Non-destructive characterization of grafted tomato root systems using the mini-horhizotron

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Abstract

Root system morphological and architectural characteristics play a critical role in a plant's ability to utilize substrate resources. Unfortunately, viewing and quantifying root system activity in potted plants is exceedingly difficult and traditionally done through destructive harvests. This method only allows for a snapshot of the plant root system at the time of harvest and gives no inference on the rate of root growth. Furthermore, this method can be highly destructive to the root system itself; many of the fine, high absorptive-capacity roots are lost during the cleaning process. The following study utilized the newly developed mini-horhizotron to non-destructively characterize root system morphology and architecture in grafted tomato (*Solanum lycopersicum* L.). Root tip density, speed of horizontal root growth, and total root length in the commercially available tomato scion ('Tribute') and two rootstock cultivars ('Maxifort' and 'RST-106') were compared. The study was conducted and repeated twice in a heated greenhouse during the months of February and March, 2014. A total of eight grafted treatments were compared: non-grafted 'Tribute', 'RST-106', and 'Maxifort'; self-grafted 'Tribute', 'RST-106', and 'Maxifort'; and 'Tribute' grafted onto 'RST-106' and 'Maxifort'. The 'Maxifort' rootstock produced root systems with up to 80% higher root tip density, 25% faster rate of horizontal root growth, and 35% increase in total root length compared to 'Tribute', with 'RST-106' rootstock being intermediate to the two. These observed differences in 'Maxifort' root systems may correlate to the increased yield and vegetative vigor reported in the literature when this rootstock is used in greenhouse and field production. Furthermore, results from this study indicate that the mini-horhizotron allows for sensitive and robust non-destructive data collection on root system traits.

Keywords: roots, rhizotron, herbaceous graft, grafting, *Lycopersicum esculentum*

INTRODUCTION

Root system morphology in tomato rootstocks has been well documented in grafted tomato (Suchoff et al., 2017). Through destructive harvesting, these studies demonstrated that tomato rootstock root systems differ in morphological traits such as average diameter, total root length, and specific root length. While the root harvesting method developed by Suchoff et al. (2017) was robust enough to find significant differences in minute root morphological traits, root loss due to harvesting and washing is inevitable; 20 to 40% of the root dry weight can be lost during the washing and storage of roots for dry weight analysis (van Noordwijk and Floris, 1979).

Non-destructive methods to analyze root morphology during plant growth have been developed through the use of rhizotrons. These containers can be filled with any substrate of choice and, due to their transparent sides, allow for visual examination of root growth. Traditional rhizotrons like those developed by Silva and Beeson (2011) or the Horhizotron™ created by Wright and Wright (2004) are excellent tools for analyzing root growth, especially horizontal growth, over time. However, both are very large and cumbersome, and more useful for working with large woody perennials (Silva and Beeson, 2011; Wright and Wright, 2004). Smaller rhizotrons are more appropriate for working with herbaceous crops like tomatoes

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when trying to capture the rate of early root growth. A mini-horhizotron has been developed that allows for the easy, non-destructive, and sensitive analysis of root systems in small herbaceous plants (Judd et al., 2014). The mini-horhizotron is composed of three chambers that form a geometric deltoid made of six transparent acrylic faces (Figure 1). These acrylic faces yield 1260 cm^2 of surface area for viewing and measuring roots and are covered with a PVC shade panel when not in use. Each mini-horhizotron has a volume of 2.1 L and rests on a triangular PVC board 907.2 cm^2 in area (Judd et al., 2014). In tests growing *Echincea purpurea* 'Prairie Splendor', *Chrysanthemum* 'Garden Alcala Red', and *Ilex crenata* 'Steeds' in the mini-horhizotrons, there were no physiological differences compared to those same plant species grown in nursery containers of similar volume (Judd et al., 2014). Utilizing the mini-horhizotron, a greenhouse experiment was conducted with the following objectives: 1) to compare the rate of early horizontal root growth in grafted, self-grafted, and non-grafted tomato plants; and 2) to compare root-tip density, its horizontal location within the substrate, and rate of growth using two different commercially available tomato rootstocks.

MATERIALS AND METHODS

This study was conducted in the Marye Anne Fox Science Teaching Laboratory Greenhouses on North Carolina State University campus, Raleigh, NC. The experiment was conducted twice, with data collection occurring in February 2014 and March 2014. The determinate tomato cultivar 'Tribute' (*Solanum lycopersicum* L.; Sakata Seed, Morgan Hill, CA, USA) was used as the scion material grafted onto two commercial rootstocks: 'Maxifort' (*S. lycopersicum* L. × *S. habrochaites* S. Knapp and D. M. Spooner; De Ruiter, St. Louis, MO, USA), and 'RST-106' (*S. lycopersicum* L. × *S. habrochaites* S. Knapp and D. M. Spooner; DP Seeds, Yuma, AZ, USA). All three cultivars were self-grafted as well as left non-grafted resulting in a total of eight grafted treatments.

Seeding of 'Maxifort' for self- and cross-grafted (combination of 'Tribute' grafted onto one of rootstock cultivars) plants occurred on December 11, 2013 follwed by seeding of 'RST-106' and 'Tribute' on December 13, 2013. This seeding date difference was due to slow germination time observed in 'Maxifort'. All three cultivars were seeded on December 20, 2013 for the non-grafted control. The one-week delay of seeding was implemented to take into account the one-week healing period.

Grafting of both cross-grafted self-grafted cultivars occurred on January 12, 2014 when seedlings had four true leaves and a hypocotyl diameter of approximately 2.0 mm. The tube-grafting technique (Rivard and Louws, 2006) was employed and plants were placed in a healing chamber for one week until the graft union was completely healed.

Transplanting of the first experiment occurred on January 24, after the grafted cultivars were completely healed. Each of the eight treatments was transplanted into mini-horhizotrons filled with a soilless substrate (Fafard® 4P mix; Sun Gro Horticulture, Agawam, MA, USA). Three replications of each of the eight treatments gave 24 mini-horhizotrons, which were placed on a greenhouse bench in a completely randomized design. Greenhouse temperatures during the day were maintained at 26.7±4°C and 18.3±3°C at night. Both watering and fertilizing (200 mg L^{-1} of 20N-4.4P-16.6K) occurred once a week.

Data collection began when roots touched the transparent acrylic walls of each chamber of the mini-horhizotron, which occurred 3 days after transplanting (DAT) for both experiments. A sheet of 20.5×10.5 cm transparency film (3M Visual Systems Division, Austin, TX, USA) was marked into five 4×10.5 cm zones and placed on each face to count the number of root tips per zone. Measurements were taken every three days and ended when five roots reached the end wall. Speed of horizontal root growth was determined and defined as the time at which root tips were first visible in each consecutive zone. For the second experiment, seeding of 'Maxifort' occurred on January 17, 2014 and 'RST-106' and 'Tribute' on January 19. All cultivars for the non-grafted controls were seeded on January 26. Grafting for the second experiment occurred on February 21. Treatments were transplanted on March 3, 2014. Watering, fertilization, and data collection were identical to the first experiment. Root length data were collected 10 DAT and 21 DAT of the second experiment. These dates correspond with one week after the first appearance of roots and the end of the experiment, respectively.

Sheets of 20.5×10.5 cm transparency film were placed on the six faces of each mini-horhizotron for each treatment and all visible roots were traced with fine point permanent marker (Sharpie®, Downers Grove, IL, USA). These tracings were then scanned (HP Scanjet G3010, Hewlett-Packard, Palo Alto, CA, USA) and saved as jpeg files. A root reading software (RootReader 2D version 4.3.1; Cornell University, USDA-ARS, Ithaca, NY, USA) measured the length of all traced roots in the file and summed the total accumulated root length.

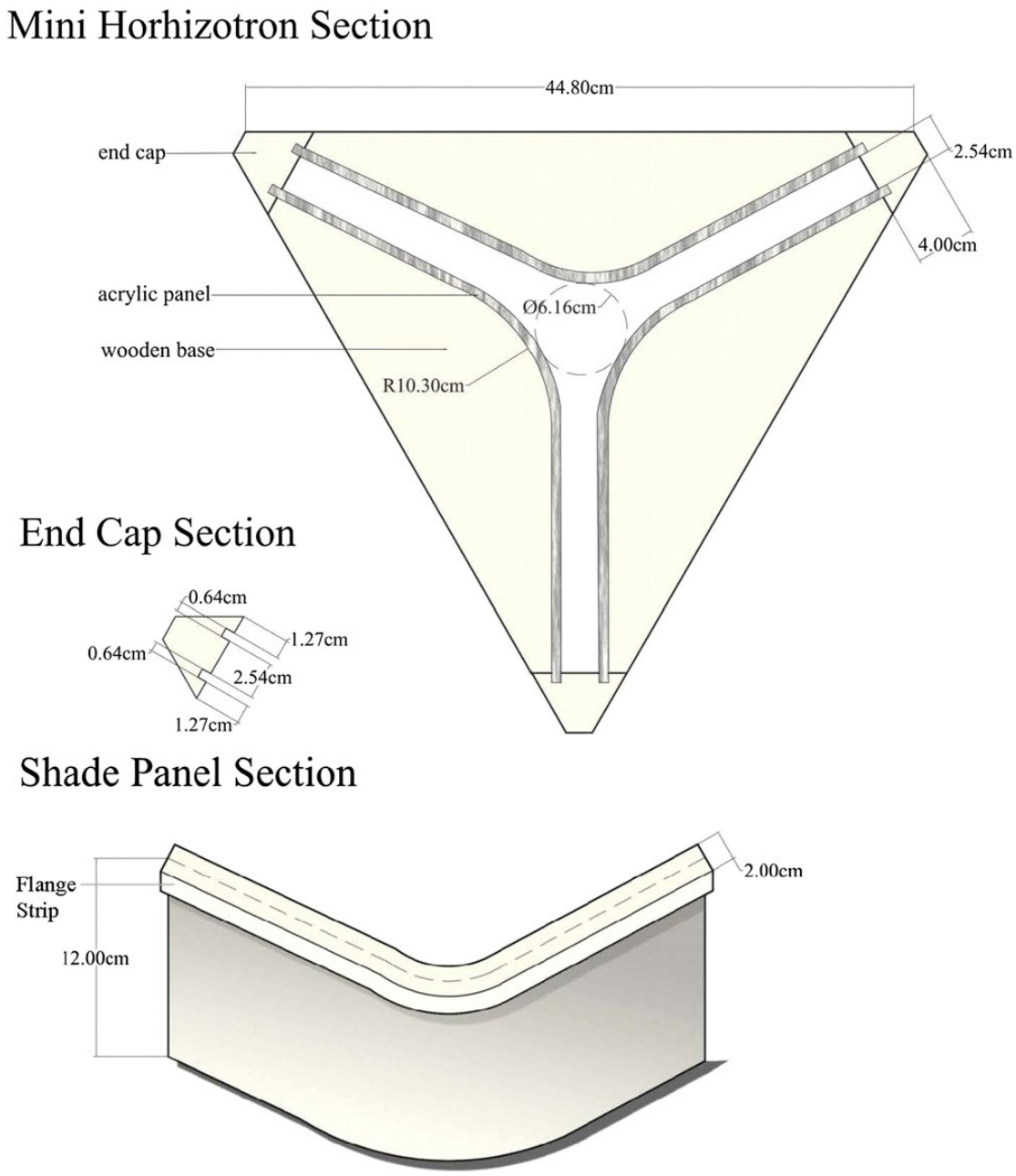

Figure 1. Schematic drawing of mini-Horhizotron. This schematic was developed by Ms. Leslie A. Judd and published in 2014 (Judd et al., 2014).

Data from experiment one and two were analyzed separately using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Total root tips, horizontal root growth rate, and total root length was analyzed with PROC GLM. Residual plots were studied for any violation of the assumptions in ANOVA such as heterogeneity and outliers. No violations of these assumptions

were found and the data were analyzed without the need for transformation. The Fisher's least significant difference test was used for mean separations.

RESULTS

Total number of visible root tips increased over time for all treatments ($P \leq 0.001$). Treatments containing 'Maxifort' tended to have the highest total number of root tips whereas non-grafted and self-grafted 'Tribute' tended to have the lowest in both experiments (Table 1). Speed of horizontal root growth followed a similar trend as seen in total number of root tips with non-grafted 'Maxifort' (M) having some of the fastest growth and self-grafted 'Tribute' (T/T) with some of the slowest growth (Table 2). At 3 DAT M had reached the second zone. This was significantly farther than non-grafted 'RST-106' (R; zone 0.7), T/T (zone 0.3), self-grafted RST-106 (R/R; zone 0.7), and 'Tribute' grafted onto 'RST-106' (R/T; zone 0.7), which did not grow past the first zone. By 6 DAT M reached zone 3.3, significantly farther than all other treatments. At this point (6 DAT) there was no significant difference between treatments other than M. At 9 DAT M roots were the farthest (zone 3.7), while T/T was slowest (zone 2.0) than all treatments except for R (zone 2.7). By 12 DAT M and self-grafted 'Maxifort' (M/M) had reached the last zone (zone 5.0). T/T was still significantly slower than these two treatments at zone 3.7. At 15 DAT all treatments had reached the fifth zone. The speed of root growth in the second experiment was more brisk than that of experiment 1. No differences were observed at 3 DAT; however, at 6 DAT M grew fastest and grew into zone 4. At 9 DAT both M and R had reached the fifth zone. All other treatments ranged in the zone between 4.0 and 4.3 and were not significantly different from the fastest or slowest treatments. By 12 DAT all treatments had reached the fifth zone.

Table 1. Total number of visible root tips in non-grafted, self-grafted, and cross-grafted tomato cultivars grown in mini-horhizotrons for experiments 1 and 2.

Treatment	Total number of root tips[a]									
	Experiment 1					Experiment 2				
	Days after transplant (DAT)									
	3	6	9	12	15	3	6	9	12	15
Tribute	2.3 ab[b]	19.3 bc	27.7 b	103.3 bc	212.0 bc	9.7 a	51.0 cd	159.3 cd	187.0 b	394.0 a
RST-106	3.3 ab	25.0 ab	38.7 ab	140.7 b	382.7 a	14.0 a	86.0 a	200.7 abc	277.7 a	391.7 a
Maxifort	6.7 a	30.7 a	47.3 a	225.7 a	382.0 a	15.3 a	83.3 a	245.3 a	261.7 a	443.0 a
Tribute/ Tribute	0.3 b	9.7 d	26.7 b	75.3 c	198.0 c	11.3 a	41.0 d	141.3 d	179.7 b	331.7 a
RST-106/ RST-106	1.0 b	11.3 cd	31.3 b	104.3 bc	277.7 abc	14.3 a	68.7 b	197.7 bc	257.7 a	360.7 a
Maxifort/ Maxifort	3.7 ab	18.7 bcd	38.0 ab	147.0 b	307.7 abc	17.7 a	68.0 b	186.0 bcd	230.0 ab	325.7 a
RST106/ Tribute	1.3 b	16.3 bcd	34.3 ab	111.0 bc	290.3 abc	17.3 a	63.3 bc	215.3 ab	218.7 ab	374.3 a
Maxifort/ Tribute	1.7 b	18.3 bcd	36.7 ab	151.3 b	309.0 ab	16.7 a	66.0 b	198.3 abc	244.3 ab	411.3 a

[a]Total number of root tips was calculated as the sum of the number of root tips counted for the entire mini-horhizotron.

[b]Means followed by the same letter within the same DAT are not significantly different based on the protected LSD ($P \leq 0.05$).

M had the longest total length of 128.1 cm ($P \leq 0.001$; Table 3). This was higher than T (75.8 cm), R (84.7 cm), T/T (65.8 cm), R/R (77.9 cm), and R/T (78.4 cm), though these treatments were not different from one another. Both M/M and M/T were intermediate and not significantly different from any of the other treatments (94.9 and 96.9 cm, respectively). At 21 DAT M/T had the longest roots (482.9 cm), significantly longer than R/R (418.7 cm), R/T (405.6 cm), T/T (349.7 cm), and T (356.9 cm). R (447.4 cm), M (442.3 cm), and M/M (436.3 cm), were shorter, but not significantly so, than M/T (Table 3).

Table 2. Speed of horizontal root growth in non-grafted, self-grafted, and cross-grafted tomato cultivars grown in mini-horhizotrons for experiments 1 and 2[a].

Treatment	Zone[b]								
	Experiment 1					Experiment 2			
	Days after transplant (DAT)								
	3	6	9	12	15	3	6	9	12
Tribute	1.3 abc[c]	2.0 b	3.0 ab	4.7 ab	5.0 a	1.3 a	2.7 b	4.0 ab	5.0 a
RST-106	0.7 bc	1.7 b	2.7 bc	4.7 ab	5.0 a	1.0 a	2.7 b	5.0 a	5.0 a
Maxifort	2.0 a	3.3 a	3.7 a	5.0 a	5.0 a	1.3 a	4.0 a	5.0 a	5.0 a
Tribute/Tribute	0.3 c	1.7 b	2.0 c	3.7 b	5.0 a	1.3 a	2.7 b	3.7 b	5.0 a
RST-106/RST-106	0.7 bc	2.0 b	2.7 bc	4.0 ab	5.0 a	1.3 a	3.0 b	3.7 b	5.0 a
Maxifort/Maxifort	1.7 ab	2.3 b	3.0 ab	5.0 a	5.0 a	1.7 a	2.7 b	4.0 ab	5.0 a
RST-106/Tribute	0.7 bc	2.0 b	3.0 ab	4.7 ab	5.0 a	1.3 a	3.0 b	4.3 ab	5.0 a
Maxifort/Tribute	1.0 abc	2.0 b	3.0 ab	4.7 ab	5.0 a	1.3 a	3.0 b	4.3 ab	5.0 a

[a]Speed of horizontal root growth was defined as the farthest zone in which a root tip was present at each DAT.
[b]Zone = 4×10.5 cm area marked on a sheet of 20.5×10.5 cm transparency film. Zone 1 was closest to the transplanted root ball, zone 5 was the farthest toward the end of the 20.5 cm wall.
[3]Means followed by the same letter within the same DAT are not significantly different based on the protected LSD (P≤0.05).

Table 3. Total root length at 10 and 21 days after transplanting[a] in non-grafted, self-grafted, and cross-grafted tomato cultivars grown in mini-horhizotrons for experiment 2.

Treatment	Total root length[b] (cm)	
	Days after transplant (DAT)	
	10	21
Tribute (T)	75.84 b[c]	356.97 c
RST-106 (R)	84.73 b	447.39 ab
Maxifort (M)	128.13 a	442.33 ab
Tribute/Tribute (T/T)	65.83 b	349.72 c
RST-106/RST-106 (R/R)	77.89 b	418.68 b
Maxifort/Maxifort (M/M)	94.99 ab	436.25 ab
RST-106/Tribute (R/T)	78.39 b	405.61 bc
Maxifort/Tribute (M/T)	96.86 ab	482.86 a

[a]10 and 21 days after transplant correspond with one week after the first appearance of roots and end of the experiment, respectively.
[b]Total root length was measured by placing sheets of 20.5×10.5 cm transparency film onto the six faces of each treatment and all visible roots were traced and the resultant tracing was scanned and analyzed with RootReader 2D version 4.3.1 software.
[c]Means followed by the same letter within the same DAT are not significantly different based on the protected LSD (P≤0.05).

DISCUSSION

The mini-horhizotron can be used as a tool for the non-destructive analysis of root architecture and growth characteristics. To date, no study has non-destructively compared rootstock root characteristics when grown in soil. This study found the 'Maxifort' genotype to have increased root length, root tip density, and faster rate of horizontal root growth compared to 'Tribute' and 'RST-106'. Destructive comparisons of tomato rootstock root system morphology demonstrated similar differences among the rootstocks. Specifically, rootstocks with similar parental lineage as 'Maxifort' ('Multifort' and 'Beaufort') had significantly longer root systems than 'RST-106' (Suchoff et al., 2017).

CONCLUSIONS

The results of the following study indicate that mini-horhizotrons can be used to non-destructively view, measure, and compare root system traits in grafted tomato. Utilization of

this technology in combination with destructive techniques such as those described by Suchoff et al. (2017) can help develop a better understanding of root system morphology and physiology in herbaceous crops.

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