

Salinity Evaluation for Watermelon (*Citrullus lanatus*) Grafted with Different Rootstocks

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ABSTRACT

Salinity is a common stress in arid and semi-arid environments and can affect quality and production of sensitive crops such as watermelon (*Citrullus lanatus*). Grafting vegetable crops has been shown to improve fruit quality as well as impart tolerance to abiotic and biotic stresses. To evaluate potential rootstocks that will minimize the impact of salinity on watermelons, a preliminary study was conducted to study the performance of watermelons grafted to experimental and commercial rootstocks. Mini watermelons (*Citrullus lanatus* ‘TAMU mini’) were grafted on four rootstocks: Strong Tosa, CTPI RS, NIZ 54-07, and smell melon. These grafted plants and ungrafted ‘TAMU mini’ controls were subjected to three levels of salinity (0, 1.5, and 3 dS·m⁻¹ irrigation water). Grafted watermelons exposed to 1.5 dS·m⁻¹ had increased dry vine biomass, flesh firmness, and soluble solids compared to plants irrigated with 0 and 3 dS·m⁻¹. Exposure to the 3 dS·m⁻¹ treatment increased brix but did not significantly change other measured variables. Increasing salinity did not reduce biomass or fruit size, and ‘TAMU mini’ watermelon grafted to ‘Strong Tosa’ rootstock was the best performer amongst the four rootstocks tested.

Additional Index Words: Watermelon, grafting, salinity, *Citrullus lanatus*, rootstocks

As of 2012, approximately 55 million acres of farmland in the U.S. were irrigated (United States Department of Commerce, 2012). Irrigated agriculture is predicted to increase in the next few decades as population and subsequent demand for food production increases (Ghassemi et al., 1995; Howell, 2001). As irrigated acreage increases, so does soil salinization, especially in areas affected by drought (Ghassemi et al., 1995; Munns, 2002). Selecting crops for tolerance to salinity stress is vital for the future of agriculture in order to meet the food needs of future populations. Most breeding programs select cultivars or rootstocks for their tolerance to abiotic stresses such as salinity, heat and cold, as well as disease resistance. However, traits related to stress tolerance may lead to reduced fruit quality or yield (Davis et al., 2008). One com-

mon method to induce stress tolerance by physical means is grafting of a productive scion on a stress tolerant rootstock. Large scale production of grafted watermelons began in Korea and Japan in the 1920’s to allow for continuous cropping in areas prone to soil-borne diseases (Davis et al., 2008). Grafting to vigorous rootstocks can enhance disease resistance, yield, nutrient acquisition, drought and cold tolerance, growth, fruit quality, and salt tolerance as reported for different crops such as watermelon, tobacco and tomato (Colla et al., 2006; Edelstein et al., 2004; Ruiz et al., 2006; Santa-Cruz et al., 2002; Uygur and Yetisir, 2009). Grafted cucumber plants have higher photosynthesis rates and stomatal conductance in saline conditions than non-grafted plants (Yang et al., 2006). Several studies have also shown that grafting led to in-

creased yield and fruit quality (Alexopoulos et al., 2007; Huang et al., 2009; Lopez-Galarza et al., 2004; Salam et al., 2002). The greater yield and fruit quality of grafted plants can be due to increased water and nutrient uptake, increased hormone production, or enhanced scion vigor (Lee et al., 2010; Lee, 1994; Ruiz et al., 1997).

Salt stress impedes growth and yield of salt sensitive crops such as watermelon (Colla et al., 2010a; Tanji, 1996). The two most common tolerance mechanisms that salt tolerant rootstocks use are 1) decreasing the amount of salt taken up by the plant and 2) accumulating salts in the rootstock tissues, thus preventing salts from moving into the scion and causing toxic effects (Edelstein et al., 2011).

Production of watermelons in the United States exceeded 56,000 ha and \$520 million in 2012 (United States Department of Agriculture, 2012a; United States Department of Agriculture, 2012b), making it a valuable specialty crop. Watermelon production requires large quantities of irrigation water, usually between 25.4 and 50.8 cm per season depending on rainfall (Dainello, 1996). However, most watermelon production takes place in areas where saline water is already a problem or may become a problem in the future where unreliable rainfall patterns and increasing population pressure reduce availability of high quality water. Thus, it is important to identify strategies to improve salinity tolerance of watermelon. The objective of this experiment was to determine if grafting on selected experimental and commercial rootstocks can improve yield, salinity tolerance, and fruit quality of personal-sized 'TAMU mini' watermelon.

MATERIALS AND METHODS

Plant materials and growing conditions. Four watermelon rootstocks: 'Strong Tosa', Cold Tolerant PI red-seed (CTPI RS), NIZ 54-07, and smell melon were selected based on previous experiments studying seed salinity exposure and germination (unpublished data, C. Simpson, 2009-2010). Several rootstock varieties were selected from commercial and experimental lines of squash, watermelon, and wild melon relatives which have not been tested for salinity tolerance and rootstock suitability. Of the commercially developed varieties, Strong Tosa and NIZ 54-07, Strong Tosa is more widely used in vegetable grafting in areas such as in Spain, Morocco, and other Asian countries where disease prevalence outweighs the costs associated with vegetable grafting. Strong Tosa has also shown promise in stress tolerance, such as salt tolerance (Colla et al., 2012; Goreta et al., 2008). Strong Tosa is a commercial interspecific hybrid (*Cucurbita maxima* Duchesne × *C. moschata* Duchesne) rootstock variety re-

leased by Syngenta® Seeds, Inc. (Boise, ID, USA) while NIZ 54-07 is a *Lagenaria siceraria* sample variety from Nickerson-Zwaan (NIZ) experimental lines (Lincolnshire, UK). *Citrullus lanatus* 'CTPI RS' is an experimental watermelon variety that has shown cold tolerance in trial studies at Texas A&M University. Smell melon (*Cucumis odoratissimus*) is an experimental melon being developed from a wild accession collected from a field in Victoria Co., TX. The scion variety 'TAMU mini' (*Citrullus lanatus* 'TAMU mini') is a personal-sized watermelon breeding line developed by Dr. Stephen King at Texas A&M University. Seeds from each rootstock variety and scion were germinated and grown in laboratory conditions under an artificial light source ($\sim 150 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) set on a 12 h cycle until the stem diameter was about 3 mm, approximately 10-14 d from seeding to grafting depending on rootstock). A 'TAMU mini' watermelon scion was cleft-grafted and held in place by a grafting clip for each rootstock, and then held in a mist chamber at 95-98% RH for 7-10 d at ambient temperature ($\sim 23^\circ \text{C}$). In addition, the TAMU mini watermelon was also left ungrafted as a control. The plants were then slowly hardened off by taking them out of the mist chamber for progressively longer periods of time over one week until wilting ceased. They were then transitioned to the greenhouse for experimentation. After two weeks (December 18, 2011) plants were transplanted to 19 L pots filled with Sunshine Mix #1 potting soil (Sun Gro® Horticulture Ltd., Agawam, MA) in a glass greenhouse. The plants were arranged in a randomized complete block design with five plants of each rootstock combination or non-grafted scion per treatment and trellised when they reached approximately 15 cm in height. Three salinity treatments were applied starting one month after transplantation (January 18, 2012), 0, 1.5 and 3 $\text{dS} \cdot \text{m}^{-1}$, with 25 plants per treatment. The plants were watered with salt solution and fertilizer four times per day with an automated drip irrigation system, 15 minutes per event, at a rate of $6.7 \text{ cm}^3 \cdot \text{s}^{-1}$. Plants receiving salinity treatments (1.5 and 3 $\text{dS} \cdot \text{m}^{-1}$) were irrigated with a salt solution, made from Instant Ocean® salt (United Pet Group, Blacksburg, VA), and fertilizer simultaneously. Salts were composed of a complex ion solution similar to that of ocean water but diluted to the experimental concentrations. These solutions were injected via MicroDos® injectors (Hydro Systems Co., Cincinnati, OH) calibrated to inject the appropriate concentrations of the salt solutions and fertilizers held in separate tanks, while the 0 $\text{dS} \cdot \text{m}^{-1}$ treatment plants received only fertilizer with the irrigation water. Fertilizer (8-16-36 Hydro-gardens, Colorado Springs, CO) was applied according to manufacturer guidelines (0.6 $\text{g} \cdot \text{L}^{-1}$, 8 oz. per 100 gal.) and additional MgSO_4 and

CaNO₃ fertilizers were added to the irrigation solutions according to recommendations for watermelons (i.e. 18-16-36, 2.27g·L; MgSO₄, 1.41g·L; CaNO₃, 2.27 g·L). Micronutrient composition of applied fertilizer consisted of 0.05% B, 0.05% Cu, 0.2% chelated Fe, 0.1% Mn, 0.01% Mo, and 0.05% Zn. When the plants reached approximately 60 cm in length, they were attached to twine supports to allow for maximum space efficiency. All lateral branches were removed from main stem periodically to minimize inter-plant competition and interference. Each plant was hand-pollinated as soon as flowers opened to avoid out-crossing with other experimental watermelon lines being grown in the greenhouse and the dates of pollination were recorded at each flower. If more than one fruit per plant set, the additional fruit was removed from the vine to obtain maximum biomass. Fruit were harvested at 45 days from pollination, stored in a walk in cooler (4 °C) and processed within one day of harvest.

Data collection. After fruit was harvested, the plants were separated into the main central vine and roots. The length of the main central vine was recorded and the plants were placed in a drying oven for at least five days at 65 °C, after which vine biomass was determined. Fruit was processed by first cutting the watermelons in half lengthwise and removing flesh from the center of each half to collect juice for soluble solid measurements. Average soluble solids were determined using a digital pocket refractometer (Pocket Refractometer PAL-1, Spectrum Technologies, Inc., Aurora, IL) on two samples from each fruit. Fruit firmness was determined by taking the average of three peak compression measurements approximately 50 mm from the terminal end of the rind using a digital force gauge (Chatillon DFM 50, Ametek Test and Calibration Instruments, Largo, FL). **Statistical analysis.** Data was analyzed using JMP[®] Pro 10.0.0 software (SAS Institute, Cary, NC). The experimental setup consisted of five replications for each rootstock per salinity treatment in a randomized complete block design. Treatment effects and interactions were analyzed using full factorial fit models. Due to the small experimental size a *P* value ≤ 0.10 was deemed statistically significant as it described 90% of the observed data. Differences in means between treatments, rootstocks, and their interactions were determined using a Students' *t* test (*P* ≤ 0.10).

RESULTS

Watermelon growth and biomass response to salinity. Above ground mass and vine length were different among rootstocks and salinity levels. Average scion vine length of watermelon grafted to the smell melon

rootstock was the shortest (4.2 m ± 0.6) and watermelon scion grafted to the Strong Tosa rootstock had the longest vines (6.5 m ± 0.9, *P* = 0.013, Table 1). However, salinity treatment did not affect vine length (*P* = 0.61). Scion dry biomass was lowest for smell melon (20.7 g ± 4.4) and Strong Tosa had the highest biomass (40.6 g ± 6.2, *P* = 0.001). Other rootstocks and control plants had scion biomass statistically similar to that of smell melon. The effect of salinity treatment on scion biomass was marginally significant (*P* = 0.054), with plants treated with 1.5 dS m⁻¹ having the highest biomass and plants receiving no salinity treatment having the lowest biomass (25.97, 32.94, 26.92 g, respectively for 0, 1.5, and 3 dS m⁻¹ treatments).

Table 1. Effect of four different rootstocks on scion ('TAMU mini' watermelon) mass, vine length, and fruit soluble solids concentration (°Brix) as compared to non-grafted 'TAMU mini'. Different letters indicate significant differences (*P* ≤ 0.10) between rootstocks as determined by Students' *t* test.

Rootstock	Mean Scion Mass (g)	Mean Vine Length (m)	Mean Fruit Soluble Solids (°Brix)
Strong Tosa	40.63a	6.45a	6.49ab
CTPI RS	27.77b	5.52ab	7.55a
NIZ 54-07	26.99b	5.15bc	6.47ab
Smell melon	20.69b	4.18c	4.98b
TAMU Mini	27.03b	5.19bc	6.09ab
non-grafted			

Fruit quality. The effect of salinity on fresh fruit mass per fruit varied by rootstock (Table 2, *P* rootstock x salinity = 0.082), 3 dS·m⁻¹ treatments reduced fruit mass in Strong Tosa compared to 0 and 1.5 dS·m⁻¹, but 1.5 dS·m⁻¹ increased fruit mass in CTPI RS compared to 0 and 3 dS·m⁻¹. However, fruit mass of NIZ 54-07, smell melon, and ungrafted TAMU mini were not affected by the salinity treatments. The effect of salinity on fruit flesh firmness also varied between rootstocks (Table 2, *P* rootstock x salinity = 0.049), Strong Tosa and CTPI RS fruit had greater firmness at 1.5 dS·m⁻¹, while NIZ 54-07 had reduced firmness at 1.5 dS·m⁻¹. Fruit firmness of smell melon and ungrafted 'TAMU mini' were not different between salinity treatments. Fruit soluble solids were significantly affected by salinity treatment (*P* = 0.003); as the fruit from the 0 dS·m⁻¹ treatment had the lowest brix values (5.15), while 1.5 and 3 dS·m⁻¹ had higher values (6.58 and 7.22, respectively). While rootstock did not show a highly significant effect on brix values (*P* = 0.109), CTPI RS showed numerically higher values than the

other varieties, with fruit from plants grafted onto smell melon rootstocks having the lowest brix values (Table 2). Despite the significant effect salinity had on brix values and the trend seen amongst rootstocks, there was no interaction effect of rootstock and salinity on brix values ($P=0.616$).

Table 2. Effect of salinity and rootstock on ‘TAMU mini’ watermelon fruit mass and firmness, and soluble solids. Different letters indicate significant differences ($P \leq 0.10$) due to salinity treatment within rootstock as determined by Students’ t test.

Rootstock	Treatment	Mean Fruit Mass (g)	Mean Fruit Flesh Firmness (N)	Mean Soluble Solids (°Brix)
Strong Tosa	0 dS·m ⁻¹	649ab	2.39cd	6.00
	1.5 dS·m ⁻¹	626ab	4.30ab	6.59
	3 dS·m ⁻¹	349c	3.68bcd	6.89
CTPI RS	0 dS·m ⁻¹	472bc	4.32abc	5.20
	1.5 dS·m ⁻¹	944a	6.65a	9.45
	3 dS·m ⁻¹	304c	2.26bcd	8.00
NIZ 54-07	0 dS·m ⁻¹	458bc	4.26abc	5.34
	1.5 dS·m ⁻¹	290c	1.81d	6.85
	3 dS·m ⁻¹	440bc	3.27bcd	7.22
Smell melon	0 dS·m ⁻¹	485bc	3.79abcd	4.18
	1.5 dS·m ⁻¹	159c	2.18bcd	3.80
	3 dS·m ⁻¹	399bc	3.23bcd	6.96
TAMU Mini	0 dS·m ⁻¹	395bc	3.63abcd	5.03
non-grafted	1.5 dS·m ⁻¹	490bc	4.30abc	6.22
	3 dS·m ⁻¹	381bc	2.88bcd	7.02

DISCUSSION

Many previous studies on grafted watermelons have shown varied results amongst rootstocks and how they affect fruit and yield parameters. Some studies suggest grafting of watermelon led to greater fruit production. For example, Colla et al. (2006) found that grafted watermelon plants grown via greenhouse channel cultivation had 81% higher yield than ungrafted plants, regardless of salinity treatment. In other greenhouse studies, grafted watermelon plants have also shown promising results with regards to performance when exposed to salinity (Colla et al., 2006; Colla et al., 2012; Colla et al., 2010b; Edelstein et al., 2011; Martinez-Rodriguez et al., 2008). Without a self-grafted control we were unable to assess the effect of grafting per se, but we were able to assess the effect of scion-rootstock combinations on fruit mass, firmness, and brix as compared to the ungrafted ‘TAMU mini’

watermelon. In our study, scion biomass was higher at salinity levels of 1.5 dS·m⁻¹, than at 0 and 3 dS·m⁻¹ ($P = 0.054$). The type of rootstock influenced vine length and scion biomass significantly, with Strong Tosa having the greatest vine length (similar to CTPI RS) and scion biomass. Smell melon rootstocks produced the smallest scions of the four rootstock varieties and may have had a slight dwarfing effect on the watermelon scion as evidenced by the lower dry mass and length at harvest. Increasing salinity to 3 dS·m⁻¹ had very little effect on most above ground growth parameters indicating that salinity effects may be more apparent in fruit quality parameters if at all.

We expected that grafting to some of the rootstocks that have shown stress tolerance in previous trials (e.g., CTPI RS, unpublished data) might lead to greater fruit mass under saline conditions than non-saline conditions. When compared across rootstocks, watermelon grafted to Strong Tosa and CTPI RS produced heavier fruit than the ungrafted TAMU mini at 1.5 dS·m⁻¹, however, there were no differences in fruit mass between rootstocks and the non-grafted plants at 3 dS·m⁻¹. The beneficial effect of grafting to Strong Tosa rootstocks was also evident under 0 dS·m⁻¹ conditions, where fruit produced by the scion grafted to Strong Tosa were 85% heavier than fruit produced by the ungrafted control. This suggests that the beneficial effect of grafting on Strong Tosa was not solely due to alleviation of salinity effects, but more likely the ability of the rootstock to produce and allocate water and sugars to the fruit. However, the fact that the ungrafted TAMU-mini watermelon fruit mass and soluble solids were not negatively affected by salinity, suggests that perhaps the scion had a certain level of salinity tolerance. A study by Mendlinger (1994) showed that increased salinity decreased fruit mass and yield in muskmelons. Our results were not that clear cut; salinity levels had variable effects on fruit mass, decreasing mass for some combinations and salinity levels and not for others. Fruit flesh firmness also reflected how salinity affected rootstocks differently ($P < 0.05$ for rootstock x salinity interaction). Strong Tosa grafted watermelons had increased mean flesh firmness at 1.5 dS·m⁻¹ by 80% compared to 0 dS·m⁻¹, while NIZ 54-07 showed a 58% decrease in flesh firmness when exposed to 1.5 dS·m⁻¹ salinity. At 3 dS·m⁻¹ none of the rootstocks showed an effect of salinity on flesh firmness compared to the 0 dS·m⁻¹ control. One of the most important fruit quality parameters is soluble solids content, many studies shown wide variability amongst fruits produced on different rootstocks. Colla et al. (2006), found that watermelons grafted to hybrid squash and *Lagenaria* spp. rootstocks irrigated with saline water produced fruit with higher soluble solids content however they found no differences between

ungrafted watermelons and rootstock species. They suggested that higher salinity reduced water uptake in salt stressed plants and thus increased fruit quality by increasing the sugar concentration. We found that fruit soluble solids content increased significantly with increasing salinity ($P = 0.003$) and showed numerically higher values depending on rootstock variety ($P = 0.109$). These results are also supported by experiments conducted by Davis and Perkins-Veazie (2006) who found that some scion-rootstock combinations increased fruit quality and yield parameters in grafted watermelons.

At moderate salinities of $1.5 \text{ dS} \cdot \text{m}^{-1}$, growth (vine length and scion biomass), fruit flesh firmness, and brix were enhanced significantly. Overall, the Strong Tosa rootstock outperformed the other rootstocks and the ungrafted control plants although CTPI RS shows promise with regards to many of the tested parameters. We conclude that a combination of Strong Tosa rootstock with 'TAMU mini' watermelon is well-suited for situations where moderately saline irrigation water must be used. In fact, mild salinity may lead to greater fruit quality under such conditions. However, in these experimental conditions, 'TAMU mini' watermelon showed some salinity tolerance without being grafted, and produced fruit of similar quality. This would suggest that unless the scion variety is susceptible to soil borne diseases in the area, that grafting may not afford any financial benefit. Additionally, fruit mass was highly variable amongst rootstocks, increasing in some cases and decreasing others. While this is of concern for commercial varieties that are classified according to strict criteria, the 'TAMU mini' watermelons that were produced remained within the size range expected for this (yet unreleased) 'personal size' watermelon variety.

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