Population genomics of *Pseudocercospora griseola* reveals new groups in the Middle American clade and the presence of the endophytic bacterium *Achromobacter xylosoxidans*

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Abstract

Angular leaf spot (ALS), caused by *Pseudocercospora griseola* is an important disease of common beans. *P. griseola*, is highly variable and has co-evolved with its host. In this study, 48 isolates of *P. griseola* from Puerto Rico, Guatemala, Honduras and Tanzania were sequenced (3RADseq), resulting in the *de novo* assembly of 42,214 contigs. Phylogenomic, population genetic structure and principal component analyses using 1,260 SNPs divided these isolates into two populations, Andean and Middle American, while the Middle American population was further divided into three sub-populations. There were moderate to high levels of differentiation between *P. griseola* populations, with pairwise Fst values ranging from 0.11 to 0.95. The Andean population was composed of isolates from Tanzania and was separated from the Middle American population (Fst = 0.95). The Middle American population was separated into 3 subpopulations including isolates from: 1. Guatemala and Honduras, 2. Tanzania, and 3. Puerto Rico. Pathogenicity testing of 27 isolates from Puerto Rico, using 12 common bean differential lines, identified ten races, but these races were not associated with SNPs found in virulence genes. DNA of an endophytic bacterium (*Achromobacter xylosoxidans*) was found in seven mildly virulent isolates suggesting a possible role of the bacterium in the observed virulence patterns. To understand the evolution and diversity of *P. griseola*, further study of the virulence genes and the interactions among the endophytic bacterium, the fungus, and the host plant is required. Such information is critical to inform breeding strategies for the development of resistant germplasm and cultivars.

Pseudocercospora griseola (Sacc.) Crous and Braun*,* is one of the most important pathogens of common and snap beans (*Phaseolus vulgaris* L.) in the tropics and subtropics (de Jesus Junior et al., 2001). Lesions of *P. griseola* on common bean are limited by leaf veins, and the typical symptoms of angular leaf spot (ALS) are observed in its necrotrophic phase when the hyphae cause necrosis without penetrating host cells (Monda et al., 2001). Several studies have demonstrated that the fungus has co-evolved with the Andean and Middle American gene pools of *P. vulgaris* and consequently has been divided into two populations: Andean and Middle American (Pastor Corrales et al., 1998; Guzman et al., 1995; Crous et al., 2006). The Andean and Middle American common bean gene pools diverged between 110,000 and 500,000 years ago (Mamidi et al., 2013). Likewise, the Andean and Middle American *P. griseola* populations diverged approximately 100,000 years ago, so co-evolution with the Middle American and Andean common bean gene pools has occurred since that divergence (Serrato-Diaz et al., 2020).

The Andean *P. griseola* population (*P. griseola* f. *griseola*) infects Andean common bean genotypes, while the Middle American population (*P. griseola* f. *mesoamericana*) infects both Andean and Middle American common bean genotypes (Guzman et al., 1995; Pastor-Corrales et al., 1998). In Africa, a rare group of Andean isolates named 'Afro-Andean' mainly infect Andean common bean genotypes but also infect a few Middle American genotypes (Mahuku et al., 2002). Analyses based on random amplified microsatellites (RAMS) and restriction fragment length polymorphisms (RFLPs) of the ribosomal intergenic spacer (IGS) suggested that the Afro-Andean isolates belong to the Andean *P. griseola* population (Mahuku et al., 2002). However, genetic analysis using four nuclear genes confirmed the existence of the Afro-Andean sister clade that diverged from the Andean population approximately 40,000 years ago (Serrato-Diaz et al., 2020).

Pseudocercospora griseola is a highly variable pathogen, and different virulence phenotypes (races) have been reported worldwide (Pastor-Corrales et al., 1998; Aytenfsu and Terefe, 2023). For instance, an analysis of 419 isolates from 23 countries including five Middle American isolates from Puerto Rico found 120 *P. griseola* races (Jara et al., 2001). Currently, the identification of *P. griseola* races is based on its virulence patterns over a set of 12 common bean differential lines (six of Andean origin: ‘Don Timoteo’, ‘G11796’, ‘Bolon Bayo’, ‘Montcalm’, ‘Amendoin’ and ‘G5686’; and six of Middle American origin: ‘Pan 72’, ‘G2858’, ‘Flor de Mayo’, ‘Mexico 54’, ‘BAT 332’, and ‘Cornell 49-242’) (Pastor-Corrales and Jara, 1995). Common bean breeding programs have been focused only on detecting resistance genes or QTLs associated with *P. griseola* races in these two gene pools (Middle American or Andean). For example: for Middle American common bean, the resistance gene *Phg-2* was identified and mapped in chromosome 8 (Pv08) in ‘Mexico 54’ and ‘BAT 332’ using the *P. griseola* races 63-19 and 63-39. Resistance genes *Phg-2* to *Phg-6* were identified in ‘Mexico 54’ and ‘MAR 2’ using the *P. griseola* races 63-19, 63-23 and 63-39. The dominant genes PhgG10909B and PhgG10909B were identified and mapped to chromosomes PV04 and Pv08, respectively, in the accession ‘G10979’ using *P. griseola* race 63-63. The Phg-3 gene, identified in the common bean ‘Ouro Negro’ and mapped to Pv04, confers resistance to *P. griseola* races 63-39, 31-55, and 63-31 (Nay et al., 2019). For Andean common beans, a resistance QTL (ALS4.1$^{\mathrm{GS.UC}}$) and three resistance genes, PhgG5686A, PhgG5686b and PhgG5686c, were identified in ‘G5686’ on chromosome Pv04 and Pv09 using *P. griseola* race 31-0. The *Phg-1*, *Phg-2*^{2}, *Phg-3*^{2}, and *Phg-4*^{2} genes were identified in ‘AND277’ on chromosome Pv01 using *P. griseola* races 31-17, 63-19 and 63-23. The QTL's ALS10.1$^{\mathrm{DG\ UC}}$, ALS4.2 $^{\mathrm{GS\ UC}}$ and ALS5.2 $^{\mathrm{UC}}$ were found in ‘CAL143’

(‘AND277’ is one of its parents), and are responsible for 16 to 22%, 10.8% and 9.4% of the resistance to ALS, respectively (Nay et al., 2019)

Restriction-associated DNA sequencing (RADseq) samples a consistent portion of the genome at a fraction of the cost of whole genome sequencing (Bayona-Vásquez et al., 2019). RADseq reduces the complexity of the genome and has been useful for understanding populations of different organisms (Masclaux et al., 2019; Liu et al., 2020). One of the advantages is that it provides thousands of polymorphic genetic markers for any species using either a reference genome or *de novo* analysis for a non-model organism (Bayona-Vásquez et al., 2019). Many variants of the RADseq approach have been developed including genotype-by-sequencing (GBS), 2-enzyme GBS, dual-digest RADseq (ddRADseq), ezRAD, and recently the 3RADseq method (Bayona-Vásquez et al., 2019). The advantage of 3RADseq is that it uses a third restriction enzyme which cuts apart adapter-dimers formed by the phosphorylated adapter, reducing chimeras and increasing the efficiency of adapter ligation to DNA (Bayona-Vásquez et al., 2019). 3RADseq is effective with low quantity and quality DNA samples (Bayona-Vásquez et al., 2019).

The understanding of the evolutionary dynamics of ALS, genetic changes that drive its virulence, and host-pathogen coevolution and adaptation is critical for developing durable ALS disease-resistant bean varieties, improving disease management strategies, reducing chemical control and ensuring sustainable crop production. Moreover, this knowledge can provide broader insights for other host-pathogen interactions and mitigate future disease outbreaks. In addition, the elucidation of the genetic variability of *P. griseola* at the country and regional scale will assist common bean breeders in selecting the most relevant isolates for ALS germplasm evaluation for targeted regional cultivar release. The purpose of this study was to evaluate the genetic diversity of *P. griseola* isolates from four countries using 3RADseq to better understand the genetic variability of *P. griseola* isolates from Puerto Rico.

Materials and Methods

Isolation of *P. griseola* and inoculation on ALS differential plants

Middle American and Andean *P. griseola* isolates from Guatemala (9), Honduras (6), Puerto Rico (28) and Tanzania (5) (Table 1) were collected from leaves of common bean showing ALS symptoms (Fig. 1A). Infected common bean leaves were incubated in humid chambers at 24°C with a 12 h photoperiod for 24 h for induction of fasciculate conidiophores (Fig. 1C). Fasciculate conidiophores were picked with a Pasteur pipette using a stereomicroscope and placed on V8 media in Petri dishes. The V8 agar was amended with 125 mg/L streptomycin and 150 mg/L ampicillin to prevent bacterial contaminant growth. Monosporic colonies of *P. griseola*, approximately 2 cm in diameter, were obtained 30 days after plating. A 500 µl drop of sterile distilled water was placed on the monosporic colony and the colony surface scraped using a sterile spatula. Spore solutions from scraped isolates were spread on fresh V8 media in Petri plates to increase the inoculum. Twenty days after incubation of this second sub-culture, conidia (Fig. 1B) were scraped again, either with or without sterile distilled water, for plant inoculations or DNA extractions, respectively. For long-term storage (approximately five years), six 1 cm^3 plugs of V8 media containing *P. griseola* were placed into 2 ml microcentrifuge tubes with 1 ml of sterile distilled water. As needed for inoculum production, *P. griseola* isolates were re-activated by placing one 1 cm^3 plug of V8 media containing *P. griseola* on fresh V8 media (amended as described above).

Virulence and race determination

Ten seeds of each ALS common bean differential line (six from the Andean gene pool – 'Don Timoteo', 'G11796', 'Bolon Bayo', 'Montcalm', 'Amendoin', and 'G5686; and six from the Middle American gene pool – 'Pan 72', 'G2858', 'Flor de Mayo', 'Mexico 54', 'BAT 332', and 'Cornell 49-242') were planted in a greenhouse on four benches, each containing raised beds six inches tall, constructed with cement boards and filled with sterilized silica sand at the U.S. Department of Agriculture, Agriculture Research Service, Tropical Agriculture Research Station (USDA-ARS-TARS). The average minimum and maximum temperatures inside the greenhouse were 23°C and 37.6°C, respectively. Relative humidity ranged from an average minimum of 55% to a maximum of 87%. For *P. griseola* race determination, the first trifoliate leaf of the common bean seedlings was inoculated with a suspension of 4×10^4 conidia/mL of a Puerto Rican *P. griseola* isolate at 20 days after germination. Differential plants were irrigated by sprinkler for 1 minute at 4-hour intervals starting 12 hours after inoculation. ALS symptoms were assessed 20 days after inoculation and *P. griseola* races were identified according to Pastor-Corrales and Jara (1995). The race is given by a binary code based on the summation of the binary numbers of the susceptible Andean and Middle American lines. For the Andean genotypes, the code numbers are 'Don Timoteo' = 1, 'G11796 = 2', 'Bolon Bayo' = 4, 'Montcalm' = 8, 'Amendoin' = 16, and 'G5686' = 32; and for the Mesoamerican genotypes the numbers are 'Pan 72' = 1, 'G2858' = 2, 'Flor de Mayo' = 4, 'Mexico 54' = 8, 'BAT 332' = 16, and 'Cornell' 49-242 = 32) (Pastor-Corrales and Jara, 1995).

DNA extraction and 3RADseq library preparation

Genomic DNA (gDNA) from the conidia of 48 *P. griseola* isolates was extracted using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer instructions (Table

1). The gDNA was normalized to 100 ng per isolate to construct 3RADseq libraries as described by Bayona-Vásquez et al. (2019) with slight modifications. Genomic DNA was digested using 10U of the restriction enzymes ClaI, BamHI-HF, and MspI (R0197S, R3136S and R0106S, respectively; New England Biolabs (NEB), Ipswich, MA). Samples were incubated at 37^{o}C for 1 hour followed by adapter ligation using i5 and i7 internal adapters provided by the Fitzpatrick Laboratory (http://web.utk.edu/~bfitzpa1/), University of Tennessee, Knoxville, TN (UTK). The ligation mix included 2 µL deionized water, 1.5 µL ATP (10mM), 0.5 µL 10x ligase buffer and 400U of T4 DNA ligase (NEB M0202S).

Ligation was completed in a thermocycler with the following conditions: 2 cycles of 22^{o}C for 20 min and 37^{o}C for 10 min followed by 80^{o}C for 10 min. Later, digested gDNA/adapters were purified using Serapure speed beads (Thermo-Scientific, Waltham, MA, USA) and resuspended in 20 µL TE. The Illumina index library was prepared using a mix of 10 ul of the ligation product, 5 µL Kapa HiFi Buffer, 0.75 µL dNTPs, 8.75 µL deionized water, 0.5 µL Kapa HiFi DNA Polymerase, and 2.5 µL of i5 and i7 primers from iTru5 96-4 and iTru7 96-1 plates from the Environmental Health Science (EHS) DNA laboratory at the University of Georgia, Athens, GA (https://baddna.uga.edu/services.html). The library was amplified in a thermocycler with the following conditions: 95^{o}C for 2 min; 98^{o}C for 20 sec, 60^{o}C for 15 sec, 72^{o}C for 30 sec for 25 cycles; followed by 72^{o}C for 5 min and was visualized under uv in a 1% agarose gel. The Adapterama 3RADseq method (Bayona-Vasquez et al., 2019) was applied across all *P. griseola* isolates. Each isolate/PCR product was cleaned using Serapure speed beads and resuspended in 25 µL TE. A library pool was made by normalizing each isolate's DNA to 55 ng/µL DNA in a single 1.5 mL tube. Automated gel extraction for approximate 150 bp fragment sizes was completed using a Pippin Prep at the UTK Genomic Core facility. The final pool was cleaned using Serapure speed beads and the DNA concentration was normalized for Illumina sequencing (Qubit, Invitrogen, Carlsbad, CA). The library was sequenced using an Illumina NovaSeq S4 at the Georgia Genomics and Bioinformatics Core of the University of Georgia (https://dna.uga.edu/).

RADseq quality filtering *denovo* assembly and SNP calling

The *P. grioseola* pair-end raw sequences were demultiplexed by the Environmental Health Science (EHS) DNA laboratory at the University of Georgia (https://baddna.uga.edu/services.html). Pre-processing used fastp (Chen et al., 2018) for trimming adapters, polyG and polyX tails. The STACKS V.2 program (Rochette et al., 2019) was used to filter sequences for read quality and to trim their lengths to 75 bp using the *process_radtags* command. The *denovo_map.pl* program of STACKS V.2 was used to build *de novo* sequence contigs without a reference genome (*ustacks*), to create a catalog of loci (*cstacks*), and to match each sample against the catalog (*sstacks*). The sequence variants [i.e. single nucleotide polymorphisms (SNPs)] in the population were identified using the *gstacks* program and summarized into the variant call format (VCF) with the *populations* program from STACKS V.2. The raw genotypes with minor allele frequency (MAF) >0.01 and missing data <0.50 were filtered in Tassel V.5 (Glaubitz et al., 2014) to retain 1,260 SNPs for downstream population genomics analysis.

Phylogenomic tree construction

Pair-wise genetic distance among the 48 *P. griseola* isolates was calculated based on identity-by-state (IBS) using the 1,260 SNPs as implemented in Tassel v5 (Glaubitz et al., 2014). The phylogenetic tree was created using the maximum-likelihood method with Model Finder software (Kalyaanamorrthy et al., 2017) as implemented in IQ-TREE 2 software (Minh et al., 2020). The non-parametric bootstrapping value for each branch was determined using the ultrafast bootstrapping method (Hoang et al., 2018) with 1,000 replicates as implemented in the IQ-TREE 2 software (Minh et al., 2020). The phylogenetic tree was visualized using Interactive Tree of Life (Letunic and Bork, 2011).

Population structure and fixation index

Population structure of the *P. griseola* isolates was determined based on the 1,260 SNPs and the admixed ancestry model-based clustering method in STRUCTURE v2.3.4 (Pritchard et al., 2000). Six independent runs using an admixture model with correlated frequencies, 50,000 burn-in periods, and 125,000 Monte Carlo Markov Chain cycles for each *k* value set from 1 to 11 were performed. The analysis output was processed using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) to determine the number of populations based on the change in the log probability data between successive *k* values (Evanno et al., 2005). These six runs were matched by permutation in CLUMPP (Jakobsson and Rosenberg, 2007). Principal component analysis was conducted using Tassel v5 and the 1,260 SNPs. The number of private SNPs and the genetic profile of each *P. griseola* population was determined based on allele frequencies. Pairwise fixation indexes (F_{ST}; Weir and Cockerham, 1984) for each population was estimated in R (R Core Team 2024) based on the 1,260 SNPs package stAMPP (Pembleton et al. 2013) using 1,000 cycles of bootstrapping (Goudet, 2005).

Genome alignments and virulence genes

To obtain insight into the genomic structure of *P. griseola,* the de-novo contigs (NCBI SRA Bioproject accession number PRJNA1174785; NCBI, Bethesda, MD) generated by Stacks V2 were aligned to the *Cercospora beticola* (Sacc.) genome sequence (CB0940.V2; Wyatt et al., 2024). The consensus sequence of each contig was aligned to the *C. beticola* genome using the standalone version 2.15 of the Basic Local Alignment Search Tool (BLAST V.2.15, NCBI). The regions with >80% identity (E-value < 3.15 E-07) were considered homologous.

Putative virulence and non-virulence genes in the *P. griseola* genome were identified using biopython in the Bio.Blast package of the NCBI module (Cock et al., 2009). Once the excel file was generated by this package, sequences are filtered by gene function in fungi reported in GenBank (NCBI). We considered genes homologous with >70% identity and Evalues <E-15. A phylogenetic tree was constructed with isolates from Puerto Rico using the 87 contigs with putative virulence function and the 1,260 SNPs data set using the maximum-likelihood method with ModelFinder (Kalyaanamorrthy et al., 2017) as implemented in the IQ-TREE program (Nguyen et al., 2015). The non-parametric bootstrapping value for each branch was determined using the ultrafast bootstrapping method (Hoang et al., 2018) with 1,000 replicates as implemented in IQ-TREE 2 (Minh et al., 2020).

The presence of *Achromobacter xylosoxidans* in the sequence of the *P. griseola* isolates was determined using the standalone BLAST V.2.15 software. The *P. grioseola* pair-end processed sequence of each isolate was BLASTed against the genome sequence of the *A. xylosoxidans* strain MN001 (CP012046.1; Badalamenti and Hunter, 2015). Subsequently, the isolates with reads that showed >80% of identity (E-value < 7.96 E-31) with *A. xylosoxidans* were identified.

Results

Genomic sequence of *P. griseola*

A total of 27.9 million reads were obtained and analyzed from the 3RADseq libraries of the 48 isolates of *P. griseola* from Honduras, Guatemala, Puerto Rico and Tanzania. These sequences were assembled into 42,214 contigs from 75 to 237 bp with an average length of 121 bp (N50 = 150 bp). Several putative virulence genes and transposable elements (retrotransposons catalog numbers 1731, 2093 and 7292) were found in the *P. griseola* genome (Table S1). The BLAST analysis found that 262 contigs were associated with virulence genes. These virulence genes were associated with cell wall degradation (glycoside hydrolase family genes), degradation of salicylic acid (salicylate 1-hydroxylase), Major Facilitator Superfamily (MFS), ATP-binding cassette (ABC) and multidrug transporters, homologues to Aflatoxins, AAL, AMT, AK, HC toxins, homologous of CEK1, YAK1, CLA4 and SSK1 protein kinases, polyketide synthases, hybrids of PKS genes and non-ribosomal peptide synthetases (NRPS), and Chitin synthases CHS1, CHS2, CHS4, CHS5, CHS6 and CHS8 genes (Table 2). A total of 2,879 contigs (6.88%) were aligned against the *C. beticola* reference genome (Fig. 2). These contigs were distributed across the 10 chromosomes of *C. beticola* showing homology with this genus.

Population genetic structure and phylogenetic analysis

Analysis of the population genetic structure identified *P. griseola* isolates in Andean (three Tanzania isolates) and Middle American (45 isolates from Tanzania, Central America and Puerto Rico) populations (K=2; Fig 3b). To understand the population structure within the Middle American population we assessed the population disruption at different K values. The analysis showed that the Middle American population comprises two subpopulations, with one consisting of isolates from Guatemala and Honduras, and the other isolates from Puerto Rico (Fig. 3). Remarkably, one isolate from Guatemala (ALS-47), three from Puerto Rico (ALS-50, ALS-45 and ALS-35), and two from Tanzania (ALS-66 and ALS-56) showed divergence from these two sub-populations. The Maximum likelihood tree supported this population structure. The Puerto Rico, Honduras and Guatemala *P. griseola* isolates were grouped in the Middle American clade (bootstrap = 100) (Fig. 3). Likewise, the Middle American subpopulation from Guatemala and Honduras were grouped into one clade (bootstrap >92) separate from the Puerto Rican clade (bootstrap >92). The Tanzanian isolates, ALS-66 and ALS-56 showed a closer genetic relationship with the isolates from Puerto Rico than the other isolates from Tanzania.

The PCA analysis also confirmed the genetic differentiation among *P. griseola* isolates from Guatemala, Honduras, Puerto Rico and Tanzania (Fig. 4). The first principal component explained 72% of the variation and mostly separate the isolates into Andean and Middle American groups. The second principal component explained 15% of the variation and defined the three subpopulations within the Middle American group. These three subpopulations were defined as Guatemala/Honduras, Puerto Rico and Guatemala/Tanzania (two isolates each). The level of genetic differentiation between the Andean and Middle American populations was high (Fst = 0.95; p-value = <0.0001). Likewise, the genetic differentiation within the Middle American populations was high between the Puerto Rico and the Guatemala/Honduras sub-populations (Fst = 0.82; p-value = <0.0001). However, the isolates from Guatemala and Honduras showed a moderate genetic differentiation (Fst = 0.11; p-value = <0.0001). The Middle American, Tanzanian isolates showed high genetic differentiation from both Puerto Rico and Guatemala/Honduras populations (Fst = 0.68; p-value = <0.0001).

Genetic diversity of *P. griseola* isolates

The allele distribution across populations provides insight into the genetic diversity of *P. griseola* (Table 3). Most of the alleles found in this study (81%) differentiated the Andean and Middle American populations. There were 51 private alleles in the Andean population. Remarkably, the three Tanzania isolates in the Andean population showed different genetic profiles. The Middle American population included 191 alleles distributed within the Puerto Rico (59 SNPs), Guatemala/Honduras (104 SNPs) and Tanzania (28 SNPs) populations. The most genetically diverse Middle American population was the Guatemala/Honduras group which had 52 private alleles, with fourteen of the isolates (93%) being genetically distinct. In contrast, most of the alleles found in the Puerto Rican population were private (43 SNPs) but there were only ten genetic profiles among the 32 isolates. The two Middle American isolates from Tanzania were genetically distinct and included seven private alleles.

To determine the possible roles of *P. griseola* genes in the population structure, we determined the putative gene function of the alleles (Table 4). The analysis found 32 putative genes of which 28 were associated with the population structure. The functions of these genes include biochemical pathway regulators, housekeeping, and virulence. In the Middle American population, 18 genes associated with the subpopulations from Puerto Rico (8), Honduras and Guatemala (9) and Tanzania (1) were identified, while in the Andean population 10 genes were identified. The analysis also found that three genes share alleles across the Middle American subpopulations, and another gene is shared between Middle American and Andean populations of *P. griseola*.

Races of *P. griseola,* virulence genes and *Achromobacter*

The virulence analysis of 27 Puerto Rican isolates against the 12 common bean differential genotypes resulted in the identification of 10 *P. griseola* races (Table 4). The most frequent races were 63-07, 63-23 and 63-31 present in both Juana Diaz and Isabela, Puerto Rico. Five races (31-07, 31-55, 47-19, 58-15 and 63-55) were found only in Juana Diaz, Puerto Rico, while another two races (63-15 and 63-63) were found only in Isabela, Puerto Rico. The most virulent race (63-63) was found in Isabela affecting the 12 common bean differential lines, while the less virulent (58-15, 47-19 and 31-07) were found in Juana Diaz, Puerto Rico, affecting 8 differential lines.

The genetic diversity of *P. griseola* from Puerto Rico included 10 genetic profiles which could not be associated with the races of the pathogen. The maximum phylogeny tree based on 59 SNPs revealed that isolates representing races 63-55, 47-19 and 63-23, found in Juana Diaz, were genetically related and were clustered into one clade (bootstrap = 85; Fig. 6). Likewise, the genetic profile was similar among three races (63-31, 58-15, 63-07) found in four isolates from Juana Diaz and two from Isabela that clustered into one clade (bootstrap = 80; Fig. 6). The results indicate that these nine isolates representing six races might be the most genetically diverse (Fig. 6). To obtain a better insight into the relationship between the genetic diversity and pathogen races, a second maximum phylogeny tree was constructed using only SNPs found in virulence genes (Fig. 5). This analysis identified four clusters with multiple pathogen races suggesting that there is not an association with virulence genes. But the two isolates from Juana Diaz, races 63-55 and 47-19, cluster within one clade (bootstrap = 79) as observed in the previous phylogenetic tree.

DNA of the endophytic bacterium *A. xylosoxidans* was found in fourteen isolates of *P. griseola* from Puerto Rico, Honduras and Guatemala. The bacterium was detected in ten isolates from Puerto Rico collected in both Juana Diaz and Isabela (Table 4). These isolates included seven races (63-31, 63-23, 31-55, 63-07, 31-07, 47-19, and 58-15) that showed low to mild virulence against the common bean differential lines. Moreover, the three most frequent races (63-23, 63-07 and 63-31) include isolates with and without the presence of the bacteria. Remarkably, the bacterium was not detected in the most virulent isolate (63-63), suggesting a possible role of the bacterium in virulence of *P. griseola.*

Discussion

Phylogenomic, population genetic structure, principal component and a phylogenetic tree based on Fst analyses of *P. griseola* isolates from Guatemala, Honduras, Puerto Rico and Tanzania all confirm the existence of Andean and Middle American populations. The Andean and Middle American populations of ALS have previously been differentiated using microsatellites, RAPDs, isoenzyme markers and nuclear genes (Crous et al., 2006; Guzman et al., 1995; Mahuku et al., 2002; Pastor-Corrales and Jara, 1995; Pastor-Corrales et al., 1998; Serrato-Diaz et al., 2020). However, the limited resolution of these previous studies did not allow for identification of subpopulations within the Middle American population. Herein, genomic analyses revealed the existence of three subpopulations in the Middle American group. 3RADseq and analyses as we have described could be extended to identify the genetic diversity of *P. griseola* in all countries that produce common beans.

Genetic diversity in fungi can be acquired in different ways, through mutations, recombination, horizontal gene transfer, transposable elements and dispersal (Fouché et al. 2018). Several authors have suggested that transposable elements (TEs) have an important role in fungal genome expansion and evolution (Dubin et al., 2018; Oggenfuss et al., 2020). For instance, TEs can provide genetic variation through chromosomal rearrangements, disruption of genes and gene order (duplicating/deleting genes or altering gene expression) and effector gene diversification (Dubin et al., 2018). In this study, TEs were found in the *P. griseola* genome, suggesting that they may play a role in its genome diversification. In addition, genetic variation within Puerto Rican *P. griseola* populations was low and could not be associated with pathogen virulence. Previous studies have found that TEs are implicated in gene expression variation through chromosomal rearrangements, the disruption of genes or the methylation of adjacent genes (Dubin et al., 2018). The actual role of TEs in the *P. griseola* genome, and the association of virulence genes with races, could not be determined in this study due to the small size of the contigs and low genome coverage (3RADseq). The information found in this study suggests that whole genome sequencing and transcriptional analysis of the *P. griseola* isolates could elucidate the role of TEs in pathogen variation, virulence genes in the *P. griseola* genome, and their interaction with resistance genes in common beans.

The virulence analysis of 27 Puerto Rican isolates against the 12 common bean differential plants resulted in the identification of 10 *P. griseola* races (63-07, 63-15, 63-23, 63-31, 31-07, 31-55, 47-19, 58-15, 63-55 and 63-63). The Andean population (*P. griseola* f. *griseola*) infects only Andean common bean genotypes, while the Middle American population (*P. griseola* f. *mesoamericana*) infects both Andean and Middle American genotypes (Guzman et al., 1995; Pastor-Corrales et al., 1998). The infections of both Andean and Middle American common bean differential plants in this study confirmed the previous identification of the Middle American population of *P. griseola* isolates in Puerto Rico (Jara et al.,1998; Serrato-Diaz et al., 2020). Several *P. griseola* races have been identified worldwide. Studies with isolates from 23 countries identified 120 races of *P. griseola* of which 68 were unique to Latin America and 31 were unique to Africa (Jara et al., 1998). Five races 63-23, 63-15, 63-31, 31-55 and 63-63 found in Puerto Rico have also been found in three Latin America countries including Brazil (31-55, 63-23, 63-31, 31-55), Colombia and Honduras (31-55, 63-15, 63-63) and have been used to detect several resistance genes in common bean genotypes (Nay et al., 2019).

Common bean is thought to have been introduced into the Caribbean and Africa at about the same time (about 400 to 500 years ago) (Myers et al., 2022), however, the Tanzanian isolates in the Middle American population showed higher genetic diversity than that found in Puerto Rican isolates. In Tanzania, Andean and Middle American common bean cultivars are planted in close proximity to each other, leading to diversity in the *P. griseola* population (Andean, Afro-Andean and Middle American) (Chilagane et al., 2016; Serrato-Diaz et al., 2020). Likewise, the genetic diversity found in the Guatemala/Honduras population was greater than that observed in the Puerto Rico population. This lower diversity in Puerto Rico could be attributed to its geographic isolation, limited production of common bean (301 MT from 320 ha) (Beaver et al., 2020) and limited number of bean types consumed. In contrast, the proximity and trade between Honduras and Guatemala, the presence of multiple hosts (largely the Mesoamerican race of the Middle American gene pool) and the larger production [162,197 ha for Honduras and 253,330 ha for Guatemala (Fewsnet, 2019; Reyes et al., 2023)] facilitates the pathogen's dispersal and gene flow between populations.

The genomic sequencing of *P. griseola* revealed the presence of an endophytic bacterium (*A. xylosoxidans*) in mildly virulent isolates, but not in a highly virulent isolate (63-63), suggesting a possible role of the bacterium in the virulence of *P. griseola.* The bacterium has been reported as a biological control agent for various plant pathogenic fungi (de Fátima Dias et al., 2022;

Dhaouadi et al., 2019; Mohamadpoor et al., 2022). The bacterium has also been found to be an endophyte in healthy leaves and roots of common bean; healthy roots of *Musa* spp., and wheat. (Chowhan et al., 2023; Jha and Kumar, 2009; Mohamadpoor et al., 2022). Therefore, it is possible that a symbiosis between the bacterium and *P. griseola* reduces the virulence of the fungus. For instance, arbuscular mycorrhizal fungi (AMF) and their immediate surroundings (hyphosphere) are colonized by specialized microbial communities which are directly recruited by the fungus (Zhou et al., 2020). The bacteria facilitate the symbiosis between the plants and the fungi and protect them from other fungi and antifungal compounds (Nazir et al., 2014). The bacterial community benefits from AMF mycelial exudates that increase bacterial growth and vitality. Likewise, the fungus *Rhizopus microsporus*, the causal agent of rice seedling blight, and its endosymbiotic bacterium *Mycetohabitans rhizoxina,* have a symbiosis where the bacterium synthesizes the phytotoxic metabolite rhizoxin which is a crucial virulence factor for rice colonization by *R. microsporus* (Partida-Martinez and Hertweck, 2005; Lackner et al., 2011). Moreover, *M. rhizoxina* secreted molecules that evade the predation of *R. microsporus* by amoebae (*Protostelium aurantium*) and the fungivorous nematode *Aphelenchus avenae* (Richter et al., 2022). This is the first report of an association between *P. griseola* and *A. xylosoxidans*. It is possible that the absence of the bacterium in some isolates was related to the low sequence coverage of the 3RADseq genotyping. However, we hypothesize *P. griseola* uses the bacterium for "clearance" or "camouflage" against antimicrobials secreted by the common bean because the bacteria promotes plant growth (Mohamadpoor et al., 2022). Thus, *P. griseola* may take advantage of this attribute to go unnoticed by the antimicrobials secreted by common bean plants. Simultaneously, the bacterium may use the fungus to facilitate colonization of common bean or to survive abiotic stress inside the mycelia or conidia of *P. griseola*. Since the bacterial infection is not lethal to *P. griseola,* symbiosis could lead to a reduction in its virulence.

Common bean breeding programs can use both the pathogen diversity information and methods from this study, and the host resistance gene structure, to determine which isolates should be used for the evaluation of germplasm for resistance to ALS. In common beans, a number of genes have been identified that provide resistance to specific isolates of *P. griseola*, a common R-Avr relationship in co-evolved species. *Phg-1*, *Phg-4*, and *Phg-5* are resistance genes from the Andean gene pool, whereas *Phg-2* and *Phg-3* are from the Mesoamerican gene pool. 'AND 277' has *Phg-1*; 'Cornell 49-242', 'Mexico 54' and 'BAT 332' have *Phg-2*; and 'Ouro Negro' has *Phg-3*. *Phg-4* and *Phg-5* are present in Andean common bean 'G5686. 'Ouro Negro' has broad resistance to pathotypes in Brazil while the small red 'G 10474' shows single gene resistance to pathotype 63-63 (Nay et al., 2019). Use of resistance genes from one gene pool to provide resistance in the other gene pool and use of tightly linked molecular markers has been proposed and implemented as an effective breeding strategy (Nay et al., 2019). The *P. griseola* races identified herein can be used to determine the presence of key resistance genes in bean germplasm and to identify new sources of resistance based on virulence patterns.

This study identified three new *P. griseola* subpopulations in the Middle American clade, identified 10 different *P. griseola* races in Puerto Rico, and associated the endophytic bacterium *A. xylosoxidans* with *P. griseola*. The *de-novo* assembly of 42,214 contigs led to the identification of several putative virulence genes and transposable elements in the *P. griseola* genome. The genetic variation of virulence genes could not be associated with *P. griseola* races found in Puerto Rico. However, we observed that the isolates of *P. griseola* with lower virulence contain the endophytic bacterium *A. xylosoxidans*. Understanding plant-pathogen interactions is imperative for the control of ALS and the results presented herein provide a better understanding of this interaction based on pathogen genetic diversity, virulence, and the presence of an endophytic bacterium. Common bean breeding programs can use the information on pathogen diversity in determining which isolates should be used for the evaluation of germplasm for ALS resistance, the key resistance genes for introgression for targeted breeding, and thus the development of new cultivars with broader resistance.

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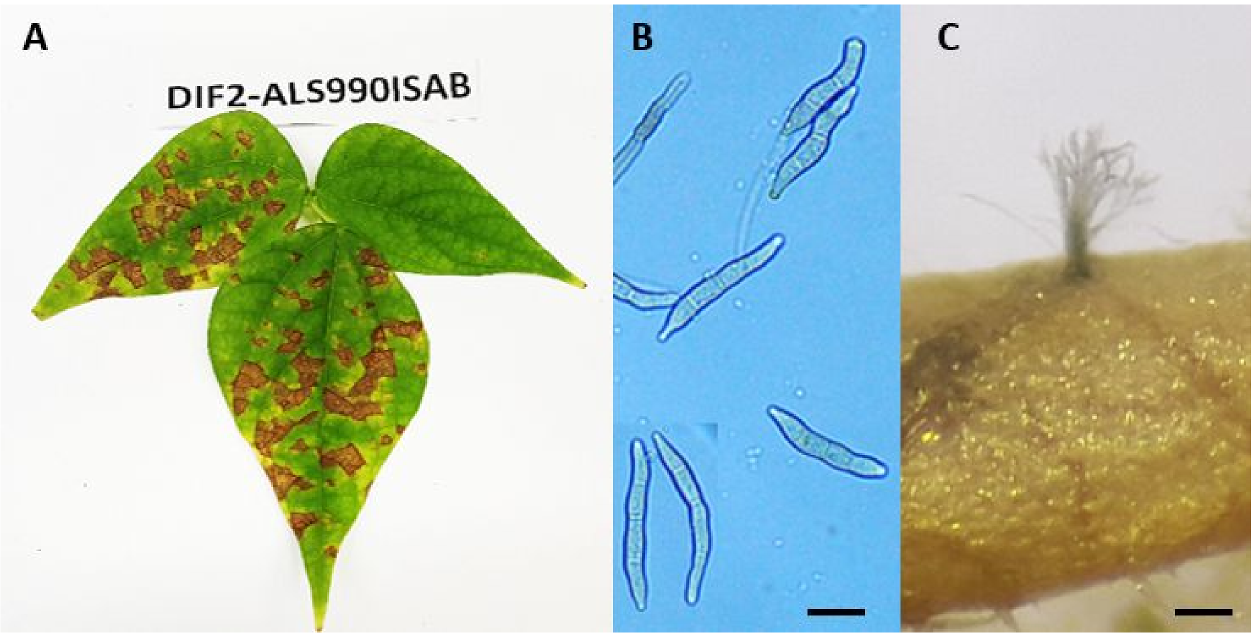

Fig. 1. Angular leaf spot (ALS) on common bean. **A.** ALS symptoms on common bean differential line G11796. **B.** Conidia of *Pseudocercospora griseola* and **C.** Fasciculate conidiophores. Scale bars: B = 25 µm and C = 150 µm.

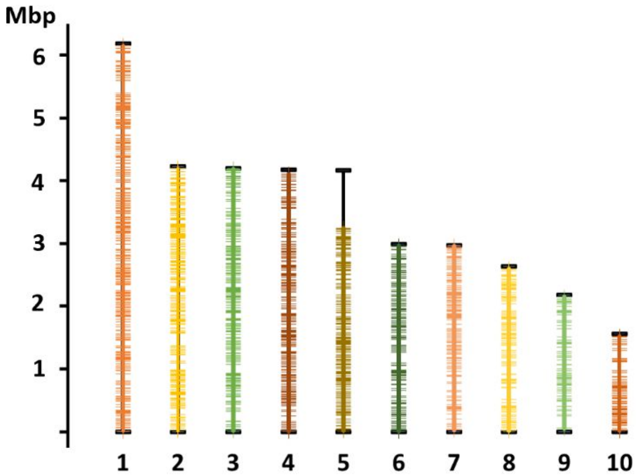

Fig. 2. Genome-wide distribution of 2,879 contigs of genomic sequence from *Pseudocercospora griseola* aligned against the *Cercospora beticola* genome.

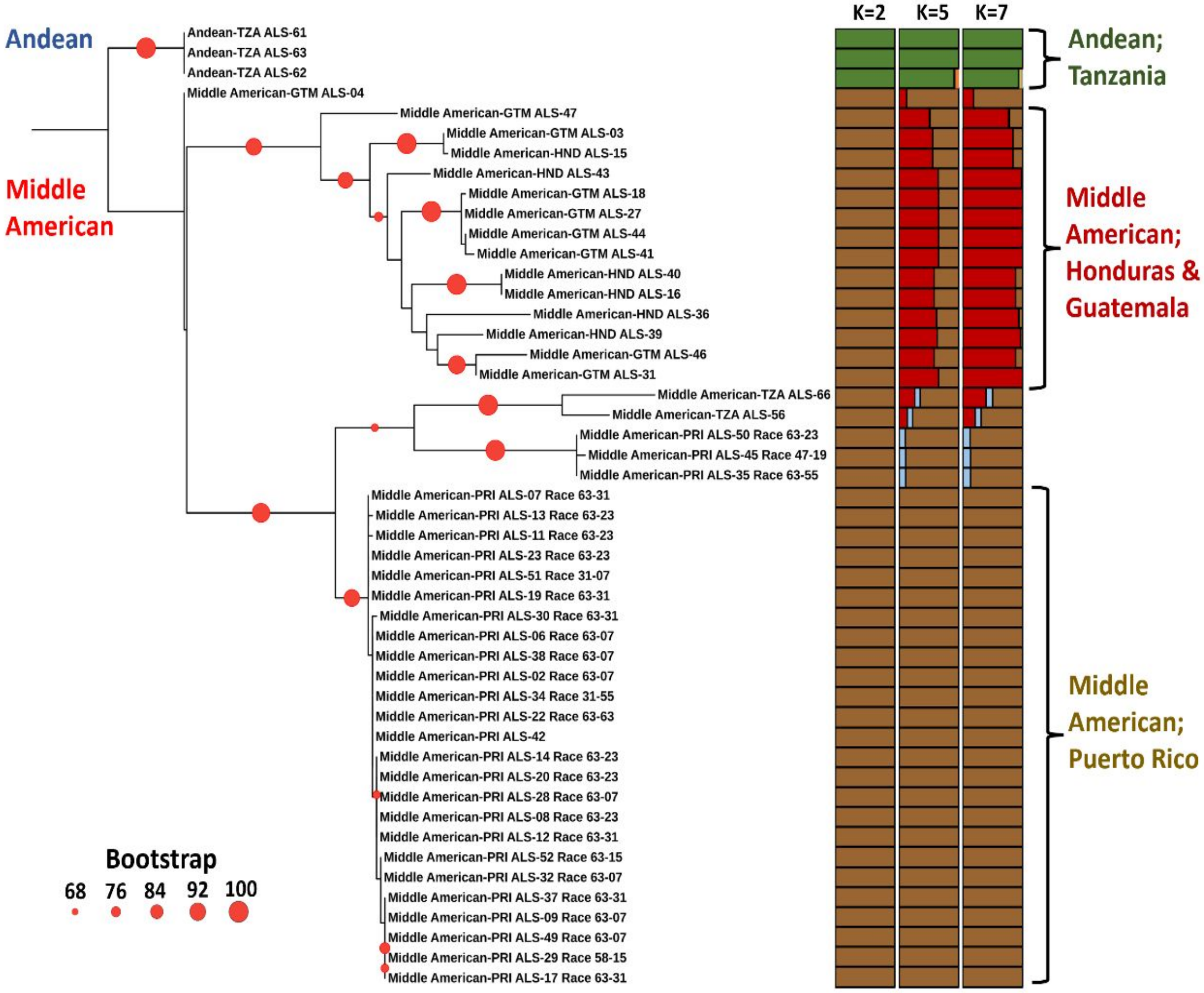

Fig. 3. Maximum likelihood tree (left) and population structure (right) based on 1,260 SNPs for 48 isolates of *Pseudocercospora griseola* collected in Tanzania (TZN), Guatemala (GTM), Honduras (HND) and Puerto Rico (PRI). Andean and Middle American refers to common bean (*Phaseolus vulgaris*) gene pools.

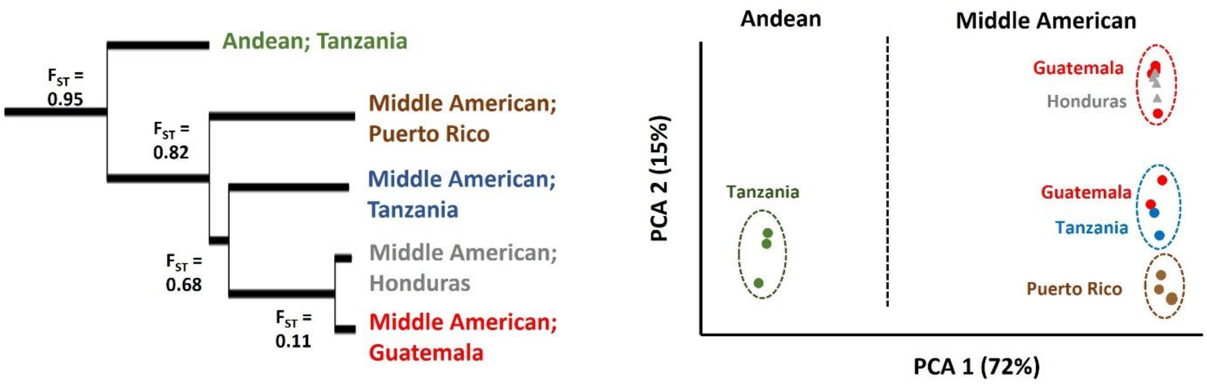

Fig. 4. Neighbor-joining tree (left) based on the F_{ST} genetic differentiation of 48 isolates of *Pseudocercospora griseola* collected in Tanzania (TZN), Guatemala (GTM), Honduras (HND) and Puerto Rico (PRI) using 1,260 SNPs. Principal component analysis (right). Each dot represents multiple isolates; dot color is consistent with the tree at left.

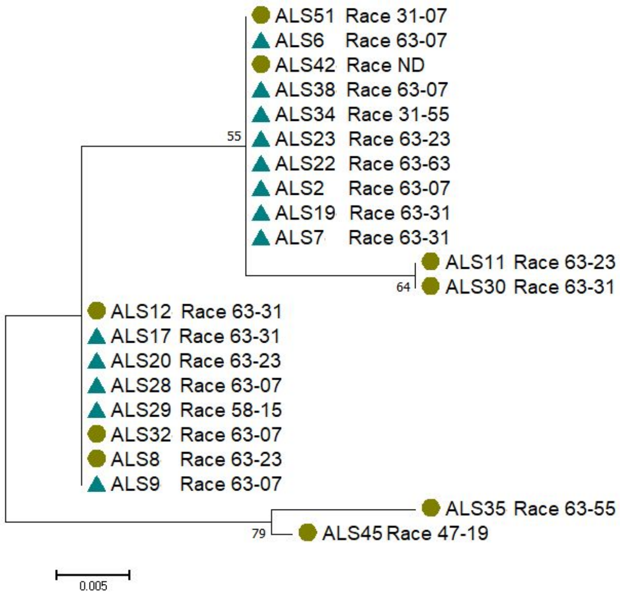

Fig. 5. Virulence vs. phylogeny._Maximum likelihood tree for 22 isolates of *Pseudocercospora griseola* collected in Isabela and Juana Diaz, Puerto Rico based on the analysis of virulence genes. Pathotype designation (races) based on virulence patterns against the 12 common bean differential lines. Green triangles show isolates from Isabela; olive circles show isolates from Juana Diaz. Bootstrap values >50% are shown at nodes.

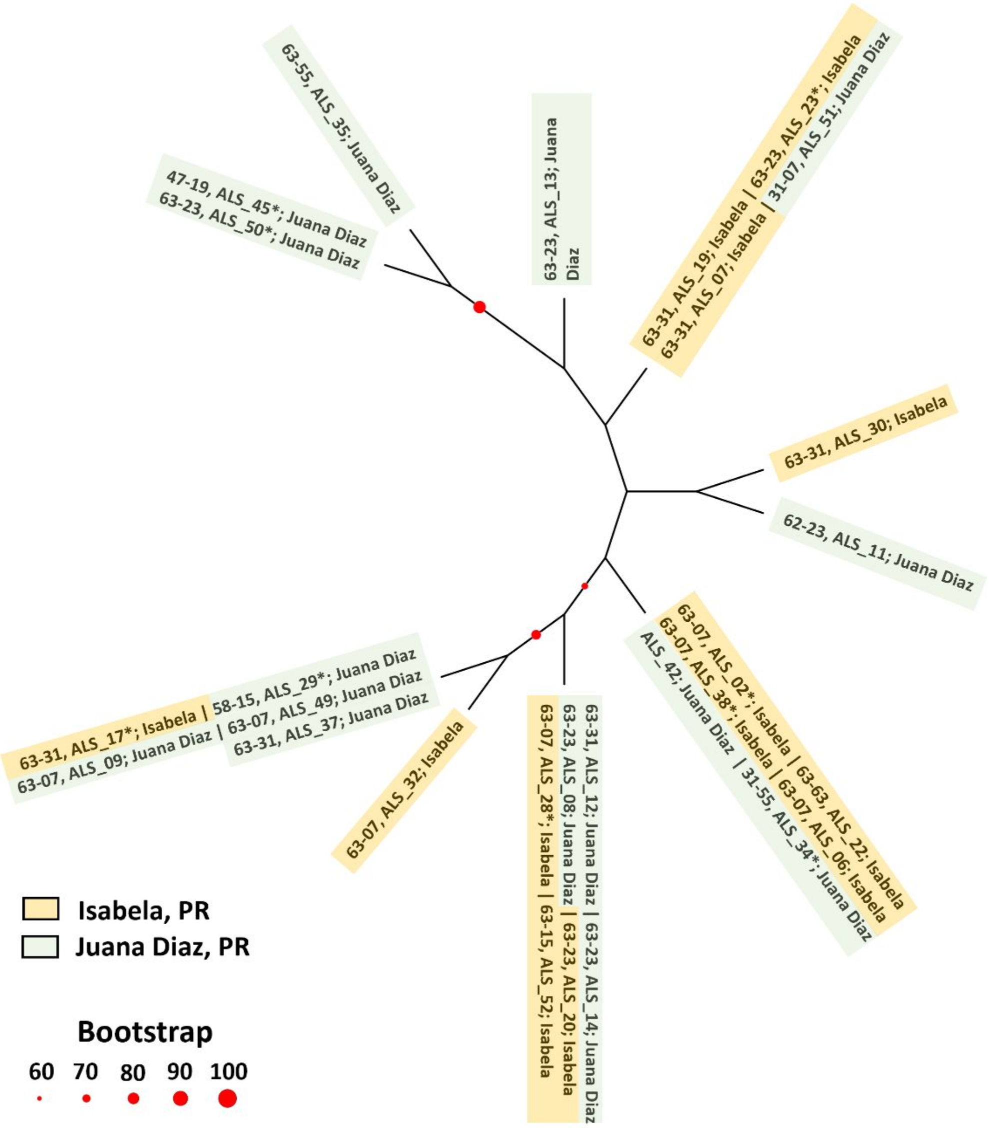

Fig. 6. Association among pathotype, phylogeny and location in *Pseudocercospora griseola* isolates from Puerto Rico. Maximum likelihood tree for 28 isolates of *P. griseola* collected in Isabela and Juana Diaz, Puerto Rico based on the analysis of 59 SNPs. Pathotype designation based on virulence patterns against 12 common bean differential lines.

TABLE 1. Origin, formae and gene pools of the bean hosts (*Phaseolus* spp.) of *Pseudocercospora griseola* isolates from Honduras, Guatemala, Puerto Rico and Tanzania.

Isolate	**Collection date**	**Location**	**Formae**	**Host**
ALS4 = FRIGUAT1C	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS18 = FRIGUAT6C	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS27 = FRIGUAT6C	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS31 = FRIGUAT1B	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS41 = FRIGUAT6B	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS44 = FRIGUAT5B	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS46= FRIGUAT1D	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS47 = FRIGUAT3D	2014	Guatemala	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS15 = TEPZAM12A	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS16 = TEPZAM3A	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS36 = TEPZAM11B	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS39 = TEPZAM2	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS40 = TEPZAM3B	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS43 = TEPZAM4B	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS3 = TEPZAM5A	2014	Honduras	mesoamericana	*P. acutifolius* A. Gray
ALS2 = ALS4124	2014	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS6 = 1146B	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Not determined
ALS7 = 1146C	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L.
ALS17 = ALSAND14E	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS19 = ALS900ISA2	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS20 = ALSAND14P	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS22 = ALS900ISAC	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS23 = ALS900ISAA	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS28 = ALS4253	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS30 = ALS3JDP8	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS32 = ALS3JDP3	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS38 = ALS990ISAE	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS52 = ALS990ISAD	2015	Isabela - Puerto Rico	mesoamericana	*P. vulgaris* L. Andean cultivar
ALS8 = ALS1JDP5	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS9 = ALS2JDP2	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS11 = ALS3JDP1	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS12 = ALS3JDP2	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS13 = ALS3JDP3	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS14 = ALS3JDP6	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS29 = ALS9029JD1	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS34 = ALS14FNS1	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS35 = ALS14FNS3	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS37 = ALS3JDP2	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS42 = ALS9029JD2	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS45 = ALS14FNS2	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS49= ALS9029JD3	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS50 = ALS1JDP5	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS51= ALS1JDP7	2015	Juana Diaz - Puerto Rico	mesoamericana	*P. vulgaris* L. Middle American cultivar
ALS56 = LUSK2	2015	Tanzania - Africa	mesoamericana	*P. vulgaris* L. Not determined
ALS61 = ALS82	2014	Tanzania - Africa	Andean	*P. vulgaris* L. Not determined
ALS62 = ALS13	2014	Tanzania - Africa	Andean	*P. vulgaris* L. Not determined
ALS63 = ALS16	2014	Tanzania - Africa	Andean	*P. vulgaris* L. Not determined
ALS66 = ALS78	2014	Tanzania - Africa	mesoamericana	*P. vulgaris* L. Not determined

TABLE 2. Putative virulence genes identified in the *Pseudocercospora griseola* genome. For sequences in catalog # see Table S1

Gene	Catalog #	Enzymatic activity	role	Fungi associated	References
Glycoside Hydrolases 1	1803, 1583, 3031 10199, 13060, 29900, 33025, 37393	β-glucosidase	Cellulose degrading enzymes	*Magnaporthe oryzae*	Quoc and Chau, 2017
Glycoside Hydrolases 2	1383, 3612, 7935, 8678, 20406, 40706	β-mannosidase β-galactosidase	Hemicellulose degrading enzymes	*M. oryzae,* *Colletotrichum fructicola*	Quoc and Chau, 2017 Seifbarghi et al., 2017 Zhang et al. 2018
Glycoside Hydrolases 3	1667, 1803, 7288, 7933, 10683, 12055, 13060, 29900, 35683, 37393	β-glucosidases, β-N-acetylglucosaminidase	Cellulose degrading enzymes	*M. oryzae*	Quoc and Chau, 2017
Glycoside Hydrolases 5	3851, 5539, 8778, 13241	Endoglucanases	Penetration of the cell wall, cellulose degrading enzymes	*Macrophomina phaseolina*	Wang and Jones. 1995
Glycoside Hydrolases 7	4272, 16216	Endo-β-1,4-glucanase	Virulence factor, cellulose degrading enzymes	*M. oryzae,*	Van et al., 2012
Glycoside Hydrolases 10	2323, 5427, 8737, 35763	Endo-1,4-β-xylanases	Virulence factor	*M. oryzae*	Nguyen et al., 2011
Glycoside Hydrolases 18	1223, 9037, 11575, 37200, 40573,	Chitinases	Virulence factor, growth, suppression of host immunity, pathogen wall modification	*M. oryzae, C. fructicola*	Han et al., 2019
Glycoside Hydrolases 27	512, 4419, 5386, 8251, 8588, 13037, 13446, 19452	α-galactosidase	Hemicellulose degrading enzymes	*M. oryzae*	Quoc and Chau, 2017

Glycoside Hydrolases 28	6782, 30397	Polygalacturonases	Pectin degrading enzymes, virulence factor	*Sclerotinia sclerotirum, C. fructicola,*	Seifbarghi et al., 2017; Zhang et al., 2018;
Glycoside Hydrolases 29	2249, 8887	α-L-fucosidase	Hemicellulose degrading enzymes	*M. oryzae*	Quoc and Chau, 2017.
Glycoside Hydrolases 31	4453, 5386, 10149, 11201, 13037, 13493, 19452, 19831, 20419, 20356	α/β-glucosidase	Normal growth and pathogenicity Cell wall degrading enzymes		
Glycoside Hydrolases 35	7082, 9164, 18833, 28721, 35987,	β-xylosidase	Hemicellulose degrading enzymes	*Magnaporthe oryzae,*	Quoc and Chau, 2017
Glycoside Hydrolases 36	368, 10680, 13486, 41516	α-galactosidase	Hemicellulose degrading enzymes	*M. oryzae,*	Quoc and Chau, 2017;
Glycoside Hydrolases 43	844, 7992, 10150, 11380, 20421	Endo-β-1,4-xylanases xylosidase/arabinosidases	Xylan degradation enzymes	*M. oryzae*	Nguyen et al., 2011
Glycoside Hydrolases 53	2453, 32468	Arabinogalactan endo-beta-1,4-galactanase, endo-1,4-β-galactosidase	Hemicellulose degrading enzymes	*C. fructicola, M. oryzae*	Quoc and Chau, 2017, Zhang et al., 2018
Glycoside Hydrolases 55	283, 3851, 8778, 10666, 13241, 19201, 36008,	Exo-β-1,3-glucanase	Plant immunity suppression, reduction in virulence	*M. oryzae,*	Liu et al. 2023a
Glycoside Hydrolases 64	16216	Endo-β-1,3-glucanase	Plant immunity suppression	*C. fructicola*	Zhang et al., 2018
Glycoside Hydrolases 78	5646, 34601	α-L-rhamnosidase	Pectin degrading enzymes	*M. oryzae*	Quoc and Chau, 2017

vanillin dehydrogenase	8556, 13224, 14485	Vanillin dehydrogenase	Degradation of lignin-derived aromatic compounds	*Trametes versicolor* *Gelatoporia subvermispora*	Del Cerro et al., 2021
Chitin synthases	615, 1715, 6742, 7546, 10204, 10395, 11029, 11046, 13560, 14814, 23518, 31988	CHS1, CHS2, CHS4, CHS6 CHS8	Virulence factor, normal growth and pathogenicity	*Magnaporthe oryzae*, *Botrytis cinerea*	Kong et al., 2012; Morcx et al., 2013
Salicylate hydroxylase	1866, 6106, 30997	salicylate 1-hydroxylase	salicylic acid degrading enzyme	*F. graminearum*	Rocheleau et al., 2019
Transporters ABC, MFS	224, 317, 584, 805,1038, 1118, 1242, 1389, 1836, 2011, 3765, 4542, 6076, 6077, 6125, 7703, 7548, 7651, 8016, 8421, 9274, 9708, 10289, 10888, 11131, 11404, 11875, 15073, 17409, 17454, 18187, 19495, 20294, 20363, 30031, 34367, 34411, 34787, 34535, 34813, 38219, 38369, 40591, 40640	ATP-binding cassette (ABC) transporter Major facilitator superfamily (MFS) transporters Multidrug transporters	Detoxification Toxin secretion	*S. sclerotiorum* *F. graminearum*	Seifbarghi et al., 2017; O'Mara et al., 2023

Toxins	1323, 3006, 7278, 7646, 10022, 13334, 17496, 39447	homologous to AAL, AMT, AK, HC toxins, Aflatoxins	Toxins, cell degradation. Suppression of host defenses. Disruption of mitochondrial activity	*Cochliobolus carbonum* *Bipolaris maydis* (syn. *C. heterostrophus*)	Stergiopoulos et al., 2013 Meena and Samal 2019.
polyketide synthases (PKS) non-ribsosomal peptide synthetases (NRPS) PKS-NRPS hybrid	678, 694, 1159, 1126, 2521, 3150, 3265, 4443, 4869, 6688, 7087, 7684, 8083, 8451, 8543, 9423, 9542, 9658, 10311, 11599, 11663, 13595, 17423, 29288, 30039, 32236, 34479, 34894, 35306, 35911, 36066, 37089, 36444, 38128, 38362, 41668	polyketide synthases non-ribsosomal peptide synthetases	Fungal secondary metabolites	*Pseudocercospora fijensis*	Thomas et al., 2021
protein kinases: PAK, Mitogen-activated protein	110, 313, 694, 722, 921, 962, 2818, 4092, 6537, 7087, 7167, 7506, 8079, 8656, 9524, 9636, 9731, 10320, 11475, 11515, 11635, 11762,	HOG, CLA4, Chm1, PKH1, STE20, YAK1, hexokinase, KIN4,	Hyphal growth, pathogenicity, osmoregulation, oxidative response, fungicide sensitivity, sexual reproduction.	*Verticillium dahliae* *Botrytis cinerea* *Cochiobolus sativus* *M. graminicola* *F. oxysporum f. sp. niveum* *Puccinia striiformis f. sp. tritici*	Li et al., 2004 Jiao et al., 2017 Jiang et al., 2018 Wang et al., 2024

	11872, 15072, 20253, 20409, 29030, 30135, 30215, 30636, 32236, 33371, 38128, 40143, 40889, 41424, 41387				
Metalloproteases, M35	208, 7898, 7924, 11596	Deuterolysin	Virulence factor	*Fusarium odoratissimum* Syn. *F. oxysporum f. sp. cubense*	Zhang et al., 2021
Deoxyhypusine hydroxylase/synthase	1369, 2212, 8949, 10002, 11122, 29654	deoxyhypusine hydroxylase/synthase	Virulence factor	*F. graminearum*	Martinez-Rocha et al., 2016

TABLE 3. Genetic profiles, private alleles, and average (X) genetic distance for four populations of *Pseudocercospora griseola* collected from common bean in Tanzania (TZN), Guatemala (GTM), Honduras (HND) and Puerto Rico (PRI).

				Number of SNPs		
Gene pool	Origin	n	Genetic profiles	Total	Private	X and S.D.
Andean	TZN	3	3	51	51	0.32 ± 0.24
Middle American	PRI	32	10	59	43	0.79 ± 0.02
	GTM & HND	15	14	104	52	0.70 ± 0.01
	TZN	2	2	28	7	*n.a.*

TABLE 4. Putative genes with known function of populations of *Pseudocercospora griseola* collected in Tanzania (TZN), Guatemala (GTM), Honduras (HND) and Puerto Rico (PRI). Black shading represents genes that are found in a single population, dark grey shading represents shared genes between populations, and light grey shading represents the absence of the gene.

	Middle American			Andean
Putative genes	**PRI**	**HND and GTM**	**TZN**	**TZN**
480_NRPS like enzyme	■	░	░	░
714_Histidine permease	■	░	░	░
1019_Amidase	■	░	░	░
1320_PHD finger protein	■	░	░	░
1549_Kinase like protein	■	░	░	░
1579_Beta-ureidopropionase 1	■	░	░	░
1823_Cytochrome P450 monooxygenase	■	░	░	░
2093_Regulator of Ty1 transposition protein 10	■	░	░	░
630_Tricalbin-1	░	■	░	░
921_Serine/threonine protein Kinase KSG1	░	■	░	░
967_DNA/RNA polymerase	░	■	░	░
1161_Beta-ketoacyl synthase	░	■	░	░
1810_NRPS-like enzyme	░	■	░	░
2112_Calcium transporting ATPase1	░	■	░	░
2935_Transcription initiation factor TFIIE	░	■	░	░
5498_Glutathione S transferase kappa 1	░	■	░	░
6573_Integrin	░	■	░	░
844_Glycoside family 43	░	░	■	░
423_ Nucleolar GTP-binding protein	░	░	░	■
636_DNA polymerase gamma	░	░	░	■
1397_PH response	░	░	░	■
1475_Glutamate cysteine ligase	░	░	░	■
1676_Myosin phospatase Rho interacting protein	░	░	░	■
2852_ Phosphatidate cytidylyltransferase	░	░	░	■
5547_CAZyme family AA1	░	░	░	■
6736_Coenzyme synthase	░	░	░	■
7350_Argonaute-binding protein 1	░	░	░	■
7589_Serine/threonine protein kinase CEK1	░	░	░	■
261_Sterol reductase	▓	▓	░	░
1605_Salicylaldehyde dehydrogenase	▓	▓	▓	░
1252_Glucan endo-1,3 beta glucosidase	░	▓	▓	░
1468_transcription factor IIA	▓	░	▓	▓

TABLE 5. Race designation of 27 isolates of *Pseudocercospora griseola* collected in Isabela (ISA) and Juana Diaz (JND), Puerto Rico based on their virulence patterns using 12 common bean differential lines and presence of *Achromobacter xylosoxidans*. Isolates are ordered from the most virulent (63-63) to the less virulent isolate (31-07).

			Differential Lines												
Isolate	Loc.	Achromobacter[1]	G11796	Montcalm	Pan72	G2858	Don timoteo	Bolon Bayo	Amendoin	Flor de Mayo	G5686	BAT332	Mexico 54	Cornell 49-242	Strain
ALS_22	ISA	-	+	+	+	+	+	+	+	+	+	+	+	+	63-63
ALS_35	JND	-	+	+	+	+	+	+	+	+	+	+	-	+	63-55
ALS_07	ISA	-	+	+	+	+	+	+	+	+	+	+	+	-	63-31
ALS_19	ISA	-	+	+	+	+	+	+	+	+	+	+	+	-	63-31
ALS_30	ISA	-	+	+	+	+	+	+	+	+	+	+	+	-	63-31
ALS_12	JND	-	+	+	+	+	+	+	+	+	+	+	+	-	63-31
ALS_37	JND	-	+	+	+	+	+	+	+	+	+	+	+	-	63-31
ALS_17	ISA	+	+	+	+	+	+	+	+	+	+	+	+	-	63-31
ALS_20	ISA	-	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_11	JND	-	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_13	JND	-	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_14	JND	-	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_08	JND	-	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_23	ISA	+	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_50	JND	+	+	+	+	+	+	+	+	+	+	+	-	-	63-23
ALS_52	ISA	+	+	+	+	+	+	+	+	+	-	-	+	-	63-15
ALS_06	ISA	-	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_38	ISA	-	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_49	JND	-	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_09	JND	-	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_02	ISA	+	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_28	ISA	+	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_32	ISA	+	+	+	+	+	+	+	+	+	+	-	-	-	63-07
ALS_29	JND	+	+	+	+	+	-	-	+	+	+	-	+	-	58-15
ALS_45	JND	+	+	+	+	+	+	+	-	-	+	+	-	-	47-19
ALS_34	JND	+	+	+	+	+	+	+	+	+	-	+	-	+	31-55
ALS_51	JND	-	+	+	+	+	+	+	+	+	-	-	-	-	31-07

[1] Presence (+) or absence (-) of genomic sequence of *Achromobacter xylosoxidans* in the sequencing analysis of the isolates.