20). 'Strong Tosa' and 'Emphasis' rootstock roots increased in total carbohydrates per plant organ at the third RLNS only, but 'Ojakkyo' and 'Tri-X 313' rootstock root organ incrementally increased in total carbohydrates at each of the three RLNS. When grafted with the root intact, 'Strong Tosa', 'Emphasis', 'Ojakkyo' and 'Tri-X 313' hypocotyl total carbohydrates per plant organ all increased at the third RLNS only. With roots excised, 'Strong Tosa' increased in hypocotyl organ carbohydrates at each change in RLNS. 'Emphasis', 'Ojakkyo' and 'Tri-X 313' however, had no increase in hypocotyl total organ carbohydrates until the third RLNS.

When comparing across root treatment, in general, rootstock hypocotyl carbohydrates decreased between 2 and 9x when roots were left intact, but not all differences appeared to be significant (Table 20). 'Strong Tosa' hypocotyl carbohydrates decreased over 8x with roots intact but at a lower rate than the second and third RLNS with roots excised at the first RLNS. At the second RLNS the carbohydrates per plant hypocotyl organ greatly decreased over 16x with roots intact, but at the third RLNS the carbohydrates per plant hypocotyl organ decreased more than 2x. This difference was much greater than the 9x decrease at the first RLNS. 'Emphasis' had more than a 16x decrease in the carbohydrates per plant hypocotyl organ at the first RLNS and more than a 22x decrease at the second RLNS with roots intact. At the third RLNS, the carbohydrates per plant hypocotyl organ decreased just over 2x.

‘Ojakkyo decreased over 9x in carbohydrates per plant hypocotyl organ at the first RLNS with roots intact and displayed more than a 3x decrease at the second RLNS but was not significant. At the third RLNS, the carbohydrates per plant hypocotyl organ decreased over 3x with roots intact. ‘Tri-X 313’ showed no difference in carbohydrates per plant hypocotyl organ at the first or second RLNS whether intact or excised. Only the third RLNS decreased over 2x in carbohydrates per plant hypocotyl organ with intact roots.

Rootstock genotype interacted with RLNS among the scion aerial carbohydrates per organ variables when pooled over root treatment (Table 21). ‘Strong Tosa’ and ‘Emphasis’ increased in scion cotyledon, hypocotyl and leaf at each increasing RLNS. When grafted on ‘Ojakkyo’, however, only the scion hypocotyl increased at each RLNS without any change to the scion cotyledon and leaf total sample carbohydrates at any of the RLNS. When grafted on ‘Tri-X 313’, scion cotyledon decreased in total sample carbohydrates at the second RLNS compared to the first RLNS, and remained unchanged through the third RLNS versus the first RLNS. The scion leaf carbohydrates remained unchanged from the first to the third RLNS.

Grafting Success

In order for grafting to be successful, success rates need to reach a rating of 9 or above (personal communication, Jim McConnell, Syngenta Seeds Inc.) on the scale in Table 1. Grafting success scores interacted with rootstocks, RLNS and root treatment (Table 11). When comparing across root treatments, cultivars at the three RLNS responded differently when the rootstock roots were left intact or excised prior to healing (Table 12). ‘Strong Tosa’ grafting success did not differ at the first or third RLNS with roots left intact compared to roots excised. At the second RLNS, grafting success increased by 39% when roots were excised. At the first RLNS, ‘Emphasis’ grafting success increased 30% with roots excised rather than with roots left intact without any improvement at the second and third RLNS.

‘Ojakkyo’ was not influenced by root treatment at any RLNS. ‘Tri-X 313’ did not react favorably to root excision prior to healing. At the first and second RLNS, grafting success dropped over 75% and 66% respectively when roots were excised. At the third RLNS, however, grafting success rate dropped only 6% when roots were excised.

The main reason for excising the roots prior to healing, was to allow for mechanization, reduce greenhouse space and facilitate commercialization that would potentially lower grafting costs. In order to add this root treatment (roots excised), all rootstocks must be able to be adapted. However, it was apparent that each rootstock reacted differently at each RLNS. Even though this reaction was not always at the grafting success desired (at least 9), it still gave us a guidelines to follow. ‘Strong Tosa’ reached the critical RLNS for root removal at the second RLNS, but this increase was not enough to reach the critical score of 9. With ‘Emphasis’ the third RLNS is critical to reach the score of 9 and the removal of the existing root didn’t impair this success rate. With ‘Ojakkyo’, the second RLNS was critical to reaching the score of 9 and the removal of the existing roots also did not impair the success rate. ‘Tri-X 313’ reacted negatively to root excision; however, this reaction was greatly diminished as the rootstock grew from the first to the third RLNS. Once the third RLNS had been reached grafting success had reached a successful level and existing roots could be removed with no significant detrimental effect.

Relationship between Hypocotyl Carbohydrates and Grafting Success

Rootstock hypocotyl total carbohydrates and grafting success varied at each RLNS, and there was an apparent relationship between total carbohydrates in the rootstock hypocotyl and grafting success scores at each RLNS depending on rootstock (Fig. 6). ‘Strong Tosa’ rootstock hypocotyl had 105.04 µg total carbohydrates at the first RLNS and a grafting success score of a low 1.5 (roots intact) and 0.8 (roots excised); however, total carbohydrates

levels increased nearly 5x (504.13 µg) at the second RLNS and grafting success score increased by 4x (6) and 10x (8.3) when roots were left intact or excised, respectively. Total carbohydrates further increased 1.3x (643.23 µg) in the rootstock hypocotyl from the 2nd to the 3rd RLNS with grafting success increased by 1.4x (8.4) and 1.1x (8.9) with roots left intact versus excised, respectively. The relationship between total carbohydrates and the grafting score appeared strong at all three RNLS.

'Emphasis' hypocotyl increased in carbohydrates at each RLNS. Total hypocotyl carbohydrates was 260.75 µg at the first RLNS when grafting success was about 3.9 and considered very low (roots intact) and 5.1 (roots excised); however, total carbohydrate levels increased 1.3x (349.43 µg) at the second RLNS where grafting success score increased by 2.2x (8.5) and 1.7x (8.4) when roots were left intact or excised respectively. Total carbohydrates further increased 2.1x (728.39 µg) in the rootstock hypocotyl from the 2nd to the 3rd RLNS where as grafting success scores also increased by 1.2x (9.8) and 1.2x (9.8) with roots intact versus excised, respectively. The relationship between total carbohydrates and the grafting score also appeared strong at the 1st and 3rd RNLS but not as strong at the 2nd RLNS.

'Ojakkyo' hypocotyl increased in carbohydrates from the first to the third RLNS but not at the second RLNS. The hypocotyl had 56.12 µg total carbohydrates at the first RLNS when grafting success score was also low being 5.8 (roots intact) and 5.0 (roots excised); however, total carbohydrates levels decreased 1.2 fold (45.47 µg) to the second RLNS where grafting success score increased by 1.7x (10) and 2.0x (10) when roots were left intact or excised respectively. Total carbohydrates then increased 9.4x (428.71 µg) in the rootstock hypocotyl form the 2nd to the 3rd RLNS where as grafting success scores remained the same

with roots intact verse excised respectively. The relationship between total carbohydrates and the grafting score also appears strong at the 1st and 3rd RNLS but weak at the 2nd RLNS.

'Tri-X 313' hypocotyl followed a similar carbohydrate levels as 'Ojakkyo' but did increase at each RLNS. The hypocotyl had 51.27 µg total carbohydrates at the first RLNS when grafting success score was high 7.5 (roots intact) and low 1.9(roots excised); however, total carbohydrates levels increased 1.3x (68.05 µg) to the second RLNS where grafting success score increased by 1.1x (8.3) and 2.0x (3.7) when roots were left intact or excised respectively. Total carbohydrates then increased 5.6x (382.08 µg) in the rootstock hypocotyl form the 2nd to the 3rd RLNS where as grafting success scores also increased by 1.1x (9.5) and 2.4x (8.8) with roots intact verse excised respectively. The relationship between total carbohydrates and the grafting score appears weak with roots intact at any of the RLNS. However the relationship appears strong when roots were excised at all three RLNS.

Grafting Success Prediction Analysis

The goal was to determine the relationship between grafting success and total hypocotyl organ carbohydrates (Fig. 6) that predicts the carbohydrate levels that coincide with a grafting success score of 9. This information allows growers and researchers to know the minimal level of carbohydrates necessary to achieve acceptable grafting success. Regression of grafting success scores with total hypocotyl organ carbohydrates predicted the model that best explained the desired total hypocotyl organ carbohydrates (Fig. 7). Each rootstock cultivar followed a different pattern suggesting different total carbohydrate levels may be required for each rootstock to achieve the minimally ideal grafting success score of 9. Values given by r^2 varied among rootstocks and whether their roots were excised or left intact. Both 'Strong Tosa' and 'Emphasis' rootstocks had high and fair r^2 values (0.90 and 0.70, respectively) regardless of whether the roots were left intact or excised. 'Tri-X 313'

rootstock had a similar high r^2 value (0.92) when roots were excised but r^2 decreased when they were left intact (0.59). 'Ojakkyo' had the lowest r^2 (0.21) of all rootstocks regardless of root treatment.

The overall carbohydrate concentration and hypocotyl dry weight values were also individually regressed with grafting success (data not shown) to determine their status at 9 grafting success score. Table 22 summarizes the scale of level of hypocotyl carbohydrates, carbohydrate concentration and dry weight that correspond to the grafting success score of 9. Overall, total carbohydrates in hypocotyl organ required to reach a success score of 9 varied with rootstock but were minimally affected by root treatment. For example the carbohydrate concentration (μg/ml) among rootstock cultivar hypocotyls ranged between 250 μg/ml and 308 μg/mg at success score of 9 and reflected smaller differences between rootstocks than the overall carbohydrates (μg) per whole hypocotyl organ. 'Strong Tosa' had the greatest amount of hypocotyl carbohydrates (μg) and overall dry weight (g/hypocotyl) at time of grafting among all rootstocks. 'Emphasis' had the second greatest amount of carbohydrates (μg) and dry weight value per hypocotyl. 'Ojakkyo' possessed the smallest amount of carbohydrates (μg) and dry weight per hypocotyl, which indicated a greater grafting success with less carbohydrates present. This could be attributed to 'Ojakkyo' having the closest family relationship with the scion than the 'Strong Tosa' and 'Emphasis' rootstocks. The self graft control 'Tri-X 313' had second to the smallest overall carbohydrates (μg) and dry weight per hypocotyl. By negating the roots excision, the carbohydrate (μg) and dry weight per hypocotyl are very close to the 'Ojakkyo' rootstock hypocotyl, and also support the idea that less carbohydrates (μg) and dry weight per hypocotyl are needed in obtaining the minimally grafting success score of 9. Hypocotyls that possess more carbohydrates are heavier. In general, hypocotyl carbohydrate concentration remained similar among rootstocks and

overall dry weight accounted for the vast increase in total carbohydrates per hypocotyl. The hypocotyl weight and size affected the overall carbohydrates present and grafting success even though the carbohydrate concentration tended to remain unchanged. This study suggests that the size of the hypocotyl increases grafting success, not only by increasing the diameter as suggested by Oda et al. (1993) but also through an increased amount of carbohydrates (μg) stored within the rootstock hypocotyl.

Aerial Growth and Carbohydrate Discussion After Grafting

Grafting success score increased as the rootstock and scion seedling matured from the first to the third RLNS not only in response to an increased diameter but also from increased carbohydrate levels present. The increase in hypocotyl length, diameter and area at each RLNS increased with grafting success score. Oda et al. (1993) suggested that the increased diameter also contributes to an increase in contact region between vascular bundles which increases grafting success. Although this may have contributed to an increased success score, it does not stand alone since the cotyledon plays a varied and vital role in grafted seedling survival, being the main source or photosynthates. Furthermore, 'Strong Tosa' had the largest diameter out of the four rootstocks but did not have the greatest grafting success score, but to the contrary, had the lowest score among the other rootstocks when grafted at the second RLNS as opposed to the first. This indicates additional factors influenced and contributed to the increased success such as carbohydrates.

Hypocotyl carbohydrates increased with overall size. The increase in hypocotyl area at each increased RLNS also suggested that a larger storage capacity is present in the hypocotyl to store reserves. Carbohydrates per gram of hypocotyl tissue did not increase with grafting success scores; however, the overall amount of total carbohydrates present in the rootstock hypocotyl organ increased from the first to the third RLNS with grafting success

scores (Fig. 6). This was true with few exceptions for most rootstocks. As the carbohydrates in the plant hypocotyl increased at each RLNS, so did grafting success scores at each RLNS for ‘Strong Tosa’ and ‘Emphasis’.

In general, the root excised hypocotyl had greater amounts of total carbohydrates versus the hypocotyl with the root intact suggesting greater amounts are needed during the healing period to maintain the roots and heal the graft (Tables 16 and 20). Removing the root allows for mechanization and increased productivity at lower costs. Mechanical equipment that is used for the one cotyledon graft method currently excises the root to facilitate the grafting procedure, and can easily be adjusted to perform this graft if the root can be removed while maintaining grafting success score. If mechanization is not available, growers should consider not excising the roots but keeping the root intact. Greater nutrient reserves remained present in the hypocotyl during healing with the removal of one major sink (growing root tips). This great depletion indicated that carbohydrates were consumed during the healing process and plays a major role in sustaining the grafted seedling. Another important point is that the scion hypocotyl tissue had greater total carbohydrate levels at each RLNS than the rootstock hypocotyl at each RLNS. This suggests perhaps, that rootstock incompatibility could be restricting the translocation of carbohydrates to the rootstock through the graft union. Further research is necessary to determine if there is a particular carbohydrate being restricted or if there are many different carbohydrates being restricted or if fertility can moderate carbohydrates and grafting success.

‘Ojakkyo’ did not follow the same carbohydrate and grafting success score trends as ‘Strong Tosa’ and ‘Emphasis’. At the first RLNS ‘Ojakkyo’ grafting success score was very close to 5 when roots were left intact or excised, being the greatest grafting success score over all rootstocks of interest at that RLNS (Table 12). ‘Ojakkyo’ is the closest related

rootstock to the scion material (besides the self graft control) and had the greatest grafting success with the lowest amount of carbohydrates present among the rootstocks. Incompatibility between the rootstock and scion is expected to be less with a closer related scion and rootstock (Andrews and Marquez, 1993). This being the case, we should also expect a greater success score in the scion self graft control, and lower carbohydrates present to obtain realistic grafting success.

'Tri-X 313' hypocotyl carbohydrates followed a similar trend as 'Ojakkyo', but not grafting success score which resembled 'Strong Tosa' and 'Emphasis' more similarly. At the first RLNS 'Tri-X 313' grafting success score was the greatest with roots left intact having greatly decreased with roots excised. The hypocotyl carbohydrate levels were low at the first and second RLNS, but increased at the third RLNS. These 'Tri-X 313' grafted plants had a lower root regeneration rate which showed a sensitive hypocotyl rerooting response (being a triploid hybrid with flat stems) which also accounts for the low grafting success score with roots excised. When roots were left present, the predicted carbohydrate (μg/ hypocotyl) level was similar to those that correspond to 'Ojakkyo'.

Rootstock genotype reacted differently; however, planting days may account for some of the differences. Each rootstock genotype was grafted and sampled in a different month, from fall through spring. Light intensity was low and varied due to cloudy weather, and shorter day length. This was done due to the limited greenhouse space available, large population size, and available man power to carry out the grafting in one day to reduce the amount of variation and potential introduced error. Future studies may want to test if the variation between rootstock genotypes was due in part to this variable since light is the contributing source of photosynthates. The decrease in carbohydrate levels at the second

RLNS for ‘Ojakkyo’ and ‘Tri-X 313’ does not sound reasonable and may be direct error of these environmental conditions.

The preferred rootstock genotype for commercial production is dependent on growers needs and resources; the RLNS at which grafting should be performed is also rootstock genotype dependant. Based on these findings and grafting scores, I recommend that ‘Strong Tosa’, and ‘Emphasis’ with scion ‘Tri-X 313’ seedlings be grown to the third RLNS before grafting (with roots left intact or excised) to maximize grafting success, and in order to successfully eliminate rootstock re-growth using the “Cotyledon Devoid Method”. ‘Ojakkyo’ grafted with scion ‘Tri-X 313’ can be grafted as early as the second RLNS to achieve optimal results. The greatest grafting success rate of all cultivars is achieved by grafting with ‘Ojakkyo’ which is the rootstock of preference for scoring 10 by the second RLNS. It is the overall weight and size that affected the carbohydrate leaves and overall grafting success sores. By allowing the hypocotyl to develop past the first RLNS to the third RLNS (rootstock dependent), the overall weight and carbohydrate levels increased sufficiently to achieve realistic grafting success.

CONCLUSIONS

In order for watermelon grafting to be successful in the United States, the cost of the transplants needs to be affordable to the grower. The costs are associated with labor both in performing the graft and then in maintaining the transplant. Current commercial methods being practiced throughout the world are both labor intensive and costly to maintain making them inadequate for our needs in the U.S. Besides labor, by not eliminating meristematic tissue causes the rootstock to regenerate the original rootstock plant causing possible scion abortion or yield reduction if not removed. With the introduction of the "Cotyledon Devoid Method" as described in this thesis, all the above concerns would be eliminated, thus reducing the costs of the transplant. Current automated equipment can be easily adapted to perform this new method. By eliminating the need for at least one cotyledon, these automated machines would not have to be constantly adjusted to remove the majority of meristematic growth while maintaining at least one cotyledon, thus reducing their costs as well. The differences found in the rootstock and scion material before and after grafting, indicated that the development of seedlings before grafting is critical for the success of the cotyledon devoid graft method.

Before Grafting

Rootstock and scion types germinate and grow at a much different rates.

Seed emergence time varied among rootstock genotypes. Rootstock genotype *Cucurbita moschata* x *Cucurbita maxima* cultivar Strong Tosa emerged the earliest followed by *Citrullus lanatus Var. Citroides* cultivar Ojakkyo and finally *Lagenaria siceraria* cultivar Emphasis. After emergence, the rate of development to the second and third RLNS also varied between rootstock genotypes. 'Strong Tosa' developed at the fastest rate followed by 'Ojakkyo' and finally 'Emphasis'. Scheduling the planting times to coincide with RLNS

development is necessary to maximize grafting success. Furthermore, scheduling the planting times will also allow greenhouse space to be maximized by only allowing the seedlings to develop to the minimum number of days necessary to achieve greatest grafting success. Scion material (Triploid watermelon seed) needs to germinate and emerge uniformly; using strong vigorous seed lots and the germination process developed by Hassell and Schulthies (2002) will insure uniformity. Insuring that the rootstock and scion develop to the same stage of growth prior to grafting is essential for grafting success. Devising a germination and developmental growth parameter recommendation that would encompass all rootstocks is impossible. However, knowing the germination and growth rates of each of the rootstock genotypes and scion material is a first step to make this grafting method a success.

Rootstock and scion aerial growth at each leaf developmental stage proved to be critical to grafting success.

Generally, the rootstock hypocotyl length, diameter, and area of most cultivars and scion material (scion cotyledon area and color and scion leaf) increased at each RLNS and related to final grafting success. As the scion cotyledon and leaf area increased at each leaf stage of development, the grafting success score increased as well. Scion material quality played a significant role in grafting success. 'Tri-X 313' grafting success increased significantly only at the 3rd leaf stage when roots were excised indicating that the scion material may not be able to contribute nutritional reserves until it has reached the third RLNS. These results suggested that the more nutrient reserves accumulated in both the rootstock and the scion, the better the chance of a successful graft. Further research is needed to find methods to increase nutrient load within the rootstock and scion material prior to grafting to insure constant success of the graft, such as hormones, nutrients, or environmental manipulation.

After Grafting

Rootstock roots influenced total hypocotyl carbohydrate concentration.

When roots were excised, the rootstock hypocotyl maintained greater levels of total carbohydrates than when roots were left intact at each of the three RLNS regardless of rootstock. This suggested that the roots required a large amount of carbohydrates as a sink (Taiz and Zeiger, 2006) while remaining active once grafting has taken place. In addition to the increasing carbohydrate levels at the three RLNS, hypocotyl senescence no longer occurred at the second and third RLNS after healing which suggested sufficient nutrients were present to maintain the root system and heal the graft at these two RLNS. The overall depletion in hypocotyl total carbohydrates before and after grafting when roots were left intact versus excised at the three RLNS, demonstrated the strength of the roots as a sink. With each increasing RLNS greater than the first RLNS, more carbohydrates accumulated in the hypocotyl so when grafting occurred, sufficient nutrients remained in the hypocotyl to maintain root activity and heal the graft.

Hypocotyl carbohydrates reserves increased from the first to the third RLNS.

Rootstock hypocotyls showed different levels of total carbohydrates at different RLNS regardless of rootstocks roots being intact or excised for all cultivars. Total carbohydrates per hypocotyl organ increased from the first to the third RLNS, suggesting a relationship between carbohydrates and grafting success. The larger the hypocotyl, the more carbohydrates accumulated and a greater success score was achieved. Previous studies by Asahina et al. (2002),Oda et al. (1993), Traka-Mavrona et al. (2000), Andrews and Marquez (1993), and Shan-fa et al. (1996) have focused on and found that gibberellic acid, cytokinins and auxins such as IBA, larger hypocotyl diameters (which increase vascular contact region between the rootstock and scion) increase grafting success. This study also relates the overall

increase in weight of the hypocotyl from the first to the third RLNS increases overall carbohydrates per hypocotyl and increases grafting success with the cotyledon excised during the grafting procedure. Further research is needed to test the predicted levels indicated to confirm these results and next identify individual carbohydrates present within the hypocotyl organ to determine which is primarily important or are there many carbohydrates that influence grafting success.

Rootstock genotype reacted differently to roots excision or left intact at different RLNS.

Grafting success was not only influenced by RLNS but also by rootstock treatment (rootstock dependent). The "Cotyledon Devoid Method" was most successful when performed at the second or third RLNS to achieve the greatest grafting success. RLNS was the main determining factor in grafting success; however, the treatment of excising rootstock roots at each of the three RLNS did not decrease grafting success. Although scion leaf area was greater with roots present this difference was negligible for most rootstocks. 'Strong Tosa' increased in grafting success whether the rootstock roots were excised at the second RLNS. 'Ojakkyo' and 'Emphasis' did not differ in grafting success whether the roots were left intact or excised. 'Tri-X 313' responded, however, just the opposite with the best grafting success rate achieved when roots were left intact.

Delay of rootstock hypocotyl root regeneration occurred with different rootstocks which decreased plant survival. Hypocotyl root regeneration occurred at acceptable rates only with 'Strong Tosa' and 'Ojakkyo'. 'Emphasis' exhibited a greater inability to re-root when the roots were excised even though the nutrient reserves were great. This is further evidence that rootstock genotypes responded independently of each other making it difficult to make a standard recommendation across all cultivars to either leave the rootstock roots intact or

excised after grafting. The reason for less root regeneration is unknown. Further research is necessary to determine techniques to effectively stimulate rooting with difficult rootstocks such as rooting hormones, nutrient loads, and optimal rooting environment (humidity and temperature). The next step in this research is use grafted seedlings using the “Cotyledon Devoid Method” in a field study to examine how well they hold up to environmental stresses upon transplanting and also if crop yield is affected by the grafting procedure.

Table 3. Sources of variation[z] in the analysis of variance (ANOVA) for aerial growth and chlorophyll color index of four rootstocks at three different RLNS[y] before grafting.

	Percent of total sums of squares						
	Hypocotyl			Cotyledon		Leaf	
Source of variation	Length	Diameter	Area	Area	Color[x]	Area	Color[x]
Replication	0.66	0.95	0.50	0.23	0.54	0.01	0.06
Rootstock (RS)	31.27**	39.45**	25.94**	73.36**	6.25**	18.98**	1.38**
RLNS	29.86**	12.18**	23.11**	14.50**	49.58**	41.24**	96.61**
RS * RLNS	30.02**	35.60**	45.66**	10.07**	40.25**	39.30**	1.57**
Error	8.20	11.83	4.79	1.84	3.38	0.48	0.37
CV	12.03	8.56	16.36	7.75	3.69	13.68	5.11

** F values significant at $P = 0.01$.

[z]The sum of squares for each factor in the ANOVA were converted to a percentage of the total sum of squares.

[y]RLNS is rootstock leaf number stage.

[x]Derived by SPAD measurements.

Table 4. Two-way interaction of four rootstocks and three different RLNS[z] at grafting on aerial growth and chlorophyll color index.

Rootstock cultivar	Rootstock genotype[x]	RLNS[w]	Hypocotyl[y]			Cotyledon[y]		Leaf[y]	
			Length (mm)	Diameter (mm)	Area (cm^2)	Area (cm^2)	Color (SPAD)[v]	Area (cm^2)	Color (SPAD)[v]
Strong Tosa	C.mo. x C.ma.	1	39.3 d[u]	3.6 b	1.3 d	18.2 b	73.5 a	0.3 f	-----
		2	48.6 c	3.3 cd	1.5 c	19.5 b	51.4 c-e	4.2 e	42.1 a
		3	73.7 a	5.3 a	4.3 a	31.3 a	36.9 i	58.7 a	38.3 b
Emphasis	L.s.	1	27.4 e	2.7 ef	0.7 f	14.8 cd	53.0 b-d	0.6 f	-----
		2	30.2 e	3.4 c	1.1 e	15.4 c	51.7 c-e	4.1 e	36.0 c
		3	57.3 b	3.1 d	1.8 b	19.6 b	46.8 g	10.9 c	35.8 c
Ojakkyo	C.l Var.c.	1	29.0 e	2.5 f	0.6 g	7.4 e	54.1 b	0.0 f	-----
		2	37.9 d	2.8 e	1.1 e	13.4 d	48.0 fg	6.5 d	28.5 e
		3	55.5 b	3.3 cd	1.8 b	14.4 cd	50.7 de	13.5 b	35.3 c
Tri-X 313	C.l. Var.l(3x)	1	28.2 e	2.3 g	0.7 fg	5.3 f	53.7 cb	0.3 f	-----
		2	31.6 e	2.8 e	0.8 f	7.1 e	49.6 ef	2.9 e	32.8 d
		3	33.2 de	3.2 cd	1.1 e	7.9 e	39.4 h	9.5 c	36.0 c

[z]RLNS is rootstock leaf number stage.
[y]Values represent a mean of a ten plants replicated five times.
[x]Genotype is C.mo. x C.ma. = *Cucurbita moschata* x *Cucurbita maxima*, L.s. = *Lagenaria siceraria*, C.l Var.c. = *Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[w]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[v]SPAD values are defined by Minolta as the relative amount of chlorophyll present in plant leaves; greater value means greener.
[u]Means within columns followed by a different letter are significant by LSD at $P = 0.05$.

Table 5. Sources of variation[z] in the analysis of variance (ANOVA) for scion aerial growth and chlorophyll color index at three different RLNS[y] before grafting.

	Percent of total sums of squares						
	Hypocotyl			Cotyledon		Leaf	
Source of variation	Length	Diameter	Area	Area	Color[x]	Area	Color[x]
Replication	0.06	0.67	3.85	6.26	0.13	0.92	0.03
Rootstock (RS)	44.41	4.91	23.08	14.90	11.07	13.46	1.80
RLNS	28.40**	18.57*	38.94**	44.44**	38.64**	67.85**	96.95**
RS * RLNS	22.86	8.25	9.13	8.98	15.64	14.78	1.01
Error	4.27	67.60	25.00	25.42	4.97	2.99	0.21
CV	6.65	10.68	17.51	7.92	3.34	18.81	3.87

*,** F values significant at $P = 0.05$ or $P = 0.01$.

[z]The sum of squares for each factor in the ANOVA were converted to a percentage of the total sum of squares.

[y]RLNS is rootstock leaf number stage.

[x]Derived by SPAD measurements.

Table 6. Main effect of RLNS[z] (pooled over rootstocks) on scion[y] aerial growth, chlorophyll color index, carbohydrate[x] concentration and total carbohydrates per scion organ before grafting initiation.

		Hypocotyl[w]			Cotyledon[w]		Leaf[w]			
Scion cultivar	RLNS[u]	Length (mm)	Dia. (mm)	Area (cm^2)	Area (cm^2)	Color (SPAD)[t]	Area (cm^2)	Color (SPAD)[t]	Carbohydrates[v] (µg/g)	Carbohydrates[v] (µg/scion)
Tri-X 313	1	33.6 c[s]	2.7 b	0.7 c	5.6 b	53.5 a	0.6 c	-----	226.8 a	48.41 b
	2	43.7 b	3.0 a	1.2 b	6.9 a	48.5 b	4.7 b	33.8 a	153.1 b	67.60 b
	3	48.3 a	3.1 a	1.3 a	6.5 a	41.2 c	10.4 a	34.7 a	212.7 a	169.92 a

[z]RLNS is rootstock leaf number stage.
[y]Scion is defined as the grafted portion including the hypocotyl cotyledons, and leaf.
[x]Carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[w]Values represent a mean of a forty plants replicated five times.
[v]Values represent a mean of two readings pooled forty plants replicated five times.
[u]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[t]SPAD values are defined by Minolta as the relative amount of chlorophyll present in plant leaves; greater value means greener.
[s]Means within columns followed by a different letter are significant by LSD at $P = 0.05$.

Table 7. Sources of variation[z] in the analysis of variance (ANOVA) for seedling total tissue carbohydrate[y] concentration for entire plant including the scion and rootstocks at three different RLNS[x] before grafting.

	Percent of total sums of squares				
Source of variation	Cotyledon	Hypocotyl	Leaf	Root	Scion
Replications	10.81	2.29	0.38	7.28	0.67
Rootstock (RS)	22.06**	49.68**	8.89**	55.28**	31.92**
RLNS	16.26**	9.10**	79.12**	10.89**	23.11**
RS * RLNS	18.77**	9.43*	5.37**	13.70**	20.59
Error	32.10	29.51	6.24	12.84	23.71
CV	20.43	9.64	23.46	11.00	19.51

[*,**] F values significant at $P = 0.05$ or $P = 0.01$.

[z]The sum of squares for each factor in the ANOVA were converted to a percentage of the total sum of squares.

[y] Total carbohydrate is defined as the sum of sucrose, glucose and fructose.

[x]RLNS is rootstock leaf number stage.

Table 8. Two-way interaction of four rootstocks and three different RLNS[z] at grafting on total tissue carbohydrate[y] concentrations.

Rootstock cultivar	Rootstock genotype[x]	RLNS[w]	Cotyledon (µg/ml)[v]	Hypocotyl (µg/ml)[v]	Leaf (µg/ml)[v]	Root (µg/ml)[v]
Strong Tosa	C.mo. x C.ma.	1	280.8 a[u]	286.8 b-e	-----	270.9 a
		2	284.1 a	290.7 a-d	253.3 ab	275.8 a
		3	276.3 a	278.6 c-f	279.7 a	282.5 a
Emphasis	L.s.	1	239.0 a-c	323.1 a	------	268.7 a
		2	236.1 a-c	298.3 a-c	186.4 cd	265.1 a
		3	139.8 d	316.2 ab	215.2 bc	278.8 a
Ojakkyo	C.l Var.c.	1	280.1 a	263.0 d-g	------	258.8 a
		2	130.2 d	195.6 h	102.8 e	127.7 c
		3	186.2 cd	256.1 e-g	179.2 cd	193.9 b
Tri-X 313	C.l. Var.l(3x)	1	252.2 ab	253.6 fg	------	202.4 b
		2	209.0 bc	232.0 g	164.5 d	141.9 c
		3	184.1 cd	267.5 c-f	174.8 d	185.0 b

[z]RLNS is rootstock leaf number stage.
[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]Genotype is C.mo. x C.ma. = *Cucurbita moschata* x *Cucurbita maxima,* L.s. = *Lagenaria siceraria,* C.l Var.c. = *Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[w]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[v]Values represent a mean of two readings pooled from ten plants replicated five times.
[u]Means within columns followed by a different letter are significant by LSD at $P = 0.05$.

Table 9. Sources of variation[z] in the analysis of variance (ANOVA) for seedling total carbohydrates per plant organ including the scion and rootstocks at three different RLNS[y] at grafting.

	Percent of total sums of squares				
Source of variation	Cotyledon	Hypocotyl	Leaf	Root	Scion
Replications	3.03	.40	0.05	1.53	1.07
Rootstock (RS)	43.03**	22.58**	15.45**	78.60**	39.74**
RLNS	46.96**	73.29**	82.93**	18.28**	55.69**
RS * RLNS	5.41**	3.52**	1.44**	0.99**	2.28**
Error	1.58	.20	0.13	0.59	1.23**
CV	37.66	17.22	22.33	16.22	45.08

[*,**] F values significant at $P = 0.05$ or $P = 0.01$.

[z]The sum of squares for each factor in the ANOVA were converted to a percentage of the total sum of squares.

[y]RLNS is rootstock leaf number stage.

Table 10. Two-way interaction of four rootstocks and three different RLNS[z] at grafting on total carbohydrates[y] per plant organ.

Rootstock cultivar	Rootstock genotype[x]	RLNS[w]	Cotyledon (µg)[v]	Hypocotyl (µg)[v]	Leaf (µg)[v]	Root (µg)[v]
Strong Tosa	C.mo. x C.ma.	1	124.61 c[u]	105.04 f	-----	248.57 e
		2	230.29 b	504.13 c	67.36 d	338.40 cd
		3	340.40 a	643.23 b	649.88 a	739.04 a
Emphasis	L.s.	1	54.09 de	260.75 e	-----	115.47 fg
		2	200.39 b	349.43 d	103.41 cd	348.32 c
		3	220.85 b	728.39 a	532.33 b	583.64 b
Ojakkyo	C.l Var.c.	1	65.27 c-e	56.12 f	-----	71.25 gh
		2	79.89 c-e	45.47 f	61.68 e	165.99 f
		3	115.99 cd	428.71 c	125.37 c	293.73 c-e
Tri-X 313	C.l. Var.l(3x)	1	36.09 e	51.27 f	-----	37.04 h
		2	98.19 c-e	68.05 f	90.50 c-e	165.60 f
		3	108.18 cd	382.08 d	116.39 c	280.60 de

[z]RLNS is rootstock leaf number stage.

[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.

[x]Genotype is C.mo. x C.ma. = *Cucurbita moschata* x *Cucurbita maxima,* L.s. = *Lagenaria siceraria,* C.l Var.c. = *Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).

[w]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.

[v]Values represent a mean of two readings pooled from ten plants replicated five times.

[u]Means within columns followed by a different letter are significant by LSD at $P = 0.05$.

Table 11. Sources of variation[z] in the analysis of variance (ANOVA) for scion aerial growth and color, carbohydrate[y] concentration and grafting success, seven days after grafting at three different RLNS[x] with rootstock root treatment[w].

	Percent of total sums of squares									
	Scion aerial growth				Carbohydrates					
	Leaf		Cotyledon		Rootstock		Scion			
Source of variation	Area	Color[v]	Area	Color[v]	Roots	Hypocotyl	Cotyledon	Hypocotyl	Leaf	Grafting success
Replication	0.19	0.38	2.27	0.03	2.95	0.62	4.42	11.34	4.94	0.14
Rootstock (RS)	11.02**	17.69**	10.53**	4.26**	14.16**	1.98*	28.72**	5.23	6.16*	12.44**
RLNS	61.04**	22.29**	34.69**	63.76**	46.60**	25.92**	0.92	1.41*	7.73	60.65**
RS * RLNS	16.48**	16.68**	5.28*	4.53**	17.38**	8.56**	13.85**	12.20	2.47*	7.07**
Root treatment (RT)	4.05**	0.27	1.60*	2.32**	-----	28.48**	1.40	8.42*	4.12*	1.40**
RS * RT	1.92**	6.25**	0.69*	0.40	-----	9.93**	8.24**	2.10	12.43**	8.53**
RLNS * RT	1.48**	1.96*	5.27**	0.31	-----	1.37*	1.40*	2.60	0.32*	0.98**
RS * RLNS * RT	0.54*	14.31**	3.50	3.32*	-----	9.53**	2.80	6.45**	4.24	3.68**
Error	3.27	20.16	36.18	21.16	18.	13.66	39.56	50.25	57.60	5.11
CV	13.47	8.51	14.94	15.60	31.75	23.37	9.26	9.19	6.75	10.84

[*,**] F values significant at $P = 0.05$ or $P = 0.01$.

[z]The sum of squares for each factor in the ANOVA were converted to a percentage of the total sum of squares.

[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.

[x]RLNS is rootstock leaf number stage.

[w]Root treatment consist of rootstock roots excised or intact following grafting.

[v]Derived by SPAD measurements.

Table 12. Three-way interaction of four rootstocks and three different RLNS[z] seven days after grafting on scion aerial growth, chlorophyll color index, and grafting success with rootstock roots intact or excised.

			Rootstock roots intact				Rootstock roots excised[y]			
			Cotyledon[x]	Leaf[x]		Grafting[w] success	Cotyledon[x]	Leaf[x]		Grafting[w] Success
Rootstock cultivar	Rootstock genotype[v]	RLNS[u]	Color (SPAD)[t]	Area (cm^2)	Color (SPAD)[t]	Score (0-10)	Color (SPAD)[t]	Area (cm^2)	Color (SPAD)[t]	Score (0-10)
Strong	C.mo. x	1	52.8 a[s]	2.6 lm	31.8 g-k	1.5 ij	42.0 b-e	1.4 mn	31.4 g-l	0.8 j
Tosa	C.ma.	2	42.6 b-e	10.8 ef	39.4 b-d	6.0 f	44.2 bc	9.0 gh	42.0 a-c	8.3 de
		3	30.8 g-i	21.0 a	28.8 lm	8.4 de	29.6 g-j	18.8 b	28.4 k-m	8.9 b-d
Emphasis	L.s.	1	49.4 ab	1.0 n	43.0 ab	3.9 h	44.4 bc	1.0 n	39.2 cd	5.1 fg
		2	43.8 b-d	8.2 hi	42.6 a-c	8.5 de	31.4 f-i	8.2 hi	38.0 de	8.4 de
		3	20.6 k	9.8 fg	30.4 i-l	9.8 ab	22.6 jk	7.6 i	34.6 e-h	9.8 ab
Ojakkyo	C.l.	1	56.0 a	3.8 kl	28.8 j-m	5.8 fg	51.2 a	1.6 mn	44.0 a	5.0 g
	Var. c.	2	36.4 d-g	10.4 f	36.8 d-f	10.0 a	35.8 e-g	5.6 j	34.0 f-i	10.0 a
		3	33.0 f-h	14.0 c	32.0 g-j	10.0 a	26.8 h-k	7.2 i	35.0 e-g	10.0 a
Tri-X 313	C.l.	1	44.6 bc	4.4 jk	34.4 e-h	7.5 e	44.6 bc	4.4 jk	34.4 e-h	1.9 i
	Var. l.(3x)	2	38.8 c-f	11.8 de	31.0 h-l	8.3 de	38.8 c-f	11.8 de	31.0 h-l	3.7 h
		3	25.2 i-k	12.6 d	32.2 g-j	9.5 a-c	21.6 k	8.2 hi	25.2 m	8.8 cd

[z]RLNS is rootstock leaf number stage.
[y]Rootstock hypocotyls were excised from the root system just below the soil line and then placed in new media to re-root.
[x]Values represent a mean taken from ten plants replicated five times.
[w]Grafting success score taken from ten plants replicated five times; defined as 0 = complete death to 10 = completely alive.
[v]Genotype is C.mo x C.ma. = *Cucurbita moschata* x *Cucurbita maxima,* L.s. = *Lagenaria siceraria,* C.l Var.c.=*Citrullus lanatus Var. Citroides, C.I* Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[u]The 1st RLNS is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as the unexpanded 2nd true leaf and the fully expanded 1st true leaf. The 3rd RLNS is defined as the unexpanded 3rd true leaf and the 1st and 2nd expanded true leaves.
[t]SPAD values are defined by Minolta and indicate relative amount of chlorophyll present in plant leaves; greater value means greener.
[s]Means within columns and rows for the same variable that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 13. Two-way interaction of four rootstocks and three different RLNS[z] (pooled over root treatment) seven days after grafting on scion: cotyledon area, cotyledon carbohydrates and leaf carbohydrates[y].

Rootstock cultivar	Rootstock genotype[w]	RLNS[v]	Cotyledon Area[x] (cm^2)	Cotyledon Carbohydrates[x] (µg)	Leaf Carbohydrates[x] (µg)
Strong	C.mo. x	1	4.2 e[u]	282.4 b-e	267.5 ab
Tosa	C.ma.	2	6.9 a	236.0 f	279.9 ab
		3	6.7 a	269.3 c-e	286.0 a
Emphasis	L.s.	1	5.5 c	285.3 b-d	262.0 bc
		2	6.8 a	300.4 ab	278.8 ab
		3	6.6 a	317.5 a	274.0 ab
Ojakkyo	C.l.	1	4.4 e	263.0 de	241.7 c
	Var. c.	2	5.7 c	259.8 e	273.7 ab
		3	5.5 c	234.9 f	261.1 bc
Tri-X 313	C.l.	1	4.8 d	289.1 bc	268.6 ab
	Var. l.	2	6.2 b	290.5 bc	284.3 ab
	(3x)	3	5.8 c	277.7 c-e	269.1 ab

[z]RLNS is rootstock leaf number stage.
[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]Values represent a mean of two readings pooled from twenty plants replicated five times.
[w]Genotype is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima*, L.s. = *Lagenaria siceraria*, C.l Var.c.=*Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[v]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[u]Means within columns followed by a different letter are significant by LSD at $P = 0.05$.

Table 14. Two-way interaction of rootstock (pooled over RLNS[z]) on scion cotyledon area with roots intact or excised seven days after grafting.

Rootstock cultivar	Rootstock genotype[w]	Scion cotyledon[y] area (cm^2)	
		Rootstock roots intact	Rootstock roots excised[x]
Strong Tosa	C.mo. x C.ma.	5.93 b[v]	5.93 b
Emphasis	L.s.	6.53 a	6.07 b
Ojakkyo	C.l. Var. c.	5.33 c	5.07 c
Tri-X 313	C.l. Var. l.(3x)	5.87 b	5.33 c

[z]RLNS is rootstock leaf number stage.
[y]Values represent a mean of thirty plants replicated five times.
[x]Rootstock hypocotyls were excised from the root system just below the soil line and then placed in new media to re-root.
[w]Genotype is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima*, L.s. = *Lagenaria siceraria*, C.l Var.c.=*Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[v]Means within columns and rows that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 15. Two-way interaction of RLNS[z] (pooled over rootstock) on scion cotyledon area with roots intact or excised seven days after grafting.

RLNS[w]	Scion cotyledon[y] area (cm^2)	
	Rootstock roots intact	Rootstock roots excised[x]
1	4.50 c[u]	4.90 c
2	6.65 a	6.15 ab
3	6.60 a	5.74 b

[z]RLNS is rootstock leaf number stage.
[y]Values represent a mean of a forty plants replicated five times.
[x]Rootstock hypocotyls were excised from the root system just below the soil line and then placed in new media to re-root.
[w]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[v]SPAD values are values defined by Minolta which indicate the relative amount of chlorophyll present in plant leaves; greater value means greener.
[u]Means within columns and rows that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 16. Three-way interaction of four rootstocks and three different RLNS[z] seven days after grafting on rootstock and scion tissue carbohydrate[y] concentrations with rootstock roots intact or excised.

			Rootstock roots intact			Rootstock roots excised[x]	
			Rootstock		Scion	Rootstock	Scion
Rootstock cultivar	Rootstock genotype[v]	RLNS[u]	Roots[w] (µg/ml)	Hypocotyl[w] (µg/ml)	Hypocotyl[w] (µg/ml)	Hypocotyl[w] (µg/ml)	Hypocotyl[w] (µg/ml)
Strong	C.mo. x	1	24.0 g[t]	34.6 j	283.0 a-c	263.0 a-c	287.4 ab
Tosa	C.ma.	2	29.5 fg	64.8 h-j	262.4 c	231.2 bc	287.8 ab
		3	158.0 a	83.8 de	297.6 ab	263.2 a-c	302.6 a
Emphasis	L.s.	1	56.5 de	51.6 ij	279.2 a-c	182.0 de	291.0 ab
		2	47.9 d-f	50.4 ij	276.4 bc	159.6 ef	292.4 ab
		3	140.8 a	261.8 a	279.8 a-c	271.8 ab	288.4 ab
Ojakkyo	C.l.	1	37.8 e-g	58.8 ij	228.4 d	224.2 cd	289.2 ab
	Var. c.	2	66.4 cd	73.8 h-j	280.8 a-c	179.6 d-f	295.2 ab
		3	65.5 cd	95.4 g-i	278.0 bc	224.8 cd	279.6 bc
Tri-X 313	C.l.	1	78.4 c	133.0 fg	283.6 a-c	94.8 g-i	297.2 ab
	Var. l.	2	100.5 b	133.2 fg	297.0 ab	111.0 gh	298.4 ab
	(3x)	3	146.9 a	169.0 ef	278.2 bc	276.0 ab	281.0 a-c

[z]RLNS is rootstock leaf number stage.
[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]Rootstock hypocotyls were excised from the root system just below the soil line and then placed in new media to re-root.
[w]Values represent a mean of two readings pooled from ten plants replicated five times.
[v]Genotype is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima,* L.s. = *Lagenaria siceraria,* C.l Var.c.=*Citrullus lanatus Var. Citroides,* C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[u]The 1st RLNS in this study is defined as first unexpanded true leaf. The 2nd RLNS is defined as the unexpanded 2nd true leaf and the fully expanded 1st true leaf. The 3rd RLNS is defined as the unexpanded 3rd true leaf and the 1st and 2nd expanded true leaves.
[t]Means within columns and rows for the same variable that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 17. Three-way interaction of rootstock (pooled over RLNS[z]) seven days after grafting on scion cotyledon and leaf tissue carbohydrate[y] concentration with rootstock roots intact or excised.

		Rootstock roots intact		Rootstock roots excised	
Rootstock cultivar	Rootstock genotype[w]	Scion leaf[x] (μg/ml)	Scion cotyledon[x] (μg/ml)	Scion leaf[x] (μg/ml)	Scion cotyledon[x] (μg/ml)
Strong Tosa	C.mo. x C.ma.	277.53 a[v]	267.67 cd	278.07 a	257.60 d
Emphasis	L.s.	276.53 a	305.40 a	266.67 a	296.73 ab
Ojakkyo	C.l. Var. c.	236.80 b	232.40 e	280.87 a	272.73 cd
Tri-X 313	C.l. Var. l.(3x)	268.07 a	279.73 bc	279.93 a	291.80 ab

[z]RLNS is rootstock leaf number stage.
[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]Values represent a mean of two readings pooled from thirty plants replicated five times.
[w]Genotype is C.mo x C.ma. = *Cucurbita moschata* x *Cucurbita 80áxima*, L.s. = *Lagenaria siceraria*, C.l Var.c.=*Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[v]Means within columns and rows for the same variable that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 18. Two-way interaction of RLNS[z] and cotyledon (pooled over rootstock) seven days after grafting on scion leaf and cotyledon tissue carbohydrate[y] concentration with rootstock roots intact or excised.

	Rootstock roots intact		Rootstock roots excised	
RLNS[w]	Scion leaf[x] (µg/ml)	Scion cotyledon[x] (µg/ml)	Scion leaf[x] (µg/ml)	Scion cotyledon[x] (µg/ml)
1	252.35 c[v]	274.50 bc	267.55 b	285.45 a
2	273.00 b	267.55 c	285.35 a	275.80 bc
3	268.85 b	271.90 bc	276.25 ab	277.90 ab

[z]RLNS is rootstock leaf number stage.
[y]Total carbohydrate is defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]Values represent a mean of two readings pooled from forty plants replicated five times.
[w]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[v]Means within columns and rows for the same variable that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 19. Sources of variation[z] in the analysis of variance (ANOVA) for total carbohydrates[y] per plant organ, seven days after grafting at three different RLNS[x] with rootstock root treatment[w].

	Percent of total sums of squares				
	Rootstock		Scion		
Source of variation	Roots	Hypocotyl	Cotyledon	Hypocotyl	Leaf
Replication	.16	.39	1.01	.10	.07
Rootstock (RS)	4.81**	2.08**	36.63**	9.61**	44.34**
RLNS	91.85**	50.07**	36.84**	73.05**	40.67**
RS * RLNS	2.74**	1.04**	22.97**	16.50**	14.42**
Root treatment (RT)	-----	37.92**	.38	.02	.09
RS * RT	-----	1.61**	1.03	.18	.16
RLNS * RT	-----	6.46**	.38	.03	.06
RS * RLNS * RT	-----	.66**	.52	.29	.16
Error	.44	.13**	.25	.21	.03
CV	50.80	33.12	18.70	19.85	11.22

** F values significant at $P = 0.01$.
[z]The sum of squares for each factor in the ANOVA were converted to a percentage of the total sum of squares.
[y]Total carbohydrates are defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]RLNS is rootstock leaf number stage.
[w]Root treatment consist of rootstock roots excised or intact following grafting.

Table 20. Three-way interaction of four rootstocks and three different RLNS[z] seven days after grafting on rootstock total carbohydrates[y] per plant organ with rootstock roots intact or excised.

Rootstock cultivar	Rootstock genotype[v]	RLNS[u]	Rootstock roots intact		Rootstock roots excised[x]
			Roots[w] (µg)	Hypocotyl[w] (µg)	Hypocotyl[w] (µg)
Strong	C.mo. x	1	8.23 fg[t]	9.52 j	83.42 h
Tosa	C.ma.	2	33.82 ef	18.79 ij	308.29 d
		3	236.10 a	152.37 g	425.41 c
Emphasis	L.s.	1	7.32 g	9.06 j	147.18 g
		2	19.80 fg	7.96 j	182.24 fg
		3	102.49 c	262.43 e	583.20 a
Ojakkyo	C.l.	1	10.91 fg	5.71 j	54.99 hi
	Var. c.	2	59.46 de	12.82 ij	46.25 h-j
		3	218.64 ab	149.02 g	490.14 b
Tri-X 313	C.l.	1	7.32 g	14.99 ij	17.25 ij
	Var. l.	2	67.28 d	26.15 ij	34.32 ij
	(3x)	3	196.64 b	200.85 f	413.53 c

[z]RLNS is rootstock leaf number stage.

[y]Total carbohydrates are defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.

[x]Rootstock hypocotyls were excised from the root system just below the soil line and then placed in new media to re-root.

[w]Values represent a mean of two readings pooled from ten plants replicated five times.

[v]Genotype is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima,* L.s. = *Lagenaria siceraria,* C.l Var.c.=*Citrullus lanatus Var. Citroides,* C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).

[u]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.

[t]Means within columns and rows for the same variable that are followed by a different letter are significant by LSD at $P = 0.05$.

Table 21. Two-way interaction of four rootstocks and three different RLNS[z] (pooled over root treatment) seven days after grafting on scion carbohydrates[y] per plant organ.

Rootstock cultivar	Rootstock genotype[w]	RLNS[v]	Scion		
			Cotyledon[x] (µg)	Hypocotyl[x] (µg)	Leaf[x] (µg)
Strong	C.mo. x	1	92.31 ef[u]	61.08 i	69.47 ef
Tosa	C.ma.	2	109.28 d	184.86 c	448.10 c
		3	155.18 b	427.02 a	692.82 a
Emphasis	L.s.	1	95.08 ef	50.53 j	66.91 ef
		2	137.39 c	107.37 h	364.14 d
		3	375.24 a	348.31 b	672.79 b
Ojakkyo	C.l.	1	85.36 f	43.76 k	68.18 ef
	Var. c.	2	93.27 ef	158.32 e	62.09 f
		3	88.44 f	172.63 d	64.04 ef
Tri-X 313	C.l.	1	102.39 de	146.86 f	78.87 e
	Var. l.	2	85.25 f	187.73 c	77.48 e
	(3x)	3	95.78 ef	140.59 g	66.87 ef

[z]RLNS is rootstock leaf number stage.
[y]Total carbohydrates are defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.
[x]Values represent a mean of two readings pooled from twenty plants replicated five times.
[w]Genotype is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima*, L.s. = *Lagenaria siceraria*, C.l Var.c.=*Citrullus lanatus Var. Citroides*, C.I Var. 1(3x) = *Citrullus lanatus Var. Lanatus* (triploid).
[v]The 1st RLNS in this study is defined as seeing the first unexpanded true leaf. The 2nd RLNS is defined as seeing the fully expanded 1st true leaf and the unexpanded 2nd true leaf. The 3rd RLNS is defined as seeing the 1st and 2nd expanded true leaves and the unexpanded 3rd true leaf.
[u]Means within columns followed by a different letter are significant by LSD at $P = 0.05$.

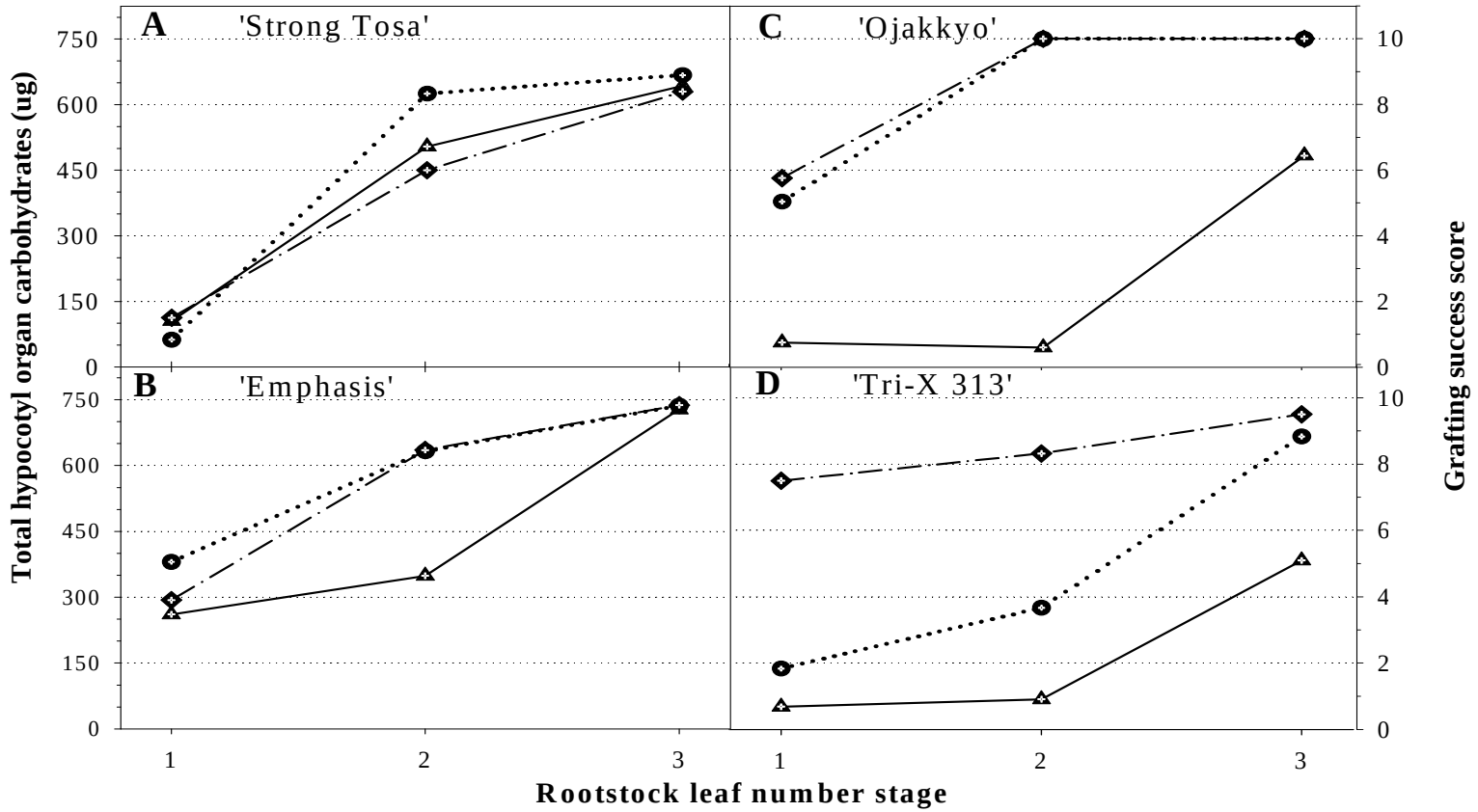

Figure 6. Relationship between rootstock hypocotyl total organ carbohydrates (—△—) measured at time of grafting with grafting success score where "0" = graft death and "10" = optimal growth (13 days after healing) with roots either excised (···●···) or left intact (··◆—) prior to healing at three RLNS on rootstocks *Cucurbita moschata* x *Cucurbita maxima* (A), *Lagenaria siceraria* (B), *Citrullus lanatus Var. Citroides* (C), and *Citrullus lanatus Var. Lanatus* (D) a triploid. The 1st RLNS is defined as the first unexpanded true leaf. The 2nd RLNS is defined as the unexpanded 2nd true leaf with the 1st fully expanded true leaf. The 3rd RLNS is defined as the unexpanded 3rd true leaf with the 1st and 2nd expanded true leaves. Values represent a mean taken from ten plants per replication, replicated five times. Total carbohydrates are defined as the sum of major carbohydrates including: fructose, glucose, sucrose, stachyose, galactose and raffinose. Rootstock hypocotyls were excised from the root system prior to healing just below the soil line and then placed in new media to re-root.

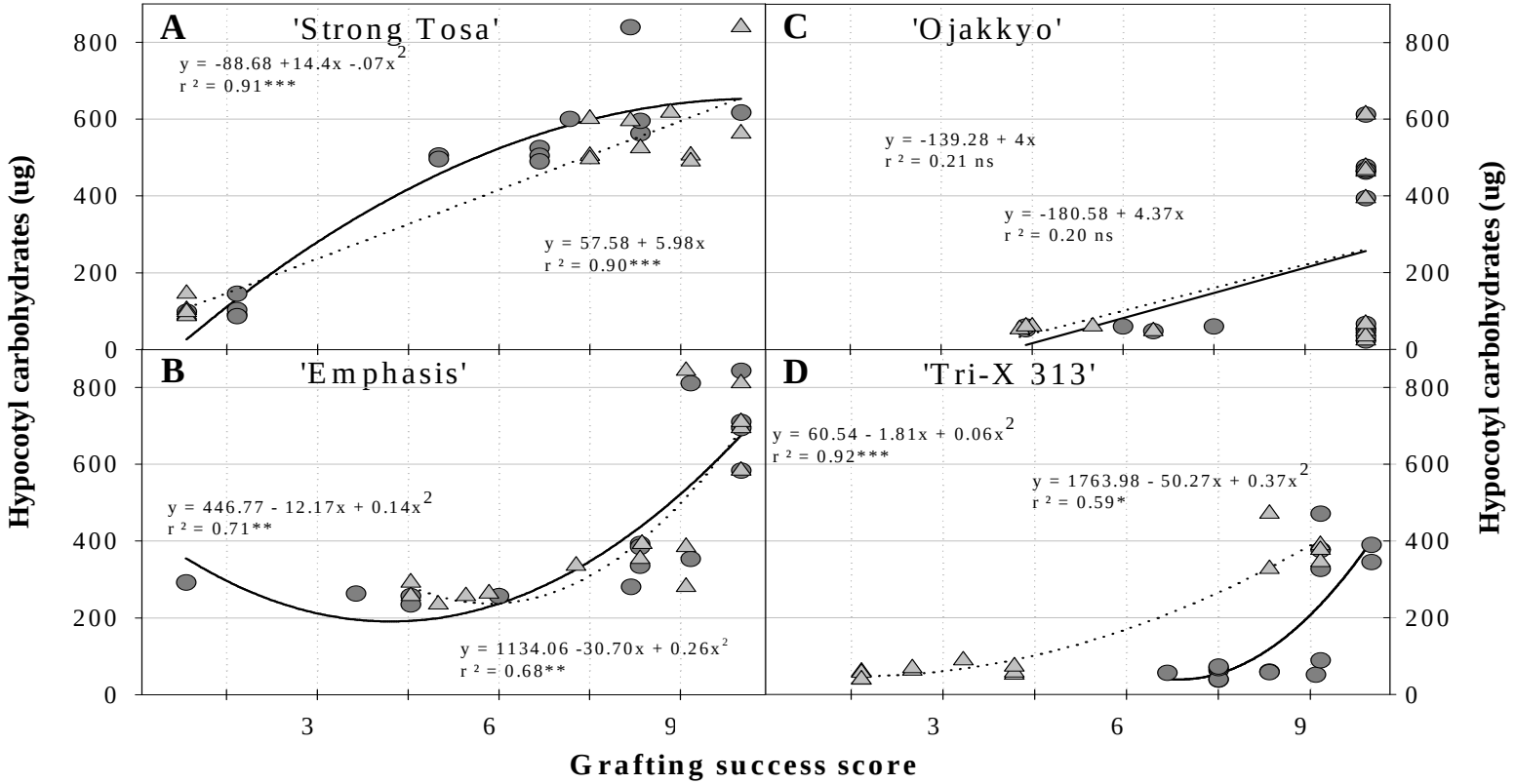

Figure 7. Regression of total hypocotyl carbohydrates levels (prior to grafting) over grafting success score (13 days after grafting) of four rootstock cultivars C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima* (A), *Lagenaria siceraria* (B), *Citrullus lanatus Var. Citroides* (C), *Citrullus lanatus Var. Lanatus* (D) a triploid. Each point represents a mean of 10 plants per replication with hypocotyl treatment after grafting of whether roots were left intact (●) or excised (▲) prior to healing. The solid (——) and broken (••••••) lines (roots left intact or excised, respectively) represent regression lines generated for the entire population data polynomial regression analysis. Total carbohydrates are defined as the sum of major carbohydrates including: fructose, glucose, sucrose, stachyose, galactose and raffinose. Grafting success score were defined as "0" = graft death and "10" = optimal growth with a score of 90 being the lowest level of acceptability. Rootstock hypocotyls were excised from the root system prior to healing just below the soil line and then placed in new media to re-root. ns, *,**,*** = not significant or significant at P=.05, .01, and .001 respectively.

Table 22. Predicted rootstock hypocotyl organ carbohydrates[z] and dry weight status at 90 grafting success score of four rootstock cultivars with roots left intact or excised[y] (prior to healing).

Rootstock cultivar	Rootstock genotype[x]	Root treatment	Carbohydrates[w] (µg/ hypocotyl)	Carbohydrates[w] (µg/ml)	Dry weight[w] (g/ hypocotyl)
Strong Tosa	C.mo. x C.ma.	Intact	640	282	2.27
		Excised	595	283	2.10
Emphasis	L.s.	Intact	485	308	1.57
		Excised	477	306	1.56
Ojakkyo	C.l. Var. c.	Intact	212	243	.87
		Excised	220	242	.91
Tri-X 313	C.l. Var. l.	Intact	236	251	.94
		Excised	383	261	1.47

[z]Carbohydrates represent a mean of two carbohydrate measurements taken from a subsample of ten plants replicated 15 times and are defined as the sum of major carbohydrates such as: fructose, glucose, sucrose, stachyose, galactose and raffinose.

[y]Rootstock hypocotyls were excised from the root system just below the soil line and then placed in new media to re-root.

[x]Genotype is C.mo x C.ma.= *Cucurbita moschata* x *Cucurbita maxima,* L.s. = *Lagenaria siceraria,* C.l Var.c.=*Citrullus lanatus Var. Citroides,* C.1 Var. 1 = *Citrullus lanatus Var. Lanatus* (triploid).

[w]Values represent a calculated number taken from the regression prediction model of hypocotyl total organ carbohydrates, carbohydrate concentration per dry weigh gram, and overall dry weight each with grafting success.

APPENDIX

Additional pictures of rootstock and scion leaf number stages at which grafting took place, grafting, inside of healing chamber, and grafted seedlings.

Fig. A-1. Scion and rootstock at first leaf stage.

Fig. A-2. Scion and rootstock at second leaf stage.

Fig. A-3. Rootstock and scion at third leaf stage.

Fig. A-4. Rootstock prepared for "Cotyledon Devoid" grafting.

Fig. A-5. Scion prepared for grafting.

Fig. A-6. Excised grafted seedling immediately following grafting but prior to healing.

Fig. A-7. Grafted seedling inside high humidity healing chamber immediately following grafting.

Fig. A-8. First rootstock leaf number stage grafted seedlings after healing

Fig. A-9. First leaf stage grafted rootstock hypocotyl and scion cotyledon senescence following healing.

Fig. A-10. Second rootstock leaf number stage grafted seedlings after healing.

Fig. A-11. Second rootstock leaf number stage grafted seedlings after healing.

Fig. A-13. Third rootstock leaf number stage grafted seedlings after healing.

Fig. A-14. Third rootstock leaf number stage grafted seedlings after healing.

Fig. A-15. Close up of grafted second rootstock leaf number stage seedling after healing.

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