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Tomato Genotype Modulates Selection and Responses to Root Microbiota

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ABSTRACT

Using microbial inoculants to enhance plant health is promising for crop improvement. However, for success, knowledge of how different cultivars within a crop species select and respond to the root microbiome is critical. The aims of this study were to (i) determine the contribution of tomato genotype to the tomato root bacterial microbiome and (ii) investigate whether closely related tomato genotypes differ in their selection of and response to root endophytes. We used 16S ribosomal RNA amplicon sequencing to examine the root bacterial communities of six *Solanum lycopersicum* (domesticated tomato) and two *S. pimpinellifolium* (wild tomato) accessions. We found that, across accessions, both the root endosphere and rhizosphere were affected by genotype. Genotype accounted for 10% of the variation in root microbiota. Two bacterial families, *Bacillaceae* and *Rhizobiaceae*, were significantly enriched in the root endosphere in at least six of the

eight tomato genotypes. To investigate whether closely related tomato genotypes differed in selection of these endosphere-enriched taxa, we profiled the root endosphere of 20 recombinant inbred lines (RILs) derived from two of the genotypes. The abundance of *Bacillaceae* and *Rhizobiaceae* isolates varied quantitatively in the root endosphere of the RILs. Inoculation of 16 RILs with a *Bacillaceae* isolate identified from the root endosphere of field-grown tomato showed that RIL responses, in terms of shoot and root growth, varied from less than 5% growth enhancement to more than 40%. Our data show that tomato genotypes have distinct but overlapping root bacterial microbiomes and respond differently to specific bacterial endophytes.

Keywords: endophytes, microbiome, rhizosphere and phyllosphere

Plant roots are intimately connected to a diversity of microbes in the soil. Some soil microbes are root pathogens and cause destructive diseases in their hosts. Others are beneficial root colonizers and contribute to plant health and function through enhancing root growth and nutrient acquisition, promoting defense responses, or augmenting

abiotic stress tolerance (Berendsen et al. 2012; Bulgarelli et al. 2013; Pieterse et al. 2014). Optimizing host selection of root-associated beneficial microbial communities holds promise for sustainably increasing crop production. However, to fulfill this promise, understanding how crop genetic variation shapes root microbial communities is needed. Here, we asked how genetic variation among cultivated and wild tomato affects selection of the root bacterial microbiome. We hypothesized that closely related tomato genotypes would differ in their selection of and responses to soil microbiota.

Root microbiome structure is determined by soil characteristics, root compartment, and plant genotype (Berendsen et al. 2012; Bulgarelli et al. 2013; Pieterse et al. 2014). Root compartments—the rhizosphere, rhizoplane (root surface) and root endosphere (inside the root)—each act as microhabitats and have a distinct assemblage of microbes (Edwards et al. 2015; Fitzpatrick et al. 2018; Lebeis et al. 2015; Lee et al. 2019; Lundberg et al. 2012; Poudel et al. 2019; Schlaeppi et al. 2014). Multiple studies have demonstrated higher levels of bacterial diversity within the rhizosphere compared with the endosphere (Edwards et al. 2015; Fitzpatrick et al. 2018; Lebeis et al. 2015; Lee et al. 2019; Lundberg et al. 2012; Poudel et al. 2019). Current models of root microbiome assembly suggest a multistep model in which each root compartment selects a subset of microbes from the surrounding compartment, resulting in lower diversity in

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the root endosphere compared with rhizosphere (Edwards et al. 2015; Reinhold-Hurek et al. 2015; van der Heijden and Schlaeppi 2015).

Although the effect of soil type on root microbiome structure is greater than that of root compartment and plant genotype (Chen et al. 2019; Lebeis et al. 2015; Lundberg et al. 2012; Schlaeppi et al. 2014, Veach et al. 2019; Yeoh et al. 2017), elucidating the role of plant genotype is important due to the potential to select for plants which recruit specific microbial taxa or functions. Evidence from *Arabidopsis* and maize supports the possibility of genetic control of host microbiome selection. Quantitative trait loci (QTL) for selection of phyllosphere microbiome taxa have been identified in *Arabidopsis* (Horton et al. 2014) and maize (Balint-Kurti et al. 2010). Maize genome-wide association study (GWAS) analysis suggests that host genetics affect leaf microbiome metabolic functions (Wallace et al. 2018). Plant selection for root microbial taxa may be due to traits that influence the root microbiome, including root exudates (Carvalhais et al. 2015; Hu et al. 2018; Stringlis et al. 2018; Zhelnina et al. 2018) and plant hormones (Carvalhais et al. 2013, 2015; Doornbos et al. 2011; Lebeis et al. 2015; Liu et al. 2017b; Long et al. 2010; Veach et al. 2019).

The effect of host plant genotype on the root microbiome is evident both between (Bulgarelli et al. 2015; Fitzpatrick et al. 2018; Schlaeppi et al. 2014; Zachow et al. 2014) and within (Cardinale et al. 2015; Lundberg et al. 2012; Peiffer et al. 2013; Poudel et al. 2019; Sharaf et al. 2019) plant species, and within root compartments (Edwards et al. 2015; Lebeis et al. 2015; Lundberg et al. 2012), although is not universal (Wagner et al. 2016).

Consistent with the idea that plant genotype alters root microbiome selection, different plant genotypes may have distinct responses to the same microbe. Understanding how different genotypes respond to the same root endophyte is important for effectively using bacterial taxa to promote crop production. For example, tomato recombinant inbred lines (RILs) differed in their response to the biocontrol strain *Bacillus cereus* UW85 (Smith et al. 1999). *Arabidopsis* accessions differed in fresh weight and root architecture after treatment with *Pseudomonas simiae* WCS417r (Haney et al. 2015) and GWAS led to the identification of several candidate genes for response to WCS417r (Wintermans et al. 2016). In *Arabidopsis*, GWAS identified a cytochrome P450 gene, *CYP71A*, that regulates responses to *Pseudomonas* sp. CH267. The two alleles of *CYP71A* resulted in differences in the ability of plants to gain fresh weight in response to CH267 (Koprivova et al. 2019). Thus, plant responses to root microbiota appear to be genetically encoded.

Here, we use eight tomato genotypes, including six genotypes of cultivated tomato *Solanum lycopersicum* and two accessions of wild tomato *S. pimpinellifolium*. We also include 20 RILs derived from one of the cultivated genotypes and one wild-type tomato. We ask how selection of root bacterial communities varies across tomato genotypes and how different tomato genotypes respond to inoculation of an isolate identified from the root endosphere of one cultivar. Tomato is the second most important vegetable crop globally (in terms of tons produced) (FAO 2018). Questions of microbiome selection and responses are important for developing microbiome management strategies, particularly in tomato, in which a multitude of different cultivars are used by commercial growers, the seed industry, small-scale farmers, and home gardeners. Our results suggest that even closely related tomato genotypes select for different but overlapping microbial assemblages and have distinct responses to a microbial inoculant.

MATERIALS AND METHODS

Soil mix. Soil mix was prepared by hand mixing autoclave-sterile potting mix and field soil in a 2:1 ratio by volume. The field soil was a sandy loam collected from the top 10 cm of a conventional agricultural field at Throckmorton Purdue Agricultural Center (40.2° N, 86.9° W) in three batches from April to June 2017, ground, sieved to 4 mm, air dried at 27°C to a constant weight, and mixed to homogenize the three batches. Potting mix was Fafard germination mix, custom blend with 56.69% sphagnum peat moss, composted bark, perlite, vermiculite, dolomite lime, wetting agent, and 0.001% silicon dioxide (SKU code 8269028, lot Q17.05). The potting mix was autoclaved for 30 min at 122.8°C. Samples of field soil and potting mix-field soil mixture were sent to A&L Great Lakes Laboratories for nutrient characterization (Supplementary Table S1). Three technical replicates were performed for each soil sample.

Plant genotypes. To investigate the effect of tomato genotype on the root microbiome, the following genotypes were used: *S. lycopersicum* ‘MoneyMaker’ (MM), ‘Bonny Best’ (BB), ‘Pearson’, ‘Castlemart II’ (CMII), ‘UC82B’, and ‘Hawaii7996’ (H7996); *S. pimpinellifolium* accessions LA2093 and West Virginia700 (WV); and 20 RILs derived from H7996 and WV. MM, BB, and Pearson are heirloom fresh-market tomato varieties. CMII and UC82B are processing tomato. H7996 and WV are the parents of a well-described RIL population that has been used to identify QTL for resistance to the soilborne pathogenic bacterium *Ralstonia solanacearum* (Carneille et al. 2006; Wang et al. 2000). LA2093 has been described for its resistance to plant pathogens (Ashrafi et al. 2009; Caldwell and Iyer-Pascuzzi 2019). RILs were in the F₉ generation. Microbiome analyses of all genotypes were performed together as described below.

Plant growth and harvest for microbiome analyses. Seed were surface sterilized by incubating with gentle rocking in 50% bleach for 10 min, then rinsing five to six times in sterile double-distilled (dd)H₂O. Seed were then stored at 4°C overnight. Surface-sterile seed were planted in randomized complete blocks in 36-pot flats with 1 unplanted cell per block to represent the bulk soil control. Three seeds were planted to each pot and thinned to one plant after germination. Eight full biological replicate blocks were planted to account for any issues with germination. Flats were fertilized once per week with 500 ml of nitrogen standard MiracleGro fertilizer at 150 ppm. Plants were grown in a light- and temperature-controlled greenhouse (temperature setting 75 to 84°F). Lights operated on a daily cycle 16 h of light and 8 h of darkness.

Four blocks were harvested for sequencing after seedlings reached the four-leaf stage (approximately 2.5 weeks). Due to filtering for low-quality sequencing results (<2,000 reads/sample; see Results), all genotypes had either three or four replicates. Rhizosphere and endosphere samples were collected from each genotype, except RILs, for which only endosphere samples were collected (see Supplementary Table S2 for all genotypes). For rhizosphere sampling, roots were removed from the pot and excess soil was removed gently under aseptic conditions until only soil within 1 mm from the root surface remained (Lundberg et al. 2012). Roots were then placed into 15-ml conical tubes containing sterile 1× phosphate-buffered saline (PBS), shaken manually, and placed in a new 15-ml conical tube for surface sterilization. Conical tubes containing the rhizosphere soil were spun at 5,000 rpm for 5 min. Excess liquid was decanted, and soil pellets were resuspended and transferred to a 1.5-ml sterile Eppendorf tube. The Eppendorf tube was spun at maximum speed for 10 min, supernatant was decanted, and rhizosphere soil was frozen in liquid nitrogen (LN₂) and stored

at -80°C until DNA extraction. These samples were designated the rhizosphere compartment samples.

Roots were cleaned by performing an additional rinse to remove any remaining soil. Subsequently, roots were surface sterilized by incubating in 5% bleach with gentle shaking for 2 min, then rinsed three times in sterile ddH₂O before freezing in LN₂ and storing at -80°C until DNA extraction. These samples were designated the endosphere compartment samples.

Root architecture and disease resistance phenotyping in RIL population. The roots of remaining RIL plants that were not harvested for 16S ribosomal RNA (rRNA) sequencing were harvested by first gently removing excess soil by hand; then, the remaining soil was removed by gently rinsing in a water-filled tub and gently brushing with a paintbrush to prevent root breakage. Roots were then imaged on a flatbed scanner and root architecture traits were analyzed with WinRhizo (regular, v.2016). Phenotypic data on resistance of the 20 RIL lines to *Ralstonia solanacearum* were collected in a separate experiment (Supplementary Table S2). For this, *R. solanacearum* was soil soak inoculated at 10^8 CFU/g of soil as described by French et al. (2018). Wilting was assessed as the percentage of leaves wilting at 8 days postinoculation. The average wilting and root architecture values from four to five individuals of each line were then compared with average values of bacterial community composition traits for each RIL via simple regression.

DNA extraction and library preparation. Frozen roots were ground under LN₂ in sterile mortars and pestles before DNA extraction. Each root sample weighed approximately 0.22 ± 0.03 g. DNA was extracted from all samples using Norgen Soil DNA (Norgen Biotek Corp., Canada) extraction kits. DNA concentration and purity were measured by Nanodrop3000. Library preparation was performed using the Illumina 16S Metagenomics Sequencing Library Preparation protocol according to the manufacturer's instructions, with slight modifications. Two-step PCR was performed to amplify the V5 through V7 region of the 16S rRNA gene with the chloroplast excluding primer pair 799F-1193R (Beckers et al. 2016; Chelius and Triplett 2001) and to add Illumina Nextera XT indices.

First, all DNA samples were standardized to 5 ng/ μl . One negative water control and one mock community DNA control (ZymoBIOMICS Microbial Community Standard; Zymo Research, Irvine, CA, U.S.A.) were included on each plate. PCR assays (25 μl each) were performed with 2.5 μl of genomic DNA, 12.5 μl of 2 $\times$ KAPA HiFi HotStart ReadyMix, and 5 μl each of the forward and reverse primers (1 μM) in two 96-well plates. Primer sequences with adapters were as follows: 799F + Nextera adapter 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GAACMGGATTAGATACCKG-3' and 1193R + Nextera adapter 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGACGTCATCCCCACCTTCC-3', which produces an approximately 480-bp product. Underlined portions indicate the 16S primer portion. PCR cycles were performed as follows: 95°C for 3 min; 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and 72°C for 5 min.

After the initial PCR step, PCR products were run on 1.5% agarose gels. The approximately 480-bp band was excised and gel extracted with the Invitrogen PureLink gel purification kit. This step was performed to exclude the larger mitochondrial band amplified by the 16S primers. Gel-purified PCR products were used for the second PCR step to attach dual indices and sequencing adapters. For this step, 50- μl PCR assays were performed with 5 μl of purified Step 1 PCR product, 25 μl of 2 $\times$ KAPA HiFi HotStart ReadyMix, 10 μl of sterile ddH₂O, and 5 μl each of the Index 1 and Index 2 Nextera XT Primers (set A and B) in two 96-well plates. The PCR cycle was as follows: 95°C for 3 min; eight cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. Standard

AmpureXP bead purification was performed on the Step 2 PCR products.

Step 2 PCR products were quantified at the Purdue Genomics Core by mixing 1 μl of each library into a pool and sequencing as 10% of a MiSeq paired-end 250 bp run. The library sizes were estimated from the number of reads obtained from each library and used to calibrate library concentrations for the final pool. All 188 samples were multiplexed into a single pool in equivalent concentrations. The pool was run on an Agilent bioanalyzer chip to confirm library size and purity. The pool was sequenced at the Purdue Genomics Core Facility using Illumina MiSeq V2 chemistry with paired-end 250-bp sequencing.

Sequence processing. Demultiplexing was performed by the Purdue Genomics Core with Illumina software; adapter removal and primer clipping was performed with Trimmomatic (v 0.36) (Bolger et al. 2014) and Cutadapt (v 1.13) (Martin 2011). All subsequent processing was performed using packages in R (v 3.5.0) and Bioconductor (v 3.7). Reads were processed through the DADA2 (v 1.8.0) pipeline by filtering and trimming based on read quality, inferring error rates, merging paired-end reads, removing chimeras, and assigning taxonomy with the Silva reference database v. 132 (Callahan et al. 2016). Likely contaminant sequences were removed with the decontam package using negative controls to infer likely contaminants (Davis et al. 2018). Very low-abundance sequences (fewer than two reads in 10% of the samples) were removed. The average number of reads per sample was 10,612 reads. The median number of reads was 9,181. Archaea represented a very low proportion of reads (approximately 350 reads) and we would not have been able to draw any meaningful conclusions by including them. Thus, they were filtered out along with chloroplast and mitochondrial contaminant reads. Samples with α diversity measurements more than 1.5 $\times$ outside the interquartile range were considered outliers and removed. The α -diversity measurements were performed with the Phyloseq (v 1.24.0) package after subsampling to the smallest library size (2,569 reads) 100 times and averaging the results (McMurdie and Holmes 2013). The β -diversity measurements and constrained analysis of principle coordinates (CAP) was performed with Phyloseq and vegan (v 2.5-2) packages, with reads proportionally scaled to the smallest library size (code courtesy of Deneff lab tutorial; <http://denefflab.github.io/MicrobeMiseq/>). Normalization and differential abundance analysis were performed with DESeq2 (v 1.20.0) (Love et al. 2014). Linear regression was performed with the lm() function in R. All plots were made with the ggplot2 (Wickham 2009) package and arranged in Inkscape (v 0.92.3). All code for analysis and figure generation can be found in the Purdue University Github to <https://github.rcac.purdue.edu/AnjaliIyerpuscuzziGroup/ Tomato-Root-16S-Sequencing> as "Tomato-Root-16S-Sequencing". The sequencing summary is provided in Supplementary Table S3.

Identification of culturable root endosphere microbes. H7996 and WV were planted in May 2017 at Throckmorton Purdue Agriculture Center (40.2°N , 86.9°W). In August, roots were harvested, washed, and sterilized as above. Root tissue was then homogenized in a sterile mortar and pestle with sterile 1 $\times$ PBS. Homogenized liquid (100 ml) was dilution plated onto three different types of media: Luria-Bertani (LB) for isolation of fast-growing copiotrophic bacteria, R2A for isolation of slow-growing oligotrophs, and King's B medium for pseudomonads. In total, 183 isolates were collected and identified by Sanger sequencing of the 16S rRNA subunit. The bacterial colony was gently picked and diluted in 100 μl of ddH₂O. Colony PCR (20 μl) was performed with 10 μl of Apex Taq RED Master Mix 2.0 $\times$ 1.5 mM MgCl₂ (Genesee Scientific, San Diego, CA, U.S.A.), 6 μl of sterile ddH₂O, 2 μl of bacterial cell solution, and 1 μl each of the forward and reverse primers (1 μM). Two 16S rRNA

universal primers 27F and 1392R were used. Primer sequences were as follows: 27F: 5'-AGA GTT TGA TCM TGG CTC AG-3' and 1392R: 5'-ACG GGC GGT GTG TAC A-3'.

The PCR cycle for amplification was performed as followed: 94°C for 5 min; 35 cycles of 94°C for 30 s, 51°C for 30 s, and 72°C for 1.5 min; and 72°C for 10 min. After the amplification step, PCR products were enzymatically purified with ExoSAP-IT PCR Product Cleanup Reagent using standard cleanup protocol from the manufacturer (Thermo Fisher Scientific). Purified PCR product was sent for Sanger sequencing and aligned to the NCBI database.

Plant growth promotion assays. In all, 18 plants of each of 16 RILs derived from H7996 and WV, as well as 18 individuals of H7996 and WV, were sown in autoclaved Metro Mix propagation mix and grown in the same greenhouse under water and temperature conditions similar to those of the plants used for root microbiome sequencing. Seed were surface sterilized for 1 day before sowing in sterile soil. Plants were organized in a random complete block design with nine blocks; both treated and mock-treated plants of all genotypes were included in each block. At 10 days old, nine seedlings of each genotype were inoculated with a *Bacillus* isolate by pouring the bacteria over the soil to achieve 10^6 CFU/g of soil. The isolate was originally identified from the root endosphere of H7996 tomato roots grown in field soil. This isolate was 97.1% identical to the high-frequency colonizing amplicon sequence variant (ASV) 397. Another nine seedlings of each RIL and the parents were inoculated with the buffer $1\times$ PBS (pH = 7.4) as controls. Plants were fertilized with Peter's Excel 15-5-15 Cal Mag at 1.6 g/plant 2 days after inoculation. All nine blocks were

harvested at 24 days old. For each genotype, plant growth promotion was determined by the effect of the isolate on fresh root and shoot weight compared with the mock-inoculated control.

Data analysis for plant growth promotion assay. Fresh shoot and root tissues were weighed upon harvesting. The plant growth promotion effect was measured by response rate, which was calculated by the change in weight of the inoculated plant compared with the mock-treated plant of the same genotype within the same block. In total, nine response rates per genotype were obtained from nine blocks. Data normalization and analysis was performed in R (v 3.5.0). Response rate data were normalized using the formula $\ln(\text{response rate}^2)$. One-way analysis of variance (ANOVA) and Tukey's test were performed on normalized response rate using the `aov()` and `TukeyHSD()` functions. Bar graphs were made with the `ggplot2` package.

RESULTS

The structure of the tomato root microbiota. To investigate the structure of the tomato root bacterial microbiome, we sequenced the V5-V7 region of the 16S rRNA from our samples by paired-end 250-bp MiSeq Illumina sequencing, resulting in 2.9 million high-quality sequences after quality filtering and removal of chimera, nontarget (mitochondria, chloroplast, and archaea), and likely contaminant sequences (see Materials and Methods and Supplementary Table S3). These sequences corresponded to 22,078 ASVs. We filtered for low-abundance ASVs (fewer than 2 reads in less than 10% of the samples), samples with fewer than 2,000 total reads, and outliers. Our

Fig. 1. Tomato root microbiome bacterial community composition across compartments at the phylum level. Stacked bar plot of relative abundance of bacterial taxa at phylum level for each genotype. Proteobacteria comprised greater than 70% of the overall dataset and so were split into classes. Phyla with less than 1% average abundance across genotypes were grouped together and represented as "Below 1%". Abbreviations: CMII = *Solanum lycopersicum* 'Castlemart II', WV = *S. pimpinellifolium* accession WestVirginia700, UC82B = *S. lycopersicum* 'UC82B', MM = *S. lycopersicum* 'Money Maker', BB = *S. lycopersicum* 'Bonnie Best', Pearson = *S. lycopersicum* 'Pearson', LA2093 = *S. pimpinellifolium* accession LA2093, and H7996 = *S. lycopersicum* 'Hawaii7996'.

final data set for analysis consisted of approximately 1.4 million reads and 901 ASVs, with an average of 10,612 reads/sample (see Materials and Methods for description of sequence processing and Supplementary Supplementary Tables S4 to S6 for raw ASV counts in each sample, sample data, and taxonomy).

We first examined the taxonomic structure of root bacterial communities in all tomato genotypes combined. Combining all genotypes, taxonomic classification of all 901 ASVs revealed 16 phyla, with 7 phyla contributing to the majority of the bacterial microbiome (Fig. 1; specific taxa are listed in Supplementary Table S6). Proteobacteria are split into classes because they made up the majority of sequences across the dataset (>70%). Taxonomic classification of the ASVs at phylum level revealed that, in the root rhizosphere, γ -proteobacteria (44.2%), α -proteobacteria (22.7%), Actinobacteria (15.6%), and δ -proteobacteria (4.1%) are most abundant, regardless of genotype. In the root endosphere, Proteobacteria are also common, and comprise approximately 70% of the abundance of most genotypic communities (γ = 46.6% and α = 22.5%, and δ = 3.4%). However, in contrast with the rhizosphere, Firmicutes (6.8%), Actinobacteria (15.0%), and Bacteroidetes (2.6%) compose the rest of the community.

The 16 phyla comprised 122 families (Supplementary Table S6). Independent of tomato genotype, 39 families were differentially abundant between the rhizosphere and endosphere,

including *Bacillaceae*, *Paenibacillaceae*, *Pseudomonadaceae*, and *Rhizobiaceae* (Fig. 2). Differential abundance results, independent of genotype, for phylum and family levels are shown in Supplementary Tables S7 and S8 (differential abundance results by genotype are in Supplementary Tables S9 to S16). Families that were abundant across all compartments included *Burkholderiaceae* (31.2%), *Sphingomonadaceae* (6.0%), *Caulobacteraceae* (4.0%), *Xanthobacteraceae* (3.8%), and *Pseudomonadaceae* (3.3%).

Impact of tomato genotype on the tomato root bacterial microbiome. Measurements of α diversity (richness and Shannon Index) revealed lower levels of α diversity in the root endosphere compared with the rhizosphere regardless of genotype (ANOVA: $F_{1,37} = 79.502$, $P < 0.001$) (Fig. 3A; Supplementary Fig. S1; Supplementary Table S17). This is consistent with other studies demonstrating lower diversity, or a “gating” effect, in the root endosphere (Bulgarelli et al. 2015; Edwards et al. 2015; Fitzpatrick et al. 2018; Lebeis et al. 2015; Lundberg et al. 2012; Poudel et al. 2019; Schlaeppi et al. 2014). There were no significant differences in either richness or Shannon Diversity among the tomato genotypes (ANOVA: $F_{7,37} = 1.592$, $P = 0.169$; average α diversity in Supplementary Table S17).

Bray-Curtis β -diversity patterns revealed a significant contribution of both microhabitat and genotype. We observed separation between the endosphere samples and the rhizosphere or bulk

Fig. 2. Log2 fold changes of significantly enriched or depleted families in the rhizosphere to endosphere compartments (genotype independent). Each point represents one family, colored by phylum. Points above the dotted line represent families significantly enriched in the endosphere compared with the rhizosphere, and points below represent depleted families.

samples along the first axis (27%) and separation between the rhizosphere and bulk soil samples along the second axis (8.8%) (Fig. 3B). Compartment accounted for 30% of overall variation (PERMANOVA, compartment: $F_{2,59} = 13.18$, $P < 0.001$). This compartmental specialization is consistent with other root microbiome studies (Bulgarelli et al. 2012; Edwards et al. 2015; Lebeis et al. 2015; Lundberg et al. 2012). Tomato genotype was responsible for approximately 10% of the variation (PERMANOVA, genotype: $F_{7,59} = 1.31$, $P = 0.048$), and there was a significant interaction between compartment and genotype (PERMANOVA,

compartment–genotype: $F_{7,59} = 1.32$, $P = 0.041$). Distance among samples was higher within the endosphere than the rhizosphere (Fig. 3B). Dispersion analysis revealed significant differences among compartments ($F_{2,59} = 99.57$, $P < 0.001$), with all three compartments having significantly different dispersions from one another by Tukey's honestly significant differences ($P < 0.05$). CAP to examine the role of compartment and genotype on bacterial community diversity revealed similar results (Supplementary Fig. S2).

We next asked whether the host genotype contributed to variation in β diversity in either the rhizosphere or endosphere. We separated

Fig. 3. Root endosphere α and β diversity varies by compartment and genotype. **A**, Boxplot of Shannon diversity of rhizospheres and endospheres of all eight genotypes and bulk (unplanted) soil. **B**, Principle coordinate analysis of Bray-Curtis distance among bacterial communities of all eight genotypes. Squares indicate rhizosphere samples, triangles indicate endosphere samples, and orange circles indicate bulk samples. Colors indicate genotype as labeled in the legend. Abbreviations: E = endosphere, R = rhizosphere, CMII = *Solanum lycopersicum* 'Castlemart II', WV = *S. pimpinellifolium* accession WestVirginia700, UC82B = *S. lycopersicum* 'UC82B', MM = *S. lycopersicum* 'Money Maker', BB = *S. lycopersicum* 'Bonnie Best', Pearson = *S. lycopersicum* 'Pearson', LA2093 = *S. pimpinellifolium* accession LA2093, and H7996 = *S. lycopersicum* 'Hawaii7996'.

the dataset and focused on the endosphere and rhizosphere compartments separately, and subsequently used CAP to quantify the effect of tomato genotype on microbial diversity within each root microhabitat. In the rhizosphere, genotype explained 34.8% of the variation (permutest, 1,000 permutations; genotype: $F_{7,27} = 1.53$, $P < 0.002$) (Fig. 4A). The clustering pattern revealed two groups: one consisted of the *S. lycopersicum* genotypes H7996 and BB and the *S. pimpinellifolium* WV and LA2093, and the other was composed of all other *S. lycopersicum* genotypes. The reason for such a clustering pattern is not clear. In the endosphere, genotype explained 32.8% of the variation (permutest, 1,000 permutations; genotype: $F_{7,24} = 1.185$, $P = 0.037$) (Fig. 4B). In the endosphere, replicates of individual genotypes tended to cluster together, although there was some overlap among genotypes.

Tomato genotypic differences in family-level abundances in the root endosphere. The characterization of microbiome β diversity revealed an important contribution of tomato genotype to the root bacterial microbiome. To further explore the role of tomato genotype, we investigated whether any taxa were enriched in the root endosphere all genotypes. We identified two bacterial families enriched in the endosphere of at least six of eight tomato genotypes. These families were called “high-frequency endosphere colonizers” (Fig. 5A). High-frequency endosphere colonizing families included *Bacillaceae*, composed of 6 ASVs (Fig. 5A), and *Rhizobiaceae*, with 26 ASVs (Fig. 5C). *Bacillaceae* was enriched in the endosphere of all eight genotypes while *Rhizobiaceae* was enriched in all genotypes except UC82B and Pearson. The abundance of high-frequency endosphere colonizers varied quantitatively across the eight tomato genotypes (Fig. 5A and C). *Bacillaceae* abundance in the endosphere ranged from 3.6% in BB to 13.3% in CMII. *Rhizobiaceae* abundance ranged from 2.4% in UC82B to 6.7% in CMII. Of the high-frequency endosphere colonizers, the most highly abundant ASV across all genotypes was ASV2, a *Bacillaceae* in the genus *Anaerobacillus*. The relative abundance

(proportion of reads for each family out of the total number of reads in each sample) of all families in the endosphere and rhizosphere for each genotype is shown in Figure S3 and Supplementary Table S18.

To investigate selection of these high-frequency endosphere colonizers by different tomato genotypes, we examined their abundance in the root endospheres of 20 RILs from a cross of *S. lycopersicum* H7996 and *S. pimpinellifolium* WV. As with other tomato genotypes, the RILs showed the highest abundance of Proteobacteria, followed by Actinobacteria, Firmicutes, and Bacteroidetes (Supplementary Fig. S4). Although the RIL parent genotypes WV and H7996 have significantly different α diversity in the endosphere by *t* test, RIL α diversity in the endosphere showed no significant differences among genotypes (ANOVA, genotype: $F_{23,86} = 1.284$, $P = 0.216$) (Supplementary Fig. S5A). The β diversity was not significantly different among RILs but showed trends toward genotypic differences (PERMANOVA, genotype: $F_{23,86} = 1.102$, $P = 0.104$) (Supplementary Fig. S5B).

Because differences in root architecture traits have been correlated with different root microbiota (Pérez-Jaramillo et al. 2017), we examined whether there was a correlation between these traits and relative abundance of high-frequency colonizers as well as overall α diversity. No relationship was found between root architecture traits and any root microbiota measures tested (Supplementary Fig. S6A and B, data shown for total root length). Plant resistance to pathogens has also previously been correlated with different root microbiota. The parents of the RIL population, H7996 and WV, are resistant and susceptible, respectively, to the soilborne bacterial pathogen *R. solanacearum*, and our RILs segregate for resistance (Supplementary Table S2). As with root architecture, we did not find a relationship between levels of immunity to *R. solanacearum* and the high-frequency colonizer families or overall α diversity (Supplementary Fig. S6C and D).

Examination of the two families in the high-frequency endosphere colonizer groups showed variation in the abundance of these

Fig. 4. Genotype plays a significant role in shaping both rhizosphere and bacterial endosphere communities. Canonical analysis of principle coordinates (CAP) of the **A**, rhizosphere and **B**, endosphere with genotype as a constraining factor. Abbreviations: E = endosphere, R = rhizosphere, CMII = *Solanum lycopersicum* ‘Castlemart II’, WV = *S. pimpinellifolium* accession WestVirginia700, UC82B = *S. lycopersicum* ‘UC82B’, MM = *S. lycopersicum* ‘Money Maker’, BB = *S. lycopersicum* ‘Bonnie Best’, Pearson = *S. lycopersicum* ‘Pearson’, LA2093 = *S. pimpinellifolium* accession LA2093, and H7996 = *S. lycopersicum* ‘Hawaii7996’.

families across the 20 RILs (Fig. 5B, *Bacillaceae*, and D, *Rhizobiaceae*). ASV 2 (genus: *Anaerobacillus*) showed the highest abundance in the RIL root endospheres, similar to the previously examined genotypes. RILs showed transgressive segregation (i.e., phenotypes more extreme than either parent) for the abundance of frequent colonizers. For example, ASV2 was found to be approximately 4% of the root endosphere in the H7996 parent and approximately 9.5% of the root endosphere of WV but varied from 2% in RIL 801 to approximately 14% in RIL 704.

A *Bacillaceae* isolate differentially promotes growth across a set of RILs. We hypothesized that high-frequency endosphere-colonizing taxa would be beneficial for tomato growth but this growth effect may differ by genotype. To examine this further, we first searched for a bacterial isolate from tomato roots that we could cultivate and which was in the same family as the high-frequency

colonizers. We isolated root endophytes from roots of six field-grown tomato plants: three H7996 and three WV. Plants were grown in spring and summer 2017 in the same farm from which soil was harvested for the microbiome greenhouse experiments described above.

We isolated 183 bacterial colonies from the inner root tissues of the six plants. Using Sanger sequencing of the 16S rDNA gene we identified an isolate (HA129) in the *Bacillaceae* family that was 97.1% identical to ASV397. Although HA129 was isolated from roots of H7996, it was also 99.5% identical to an isolate from WV. To test whether tomato genotypes exhibited variation in their response to *Bacillaceae*, we tested the response of 16 RILs plus the parents H7996 and WV to isolate HA129. Ten days after germination, nine replicates of each RIL, H7996, and WV were inoculated with HA129 and nine with sterile 1× PBS (pH = 7.4) as a

Fig. 5. High-frequency colonizer families vary quantitatively in abundance across genotypes. Abbreviations: BB = *Solanum lycopersicum* 'Bonnie Best', H7996 = *S. lycopersicum* 'Hawaii7996', LA2093 = *S. pimpinellifolium* accession LA2093, UC82B = *S. lycopersicum* 'UC82B', Pearson = *S. lycopersicum* 'Pearson', MM = *S. lycopersicum* 'Money Maker', WV = *S. pimpinellifolium* accession WestVirginia700, and CMII = *Solanum lycopersicum* 'Castlemart II'. Relative abundance of *Bacillaceae* across **A**, all eight genotypes and **B**, 20 recombinant inbred lines (RILs) derived from H7996 and WV. Relative abundance of *Rhizobiaceae* across **C**, all eight genotypes and **D**, RILs derived from H7996 and WV. Numbered genotypes indicate RILs. BB and LA2093 are included to show variation due

control. After 24 days, plants were harvested. Fresh weights of roots and shoots were compared with that of mock-inoculated controls. RILs and parental lines significantly differed in their response to HA129 for both root and shoot growth (Fig. 6). Two RILs and WV showed less than 5% growth promotion in response to HA129 compared with mock-inoculated controls. In contrast, H7996 and three RILs showed more than 30% shoot growth promotion. Response rates for roots and shoots were similar to each other across the RILs. Although RILs varied in their growth response to HA129, there was no relationship (adjusted $R^2 = 0.05123$) between the growth response rate of an RIL to the *Bacillaceae* HA129 and the relative abundance of *Bacillaceae* in an RIL's root endosphere in the microbiome experiment.

DISCUSSION

Tomato genotypes have distinct but overlapping root bacterial microbiomes. We showed that genetic variation in cultivated

Fig. 6. Recombinant inbred lines (RILs) show quantitative differences in their response to *Bacillaceae* isolate HA129. Response rate was measured in terms of the change in **A**, shoot or **B**, root weight of inoculated RILs compared with mock-treated plants of the same genotype. Bar plot showing response rate of different genotypes is color-coded in orange (RILs), blue (parent *Solanum lycopersicum* 'Hawaii7996' [H7996]), and yellow (parent *S. pimpinellifolium* accession WestVirginia700 [WV]) ($n = 9$ per genotype; different letters indicate significantly different at $P < 0.05$).

and wild tomato affects selection of the root bacterial microbiome, and that tomato genotypes differentially respond to a bacterial endophyte. We found that, within *S. lycopersicum* and *S. pimpinellifolium*, tomato genotype significantly affected microbial community diversity in both the root endosphere and rhizosphere. *Bacillaceae* and *Rhizobiaceae* were enriched in the endospheres of at least six of the eight genotypes and varied quantitatively in abundance among a set of RILs. Inoculation of 16 RILs and their parents with an isolate in the same family as the high-frequency colonizers revealed that this isolate promoted both root and shoot growth in a genotype-dependent manner. Together, these data suggest a genetic underpinning to tomato selection and responses to root microbiota and that microbiome strategies to improve tomato production should consider the specific tomato cultivar utilized.

The tomato rhizosphere displayed a rhizosphere effect, with significantly different bacterial communities compared with bulk soil. Proteobacteria and Actinobacteria, with smaller abundances of Bacteroidetes, composed a significant portion of the tomato root rhizosphere microbiota. These phyla were also present in the rhizosphere of barley (Bulgarelli et al. 2015), *Arabidopsis* (Bulgarelli et al. 2012; Lebeis et al. 2015), maize (Fitzpatrick et al. 2018; Peiffer et al. 2013), and other tomato rhizosphere microbiomes (Cheng et al. 2020; Lee et al. 2019; Poudel et al. 2019). Similar to tomato rootstocks (Poudel et al. 2019), Acidobacteria were not abundant in our study of tomato root rhizospheres. However, Acidobacteria were abundant in rhizospheres of tomato grown in Chinese and South Korean soils; therefore, this difference may be location or soil dependent (Cheng et al. 2020; Lee et al. 2019).

The tomato root endosphere microbiota was dominated by Proteobacteria, Actinobacteria, and Firmicutes, with lower levels of Bacteroidetes. Firmicutes were enriched in the endosphere compared with the rhizosphere. This is in contrast to the bacterial communities of grafted tomato root stocks (Poudel et al. 2019). The difference could have been due to soil or age of plants, or to differences in the type of root examined. We sampled whole roots of seedlings, whereas Poudel et al. (2019) harvested small lateral roots at the time of tomato harvest. Different types of roots can have significant differences in their microbial communities (Kawasaki et al. 2016).

Consistent with other root bacterial microbiome studies, distinct microbial communities exist in each tomato root compartment, with a decrease in microbial diversity from rhizosphere to endosphere (Bulgarelli et al. 2015; Caradonia et al. 2019; Edwards et al. 2015; Fitzpatrick et al. 2018; Lebeis et al. 2015; Lee et al. 2019; Poudel et al. 2019). Differences between compartments explained 30% of the variation in tomato root bacterial communities, very similar to those of tomato rootstocks (Poudel et al. 2019) and field-grown tomato roots treated with different nitrogen fertilizers (Caradonia et al. 2019).

Tomato genotype contributes to both rhizosphere and endosphere microbiome assembly. The rhizosphere and root endosphere are distinct microenvironments that each exert unique pressures on microbiota. We identified a compartment-genotype interaction and, when compartments were examined separately, CAP revealed that genotype contributed more than 32% of the variation in rhizosphere samples, and more than 33% to the variation among endosphere samples. Within compartments, the impact of genotype appears to differ according to species. In a study of six rice cultivars, the rhizosphere had a greater genotypic effect on the root bacterial communities compared with the endosphere (30% compared with more than 12%) (Edwards et al. 2015) whereas, among 36 olive cultivars, genotype explained 42% of the variation in the endosphere and more than 53% in the rhizosphere (Fernández-González et al. 2019).

Previous studies have shown that more genetically dissimilar hosts have more dissimilar root bacterial microbiomes (Bouffaud et al. 2014; Naylor et al. 2017). The root bacterial microbiomes of wild species tend to be distinct from those of their domesticated relatives (Bulgarelli et al. 2015; Chaluvadi and Bennetzen 2018; Shenton et al. 2016). We examined both fresh-market and processing cultivated tomato (*S. lycopersicum*) and *S. pimpinellifolium*, the closest wild relative to *S. lycopersicum*. We did not identify clustering patterns in β diversity based on the distinction between market and processing tomato, or on wild versus domesticated species. Our CAP revealed a clustering pattern in the rhizosphere that was unrelated to species, and no clear clustering in the root endosphere. The lack of distinction between our wild and domesticated species could be because they are close relatives.

Although we found that tomato genotype contributed more than 30% of the variation in each compartment, there was greater dispersion among endosphere samples from different genotypes compared with those of the rhizosphere. This has previously been observed among cultivars of many other species, including grapevine rootstocks (Marasco et al. 2018), olive (Fernández-González et al. 2019), poplar (Beckers et al. 2017), and cotton (Wei et al. 2019), among others. Models of microbiome assembly posit that the root endosphere acts as a gate which allows only a subset of rhizosphere and rhizoplane microbes to enter inner root tissues (Bulgarelli et al. 2013; Edwards et al. 2015). The dissimilarity among endosphere microbiomes of different genotypes could suggest that such a gate operates differently in distinct genotypes. The inside of the root exerts unique pressures on bacteria, which may contribute to the decreased species diversity of this compartment and result in greater dispersion among genotypes. Endophytes must be able to suppress the plant immune system (Liu et al. 2017a; Yu et al. 2019) and directly withstand host metabolites, nutrients, and signaling molecules, all of which may vary by genotype. For example, a screen of diverse tomato accessions revealed extensive natural variation in their responses to bacterial mitogen-activated protein kinases such as flg22 and flgII-28 (Roberts et al. 2019; Veluchamy et al. 2014). Thus, root endophytic bacteria may differentially repress host immune responses depending on host genotype. Such direct and distinct genetic pressures could result in the greater dispersion among endosphere bacterial microbiomes of different tomato genotypes.

Tomato rhizosphere communities of different genotypes also differed from each other, though to a lesser extent compared with those of the endosphere. In the rhizosphere, chemical cross-talk, mediated largely through root exudation and microbial activity, is critical for root–microbe interactions. Root exudates include a large and diverse range of primary and secondary metabolites that mediate rhizosphere microbiome structure and activity in tomato and other species (Rajniak et al. 2018; Sasse et al. 2018; Stringlis et al. 2018; Zhelnina et al. 2018). Root exudate secretion varies by soil environment, root developmental stage, and plant species (Sasse et al. 2018; Badri and Vivanco 2009). Differences among genotypes in their root exudation profiles, root cell release, and root mucilage production likely lead to differences in rhizosphere microbiota among genotypes, and may account for the genotype effect we observed in the tomato rhizosphere.

The similarity of rhizosphere samples among our genotypes relative to the endosphere may be a result of the young age of the plants used in our study. Plant age is an important determinant of the rhizosphere microbiome (Chaparro et al. 2014; Edwards et al. 2018; Hu et al. in press; Lundberg et al. 2012; Walters et al. 2018) and may result from changes in root exudation over the course of plant development (Chaparro et al. 2014). It is possible that, at such an early stage of development, tomato root rhizodeposits may not exert

a heavy selection pressure on the rhizosphere, while the root endosphere exerts a heavier pressure on potential endophytic colonizers. Consistent with our data showing small but distinct community differences between the bulk and rhizosphere soil at an early vegetative stage, a recent study examining temporal variation in the tomato rhizosphere microbiome showed increasing differences between bulk soil and rhizosphere samples with plant developmental stage (Hu et al. in press). Further examination of compartment–plant age effects on the root microbiome will be important to further understand how roots recruit and gate their microbial communities.

The potential for tomato genotype to select for root microbiota.

One agricultural microbiome management strategy is to design inoculants consisting of microbial consortia optimized for specific plant genotypes. This assumes that host-selected microbes have a beneficial effect on the host plant, and that host selection and responses are genetically encoded with sufficient heritability to make breeding effective.

Several studies, including this work, suggest that this is possible. In a large study of the rhizosphere microbiota of 27 maize inbred lines and approximately 4,800 samples across three fields, the abundance of nearly 150 operational taxonomic units was significantly regulated by genotype (Walters et al. 2018). Heritability for these microbes was low, and it is not known whether these taxa provide beneficial functions. Here, we found that multiple tomato genotypes recruit members of the families *Bacillaceae* and *Rhizobiaceae*, and that a panel of related tomato genotypes varied in growth responses to a *Bacillaceae* isolate identified from the tomato root endosphere. Variation in growth responses among these genotypes may indicate an element of genetic control, although additional experiments with larger populations are necessary. Genetic control was also postulated in a previous study that identified three tomato QTL for biocontrol of *B. cereus* UW85 to the tomato root rot pathogen *Pythium torulosum* (Smith et al. 1999).

Notably, our work was performed under nonstress conditions. Additional work is necessary to understand the impact of environment. Given the range of variation in abiotic stress responses in cultivars, particularly drought stress responses, one may hypothesize that the impact of genotype on root bacterial communities would be greater under stress. However, Naylor et al. (2017) found that, among 18 grass species, the root endosphere bacterial microbiota became more similar in drought compared with plants grown in well-watered conditions. Perhaps in some environments the impact of the soil environment overrides that of genotype. In such cases, breeding for genotype-specific host–microbiome relationships may be less important than identifying environment-specific microbiomes.

The success of using genotype-specific microbiomes in agriculture will also depend on which plant traits significantly affect root microbiota, and how much we can manipulate such traits without deleteriously affecting other aspects of plant growth and development. Although root bacterial microbiome communities may affect root architecture (Pérez-Jaramillo et al. 2017), the degree to which plants with distinct types of roots or root architectures select for specific taxa remains uncertain. We did not find a relationship between the root endosphere microbiome and root architecture in our tomato RILs, suggesting that, at least in this population, selecting for specific root architecture traits would not select for specific taxa. Similarly, we did not observe a relationship between microbiota and immune responses. The RILs we used were derived from genotypes that are resistant (H7996) and susceptible (WV) to a soilborne bacterial pathogen, *R. solanacearum*. We did not identify a relationship between specific taxa or α diversity of RIL root endosphere microbiomes with the level of RIL resistance, although this could be because our soils were not infected with *R. solanacearum*. Additional work is needed to understand how

plant traits such as root architecture and disease resistance affect selection of microbial communities.

Finally, another important aspect when considering the role of host genotype is the importance of understanding the genetics underlying both host selection and responses. We did not see a relationship between genotypic variation in relative abundance of *Bacillaceae* and growth response to inoculating with a *Bacillaceae* isolate. This may be because isolate HA129 is not identical to any of the *Bacillaceae* isolates identified in the greenhouse microbiome experiment, or because other factors besides isolate abundance contribute to growth promotion. Alternatively, we know little about the relationship between colonization levels of root-associated microbiota and plant growth responses, or whether this relationship varies across genotypes. Perhaps different cultivars require less bacterial colonization for similar growth responses. Although we did not measure colonization levels of HA129 here, future work aiming to understand the relationship between recruitment, bacterial colonization and plant growth responses is necessary.

Conclusions. Our results reveal the structure of the tomato root bacterial microbiome, and show that tomato genotype affects the selection of both rhizosphere and endosphere bacterial taxa. These data suggest that taxa present in the microbiome of multiple tomato genotypes promote growth, and hint at a genetic basis for response to root endophytic bacterial taxa. This suggests that breeding tomato for improved associations with root microbial communities is possible, although future work with RIL or GWAS tomato populations is needed to investigate this fully.

Many aspects of genotype–microbiome interactions remain to be understood if we are to use microbiomes to benefit crop production. For example, the effect of genotype varies among species, plant developmental stage, root compartment, and abiotic stress. Interactions among these add additional complexity, and how much one could rely on breeding as a strategy for optimal microbiome use is unclear. Because plants likely select for specific microbial functions regardless of taxa, additional studies examining the role of host genotype in selecting microbial function are needed. Future work is also needed to understand the heritability of QTL which select for the microbiome, and how such QTL could be used as part of a multipronged microbiome management approach. For example, combining these QTL with field inoculation of taxa selected by the host may improve production. Although much work is still needed, our work suggests that optimizing host genetics may be one way to select for beneficial microbiomes in tomato.

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