A New Method to Estimate Vegetable Seedling Vigor, Piloted with Tomato, for Use in Grafting and Other Contexts

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SUMMARY. The primary objective of this study was to test an improved method for estimating vegetable seedling vigor, which is important in grafting and other contexts. The study was also designed to test correlations between destructive and nondestructive measures of seedling growth and the effect of tomato (*Solanum lycopersicum*) rootstock and scion seedling vigor on graft success. Emergence and biomass accumulation and distribution of 18 tomato rootstock and five scion cultivars were monitored in the greenhouse through 18 days after sowing using seven destructive and nondestructive measures; growing conditions were also monitored. Plant and environmental data were used: 1) to develop cultivar growth curves, rank-sum values, and multicomponent seedling vigor values, and 2) to test correlations between percent canopy cover and other foliar measures. Also, seedlings representing all 90 rootstock–scion combinations and their associated seedling vigor values were cleft-grafted using accepted methods and grafted-plant survival was evaluated 2 weeks later. The experiment was conducted twice. Overall seedling vigor and its components differed significantly between runs of the experiment and among cultivars, although most cultivars had similar rankings (relative vigor) in both runs. Rank-sum and seedling vigor values ordered cultivars similarly. However, the range of cultivar seedling vigor values (3–11,504) greatly exceeded the range of rank-sum values (4–92). Correlations between destructive and nondestructive measures were significant. Graft success did not differ among cultivar combinations. We conclude that 1) the method to estimate seedling vigor described herein is useful in grafting and other contexts, including when discerning cultivar and other treatment effects, 2) nondestructive measures can substitute for some destructive ones, and 3) graft success in tomato is unrelated to rootstock and scion seedling vigor, provided proper grafting and healing techniques and commercial cultivars are used.

Seed and seedling vigor influence horticultural operations significantly and much has been done to establish operational definitions of

and methods to assess both. For many, the line between seed vigor and seedling vigor is the transition of new seedlings from hetero- to autotrophy; i.e., from relying on seed reserves to photosynthesis for growth. Global, standardized protocols for estimating seed vigor define it as the inherent potential of seed from different seed lots to develop normal seedlings rapidly and uniformly, and to tolerate biotic and abiotic stresses (Baalbaki et al., 2009). These protocols involve monitoring germination and

early-stage seedling development for crop-specific periods, but rarely beyond the initial expansion of radicle, hypocotyl, and cotyledon(s) (Marcos-Filho, 2015). Lengthier evaluations often signal an interest in seedling vigor, generally accepted as the capacity of seedlings to convert growth factors into biomass once they become autotrophic (Whalley et al., 1966). As such, seedling vigor assessment requires different approaches but, presumably, similar levels of standardization (at least in reporting). Much has been written about the value of seedling vigor (Hernández-Herrera et al., 2014; Rebetzke et al., 2014; Spielmeier et al., 2007); still, assessments of it remain less structured than seed vigor protocols.

Vegetable grafting is one globally significant enterprise likely to benefit from improved methods of estimating and reporting seedling vigor. Preparing, using, and evaluating grafted vegetable plants interest horticulturists, researchers, and educators worldwide (Albacete et al., 2015; Lee et al., 2010). Grafted plants have outyielded ungrafted ones, especially when abiotic or biotic stress is prevalent, in multiple regions (Colla et al., 2010; Louws et al., 2010; Savvas et al., 2010; Schwarz et al., 2010). Grafting has also been a research tool in areas such as breeding and plant physiology (Kiihl et al., 1977; Simons et al., 2007). Millions of grafted tomato plants representing dozens of rootstock–scion combinations are prepared annually by hand and with machine assistance, with success rates often exceeding 90%. Still, additional information, on seedling vigor and graft success, would benefit propagators and crop scientists.

Presowing estimates of expected cultivar vigor can help schedule propagation operations. For example, stem diameter is among the most important indicators of grafting

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Use of trade names does not imply endorsement of the products named or criticism of similar ones not named.

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274 × 10 ⁻⁵
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

readiness since rootstock and scion seedling stem diameters must not only be within a certain range but also be similar at the time of grafting (Oda et al., 1993; Yetişir and Sari, 2004). Stem diameters increase with age but at unknown rates, particularly among rootstock cultivars. To create the desired number of graft-eligible seedlings, propagators currently repeat sowings and work to speed or slow seedling growth through environmental manipulation. Both approaches are difficult and resource demanding and lower the efficiency of grafted-plant production. Well-founded estimates of seedling growth (vigor) would allow for sowing and grafting periods to be scheduled more reliably and, thereby, limit the number of mismatched or unusable seedlings and investments in environmental or cultural manipulation. It may also limit the need to sort seedlings by stem diameter immediately before grafting, which is common. In fact, matching seedlings at grafting is more difficult with some rootstocks. Although hybrid scion cultivars tend to be products of intense breeding and selection schemes emphasizing consistency, rootstock cultivars may be less consistent in emergence and growth. Some rootstock cultivars are open-pollinated and products of screening pre-existing germplasm, with less breeding and selection (King et al., 2010). Components of rootstock phenotype may vary, challenging users.

Seedling vigor information can also assist in cultivar selection. First, the number of tomato rootstocks commercially available in the United States increased from six to 60 between 2010 and 2016 (Kleinhenz and Short, 2016). New rootstock cultivars are released faster than research-based information on their performance before or after grafting, especially compared to prominent scion cultivars, is developed. Leonardi and Romano (2002) cautioned against allowing this discrepancy to persist. Second, the number of commercial and hobbyist grafted-plant producers and range of conditions under which their plants are grown are also increasing. Propagators currently work to shorten intervals between seeding and shipping as one way to increase production efficiency and profit potential. Faster-growing cultivars assist in that

objective but should be avoided if high vigor conflicts with other desirable traits, as may occur between rootstock and scion cultivars. Third, rootstock and scion seedling vigor may be related to grafted-plant vegetative and fruiting characteristics. Overall, estimates of cultivar vigor have obvious potential to increase the efficiency of grafting operations and the reliability of rootstock and scion selection.

When multiple traits are measured, a large number of cultivars can be compared using rank-sum approaches (Kleinhenz, 2003; Osborne and Simonne, 2002). These approaches rely on ranking cultivars for each trait and then summing the ranks to develop a single value for each cultivar or cultivar-site combination. However, their underlying mathematics prevents rank-sum approaches from quantifying cultivar relative seedling vigor reproducibly since the range of rank-sum scores fluctuates with the number of cultivars involved. This fluctuation creates study-to-study variation. Also, rank-sum approaches do not quantify absolute growth rates, which is essential when quantifying and expressing seedling vigor. A method lacking the pitfalls of rank-sum approaches will allow investigators and professional horticulturists to obtain and use estimates of seedling vigor more reliably and widely.

Therefore, the first objective of this study was to test a method for estimating seedling vigor that 1) incorporates plant and environmental variables and 2) differentiates cultivars and describes their responses to growing conditions early in development. Estimates based on this method will be useful in grafting and other contexts. Emergence and growth of 23 tomato cultivars (18 rootstocks, five scions) under different environmental conditions were recorded using destructive and nondestructive measurements and cultivar-specific seedling vigor values were calculated using a straightforward formula. The relative seedling vigor of each cultivar was also calculated using the rank-sum approach described earlier (Kleinhenz, 2003; Osborne and Simonne, 2002).

Secondarily, the study was also designed to test correlations between destructive and nondestructive measures of seedling growth and

relationships between rootstock and scion seedling vigor and graft success. Per standard protocol, grafted plants were prepared using seedlings with similar stem diameters. However, the seedlings represented cultivars expected to differ in seedling vigor. A total of 90 rootstock–scion combinations representing all combinations of seedling vigor resulted from grafting the 23 cultivars. Albacete et al. (2015) saw a need to study rootstock and scion traits, including vigor, more thoroughly and to use new tools in the process.

Materials and methods

PLANT MATERIALS AND GROWING CONDITIONS. Eighteen commercial rootstock and five scion tomato cultivars were selected using grower input and publicly available information. The 18 selected rootstock cultivars were nominated by growers in three states, represent 12 developers, and contain a range of advertised disease resistance traits. The five selected scion cultivars are hybrid and heirloom and round- and oblong-fruited types. The cultivars used in this study and their developers/distributors are listed in Table 1.

The study was conducted twice [Run 1 (Feb.–Mar. 2014), Run 2 (Mar.–Apr. 2014)]. Both runs employed a completely randomized design with cultivar as the treatment. They were completed in an environmentally controlled greenhouse at the Ohio Agricultural Research and Development Center in Wooster, OH. Rootstock and scion seed was sown on the same date (27 and 28 Feb. 2014 in Run 1 and 28 Mar. 2014 in Run 2) in 96-cell trays (cut into two halves) with cells measuring 1.13 inch wide, 1.5 inch long, and 2.25 inch deep. Half-tray units were preloaded with growing medium (Pro-Mix® MP Mycorrhizae™ Organik™; Premier Tech, Rivière-du-Loup, QC, Canada), and then sown with 48 seeds of a single cultivar (three half-trays per cultivar). All half-trays were placed on a capillary mat (Kapmat; Buffalo Felt Products Corp., West Seneca, NY) underlain by 4-mm-thick plastic on elevated benches in the greenhouse. Environmental conditions were monitored hourly throughout the study using data loggers (Hobo ProV2 version 2.5.0; Onset Computer Co., Pocasset, MA) and an automatic control system (Argus

Table 1. List of 18 commercial tomato rootstock and five scion cultivars used in this study for documenting their seedling vigor.

Rootstock cultivar ^a	Seed company/distributor	Rootstock cultivar	Seed company/distributor	Scion cultivar ^b	Seed company/distributor
Aiboh	Asahi Industries (Arakawa-Ku, Japan)	Kaiser	Rijk Zwaan (De Lier, Netherlands)	Brandywine	NE Seed (Hartford, CT)
Akaoni	Asahi Industries	Maxifort	DeRuiter Seeds	Better Boy	NE Seed
Aooni	Asahi Industries	Resistar	Hazera Seeds (Berurim, Israel)	Celebrity	NE Seed
Arnada	Takii Seed (Kyoto, Japan)	RST-04-105	DP Seeds (Yuma, AZ)	Cherokee Purple	NE Seed
Arnold	Siegers Seed Co. (Holland, MI)	RST-04-106	DP Seeds	San Marzano 2	NE Seed
B.B.	Takii Seed	Shield	Rijk Zwaan		
Beaufort	DeRuiter Seeds (Cambourne, United Kingdom)	Stallone	Rijk Zwaan		
Cheong Gang	Seminis Vegetable (St. Louis, MO)	Supernatural	A.P. Whaley Seeds (Mount Horeb, WI)		
Estamino	Enza Zaden (Enkhuizen, Netherlands)	Trooper	Seedway (Elizabethtown, PA)		

^aSeed of only 'Kaiser' and 'Stallone' was pelleted; all other seed was not pelleted. Seed of only 'Arnold', 'Beaufort', 'Maxifort', 'Shield', and 'Stallone' was primed; all other seed was not primed.
^b'Celebrity' is determinate, the other four scions are indeterminate.

Control Systems, Surrey, BC, Canada). The daily averages of the recorded conditions in Runs 1 and 2, respectively, were 23.2 and 23.7 °C temperature, 32% and 48% relative humidity, and 9.1 and 14.8 mol·m⁻²·d⁻¹ daily light integral (DLI) supplied by sunlight, 1000-W metal-halide lamps (Multi-Vapor®; GE Lighting, East Cleveland, OH), and 1000-W high-pressure sodium lamps (Ultra Sun®; Sunlight Supply, Vancouver, WA). Trays were hand-misted to wetness immediately after sowing; a benchtop, automated irrigation system was used thereafter to maintain soil moisture. Forty-four drippers (each with a flow rate of 1.5 gal/h) were distributed evenly among the trays on the capillary mat; each pulsed on for 10 min at 0600, 0900, 1200, 1500, 1800, and 2100 HR. Emitters were supplemented by seven foggers (each with a flow rate of 8.1 gal/h) that pulsed on for 10 s every 15 min. Supplemental fertilization and pest and disease management were not applied.

EXPERIMENTAL DESIGN AND DATA COLLECTION. Experimental units (replicates) within each experimental run consisted of a single half-tray sown with 48 seeds of one cultivar. A total of three half-trays of each cultivar by 23 cultivars were prepared for each experimental run. All replicates were used for nondestructive measures and two were used for destructive measures. The three half-trays (replicates) of each cultivar were distributed randomly on a 5.4 × 1.8-m bench within the greenhouse room.

Emergence was determined by the presence of a hypocotyl hook above the surface of the rooting medium. Emergence counts were recorded daily from days 4 to 14, and days 4 to 13 after sowing in Runs 1 and 2, respectively (beginning with the appearance of at least one hypocotyl hook and concluding when counts did not increase for two consecutive days for all cultivars).

Nondestructive canopy analysis was completed using an approach similar to that reported earlier (Bumgarner et al., 2012). Digital images collected with a tripod-mounted camera (Powershot A2000; Canon USA, Lake Success, NY) situated plumb 0.5 m over the center of a half-tray were analyzed with WinCAM software (Regent Instruments, Ville de

Québec, QC, Canada). Images of three half-trays of each cultivar were collected on days 12, 15, and 18, and days 9, 12, and 15 after sowing in Runs 1 and 2, respectively. WinCAM separated target colors (canopy) from background and calculated the percentage of the area bounded by the half-tray associated with specific colors (percent canopy cover). The raw percent canopy cover value was then divided by the number of emerged seedlings in the half-tray at the time of image capture to provide an adjusted percent canopy cover value.

Three representative plants were harvested from each unit (replicate) on days 12, 15, and 18 after sowing in both experimental runs. Seedling stems were cut at the rooting medium surface followed by five measures: 1) plant height from the cut surface to apical meristem measured by a ruler (centimeters), 2) aboveground fresh weight by a balance [milligrams (MS3002S Precision Balance; Mettler Toledo, Greifensee, Switzerland)], 3) stem diameter 1 cm below the cotyledons by a digital caliper [millimeters (Traceable®; Control Co., Friendswood, TX)], 4) leaf area by an area meter [square centimeters (LI-3100C; LI-COR Biosciences, Lincoln, NE)], and 5) aboveground dry weight by a balance [milligrams (MS3002S Precision Balance; Mettler Toledo)] after drying in an oven (Isotemp™; Fisher Scientific, Waltham, MA) at 50 °C for 2 d.

Plants representing 90 rootstock-scion combinations (18 rootstocks × 5 scions) were cleft-grafted as outlined previously (Bumgarner and Kleinhenz, 2013) when they reached 1.5 to 2.5 mm in stem diameter. Not knowing the vigor of the cultivars used before sowing but expecting it to differ, scion and rootstock cultivars were sown on multiple days. This approach allowed us to select seedlings containing similar stem diameters—but representing cultivars having different seedling vigor values—when grafting all 90 rootstock-scion combinations. Immediately after grafting, plants were placed in a healing chamber located in the experimental greenhouse room and constructed using a polyvinyl chloride frame covered by single layers of clear plastic sheeting and black knitted shade cloth (50%

photosynthetic active radiation transmission; Tek, Janesville, WI). Moisture was maintained using the same type of irrigation system as described before; however, this system was placed inside the chamber and contained 48 automated drippers (each with a flow rate at 1.5 gal/h) that pulsed on for 15 min at 0300 HR and every hour from 0600 to 2100 HR, and six foggers (each capable of delivering 8.1 gal/h) pulsed on for 10 s every 15 min. The average temperature and relative humidity in the healing chamber was 22.8 °C and 87% in Run 1 and 23.3 °C and 88% in Run 2, respectively. Two weeks after grafting, graft survival was rated using an approach described earlier (Johnson and Miles, 2011); plants with a completely wilted scion were rated as dead and all others were rated as living (successful grafts).

DATA ANALYSIS. The cumulative number of emerged seedlings as a percentage of the final count was fit to a three-parameter sigmoid model using SigmaPlot (version 12.5; Systat Software, San Jose, CA). Using estimated parameters provided by the model, the number of days to reach 90% of final emergence (T_{90}) were calculated in Microsoft Excel (2010; Microsoft, Redmond, WA). All calculated days smaller than four were adjusted to four, since emergence started 4 d after sowing.

Statistical analyses completed in SAS (version 9.3; SAS Institute, Cary, NC) included replication as a random effect, explored the separate and interactive effects of cultivar and run on dependent variables, and were performed with by-run and pooled data. The GLIMMIX procedure and its LSMEANS statement with LINES and BYLEVEL options provided analyses of variance and multiple comparisons among cultivars (the latter with $\alpha = 0.05$). Leaf area, stem diameter, and aboveground dry

weight data recorded 12, 15, and 18 d after sowing were fit to a linear model using Proc Reg in SAS and to a quadratic model using the analysis options in SigmaPlot.

Correlations between nondestructive (percent canopy cover analyzed from digital images) and destructive measures (aboveground fresh and dry weights, stem diameter, leaf area, and plant height) were calculated using replicate data and the CORR procedure in SAS.

Further, seedling vigor values were calculated for each cultivar using a formula including four plant and two environmental variables and one constant:

$$\text{Vigor} = \frac{\text{Aboveground dry weight(mg)} \times \text{stem diameter(mm)} \times \text{leaf area(cm}^2\text{)} \times (1 \times 10^5)}{(T_{90} \times \text{GDD} \times \text{DLI})}$$

where T_{90} represents days to reach 90% of final emergence; all biomass values are measures taken 18 d after sowing; growing degree days (GDD) and DLI represent these variables accumulated by 18 d after sowing; and daily GDD is calculated using a base and ceiling temperature of 10 and 27 °C, respectively.

A rank-sum approach after Kleinhenz (2003) and Osborne and Simonne (2002) was also used to compare the relative seedling vigor of the cultivars. In brief, cultivars were ranked based on their mean value of each of the individual plant-based variables also used in calculating vigor as described above. Then, individual rank values were summed to create a single value for each cultivar.

Results

Mean values of all measured plant variables differed between experimental runs (Table 2). Overall, seedlings

grew faster in Run 2 than in Run 1 but at cultivar-specific rates. Run by cultivar interactions were significant for all variables except for emergence T_{90} . As an example, mean aboveground dry weight values of ‘Arnold’ and ‘B.B.’ 18 d after sowing were nearly 1.9 and 3.5 times greater in Run 2 than in Run 1, respectively (Table 3). Ordering the 23 cultivars based on mean aboveground dry weight showed that the positions of six cultivars differed by more than five places between runs, whereas the rank of other cultivars changed less dramatically.

As expected, cultivar had a significant ($P < 0.0001$) effect on all seedling emergence and growth variables in pooled and by-run analyses of variance and in mean separation tests (Tables 2 and 3). The majority of cultivars displayed consistent levels of relative growth between experimental runs. For example, ‘Trooper’, ‘Aooni’, and ‘Estamino’ emerged most slowly, requiring 8.7 to 10.1 d to reach emergence T_{90} in Run 1 and 7.9 to 8.7 d in Run 2. ‘Arnold’, ‘Beaufort’, ‘Kaiser’, ‘Maxifort’, and ‘Stallone’ emerged most rapidly, reaching emergence T_{90} by 6.1 d in Run 1 and 5.3 d in Run 2. Emergence rates of other cultivars were intermediate, with T_{90} values ranging from 5.8 to 8.0 in Run 1 and 5.4 to 7.7 in Run 2. Emergence T_{90} values of the five scion cultivars were intermediate and relatively more stable than those of the 18 rootstock cultivars; scion T_{90} values registered 5.5 to 7.0 in both runs. Similar trends were evident in other variables. In Run 1, ‘Beaufort’ had the highest percent canopy cover, ‘Arnold’ had the largest leaf area, aboveground fresh and dry weights, and stem diameter, and ‘Maxifort’ had the largest plant height. In Run 2, ‘Kaiser’ and ‘Maxifort’ were among the cultivars displaying the fastest growth. ‘Trooper’

Table 2. Probability values of Type III tests of the effects of cultivar and run on seedling emergence and growth variables of 18 tomato rootstock and five scion cultivars as recorded in a greenhouse in Wooster, OH, in Feb.–Apr. 2014.

Source of variance	Emergence T_{90} ^z	Canopy cover (%)	Leaf area	Aboveground fresh wt	Aboveground dry wt	Plant ht	Stem diam
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Run	0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar × Run	0.2	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar (Run 1) ^y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar (Run 2) ^y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^zDays to reach 90% of final emergence.

^yRun 1 (27 Feb.–17 Mar. 2014); Run 2 (28 Mar.–15 Apr. 2014).

Table 3. Seedling emergence and growth variables 18 d after sowing of 18 tomato rootstock and five scion cultivars in a greenhouse in Wooster, OH, in Feb.–Apr. 2014.

Cultivar ^z	T ₉₀ ^y		Canopy cover (%)		Leaf area (cm ²) ^x		Aboveground fresh wt (g) ^x		Aboveground dry wt (mg) ^x		Plant ht (cm) ^x		Stem diam (mm) ^x	
	Run 1 (27 Feb.–17 Mar. 2014)													
Aiboh	6.8	de ^w	58	gh	10	h	0.31	h–k	21	f–h	4.27	b–g	1.37	e–g
Akaoni	6.0	fg	64	e–h	10	gh	0.26	jk	14	h	3.52	hi	1.29	e–g
Aooni	8.7	b	59	gh	13	e–g	0.37	e–i	21	f–h	3.97	d–h	1.34	e–g
Armada	6.1	fg	67	d–h	15	ef	0.39	d–h	24	e–g	3.67	gh	1.48	c–e
Arnold	4.0	i	88	ab	29	a	0.80	a	57	a	4.38	a–e	1.81	a
B.B.	5.8	g	72	c–f	15	ef	0.42	d–g	26	d–f	3.92	e–h	1.47	c–e
Beaufort	4.2	i	96	a	21	bc	0.63	b	50	a	4.58	a–d	1.71	ab
Better Boy	6.1	fg	63	e–h	12	f–h	0.35	f–j	25	e–g	3.95	e–h	1.38	d–g
Brandywine	6.2	fg	66	e–h	12	e–h	0.37	e–i	22	f–h	3.72	f–h	1.38	d–g
Celebrity	7.0	d	74	c–e	13	e–h	0.35	f–j	22	fg	4.03	c–h	1.38	d–g
Cheong Gang	6.2	fg	65	e–h	16	de	0.45	c–f	23	fg	4.70	ab	1.39	d–g
Cherokee Purple	5.9	fg	62	f–h	14	ef	0.45	c–f	28	c–f	4.65	a–c	1.47	c–e
Estamino	9.2	b	61	f–h	13	e–h	0.24	k	18	gh	2.95	ij	1.09	h
Kaiser	5.1	h	87	ab	23	b	0.48	cd	36	bc	3.50	hi	1.36	e–g
Maxifort	4.8	h	88	ab	20	bc	0.53	bc	41	b	4.93	a	1.56	b–d
Resistar	6.9	de	78	b–d	19	cd	0.53	bc	32	c–e	3.93	e–h	1.56	b–d
RST-04–105	8.0	c	64	e–h	13	e–h	0.32	g–k	21	f–h	2.75	j	1.26	f–h
RST-04–106	6.2	fg	72	c–f	14	ef	0.43	c–g	25	e–g	4.02	d–h	1.45	d–f
San Marzano 2	6.4	ef	69	d–g	15	d–f	0.44	c–f	31	c–e	4.23	b–g	1.65	a–c
Shield	7.1	d	55	H	10	H	0.29	i–k	17	gh	3.53	hi	1.23	gh
Stallone	6.1	fg	83	bc	20	bc	0.47	c–e	33	b–d	4.32	a–f	1.39	d–g
Supernatural	7.7	c	59	gh	13	e–h	0.35	f–j	26	d–f	4.32	a–f	1.41	d–g
Trooper	10.1	a	34	i	4	i	0.06	l	4	i	1.92	k	0.78	i
<i>P</i> value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Run 2 (28 Mar.–15 Apr. 2014) ^y														
Aiboh	6.1	e–j	45	fg	20	fg	0.61	ij	51	g–i	5.53	i	1.84	gh
Akaoni	7.2	b–e	61	a–e	44	cd	1.38	b–f	108	b–e	8.60	a–c	2.42	b–e
Aooni	8.4	ab	50	e–g	29	d–g	0.88	f–i	65	e–i	7.98	b–e	1.91	g
Armada	6.2	d–i	65	a–d	39	cd	1.74	ab	121	b–d	8.73	a–c	2.81	ab
Arnold	4.0	k	61	a–e	43	cd	1.13	d–i	108	b–f	7.25	c–g	2.26	c–g
B.B.	6.0	e–j	67	a–c	40	cd	1.48	b–e	90	c–h	9.80	a	2.81	ab
Beaufort	4.2	k	73	ab	36	c–e	0.83	g–j	83	c–i	6.48	e–i	2.16	d–g
Better Boy	6.8	c–h	53	d–g	31	d–g	1.03	e–i	83	c–i	7.20	c–h	2.05	e–g
Brandywine	6.2	d–i	55	c–f	32	d–g	1.10	d–i	67	e–i	7.52	c–g	2.41	b–f
Celebrity	6.9	c–g	51	e–g	36	c–e	1.03	e–i	78	d–i	6.98	d–i	2.21	c–g
Cheong Gang	5.4	g–k	60	b–e	39	cd	1.21	c–g	93	c–g	8.40	a–d	2.23	c–g
Cherokee Purple	5.5	f–k	43	fg	19	fg	0.67	h–j	44	hi	6.68	e–i	1.85	gh
Estamino	7.9	a–c	62	a–e	34	c–f	0.75	g–j	52	g–i	7.18	c–h	1.87	gh
Kaiser	4.6	jk	74	a	76	a	2.12	a	185	a	9.83	a	2.62	a–c
Maxifort	5.3	h–k	74	a	61	ab	1.61	a–d	146	ab	9.32	ab	2.56	a–d
Resistar	6.0	e–j	61	a–e	30	d–g	0.75	g–j	57	g–i	6.40	e–i	2.26	c–g
RST-04–105	7.7	a–d	56	c–f	44	cd	1.04	e–i	78	d–i	6.33	f–i	2.34	c–f
RST-04–106	6.6	c–i	53	d–g	39	cd	1.16	d–h	84	c–i	7.90	b–f	2.43	b–e
San Marzano 2	6.5	c–i	41	g	49	bc	1.71	a–c	128	bc	8.35	a–d	2.95	a
Shield	6.8	c–h	68	a–c	22	e–g	0.65	h–j	46	g–i	5.97	g–i	1.98	fg
Stallone	5.2	i–k	72	ab	41	cd	1.16	d–h	109	b–e	7.65	c–f	2.39	b–f
Supernatural	7.0	b–f	46	fg	23	e–g	0.66	h–j	60	f–i	5.60	hi	1.90	g
Trooper	8.7	a	26	h	17	g	0.34	j	37	i	2.45	j	1.46	h
<i>P</i> value	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

^zFive cultivars in bold are scions (Better Boy, Brandywine, Celebrity, Cherokee Purple, and San Marzano 2). The other 18 cultivars are rootstocks. ‘Kaiser’ and ‘Stallone’ seed was pelleted. ‘Arnold’, ‘Beaufort’, ‘Kaiser’, ‘Maxifort’, ‘Shield’, and ‘Stallone’ seed was primed.

^yDays to reach 90% of final emergence.

^x1 cm² = 0.1550 inch², 1 g = 0.0353 oz, 1 mg = 3.5274 × 10^{–5} oz, 1 cm = 0.3937 inch, 1 mm = 0.0394 inch.

^wMeans within the same column in each run followed by the same letter are not significantly different ($P < 0.05$) as analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure (SAS version 9.3; SAS Institute, Cary, NC).

^vPercent canopy cover in Run 2 was measured 15 d after sowing.

had the lowest mean values of all growth variables in both experimental runs.

Individual cultivar seedling vigor values (Table 4) ranged from 3 ('Trooper') to 1727 ('Arnold'), with a mean, median, and standard deviation of 304, 190, and 382 in Run 1; from 145 ('Trooper') to 11,504

('Kaiser'), with a mean, median, and standard deviation of 2314, 1593, and 2454 in Run 2, respectively. Plotting vigor values revealed that their distribution was skewed, with most values below the midpoint of the range (865 in Run 1 and 5824 in Run 2) and a smaller number of cultivars

displaying values well above average. The two lowest and highest vigor values belonged to rootstock cultivars. Seedling vigor values for most cultivars relative to other cultivars were similar in both runs. However, rankings based on vigor differed by more than five places between runs for five cultivars.

Study-wide rank-sum values ranged from four to 92 across all cultivars (data not shown), a narrower range than found for seedling vigor (Table 4). Seedling vigor values (3–11,504) and summed rank scores (4–92) ordered the 23 cultivars similarly low to high in terms of emergence and seedling growth rates.

Correlations between direct (destructive) and indirect (nondestructive) assessments of seedling vigor were strong (Table 5). WinCAM-mediated measures of canopy cover calculated from digital images were significantly ($r^2 = 0.47$ – 0.95) related to direct measures of aboveground fresh and dry weights, stem diameter, and leaf area in both runs and among rootstock and scion cultivars. Relationships between percent canopy cover and direct measures of plant height were inconsistent, but significant on days 18 and 15 after sowing in Run 1 and Run 2, respectively.

Leaf area, stem diameter, and aboveground dry weight values displayed significant ($P < 0.001$) linear and quadratic tendencies [Fig. 1 (linear data not shown)]. Quadratic r^2 values ranged from 0.63 to 0.94 for leaf area, 0.64 to 0.94 for stem diameter, and 0.50 to 0.71 for aboveground dry weight. Within each run, overall rootstock and scion regression lines were similar in shape and placement. Rootstock and scion lines retained their shape across runs.

Graft survivorship among the 90 rootstock–scion combinations ranged from 92% to 100%, and did not differ

Table 4. Seedling vigor values calculated for 18 tomato rootstock and five scion cultivars in a greenhouse in Wooster, OH, using a formula including four plant and two environmental variables and one constant.

Cultivar ^a	Vigor ^b		
	Run 1 (27 Feb.–17 Mar. 2014)		Run 2 (28 Mar.–15 Apr. 2014)
Aiboh	106	f–h ^x	401 c
Akaoni	72	gh	2,292 bc
Aooni	102	f–h	878 bc
Armada	198	e–h	3,554 bc
Arnold	1,727	a	4,008 bc
B.B.	230	e–h	2,218 bc
Beaufort	1,024	b	2,437 bc
Better Boy	154	e–h	1,593 bc
Brandywine	130	e–h	1,134 bc
Celebrity	141	e–h	1,316 bc
Cheong Gang	190	e–h	2,256 bc
Cherokee Purple	234	e–h	393 c
Estamino	65	h	606 c
Kaiser	513	cd	11,504 a
Maxifort	610	c	5,244 b
Resistar	315	d–f	956 bc
RST-04-105	99	f–h	1,557 bc
RST-04-106	191	e–h	1,844 bc
San Marzano 2	305	d–g	3,751 bc
Shield	73	gh	391 c
Stallone	357	de	4,189 bc
Supernatural	154	e–h	544 c
Trooper	3	h	145 c
P value	<0.0001		0.017

^aFive cultivars in bold are scions (Better Boy, Brandywine, Celebrity, Cherokee Purple, and San Marzano 2). The other 18 cultivars are rootstocks. 'Kaiser' and 'Stallone' seed was pelleted. 'Arnold', 'Beaufort', 'Kaiser', 'Maxifort', 'Shield', and 'Stallone' seed was primed.

^bVigor = $\frac{\text{Aboveground dry weight (mg)} \times \text{stem diameter (mm)} \times \text{leaf area (cm}^2\text{)} \times (1 \times 10^5)}{(T_{90} \times \text{GDD} \times \text{DLI})}$ where T_{90} represents days to reach 90% of final emergence; all biomass values are measures taken 18 d after sowing; growing degree days (GDD) and daily light integral (DLI) represent these variables accumulated by 18 d after sowing; and daily GDD is calculated using a base and ceiling temperature of 10 and 27 °C, respectively. In this experiment and calculation, GDD and DLI were 257 and 172 mol·m⁻², respectively, in Run 1, and 262 and 282 mol·m⁻², respectively, in Run 2; 1 mg = 3.5274 × 10⁻⁵ oz, 1 mm = 0.0394 inch, 1 cm² = 0.1550 inch², (1.8 × °C) + 32 = °F.

^xMeans within the same column in each run followed by the same letter are not significantly different ($P < 0.05$) as analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure (SAS version 9.3; SAS Institute, Cary, NC).

Table 5. The relationship between percent canopy cover based on digital image analysis and direct measures of seedling aboveground fresh and dry weights, stem diameter, leaf area, and plant height for 18 tomato rootstock and five scion cultivars grown in a greenhouse in Wooster, OH, in Feb.–Apr. 2014.

Correlation	Run 1 ^a			Run 2 ^a	
	Day 12	Day 15	Day 18	Day 12	Day 15
Percent canopy cover: aboveground fresh wt	0.71 ^y (0.0002)	0.80 (<0.0001)	0.86 (<0.0001)	0.76 (<0.0001)	0.65 (0.0009)
Percent canopy cover: aboveground dry wt	0.47 (0.02)	0.84 (<0.0001)	0.88 (<0.0001)	0.79 (<0.0001)	0.73 (<0.0001)
Percent canopy cover: stem diam	0.56 (0.005)	0.57 (0.005)	0.78 (<0.0001)	0.59 (0.003)	0.71 (0.0002)
Percent canopy cover: leaf area	0.95 (<0.0001)	0.90 (<0.0001)	0.90 (<0.0001)	0.85 (<0.0001)	0.69 (0.0003)
Percent canopy cover: plant ht	0.07 (0.8)	0.14 (0.5)	0.59 (0.003)	0.15 (0.5)	0.51 (0.01)

^aRun 1 (27 Feb.–17 Mar. 2014); Run 2 (28 Mar.–15 Apr. 2014). Digital images were not taken on day 18 in Run 2.

^yPearson correlation coefficient (r^2) followed by probability value in parentheses (N = 23).

significantly among combinations (data not shown).

Discussion

Seedling vigor differed between experimental runs but to extents depending on cultivars (Tables 2 and

3). The daily average of recorded relative humidity and the total accumulated GDD and DLI 18 d after sowing was 32%, 257 and 172 mol·m⁻² and 48%, 262 and 282 mol·m⁻² in Runs 1 and 2, respectively. Total accumulated DLI was similar between

runs through the first 7 d after sowing, but not over the remaining 11 d of each study period. Plants received 2.25 times more total accumulated DLI over days 8–18 in Run 2 than in Run 1. Growth responses to this difference were cultivar specific and expected given previous reports of tomato emergence and seedling growth rates (Gent, 1986; Gogo et al., 2012; Hussey, 1963, 1965). Although the majority of cultivars reached T₉₀ faster and grew more rapidly in Run 2 than Run 1, several cultivars displayed the opposite behavior. Although growth differed more widely within the rootstock vs. the scion cultivar group, the shapes of rootstock and scion growth curves were similar (Fig. 1). Cultivars grown from pelleted and primed seed (except for Shield) tended to have larger seedling vigor values. Fully testing the effects of priming, pelleting, or other seed treatments on seedling vigor was beyond the scope of this study. However, the process of estimating seedling vigor described here clearly differentiated cultivars and their growth responses to environmental conditions. We also expect it to be useful in differentiating other treatment effects.

Plant and environmental data can be used to calculate seedling vigor. Like other complex traits, seedling vigor has objective foundations, but is often evaluated subjectively. Here, we asked whether a process for estimating seedling vigor could be established and used to compare cultivars or other experimental units, much like internationally recognized methods contribute to assessing and reporting seed vigor (Association of Official Seed Analysts, 2002; Hoffmaster et al., 2003; Sako et al., 2001). Data representing four plant and two environmental variables were used to calculate seedling vigor values for 23 tomato cultivars (Table 4). The vigor formula assigns equal weight to all input values. Also, its structure assures that changing one or more input values leads to an equal-sized change in vigor value. However, to verify this, we used a large speculative dataset encompassing wide ranges of the six input variables. Outcomes of the test (data not shown) clearly demonstrated that changing an input value(s) results in an equal, by percent, change in vigor value. So, the more values (variables) included in

Fig. 1. Quadratic regression of (A) leaf area, (B) stem diameter, and (C) aboveground dry weight of 18 tomato rootstock and five scion cultivars grown in a greenhouse in Wooster, OH. Run 1 (27 Feb.–17 Mar. 2014); Run 2 (28 Mar.–15 Apr. 2014). Each data point is the average of a cultivar on the specified day; 1 cm² = 0.1550 inch², 1 mm = 0.0394 inch, 1 g = 0.0353 oz.

the calculation, the greater the potential range of vigor values and their sensitivity to plant physiology and growing conditions. Four plant variables were used here; however, using a smaller number along with environmental variables still could enhance the reporting of seedling vigor and investigators' abilities to compare results among studies. Current reports vary widely in their description of growing conditions. Therefore, identifying trends in plant responses to growing conditions across studies can be difficult. A formula incorporating plant and environmental data helps standardize the description of plants' conversion of growth factors into biomass. High levels of standardization have been essential to fostering the widespread use of seed vigor tests and their results.

Nevertheless, improving the approach used in this study to obtain seedling vigor values that can be compared across studies will widen its application. For example, alternative methods could begin before emergence with measures taken in companion benchtop protocols. Likewise, methods could include measures of root biomass taken on seedlings after emergence. Regardless, seedling vigor values are likely to be useful in comparing across studies, especially when relative values are used and growing conditions are clearly reported. Understanding seedling vigor more completely may also help explain the vegetative and fruiting characteristics of rootstock and scion combinations, which clearly vary (e.g., Davis et al., 2008; Leonardi and Giuffrida, 2006), but for reasons that remain largely unknown (Albacete et al., 2015).

When multiple traits are measured, a large number of cultivars can also be compared using rank-sum approaches (Kleinhenz, 2003; Osborne and Simonne, 2002). Here, study-wide rank-sum values registered 4 to 92 across all cultivars (data not shown), a narrower range than for seedling vigor, which equaled 3 to 11,504 (Table 4). Rank-sum and seedling vigor values ordered the 23 cultivars similarly low to high in terms of emergence and seedling growth rates. However, seedling vigor values are more sensitive to variations in plant and environmental data and, therefore, more useful in research and commercial settings.

Seedling growth is tracked with destructive measures, machine vision systems (Conrad, 2004; Giacomelli et al., 1996), and plant image analysis (Bumgarner et al., 2012). Image analysis may complement or reduce the need for destructive sampling if data obtained from digital images are significantly correlated with data from direct, destructive measures. However, correlations decline with canopy closure when leaves overlap and are underrepresented in image analysis (Lin et al., 2002). In this study, WinCAM software-mediated estimates of canopy size based on digital images correlated significantly with direct measures of aboveground fresh and dry weights, leaf area, stem diameter, and, less often, plant height when tested 12, 15, and 18 d after sowing (Table 5). Therefore, rapid and inexpensive nondestructive measures early in plant growth have applications in research and production settings.

These results are important whenever large numbers of tomato cultivars are sown, especially if the goal is to graft them. Graft success is more likely when stem diameters are similar (Oda et al., 1993; Pofu et al., 2013; Yetişir and Sari, 2004), especially when machines are involved. Optimizing the process demands seedlings to be ready for grafting simultaneously. Using data and equations depicted in Fig. 1B but with more time points, we can calculate the number of days seedlings of individual cultivars may have required to reach 1.5 mm in stem diameter, the generally accepted minimum for grafting. Expected windows of graft eligibility (1.5 to 3.0 mm stem diameter) could be calculated from similar datasets provided they were developed using more time points and over a sufficient period of time (regression in Fig. 1B ended at ≈ 1.5 and 2.5 mm stem diameter in Runs 1 and 2, respectively).

All 90 rootstock–scion combinations had at least 92% survival 14 d after grafting. High rates of graft success are expected in tomato (Johnson and Miles, 2011) when proper techniques (e.g., pairing rootstock and scion seedlings by stem diameter) are used. Data reported here suggest that graft success rates are unaffected by relative rootstock and scion seedling vigor, provided accepted grafting

and healing techniques and commercial cultivars are used.

We conclude that 1) tomato seedling vigor is genetically predisposed but environmentally modulated, differing widely among cultivars; 2) seedling vigor can be estimated reliably and reproducibly with plant and environmental data; 3) direct and indirect measures of selected plant traits are strongly correlated, enhancing opportunities to employ rapid and inexpensive nondestructive measures in research and production settings; 4) new tools, such as growth curves and seedling vigor values, can assist in cultivar and other comparisons and in scheduling grafting and other operations; and that 5) graft success in tomato is unrelated to rootstock and scion seedling vigor, provided accepted grafting and healing techniques and commercial cultivars are used.

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