RESULTS

To ensure that genetic inference was independent of the GWA method or SNP diversity reference, we repeated genetic analysis with a second association method (GEMMA) using SNPs called in comparison to the most recently published *B. cinerea* genome (B05.10). All methods converged on the same image of genetic architecture; we found that the genetic architecture of virulence of B. cinerea is highly quantitative, with hundreds of significant SNPs with small effect sizes associated with lesion area on each tomato genotype.

To identify variable pathogen genes controlling differential virulence across plant genotypes, we conducted GWA mapping analysis within the pathogen by two approaches, using 272,672 SNPs compared to the *B. cinerea* T4 reference genome (Table AX). Due to the large effect of plant genotype on resistance to *B. cinerea*, we performed GWA using the model-corrected least-squared mean virulence measured on each tomato genotype as separate traits. We first used a ridge-regression approach (bigRR) in combination with 272,672 SNPs from *B. cinerea* compared to the T4 reference genome to estimate the phenotypic effects across the genome {Shen, 2013; Corwin, 2016; Corwin, 2016; Francisco, 2016}. To verify these patterns, we also implemented a Genome-wide Efficient Mixed-Model Association (GEMMA) analysis with a centered relatedness matrix to control for the effects of population structure {Zhou, 2012}. In GEMMA, we included 237,878 SNPs from *B. cinerea* compared to the B05.10 reference genome. To determine significance of SNP effects under both GWA methods, we permuted phenotypes 1000 times to calculate 95, 99, and 99.9% effect size thresholds within each plant host. Under both methods, GWA analysis showed that the genetic basis of *B. cinerea* virulence on tomato is highly polygenic. Consistent with a polygenic structure of this trait in the pathogen, neither method of GWA identified large-effect SNPs (Ch1, Figure 4).

The model accounting for population structure (GEMMA) confirmed our finding of a highly polygenic nature of lesion size in the pathogen (Supplemental Figure 3), with 2,530 to 8,221 SNPs significantly associated with virulence at the 99% threshold, and 288 to 1,361 SNPs at the 99.9% threshold (significance was determined using an empirically determined 1000-permutation threshold).

To focus on the small-effect genes linked to *B. cinerea* virulence, we classified a gene as significantly associated if there was 1 SNP linked to a trait using a 2kbp window surrounding the start and stop codon for a given gene.

At the 99.9% SNP significance threshold, GEMMA identified 23 genes across 7 to 9 of the tomato accessions (Supplemental Figure 4).

To identify genes consistently associated with *B. cinerea* virulence on tomato across GWA methods, we examined the gene overlap between significant associations identified by GEMMA on the B05.10 genome and bigRR on the T4 genome. We conservatively identified genes within 2kb of significant SNPs at the 99% permutation threshold for bigRR, and at the 99.9% permutation threshold for GEMMA. Among these, 263 genes were linked to at least two plant genotypes by both methods (Supplemental Data 2 a). These genes include transporters and enzymes that can be important for *Botrytis* toxin production and/or detoxification of plant defense compounds and are key to virulence. Other known and predicted pathogen virulence functions were largely not identified (Supplemental Data 2 a, c).

At the SNP level, fewer loci contribute to virulence across all hosts and both GWA methods. We found five B. cinerea SNPs significantly linked to altered lesion size on all 12 tomato accessions using the bigRR analysis (Figure 4b). 215 SNPs were called in at least ten hosts, and 3.3k SNPs were called in at least half of the hosts while 27% (46,000) of the significant SNPs were linked to virulence on only a single host tomato genotype. These levels of overlap exceed the expected overlap due to random chance (Figure 5a). GEMMA analysis also found significant SNP overlap between hosts at the 99% permutation threshold, with 89 SNPs in at least ten hosts, 859 SNPs in at least half of the hosts, and 63% (19,270) of significant SNPs unique to a single host. SNP calling between hosts was lower for GEMMA at the 99.9% permutation threshold, with 78% of significant SNPs (4269) in a single host, and 38 SNPs significant across at least half of the hosts (Supplemental Figure 4 a). While only a small subset of these B. cinerea SNPs were linked to virulence on all the tomato genotypes, we obtained better overlap across host genotypes by focusing on gene windows.

The identification of two isolates that distinctly respond to tomato domestication suggests that there is some natural genetic variation in *B. cinerea* that is affected by tomato domestication. To directly *map B. cinerea* genes that control differential virulence on wild versus domesticated tomatoes, we used the least-squared mean virulence of each isolate across all wild and all domesticated tomato genotypes as two traits. We also calculated a domestication sensitivity trait; the relative difference in lesion size for each isolate between domesticated and wild hosts. Using these three traits, we conducted bigRR GWA within *B. cinerea* to map genes in the pathogen that respond to domestication shifts in the plant.

GWA of these domestication traits by GEMMA identified similar patterns of polygenic structure, high overlap between SNPs and genes on wild or domesticated tomato hosts, and rare overlap with Domestication Sensitivity (Supplemental Figure 5). To begin querying the underlying gene functions for these various *B. cinerea* loci, we called genes as significant if there was one SNP within 2kb of the gene (Figure 7c). We also examined the genes associated with these domestication virulence traits found by both bigRR and GEMMA. This overlap identified 200 unique genes including several transporters and enzymes, with few predicted virulence genes (Supplemental Data 2 b). One gene from this overlap list (Bcin01g05800) contains TPR repeats, which are common in bacterial virulence proteins (Cerveny, Straskova et al. 2013) and are among the proteins secreted by the plant pathogen Ustilago maydis (Lo Presti, López Díaz et al. 2016).

We also examined functional enrichment for the genes associated with domestication traits by both GEMMA and bigRR. We found 41 significantly overrepresented biological functions (Supplemental Data 2 d). In both datasets, the The enrichments were largely surrounding enzyme and transport functions, which are known to be key components of how the pathogen produces toxic metabolites and conversely detoxifies plant defense compounds.

DISCUSSION

We also identified a conservative subset of genes whose association to differential *B. cinerea* virulence is consistent across GWA methods and reference genomes (Supplemental Data 2 a, b, c, d).

METHODS

For GEMMA mapping, we used 91 isolates with a total of 237,878 SNPs against the *B. cinerea* B05.10 genome with MAF 0.20 or greater and less than 10% missing calls. The overall SNP number was similar when using either reference genome.

The *B. cinerea* GEMMA used 237,878 SNPs at MAF 0.20 or greater, and less than 10% missing SNP calls as described above. To determine significance of SNPs by GEMMA, we used 1000 permutations to determine p-value significance at the 99%, and 99.9% thresholds (Doerge and Churchill 1996, Shen, Alam et al. 2013, Corwin, Copeland et al. 2016). SNPs were annotated using a custom R script linking the SNP to genes within a 2kbp window from the gene transfer format file construction from the B05.10 gene models for genomic DNA (Staats and van Kan 2012, Zerbino, Achuthan et al. 2017). A table of gene name translations across genome annotations was pulled from the gene overlap between the bigRR T4 annotation and GEMMA B05.10 annotation using a custom R script and gene name translations pulled from the INRA Botrytis cinerea Portal (Choquer, Fournier et al. 2007, Viaud, Adam-Blondon et al. 2012).

FIGURES

Figure A1. GWA by GEMMA of *B. cinerea* lesion size on individual tomato genotypes.

Figure A2. Frequency of overlap in *B. cinerea* GEMMA GWA significance across tomato accessions.

Figure A3. GEMMA GWA analysis of domestication sensitivity in *B. cinerea*.

**Table A1. Gene and Function Annotation from B05.10 and T4 GWA Results**

a) Genes with significant SNPs on at least two tomato accessions by both bigRR on T4 and GEMMA on B05.10.

b) Genes with significant SNPs linked to *Botrytis* virulence response to tomato domestication by both bigRR on T4 and GEMMA on B05.10.

c) Functional categories significantly overrepresented in genes linked to *Botrytis* virulence response to tomato by both bigRR on T4 and GEMMA on B05.10.

d) Functional categories significantly overrepresented in genes linked to *Botrytis* virulence response to tomato domestication by both bigRR on T4 and GEMMA on B05.10.