Vascular anatomy changes in tomato stems grown under simulated microgravity

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Abbreviations: ANOVA, analysis of variance; dap, days after planting; FOL, *Fusarium oxysporum* f. sp. *Lycopersici*; H7996, Hawaii7996; ISS, International Space Station; MM, MoneyMaker; PCA, Principal Component Analysis; PC, Principal Component; PDB, potato dextrose broth; RPM, revolutions per minute; TBA, tert-butyl alcohol

ABSTRACT

Gravity is a pervasive cue that directs the growth and development of living systems on Earth and in space environments. However, little is understood about how gravity shapes living systems. This study reports a set of large-scale 2D clinostats that support the growth of plants beyond the seedling stage. Using these clinostats, five replicate experiments containing two tomato cultivars were grown under upright control and simulated microgravity conditions. We showed a variable response to simulated microgravity that was impacted by replicate experiment, but not cultivar. This variable response showed an increase in shoot and root traits under simulated microgravity in some replicate experiments and a reduction in shoot and root traits under simulated microgravity in other replicate experiments. We additionally report a cultivar-specific change in stem vascular anatomy in response to simulated microgravity, which was not impacted by replicate experiment. This work demonstrates the potential to promote plant growth under simulated microgravity and reports a novel modulation of vascular anatomy in response to simulated microgravity.

INTRODUCTION

The presence of gravity shapes the growth and development of organisms on Earth. Beyond Earth, lower gravity conditions, such as those found on the International Space Station (ISS), have been shown to have a multitude of impacts on growth and development [1]. However, space environments are complex in that they introduce many environmental stressors in addition to changing gravity. Thus, it is difficult to parse out the impacts of the overall space environment from impacts specific to changing gravity [2,3]. Despite the pervasiveness of gravity as a signal for all living things, little is understood about how gravitational forces, or lack thereof, directly impact growth and development.

Crop plants have been optimized for performance within an Earth gravitational environment. Yet, as we continue to push beyond Earth's atmosphere with prospectives to grow plants on the Moon or Mars, there is a need for plant production in alternative gravity environments. This need includes production during space flight to reach other locations, and production in those other locations upon arrival. Growing crops in-flight provides essential nutrients and minerals that would otherwise degrade within 1–3 years for the typical pre-packaged, dehydrated food [4]. The degradation of essential nutrients in pre-packaged foods in combination with limited choices causing "menu fatigue" poses a threat to astronauts during long-term space travel and could result in malnutrition [4,5]. Freshly grown vegetables during space flight address this by enhancing the nutritional and mental well-being of astronauts [6]. However, growing crops in-flight poses its own risks. Crop and human pathogens that may occur within an enclosed environment like the ISS can lead to compromised crops, or illness among astronauts. Thus, there have been concerted efforts to understand how plants grow and defend themselves in space environments [7].

Among the crops considered for production in space, tomato is a top choice [8]. Tomatoes are a staple in many global cuisines, and provide vitamin C, carotenoids, and polyphenols, and their phytochemicals have enhanced beneficial effects when part of a complete diet [9]. While several studies have utilized tomato to understand plant growth and development under different space stressors [10–12], and there are even efforts to breed tomato plants for space-production [13], understanding the specific impacts of gravity have been challenging.

Research aimed at isolating the effects of gravity relies on ground-based microgravity simulators. Among these simulators are the 2D clinostat systems, which keep plants under slow rotation perpendicular to gravity, thus randomizing the perceived gravity vector. While these systems are pervasive, they are predominantly designed for smaller plants grown on plates [14–16]. Although these experiments provide vital insights on plant germination and initial growth, there are only a few reports that focus on the effects of microgravity beyond the seedling stage. One study using the model plant *Arabidopsis thaliana* showed that simulated microgravity inhibited plant reproduction to varying degrees among different ecotypes but had no impact on the earlier developmental stages [17]. A separate study grew the dwarf tomato cultivar, Micro-Tom, to maturity on a clinostat and showed inhibited vegetative (i.e., leaf area; dry weight) and

reproductive (i.e., fruit yield; fruit weight) characteristics [18]. These studies emphasize the importance of studying the impact of simulated microgravity across the reproductive life cycle, and across different cultivars. However, there are limited 2D clinostat systems that allow crop plant growth to maturity that enable these studies.

Tomatoes that have been grown in space or under microgravity simulation show a multitude of impacts on cellular and anatomical characteristics. For example, tomatoes grown for 17 days in spaceflight showed cell and organelle deformation, and cellular rearrangement and increased plasmodesmata in leaves [19]. Similar studies using ground-based microgravity simulators showed fewer cell sub-microstructure changes, highlighting the disconnect between ground and flight experiments [19]. Another study suggested that as soon as 72 hours of clinorotation induces anatomical changes in tomato roots [20]. Despite this foundational work, there are many open questions about the timing and underlying mechanisms of cellular architecture changes under simulated microgravity and whether these changes are cultivar specific.

In this study, we designed and built two large-scale 2D clinostats, one rotating perpendicular to gravity to simulate microgravity and one rotating parallel to gravity as a control. These systems were used to study the effects of simulated microgravity on the growth and development of two tomato cultivars. To quantify the effects of simulated microgravity on each cultivar, whole-plant measurements, root map imaging, and anatomical analyses were used. While there were distinct morphological responses to simulated microgravity, there was a significant effect of replicate experiment on the results. While the exact cause of the replicate experiment differences remains to be determined, these results suggest that environmental conditions determine growth responses to simulated microgravity. We further show that there were distinct anatomical changes of tomato stem vasculature under simulated microgravity, which varied by cultivar. These vascular anatomical changes likely have an impact on the overall physiology of the tomato plants. Collectively, these results demonstrate the utility of large-scale clinostats to investigate the role of simulated microgravity in plant growth and development and report a novel anatomical impact of tomato growth under simulated microgravity.

MATERIALS AND METHODS

Clinostat Design and Construction

Two clinostats were designed and constructed: one for upright rotational control and one for simulated microgravity. A full parts list can be found in **Table S1**. For both clinostats, frames were constructed from 80-20 T-slot aluminum extrusion framing. Attachments between extrusions were achieved by L-brackets. In total, each frame measured 1.5748 m x 2.1336 m x 1.5748 m. A center shaft of 50.80 mm diameter aluminum round stock with a wall thickness of 9.525 mm was run through the center of the frames. The top of the frame contained LED light bars, each attached to a heatsink. Four rhizoboxes were attached to the center shaft, and each

rhizobox was divided in half. Rotation was achieved by pairing a 12-volt DC motor to a spur gear box that was designed to have a sub 5 revolutions per minute (RPM) spin rate.

Experimental Design

Five replicate experiments were conducted between 29 January 2024 and 12 June 2024 at the University of Delaware, Newark, Delaware, USA. Each replicate experiment had a duration of 29 days from seed sterilization to harvest. Within each replicate experiment, two tomato (*Solanum lycopersicum*) cultivars, Moneymaker (MM) and Hawaii7996 (H7996), were grown with four plants for each cultivar subject to upright control rotation and four plants for each cultivar subject to clinorotation for simulated microgravity. Each cultivar and clinorotation combination were further split into *Fusarium oxysporum* or mock treatments, with two plants per cultivar and clinostat combination being exposed to *Fusarium oxysporum* inoculation and two plants mock- inoculated. However, no *F. oxysporum* infection or disease symptoms were observed in either upright control or clinorotated plants. Additionally, there was complete overlap of plants inoculated with *F. oxysporum* and those with mock inoculation (**Fig S1**). Therefore, *F. oxysporum*- inoculated and mock-inoculated plants were grouped together in analyses. Treatments were randomly assigned to rhizoboxes within each replicate experiment and each rhizobox contained the same *F. oxysporum* treatment (inoculation or mock) to limit any potential cross-over contamination.

F. oxysporum Spore Production

Fusarium oxysporum f. sp. *Lycopersici* (FOL) was obtained from the National Center for Agricultural Utilization Research Culture Collection (NRRL #26202). 1 mL of a glycerol stock of FOL was inoculated into either a starter culture for 48h or directly into 500 mL of fresh 0.5X potato dextrose broth (PDB) and incubated on an orbital shaker under continuous light at 130 rpm for 72 h. Spores were filtered through sterile cheese cloth and counted on a hemacytometer following Caldwell and Iyer-Pascuzzi 2019 [21]. Plants were inoculated with *Fusarium* spores in PDB or PDB alone as described below.

Seed Sterilization and Germination

To ensure plantlets were at the same growth stage when starting the experiment, MM seeds were started 3-days before H7996 seeds. Seeds were sterilized with a 50% household bleach solution (1:1 solution of 8.3% sodium hypochlorite and water), and washed in ultrapure water before overnight imbibition at 4 °C. A 72-cell tray was filled with soil media (LM-1 Germination Mix, Lambert, Canada). Sterilized MM seeds were added to half of the tray and H7996 added to the second half after the 3-day offset. Seeds were germinated in a growth chamber (Percival Scientific, USA). Fluorescent and white LED bulbs were set to a 16-hr photoperiod at 198 $\mu mol/m^2/s$. Day and night temperatures were set to 28 °C and 25 °C, respectively. Seedlings were watered with a two-part nutrient solution (5-12-26 Part A and Cal Nit Part B, JR Peters Inc,

USA) at 9 days after planting (dap). At 13 dap the seedling tray was transferred from the growth chamber to the greenhouse for acclimatization.

Plant Growth on Clinostats and Fusarium Inoculation

Plants were moved to a greenhouse with high-pressure sodium lamps providing supplemental lighting for a 16-hr photoperiod. Day and night temperatures were set to 26.7 °C and 21.1 °C, respectively. Plants were allowed to acclimate to greenhouse conditions for 1-day before being transferred to the clinostats at 14 dap. Seedlings were selected for uniformity before transfer to the clinostats.

For each rhizobox panel, 300 g of soil and 300 mL autoclaved half-strength PDB or 300 mL half-strength PDB with 2.4×10^6 – 10.5×10^6 *F. oxysporum* spores per mL were mixed and packed into the upper 1/3 of each panel. The lower 2/3 of each rhizobox panel was filled with a mix of 600 g of soil and 400 mL of nutrient solution. An additional 50–100 g of soil was used to fill any gaps. An additional 250 mL of nutrient solution was added to saturate the upper half of the rhizobox panel. A piece of rockwool (1”x1.5”x1.25”, Gro-Slab, The Netherlands) was cut in half, placed at the hypocotyl-root junction of a seedling, and inserted into the top of the rhizobox panel. Rhizoboxes were closed and secured to the clinostat frames. Plants were grown for 15 days on the clinostats, with the final data collection at 29 dap.

Data Collections

Root and shoot images were obtained at the end of each experiment, and manual measurements were taken. Manual measurements included apical bud height (cm), shoot fresh mass (g), shoot dry mass (g), average stem diameter (mm), and shoot coloration (score). Coloration was scored 1-5 for chlorosis and purpling with 5 being the most severe coloration. The same material used for fresh mass was dried in a 65 °C incubator for 7 d and weighed for dry mass. Root images were assessed for any evidence that the soil was disrupted due to the clinorotation (**Fig S2**)**.** Plant data was removed for any plant where there was evidence of soil disruption that might confound the results.

Histology and Imaging

A 1 mm section of the shoot was taken from 2 cm above the rhizobox. Tissue samples (2–5 mm in size) were carefully excised and immediately immersed in a primary fixative containing 1.5% glutaraldehyde and 2% paraformaldehyde prepared in 0.1 M sodium cacodylate buffer (pH 6.8). Fixation was performed under a low vacuum for 2 hours, following protocols adapted from Caldwell et al. [22] and Caldwell & Iyer-Pascuzzi [21]. Post-fixation, samples were rinsed in the same buffer under vacuum and gradually dehydrated through a series of ethanol and tert-butyl alcohol (TBA) steps, with intermittent vacuum application to enhance reagent infiltration. Tissues were treated with three successive changes of 100% TBA over a 72-hour period before being infiltrated with molten paraffin at 54°C. Samples were then embedded in molds with their longitudinal axis oriented perpendicularly and allowed to solidify at room temperature.

Paraffin blocks were attached to embedding rings, trimmed, and sectioned using a rotary microtome at a thickness of 12 µm. Ribbons were floated on a 34°C water bath, mounted onto microscope slides, air-dried, deparaffinized and stained with 0.05% toluidine blue using a graded ethanol–xylene series. After staining, slides were mounted with Permount and sealed with coverglass. Imaging was carried out using an Olympus BX43 compound microscope equipped with a DP80 Dual CCD camera.

Environmental Data

A HOBO Sensor (MX1101, Onset, USA) was placed in the greenhouse room and recorded temperature and humidity every ten minutes during each replicate experiment. Temperature and humidity data was subset and analyzed for all data points between 10:30:00 on day 1 and 13:30:00 on the last day of each replicate experiment. Day 1 was considered the day plants were transferred to the clinostats (14 dap) and the last day was considered the day plants were harvested (29 dap). Additionally, solar radiation data was obtained from the Delaware Environmental Observing System (DEOS; www.deos.udel.edu) using the Newark, DE-Ag Farm station.

Statistical Analyses

All statistical analyses were completed in R *version* 4.4.0 [23] and all figures were generated with the ggplot2 library *version* 3.5.1 [24]. Principal component analyses (PCAs) were used to reduce the dimensionality of the data and identify the traits that best explain the variability in the data. Two different PCA approaches were taken (1) a single PCA with all traits from all replicate experiments combined, and (2) five individual PCAs (one for each replicate experiment). We investigated data within each replicate experiment separately to reduce noise from experiments spanning almost 6 months and determined if the same patterns were observed in each replicate experiment. In all the PCAs, the data was centered and scaled, and the following phenotypes were included: apical bud height, average stem diameter, fresh shoot mass, dry shoot mass, chlorosis, and purpling. To visualize separation of groups, convex hulls were generated around samples by both cultivar and clinostat rotation separately. PCAs were visualized with ggplot2 and the cowplot library *version* 1.1.3 [25]. To determine if significant differences were observed between replicate experiments or cultivars, two-way analysis of variance (ANOVA) models were used for each dependent variable (shoot fresh mass, shoot dry mass, apical bud height, average stem diameter, chlorosis, and purpling). Within each model, clinostat and cultivar were included as independent variables. For each model, if residuals were not normally distributed, a Tukey's ladder of powers (rcompanion package *version* 2.5.0 [26]) transformation was used prior to running the ANOVA. A post-hoc Tukey honest significant difference test was used to test all pairwise comparisons if a P value less than 0.05 was found with the ANOVA. To determine if the aboveground biomass mirrored the root system size, a Pearson correlation analysis was used.

RESULTS

A set of large scale 2D clinostats were built to allow paired plant growth beyond the seedling stage (**Fig 1A-B**). One clinostat rotated at 3 RPM parallel to the gravity vector (upright control conditions; **Fig 1C**), while a second clinostat rotated at 3 RPM perpendicular to the gravity vector (simulated microgravity conditions; **Fig 1D**). This slow rotation imparted negligible centripetal acceleration (0.05 m/s^2; 0.004 g) or force (0.04 m/s^2; 0.004 g). These clinostats contained modifications to enable plant growth beyond the seedling stage within a greenhouse. Specifically, an integrated LED light panel was included above the plants to limit competing tropisms. Further, rhizoboxes were used for plant growth (**Fig 1B**), which enabled the use of soil substrates and visualization of the root systems after rotation.

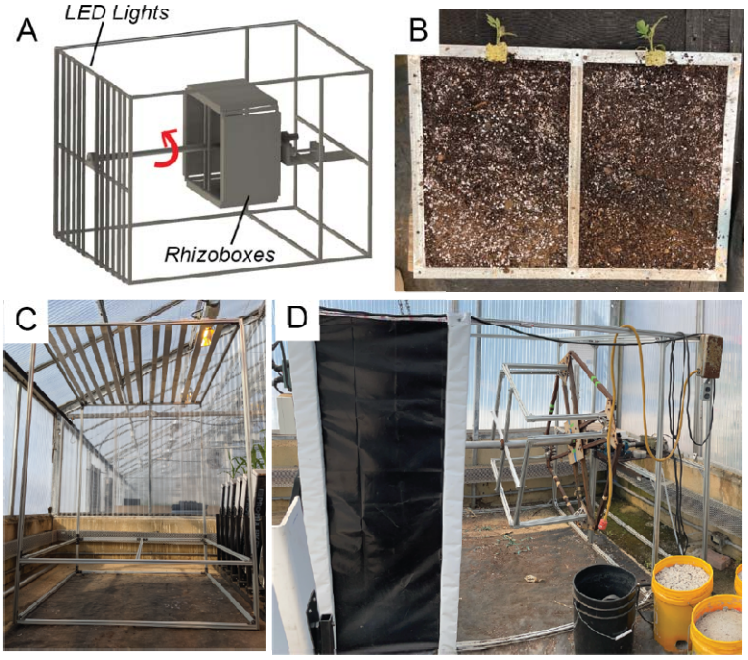

Figure 1. Large-Scale Paired 2D Clinostats. (A) Design of a modular frame for a 2D clinostat that supports plant growth beyond the seedling stage. (B) Soil-filled rhizoboxes were attached to the frame for rotation. (C) Clinostat in upright control position. (D) Clinostat in simulated microgravity position.

To evaluate the effects of simulated microgravity on plant growth, two tomato cultivars were grown in rhizoboxes on the paired clinostats for 15 days. Data from five individual replicate experiments were combined and the aboveground phenotypic data were evaluated (**Fig 2, Table S2**). The aboveground plant traits included apical bud height, fresh mass, dry mass, shoot diameter, chlorosis, and purpling. PCA for these aboveground traits demonstrated some separation between tomato cultivars (**Fig 2A**), as well as between control and simulated microgravity conditions (**Fig 2B**). However, there was substantial overlap between the cultivars

and gravitational treatment when considering the entire dataset, and neither condition separated along Principal Component (PC) 1, which describes 60.59% of the variation in the data.

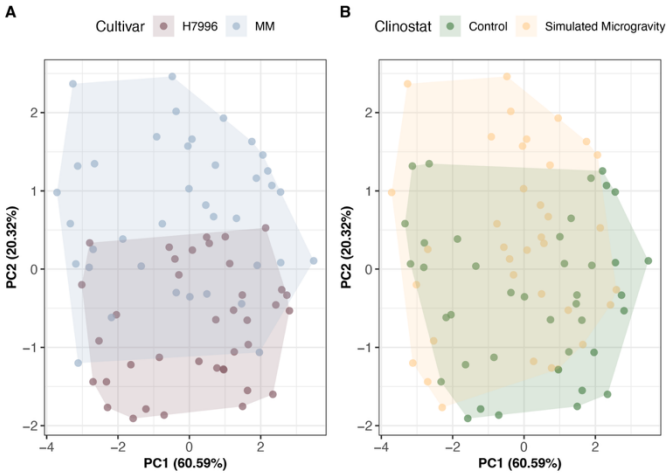

Figure 2. PCA of aboveground traits shows overlap by cultivar and gravity treatment. A PCA shows that PC1 and PC2 explain approximately 81% of the phenotypic variance. Each point represents a single plant from all five replicate experiments combined. Points were colored to determine if plants clustered by (A) cultivar, or (B) gravity treatment. While some clustering is evident, there is overlap among groups, regardless of cultivar or clinostat, and neither separates along PC1.

We hypothesized that PC1 described variation among the different replicate experiments. Specifically, the size of the clinostats necessitated the use of a greenhouse, and the long duration of each replicate led to the 5 replicate experiments spanning almost 6 months in total. Indeed, analysis of environmental sensor data with the greenhouse showed that there was substantial variation in the daily temperature (**Fig S3A**) and relative humidity (**Fig S3B**) between replicate experiments. We additionally evaluated the external solar radiation, which would have provided additional light beyond the LED lighting supplied by the clinostats and high-pressure sodium lamps in the greenhouse. Again, the external solar radiation varied between replicates (**Fig S3C**). We investigated the possibility that the greenhouse environment had an impact on the data separation, and indeed PC1 appears to separate by replicate experiment (**Fig S3D**).

To limit the impact of these environmental differences on the analysis, we separated the data by replicate experiment. We again employed a dimensionality reduction approach and showed that, within each replicate experiment, PC1 separates by gravity condition, and explains between 45-65% of the variation in the data (**Fig 3, Table S3**). Whereas PC2 separates by cultivar and explains between 15-31% of the variation in the data (**Fig 3, Fig S4, Table S4**). Together, these two PCs explain between 69-85% of the variation in the aboveground phenotypes. These data demonstrate that, although environment introduces variation in the data across replicate experiments, within a replicate environment, plant phenotypes are clearly separable by both gravity condition and cultivar.

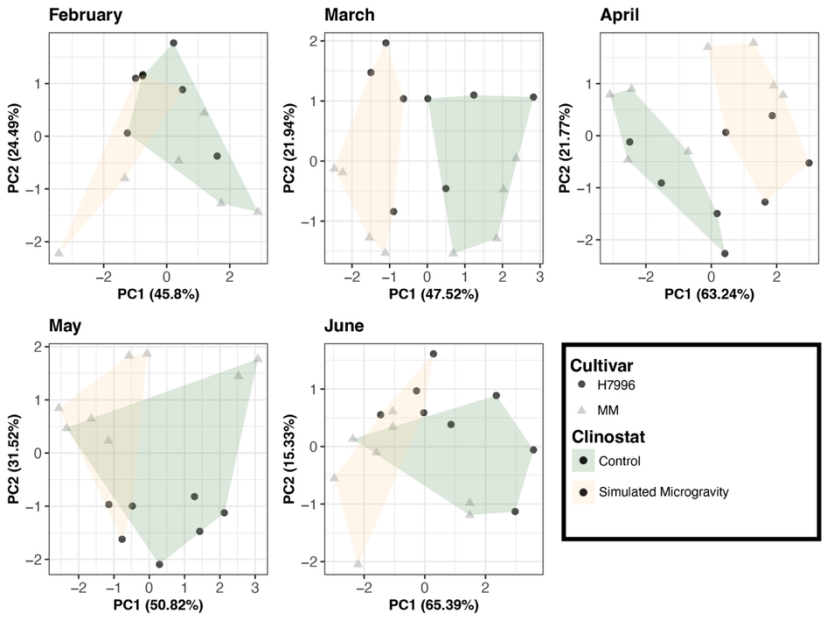

Figure 3. PCA of aboveground traits by replicate experiment reveals separation by cultivar and gravity condition. Within each replicate experiment, principal component analysis (PCA) of aboveground traits shows clear separation by gravity condition along PC1 and by cultivar along PC2. Across replicate experiments, PC1 accounts for 45–65% of the phenotypic variation and PC2 explains 15–31%.

To determine what is driving the separation within a replicate experiment, we again assessed the aboveground plant traits. Considering just the upright control conditions, the replicate experiments could be separated into two distinct groups, with plants from February, March, and May having similar aboveground characteristics, and plants from April and June having similar aboveground characteristics (**Fig 4, Table S5**). Between these groups, plants from the February, March and May replicate experiments were significantly larger than the plants from the April and June replicate experiments (**Fig 4, Table S5**). The biology underlying these replicate-specific differences is unclear as the environmental conditions in the greenhouse do not seem to clearly explain the division between these two groups (**Fig S3**).

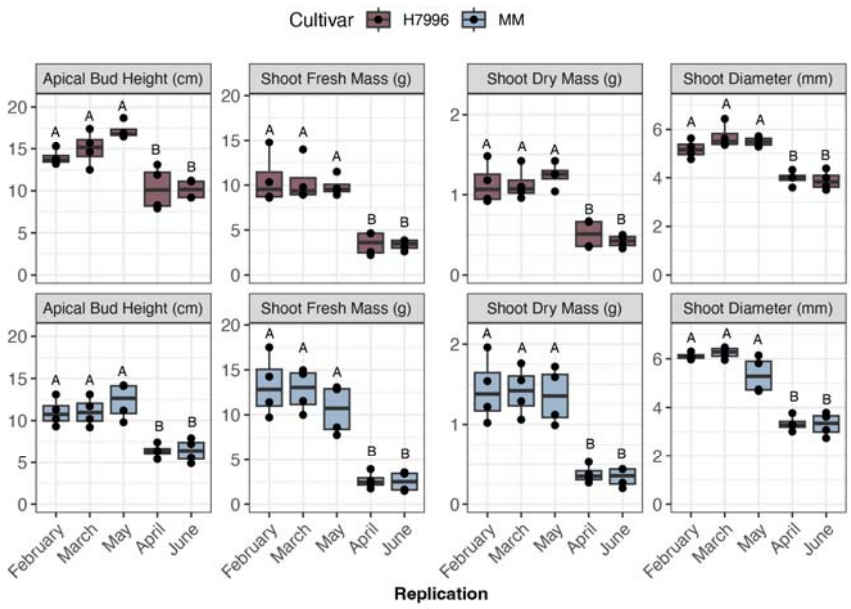

Figure 4. Within upright control conditions, replicate experiment had a significant effect on aboveground traits. For both cultivars, H7996 and MM, plants from February, March, and May had overall higher values for aboveground traits compared to plants from April and June. Within each trait, replicate experiments that share a letter are not statistically different from one another ($P < 0.05$).

In contrast to the upright control conditions, the aboveground traits for plants grown under simulated microgravity did not group by the same replicate experiments, although replicate experiment still had an effect (**Fig 5, Table S6**). For these data, plants from February, March, and April were more similar, while the plants from May and the plants from June were unique. Specifically, the May plants showed overall higher values, and the June plants showed overall lower values than the plants from February, March, and April (**Fig 5, Table S6**).

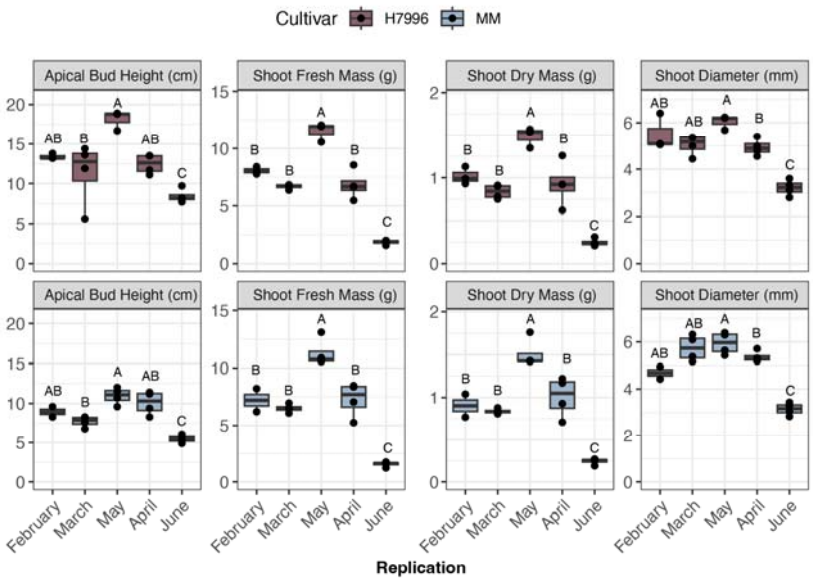

Figure 5. Within simulated microgravity conditions, replicate experiments had a significant effect on aboveground traits. For both cultivars, H7996 and MM, replicate experiment had a significant effect on aboveground traits. Overall, the plants from the May replicate experiment had overall greater aboveground traits and the plants from the June replicate experiment had overall smaller aboveground traits. Within each trait, replicate experiments that share a letter are not statistically different from one another ($P < 0.05$).

Direct comparison between the upright control and simulated microgravity conditions at each replicate experiment showed further variation by replicate experiment. For three of the replicate experiments (February, March and June), both cultivars were smaller under simulated microgravity, whereas for the other two replicates experiments (April and May) both cultivars were overall larger under simulated microgravity (**Fig 6**). These groupings did not match either of the response groupings for the upright control plants alone or the simulated microgravity plants alone. Given that both cultivars responded similarly within each replicate experiment, this suggests the environment has a role in determining these responses. Further, these data suggest that growth responses within each condition and relative growth responses between conditions are impacted by this environmental cue.

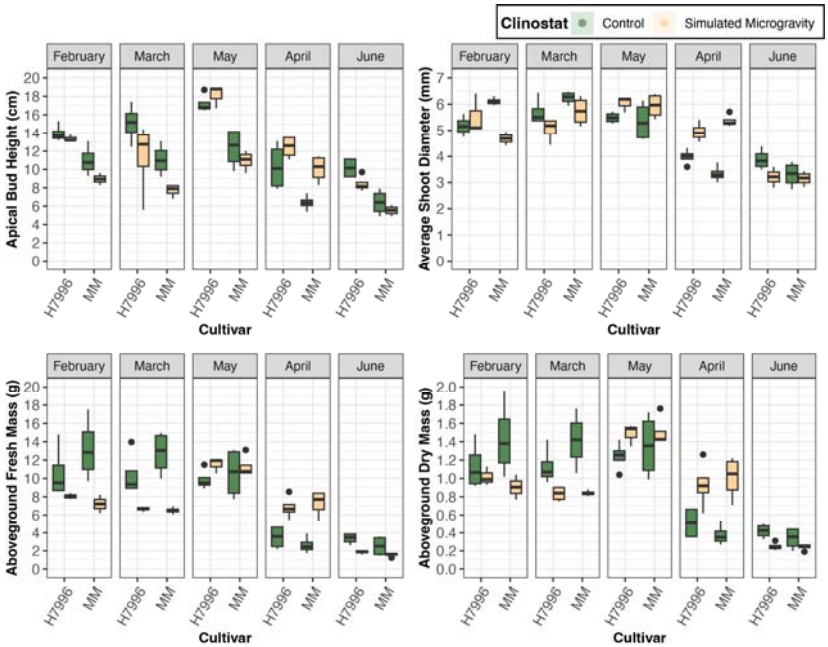

Figure 6. Responses to simulated microgravity were variable across replicate experiments. For both cultivars, H7996 and MM, replicate experiment had a significant effect on the aboveground trait responses to simulated microgravity. For the February, March, and June replicate experiments, aboveground traits were reduced under simulated microgravity, whereas the April and May cultivars showed increased aboveground traits under simulated microgravity.

We hypothesized that one explanation for the variable response could be differential soil moisture. Although experiments were set up with the aim of controlling moisture, variation in the soil batches could have contributed to differences in soil moisture between experiments. If soil moisture was a factor, we would expect to see a greater investment into root biomass corresponding with a reduced investment in shoot biomass. However, analysis of the root systems did not show this response. Instead, the overall root area (**Fig 7A**) and average size of the root system (**Fig 7B**) mirrored the outcomes of the shoot data. In other words, a larger shoot system was associated with a larger root system. However, there was a stronger correlation in root and shoot size for the upright control plants (r = 0.69) than the simulated microgravity plants (r = 0.33) (**Fig S5**).

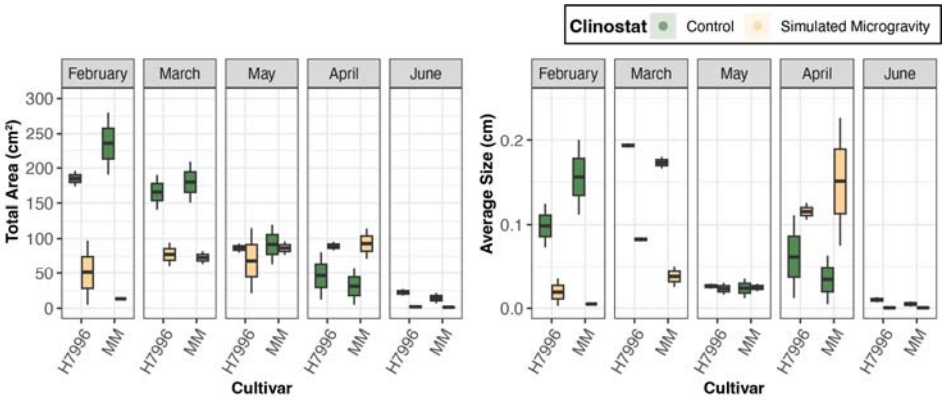

Figure 7. **Root trait responses to simulated microgravity were variable between replicate experiments but mirrored the aboveground responses.** For both cultivars, H7669 and MM, the belowground traits varied by replicate experiment. In February, March, and June, root traits were reduced under simulated microgravity, whereas in April and May, root traits increased under simulated microgravity. These changes mirrored the aboveground responses to simulated microgravity within replicate experiments and cultivars.

Since microgravity impacted plant growth, we wanted to explore if there were any anatomical changes occurring. Analysis of stem anatomy in upright control conditions showed the expected discrete array of vascular bundles encircling the central pith (**Fig 8A, C**). Thus, demonstrating that the rotation of plants was not sufficient to cause anatomical changes. In contrast, the vasculature of tomato stems grown under simulated microgravity demonstrated a loss of discrete patterning (**Fig 8B, D**). Although both cultivars showed the loss of discrete bundles, the overall vasculature was different between cultivars. For H7996, the vasculature was a continuous ring around the pith of even thickness (**Fig 8B**). For MM, the vasculature was diffuse and irregular around the pith (**Fig 8D**). These vascular changes were observed in at least two replicates with different responses to microgravity. No notable differences in vascular anatomy were found for the corresponding root samples (**Fig S6**), showing that this response is stem specific.

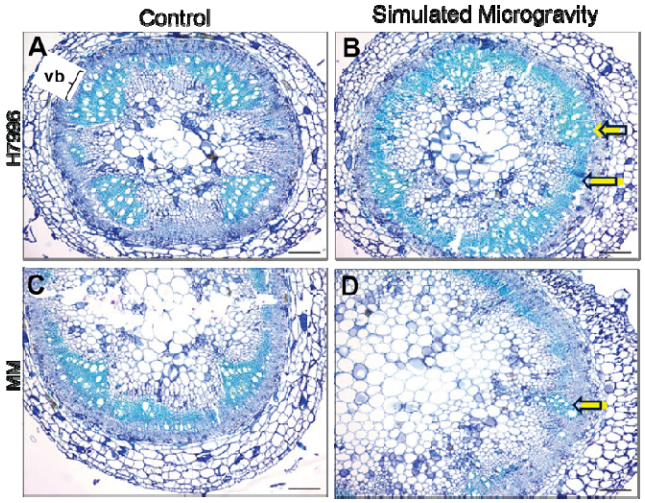

Figure 8. Cultivar-specific changes in stem vascular patterning under simulated microgravity. Stem sections from (A, B) H7996 and (C, D) MM showed altered vascular patterning under simulated microgravity. Under control

conditions, discrete vascular bundles (vb) can be observed around the central pith of the stem for both cultivars. In H7996, the discrete bundles are lost under simulated microgravity, and a continuous vascular ring is observed (yellow arrows). In MM, the discrete bundles are also lost, but the vascular ring is discontinuous and irregular (yellow arrows). Toluidine blue staining differentiates lignified tissues (teal) from non-lignified tissues (purple). Scale bar = 30 µm

DISCUSSION

Despite the pervasiveness of gravity as a signal for growth and development of living systems, untangling the role of gravity in these processes is exceedingly difficult. Spaceflight is the most ideal solution to eliminate the impact of gravity, but comes with a multitude of additional stressors, making the specific role of gravity difficult to parse out. Ground-based microgravity simulators are one approach to isolate the effects of gravity but are often limited in size and scope. This study reports a set of 2D clinostats that support plant growth beyond the seedling stage to expand the capacity to study gravitational impacts on plant growth and development. Using these paired clinostats, we showed a variable response to simulated microgravity that was impacted by replicate experiment, but not cultivar. The specific environmental conditions that varied between replicate experiments to enable these different responses remain a mystery. There was no clear relationship with the temperature, relative humidity or solar radiation. There was further no indication of water stress, as both root and shoot sizes were highly correlated. Future experiments should focus on growing in better controlled conditions, such as a phytotron or walk-in growth chamber, where specific environmental parameters could be systematically tested. Regardless, these results demonstrate that plants can display a positive or negative response to simulated microgravity under different conditions. Thus, defining the specific environment stimuli that enables this switch will be critical for tailoring plant production in limited gravity environments.

Further, while cell wall changes have been extensively reported in response to spaceflight and simulated microgravity [27–33], to our knowledge this is the first report of stem vascular anatomical changes. One previous report identified differences in root anatomy under clinorotation [20], which we do not observe. The anatomical changes in the stem are likely to have significant functional consequences for the plants, which may benefit the plant. A continuous vascular ring may increase the radial transport of water and nutrients or provide enhanced structural support. Future studies aimed at unraveling the impacts of this vascular anatomy will provide significant insights into physiology in space and the role of gravity in vascular development.

These vascular changes are also likely to alter the interaction with microorganisms. In this study, we inoculated plants with *F. oxysporum* to test this hypothesis. However, we were unable to observe disease symptoms or colonization in either upright control or simulated microgravity conditions. We initially hypothesized that this may be due to the light-impenetrable rhizoboxes, however we were able to elicit disease symptoms in the rhizoboxes when not rotating (data not

shown). This outcome has two significant implications. The first is that it emphasizes the use of upright rotating controls to compare with clinorotated plants. Many studies use non-rotated upright control plants as their comparator, but our results suggest that all three conditions are needed – upright non-rotated, upright rotated, and clinorotated – to make strong conclusions about responses to rotation-alone versus simulated microgravity. The second implication is that *F. oxysporum* spores may not be able to "find" roots during rotation – be it upright or clinorotated. This could be due to reduced spore survival or altered root exudation under rotated conditions. Future studies aimed at understanding the implications of vascular patterning for vascular pathogens should consider infection after a period of simulated microgravity.

Collectively this work demonstrates the utility of using large-scale 2D clinostats to study crop growth and development beyond the seedling stage. This work has revealed the potential to promote plant growth under simulated microgravity and reported a novel modulation of stem vascular anatomy in response to simulated microgravity. Lastly, this work highlighted the need to utilize two distinct control conditions – upright non-rotated and upright rotated – to fully elucidate the responses specific to simulated microgravity.

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FIGURE LEGENDS

Figure S1. PCA of aboveground traits shows overlap by *Fusarium oxysporum* inoculation and mock inoculation. A PCA shows that PC1 and PC2 explain approximately 81% of the phenotypic variance. Each point represents a single plant across one of the five replicate experiments. There was complete overlap in phenotypic profiles for plants inoculated with *F. oxysporum* and those with mock inoculation.

Figure S2. Example root images for two rhizoboxes. An example image of plant data that was excluded from analysis due to soil disruption due to clinorotation.

Figure S3. Environmental data shows variation in environmental conditions across replicate experiments. (A) The temperature of greenhouse rooms (°C) was investigated across hours 0:00:00-24:00:00 for each day within each replicate experiment. Boxplots illustrate the variation in temperature within each replicate experiment and across replicate experiments. (B) The relative humidity of greenhouse rooms (%) was investigated across hours 0:00:00-24:00:00 for each day within each replicate experiment. Boxplots illustrate the variation in relative humidity within each r replicate experiment and across replicate experiments. (C) The solar radiation (W/m2) was investigated across hours 0:00:00-24:00:00 for each day within each replicate experiment. Boxplots illustrate the variation in solar radiation for each replicate experiment and across replicate experiments. (D) A PCA shows that PC1 and PC2 explain approximately 81% of the phenotypic variance. Each point represents a single plant across one of the five replicate experiments and is illustrated by color and grouping.

Figure S4. PCA of aboveground traits by replicate experiment reveals separation by cultivar. Within each replicate experiment, PCA of aboveground traits shows clear separation by cultivar along PC2. PC2 explains 15-31% of the phenotypic variation and is dependent on replicate experiment.

Figure S5. The size of the aboveground biomass and size of the belowground area is correlated. A Pearson correlation analysis within each gravity condition showed that the size of the aboveground biomass was correlated with the size of the belowground area. For upright control conditions r = 0.69 and for simulated microgravity conditions r = 0.33.

Figure S6. Root vascular anatomy was unaltered under simulated microgravity. Tomato root sections from control and simulated microgravity conditions. Neither H7996 nor MM roots showed vascular patterning changes in response to simulated microgravity.

Table S1. Parts List. List of all the parts required to construct one 2D clinostat for supporting plant growth beyond the seedling stage.

Table S2. Phenotypic traits and their eigenvector loadings from a PCA on the entire dataset. A principal component analysis was performed on six aboveground traits. The eigenvector loadings for the first two principal components (PC1 and PC2) were extracted for

each of the aboveground traits. PC1 was primarily associated with aboveground traits whereas PC2 was primarily associated with stress response.

Table S3. Phenotypic traits and their eigenvector loadings on each replicate experiment for PC1. A principal component analysis was performed on six aboveground traits. The eigenvector loadings for PC1 were extracted for each replicate experiment. Regardless of replicate experiment, PC1 was consistently associated with aboveground traits.

Table S4. Phenotypic traits and their eigenvector loadings on each replicate experiment for PC2. A principal component analysis was performed on six aboveground traits. The eigenvector loadings for PC2 were extracted for each replicate experiment. Generally, PC2 was consistently associated with stress traits.

Table S5. Replicate experiment had a significant impact on aboveground traits within control conditions. To determine if significant differences were observed between replicate experiments or cultivars within upright control conditions, a two-way analysis of variance (ANOVA) was used for each aboveground trait. Within each model, cultivar and replicate experiment were included as independent variables. A Tukey's ladder of powers transformation was used if residuals were not from a normal distribution.

Table S6. Replicate experiments had a significant impact on aboveground traits within simulated microgravity conditions. To determine if significant differences were observed between replicate experiments or cultivars within simulated microgravity conditions, a two-way analysis of variance (ANOVA) was used for each aboveground trait. Within each model, cultivar and replicate experiment were included as independent variables. A Tukey's ladder of powers transformation was used if residuals were not from a normal distribution.

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